

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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SOLICITUD

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METODO PARA TRATAR VASCULITIS

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71. SOLICITANTE

GENENTECH, INC.

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SOUTH SAN FRANCISCO, CALIFORNIA, ESTADOS UNIDOS

74. APODERADO

JUAN PABLO CADENA SARMIENTO

22. SANTAFE DE BOGOTA, D.C.

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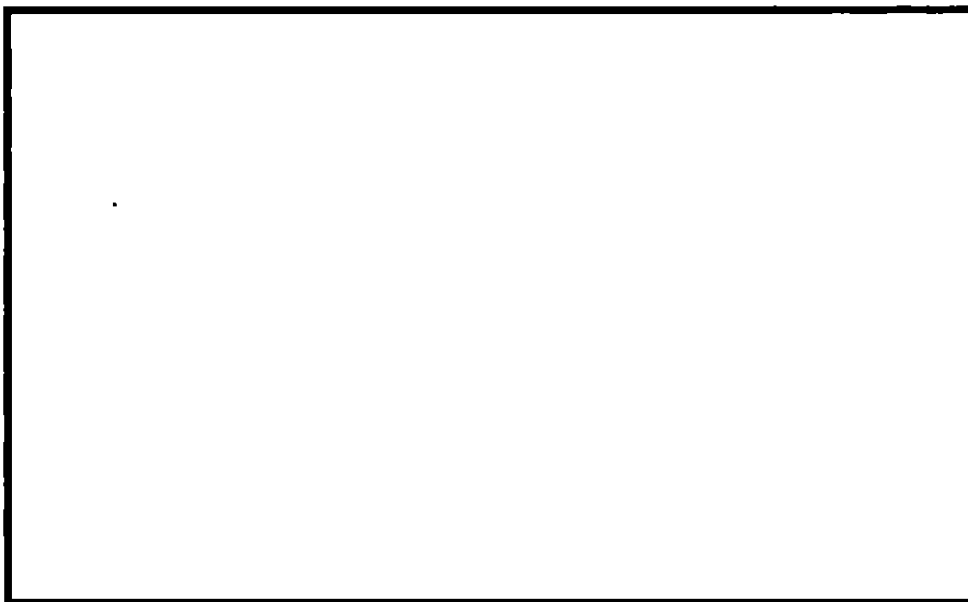
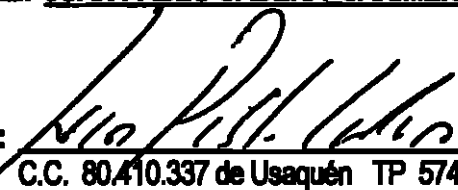
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FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
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1 SOLICITUD DE:			
☒ Patente de Invención.		☐ Patente de Modelo de Utilidad.	
☒ Capítulo I.		☐ Capítulo II.	
2 SOLICITANTE (71)	Nombre: GENENTECH, INC. Dirección: 1 DNA Way, South San Francisco, CA 94080-4990, US Nacionalidad o Domicilio: Estados Unidos Lugar de Constitución: Estados Unidos Teléfono: Fax: E-mail:		IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número _____
3 REPRESENTANTE O APODERADO	Nombre: JUAN PABLO CADENA SARMIENTO Dirección: Carrera 7 # 71 - 21 Torre B - Piso 4 Teléfono: 744-2200 Fax: 744-2700 E-mail:		IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número <u>80.410.337 TP 57492</u>
4 INVENTOR (ES) (72)	Nombre: Paul G. BRUNETTA Dirección: 1257 Hampshire Street, San Francisco, California 94110, Estados Unidos de América Nacionalidad o Domicilio: Estados Unidos de América		
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2020-F06 (01-11-15)

- ☒ Comprobante de pago de la tasa de presentación de la solicitud. \$482.000
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso. Fotocopia Autenticada
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ 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.
- ☒ Otros: Listado de Secuencias
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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(54) Title: METHOD FOR TREATING VASCULITIS

Heavy Chain Alignment

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 10207.v16
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 10207.v511
 383
 10207.v16
 10207.v511
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(57) Abstract: A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient eligible for treatment is provided involving administering an antagonist that binds to a B-cell surface marker, such as CD20 antibody, to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month. Another method of treating ANCA-associated vasculitis in a subject eligible for treatment is provided involving administering an effective amount of an antibody that binds to a B-cell surface marker to the subject to provide an initial exposure and a subsequent exposure to the antibody within certain dosing regimens. Further provided are articles of manufacture useful for such methods.

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FR, GB, GR, HU, IE, IS, IT, LU, LV, MC, NL, PL, PT, RO, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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METHOD FOR TREATING VASCULITIS**Related Applications**

10 This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application number 60/616,104 filed October 5, 2004, the contents of which are incorporated herein by reference.

Field of the Invention

15 The present invention concerns methods for treating anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis in a subject, and kits with instructions for such uses.

Background of the Invention**Vasculitis**

20 Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, vasculitis, and lupus, among others, remain clinically important diseases in humans. As the name implies, autoimmune diseases wreak their havoc through the body's own immune system. While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) present.

25 Vasculitis is defined by inflammation of the blood-vessel wall and forms the pathological foundation of a diverse group of individual disease entities. Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, which is a common primary systemic vasculitis, includes microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome, renal-limited vasculitis (idiopathic necrotizing crescentic glomerulonephritis) (Falk *et al.* *N. Engl. J. Med.*, 318: 1651-1657 (1988)), and certain types of drug-induced vasculitis. Jennette *et al.* *Arthritis Rheum* 37:187-92 (1994); Jennette and Falk, *N. Engl. J. Med.* 337:1512-1523 (1997). The diseases mentioned above affect people of all ages but are most common in older adults in their 50s and 60s, and they affect men and women equally. Pettersson *et al.* *Clin. Nephrol.*, 43: 141-149 (1995); Falk *et al.* *Ann. Intern. Med.* 113: 656-663 (1990).

30 ANCA are specific antibodies for antigens in cytoplasmic granules of neutrophils and monocyte lysosomes, first reported in 1982. Niles *et al.*, *Arch. Intern. Med.*, 156:440-445 (1996). ANCA were originally detected by indirect immunofluorescence on ethanol-fixed neutrophils. Wiik, "Delineation of a standard procedure for indirect immunofluorescence detection of ANCA" *APMIS Suppl.* 6: 12-13 (1989). At least three different patterns of fluorescence have been distinguished: a cytoplasmic/classic pattern (cANCA) with accentuation of the fluorescence intensity in the area with the nuclear lobes, a perinuclear pattern (pANCA), and a more diffuse cytoplasmic staining pattern (atypical ANCA). Approximately 90% of the sera that produce a cANCA pattern react with proteinase 3 (PR3), a serine protease from the azurophilic granules of myeloid cells. Jennette and Falk, *N. Engl. J. Med.*, *supra*. In patients with primary systemic vasculitis predominantly affecting medium- and small-sized blood vessels, approximately 75% of the sera producing a perinuclear pattern (pANCA) react with myeloperoxidase (MPO), a myeloid lysosomal enzyme. Cohen

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Tervaert *et al.*, *Am. J. Med.* 91: 59-66 (1991). In ANCA-positive patients with other non-vasculitic diseases, often antigenic specificities are recognized. The diagnostic potential of PR3-ANCA and MPO-ANCA is now fairly well established. In a patient with signs and symptoms of vasculitis, ANCA with specificity for PR3 (PR3-ANCA) suggests a diagnosis of Wegener's granulomatosis, whereas ANCA with a specificity for MPO (MPO-ANCA) is highly sensitive for microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis, or active Churg-Strauss syndrome. Cohen Tervaert *et al.*, *Sarcoidosis Vasc. Diffuse Lung Dis.* 13: 241-245 (1996). See also Xiao *et al.*, *J. Clin. Invest.*, 110: 955-963 (2002), which describe an animal model that offers strong support for a direct pathogenic role for ANCA IgG in human glomerulonephritis and vasculitis, and Popa *et al.*, *J. Allergy Clin. Immunol.*, 103: 885-894 (1999) showing that in Wegener's granulomatosis, B-cell activation is related to active disease, whereas T-cell activation persists during remission of the disease, which points to an intrinsic disordered immune system in this disease. See also Cupps *et al.*, *J. Immunol.*, 128: 2453-2457 (1982) regarding the role of cyclophosphamide in suppressing human B lymphocyte function.

Within the spectrum of primary vasculitic syndromes, the ANCA-related syndromes form a distinct group with overlapping features. Most patients have a prodromal flu-like onset consisting of malaise, myalgias, arthralgias, fever, and weight loss. This flu-like onset appears within days to weeks before the onset of overt vasculitic or nephritic disease. Wegener's granulomatosis is differentiated from the others by the presence of necrotizing granulomatous inflammation of the upper and lower respiratory tract, which is usually accompanied by systemic necrotizing small vessel vasculitis and glomerulonephritis. Churg-Strauss syndrome is differentiated by the presence of (a history of) asthma, allergic rhinitis, systemic eosinophilia, in addition to systemic vasculitis with or without glomerulonephritis. Microscopic polyangiitis is characterized by necrotizing and/or crescentic glomerulonephritis and a multi-system vasculitis involving small vessels. Microscopic polyangiitis shares many features with Wegener's granulomatosis and Churg-Strauss syndrome, but lacks necrotizing granulomatous inflammation of the respiratory tract and asthma. Jennette *et al.*, *Arthritis Rheum.*, *supra*. In idiopathic necrotizing and/or crescentic glomerulonephritis the vasculitic process is limited to the kidneys. Because the treatment of patients with microscopic polyangiitis or Wegener's granulomatosis is essentially the same when there is major organ injury, it is unnecessary to distinguish conclusively between these closely related variants of ANCA-associated vasculitis before initiating treatment. Jennette *et al. Arthritis Rheum.*, *supra*.

Before treatment became available, patients with generalized Wegener's granulomatosis had a median survival of five months. In the early 1970s, Fauci and Wolff introduced a regimen combining daily cyclophosphamide therapy given for one year after remission was achieved with prednisone therapy initiated at a dose of 1 mg per kilogram of body weight per day and tapered on an alternate-day schedule. This treatment has reproducibly been found to induce remission in 80 to 100 percent of patients and can result in long-term survival. In fact, prolonged immunosuppressive therapy (greater than 1 year) with cyclophosphamide and steroids is effective in inducing disease remission and preventing early relapses in most vasculitic disorders. Balow *et al.*, "Vasculitic diseases of the kidney, polyarteritis, Wegener's granulomatosis, necrotizing and crescentic glomerulonephritis, and other disorders." In: Schrier and Gottschalk (eds): *Diseases of the kidney*, 5th edition, (Little, Brown and Company, Boston, 1993), pp. 2095-2117; Jayne *et al.*, *N. Engl. J. Med.*, 349: 36-44 (2003); Gaskin *et al.*, "Systemic vasculitis" In: Cameron *et al.*

(eds): *Oxford textbook of clinical nephrology*. (Oxford University Press, Oxford, 1992), pp. 612-636; Fauci *et al*, *Ann. Intern. Med.* 89: 660-676 (1978); Fauci *et al.*, *Ann. Intern. Med.*, 98: 76-85 (1983); Hoffman *et al.*, *Ann. Int. Med.*, 116: 488-498 (1992); and Andrassy *et al.*, *Clin. Nephrol.*, 35: 139-147 (1991).

However, when therapy is tapered and discontinued, relapses are common. In one study, in which patients with Wegener's granulomatosis were followed for a mean of eight years, relapse occurred in 50 percent of patients. Further, continuous use of cyclophosphamide to sustain remission is not recommended, since this treatment regimen is associated with severe and potentially lethal adverse effects such as the occurrence of opportunistic infections and the development of malignancies. For example, repeated courses of cyclophosphamide are associated with bone-marrow suppression, infection, cystitis, infertility, myelodysplasia, and transitional-cell carcinoma of the bladder. In some instances, such toxic effects preclude further use of cyclophosphamide. Stillwell *et al.*, *Arthritis Rheum.*, 31: 465-470 (1988); Radis *et al.*, *Arthritis Rheum.* 38: 1120-1127 (1995). Therefore, cyclophosphamide is tapered or stopped and replaced by azathioprine once remission is achieved to prevent adverse effects, a policy tested in a rigorous multi-center trial and proven to be equally effective in the follow-up for 18 months. Gaskin *et al.*, *supra*, 1992; Jayne, *Rheumatology* 39: 585-595 (2000). Azathioprine is considered less effective in inducing remission than cyclophosphamide, but its long-term toxicity is much lower. Bouroncle *et al.*, *Am. J. Med.*, 42: 314-318 (1967); Norton *et al.*, *Arch. Intern. Med.*, 121: 554-560 (1968).

Other alternative maintenance therapy regimens include methotrexate ((de Groot *et al.*, *Arthritis Rheum.*, 39: 2052-2061 (1996)), cyclosporine A (Haubitz *et al.*, *Nephrol. Dial. Transplant*, 13: 2074-2076 (1998)), mycophenolate (Nowack *et al.*, *J. Am. Soc. Nephrol.*, 10: 1965-1971 (1999)), or trimethoprim-sulfamethoxazole (Stegeman *et al.*, *N. Engl. J. Med.*, 335: 16-20 (1996)). See also Sanders, *et al.* *N. Engl. J. Med.* 349: 2072-2073 (2003). Since, however, relapses are frequently observed in ANCA-associated vasculitis, treatment in such cases has to be intensified or reinstituted. Hoffman *et al.*, *supra*; Gordon *et al.*, *Q J. Med.*, 86: 779-789 (1993); Nachman *et al.*, *J. Am. Soc. Nephrol.*, 7: 33-39 (1996); Guillevin *et al.*, *Medicine* 78: 26-37 (1999); Reinhold-Keller *et al.*, *Arthritis. Rheum.* 43: 1021-1032 (2000); Langford, *New Eng. J. Med.*, 349: 3-4 (July 2003).

Tumor necrosis factor-alpha (TNF-alpha) blockade with infliximab is a potential therapy for ANCA-associated vasculitis, both for initial therapy and in the management of refractory disease. Infliximab was effective at inducing remission in 38% of patients with ANCA-associated vasculitis and permitted reduction in steroid doses. Booth *et al.*, *J. Am. Soc. Nephrol.* 15:717-721 (2004). In addition, Stone *et al.*, *Arthritis and Rheumatism*, 44: 1149-1154 (2001) disclosed that the TNF-alpha inhibitor etanercept (ENBREL®), given 25 mg subcutaneously twice weekly in combination with standard treatment for Wegener's granulomatosis, was well-tolerated in the patients with few adverse events, but intermittently active disease (occasionally severe) was common.

Patients with Churg-Strauss syndrome usually respond to high-dose corticosteroid therapy alone, although some cases may require the addition of cytotoxic drugs. Jayne and Rasmussen, *Mayo Clin. Proc.* 72:737-47 (1997). Co-morbid conditions that accelerate vascular damage, *e.g.*, hypertension, diabetes, hypercholesterolemia, and smoking, should be appropriately controlled.

In drug-induced vasculitis, the offending agent should be stopped. Antihistamines and non-steroidal anti-inflammatory drugs help alleviate skin discomfort and reduce associated arthralgias and myalgias. Severe cutaneous disease may warrant oral corticosteroid therapy. Jennette *et al.*, *Arthritis Rheum.* *supra*.

The persistence or reappearance of ANCA is a risk factor for the development of a relapse of disease activity, suggesting a pathophysiological role *in vivo* for these autoantibodies. Stegeman *et al.*, *Ann. Intern. Med.*, 120: 12-17 (1994); De'Oliviera *et al.*, *Am. J. Kidney Dis.*, 25: 380-389 (1995); Jayne *et al.*, *Q. J. Med.*, 88: 127-133 (1995). Relapses of Wegener's granulomatosis are frequently preceded by rises in the titer of cANCA as detected by indirect immunofluorescence (Cohen Tervaert *et al.*, *Arch. Intern. Med.*, 149: 2461-2465 (1989)), and can be prevented by treatment with immunosuppressives based on rises in cANCA (Cohen Tervaert *et al.*, *Lancet*, 336: 706-711 (1990)).

For a general discussion on ANCA-associated vasculitis, see Lhote and Guillevin, *Rheum. Dis. Clin. North Am.* 21:911-947 (1995); "ANCA-associated vasculitis: occurrence, prediction, prevention, and outcome of relapses" by Maarten Boomsma, PhD Thesis, Thesis University Groningen, ISBN 90-367-1451-6 (M.M. Boomsma, Groningen, 2001) (<http://www.ub.rug.nl/eldoc/dis/medicine/m.m.boomsma/thesis.pdf>); Kamesh *et al.*, *J. Am. Soc. Nephrol.* 13:1953-1960 (2002); and Jayne, *Kidney & Blood Pressure Research* 26:231-239 (2003).

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 naïve 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 molecules 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.*). Rituximab 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 rituximab 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, rituximab has been shown to have anti-proliferative effects in tritiated thymidine-incorporation assays and to induce apoptosis directly, while other anti-CD19 and anti-CD20 antibodies do not. Maloney *et al.* *Blood* 88(10):637a (1996). Synergy between rituximab and chemotherapies and toxins has also been observed experimentally. In particular, rituximab 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 rituximab 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* 83:435-445 (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 NHL at a dose of 375 mg/m² weekly for four doses. In April 2001, the Food and Drug Administration (FDA) approved additional claims for the treatment of low-grade NHL: re-treatment (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.* 21:1746-1751 (2003); Hainsworth *et al.*, *J. Clin. Oncol.* 20:4261-4267 (2002). Also, rituximab has been used in the treatment of malignant and nonmalignant plasma cell disorders. Treon and Anderson, *Semin. Oncol.* 27: 79-85 (2000).

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.* 30:824-828 (2002). Rituximab has been reported to potentially relieve signs and symptoms of, for example, rheumatoid arthritis (RA) (Leandro *et al.*, *Ann. Rheum. Dis.* 61:883-888 (2002); Edwards *et al.*, *Arthritis Rheum.*, 46 (Suppl. 9): S46 (2002); Stahl *et al.*, *Ann. Rheum. Dis.*, 62 (Suppl. 1): OP004 (2003); Shaw *et al.* *Ann. Rheum. Dis.*, 62 Suppl 2:ii55-ii59 (2003); Weyand and Goronzy, *Ann. N.Y. Acad. Sci.* 987: 140-149 (2003); Emery *et al.*, *Arthritis Rheum.* 48(9): S439 (2003)), lupus (Eisenberg, *Arthritis. Res. Ther.* 5:157-159 (2003); Anolik *et al.*, *Arthritis Rheum.* 48: 455-459 (2003); Leandro *et al.* *Arthritis Rheum.* 46: 2673-2677 (2002); Gorman *et al.*, *Lupus*, 13: 312-316 (2004); Tomietto *et al.*, *Thromb. Haemost.* 92: 1150-1153 (2004)), immune thrombocytopenic purpura (D'Arena *et al.*, *Leuk. Lymphoma* 44:561-562 (2003); Stasi

et al., *Blood*, 98: 952-957 (2001); Saleh et al., *Semin. Oncol.*, 27 (Supp 12):99-103 (2000); Zaja et al., *Haematologica*, 87: 189-195 (2002); Zaja et al., *Haematologica* 88: 538-546 (2003); Cooper et al., *Br. J. Haematol.* 125: 232-239 (2004); Ratanatharathorn et al., *Ann. Int. Med.*, 133: 275-279 (2000)), pure red cell aplasia (Auner et al., *Br. J. Haematol.*, 116: 725-728 (2002)); autoimmune anemia (Zaja et al., *Haematologica* 87:189-195 (2002) (erratum appears in *Haematologica* 87:336 (2002); Raj et al., *J. Pediatr. Hematol. Oncol.* 26: 312-314 (2004); Zecca et al., *Blood* 101: 3857-3861 (2003); Quartier et al., *Lancet* 358: 1511-1513 (2001)), autoimmune cytopenias (Robak, *Eur. J. Haematol.* 72: 79-88 (2004)); cold agglutinin disease (Layios et al., *Leukemia*, 15: 187-8 (2001); Berentsen et al., *Blood*, 103: 2925-2928 (2004); Berentsen et al., *Br. J. Haematol.*, 115: 79-83 (2001); Bauduer, *Br. J. Haematol.*, 112: 1083-1090 (2001); Damiani et al., *Br. J. Haematol.*, 114: 229-234 (2001); Lee and Kueck, *Blood* 92: 3490-3491 (1998)), type B syndrome of severe insulin resistance (Coll et al., *N. Engl. J. Med.*, 350: 310-311 (2004)), mixed cryoglobulinemia (DeVita et al., *Arthritis Rheum.* 46 Suppl. 9:S206/S469 (2002); Zaja et al. *Haematologica* 84: 1157-1158 (1999)), myasthenia gravis (Zaja et al., *Neurology*, 55: 1062-63 (2000); Wylam et al., *J. Pediatr.*, 143: 674-677 (2003)), Wegener's granulomatosis (Specks et al., *Arthritis & Rheumatism* 44: 2836-2840 (2001)), refractory pemphigus vulgaris (Dupuy et al., *Arch Dermatol.*, 140:91-96 (2004)), dermatomyositis (Levine, *Arthritis Rheum.*, 46 (Suppl. 9):S1299 (2002)), Sjogren's syndrome (Somer et al., *Arthritis & Rheumatism*, 49: 394-398 (2003)), active type-II mixed cryoglobulinemia (Zaja et al., *Blood*, 101: 3827-3834 (2003)), pemphigus vulgaris (Dupuy et al., *Arch. Dermatol.*, 140: 91-95 (2004)), autoimmune neuropathy (Pestronk et al., *J. Neurol. Neurosurg. Psychiatry* 74:485-489 (2003); Nobile-Orazio, *Curr. Opin. Neurol.* 17: 599-605 (2004); Rojas-Garcia et al., *Neurology* 61: 1814-1816 (2003); Renaud et al. *Muscle Nerve* 27: 611-615 (2003)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli et al. *Neurology* 60(Suppl. 1) PO5.128-A395 (2003)), acquired factor VIII inhibitors (Wiestner et al. *Blood* 100: 3426-3428 (2002); and relapsing-remitting multiple sclerosis (RRMS). Cross et al. (abstract) "Preliminary Results from a Phase II Trial of Rituximab in MS" Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis, 20-21 (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, and rituximab plus cyclophosphamide (CTX). The treatment regimen of rituximab was one gram 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 was considered to be mild to moderate in severity and was 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 4.66 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.* 46:2287-2293 (2002).

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The reported safety profile of rituximab in a small number of patients with neurologic disorders, including autoimmune neuropathy (Pestronk *et al.*, *supra*), opsoclonus-myoclonus syndrome (Pranzatelli *et al.*, *supra*), and RRMS (Cross *et al.*, *supra*), was similar to that reported in oncology or RA. In an ongoing investigator-sponsored trial (IST) of rituximab in combination with interferon-beta (IFN- β) or glatiramer acetate in patients with RRMS (Cross *et al.*, *supra*), 1 of 10 treated patients was admitted to the hospital for overnight observation after experiencing moderate fever and rigors following the first infusion of rituximab, while the other 9 patients completed the four-infusion regimen without any reported adverse events.

Patents and patent publications concerning CD20 antibodies and CD20-binding molecules 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 and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); WO 2000/27433 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO 2001/10461 (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, WO 2001/74388 and 6,896,885B5 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2005/0123540 (Hanna *et al.*); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858; US 2005/0112060, and US Patent No. 6,846,476 (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.); US Patent Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721, (Kaminaki *et al.*); US Patent Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, 6,632,852, 6,893,625 (Robinson *et al.*); US Patent 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.); WO 2000/67795 (Goldenberg); US 2003/0133930 and WO 2000/74718 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO 2004/058298 (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 (Laung, S.); WO 2003/049694, US 2002/0009427, 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.*); US 2002/0136719 (Shenoy *et al.*); WO 2004/032828 and US 2005/0180972 (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.*); US 2001/0056066 (Bugalski *et al.*); WO 1995/03770 (Bhat *et al.*); US 2003/0219433 A1 (Hansen *et al.*); WO 2004/035607 (Teeling *et al.*); WO 2004/056312 (Lowman *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.*); WO 2005/016969 (Carr *et al.*); US 2005/0069545 (Carr *et al.*); WO

2005/014618 (Chang *et al.*); US 2005/0079174 (Barbera-Guillem and Nelson); US 2005/0106108 (Leurig and Hansen); WO2005/044859 and US 2005/0123546 (Umana *et al.*); WO 2005/070963 (Allan *et al.*); US 2005/0186216 (Ledbetter and Hayden-Ledbetter); and US Patent No. 6,897,044 (Braslawski *et al.*).

Publications concerning treatment 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); Perotta *et al.*, "Rituxan in the treatment of chronic idiopathic thrombocytopenic purpura (ITP)", *Blood*, 94: 49 (abstract) (1999); 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*, *supra*; 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 and Rheumatism*, 46:2673-2677 (2002), wherein during a 2-week period, each patient received two 500-mg infusions of rituximab, two 750-mg infusions of cyclophosphamide, and high-dose oral corticosteroids, and wherein two of the patients treated relapsed at 7 and 8 months, respectively, and have been retreated, although with different protocols; "Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy" Weide *et al.*, *Lupus*, 12: 779-782 (2003), wherein a patient was treated with rituximab (375 mg/m² x 4, repeated at weekly intervals) and further rituximab applications were delivered every 5-6 months and then maintenance therapy was received with rituximab 375 mg/m² every three months, and a second patient with refractory SLE was treated successfully with rituximab and is receiving maintenance therapy every three months, with both patients responding well to rituximab therapy; Edwards and Cambridge, "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rheumatology* 40:205-211 (2001); Cambridge *et al.*, "B lymphocyte depletion in patients with rheumatoid arthritis: serial studies of immunological parameters" *Arthritis Rheum.*, 46 (Suppl. 9): S1350 (2002); Cambridge *et al.*, "Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis" *Arthritis Rheum.*, 48: 2146-2154 (2003); Edwards *et al.*, "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem Soc. Trans.*, *supra*; 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); Edwards *et al.*, "Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis" *N Engl. J. Med.* 350:2572-2582 (2004); Pavelka *et al.*, *Ann. Rheum. Dis.* 63: (S1):289-290 (2004); Emery *et al.*, *Arthritis Rheum.* 50 (S9):S659 (2004); Levine and Pestronk, "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using rituximab" *Neurology* 52: 1701-1704 (1999); Uchida *et al.*, "The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy" *J. Exp. Med.* 199: 1659-1669 (2004); Gong *et al.*, "Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy" *J. Immunol.* 174: 817-826 (2005); Hamaguchi *et al.*, "The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice" *J. Immunol.* 174: 4389-4399 (2005); Cragg *et al.*, "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.* 8:140-174 (2005); Eisenberg, "Mechanisms of autoimmunity" *Immunol. Res.* 27: 203-218 (2003); DeVita *et al.*, "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hidaishida *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; Tusciano, 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 and published Tusciano, *Arthritis Rheum.* 46: 3420 (2002); "Pathogenic roles of B cells in human autoimmunity: insights from the clinic" Martin and Chan, *Immunity* 20:517-527 (2004); Silverman and Weisman, "Rituximab therapy and autoimmune disorders, prospects for anti-B cell therapy", *Arthritis and Rheumatism*, 48: 1484-1492 (2003); Kazkaz and Isenberg, "Anti B cell therapy (rituximab) in the treatment of autoimmune diseases", *Current opinion in pharmacology*, 4: 398-402 (2004); Virgolini and Vanda, "Rituximab in autoimmune diseases", *Blomeditine & pharmacotherapy*, 58: 299-309(2004); Klemmer *et al.*, "Treatment of antibody mediated autoimmune disorders with a AntiCD20 monoclonal antibody Rituximab", *Arthritis And Rheumatism* , 48: (9) 9,S (SEP), page: S624-S624 (2003); Kneitz *et al.*, "Effective B cell depletion with rituximab in the treatment of autoimmune diseases", *Immunobiology*, 206: 519-527 (2002); Arzoo *et al.*, "Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal antibody (rituximab)" *Annals of the Rheumatic Diseases*, 61 (10), p 922-924 (2002) *Comment In Ann Rheum Dis.* 61: 863-866 (2002); "Future strategies in immunotherapy" by Lake and Dionne, in *Burger's Medicinal Chemistry and Drug Discovery* (2003 by John Wiley & Sons, Inc.) Article Online Posting Date: January 15, 2003 (Chapter 2 " Antibody-Directed Immunotherapy"); Liang and Tedder, *Wiley Encyclopedia of Molecular Medicine*, Section: CD20 as an Immunotherapy Target, article online posting date: 15 January, 2002 entitled "CD20"; Appendix 4A entitled "Monoclonal Antibodies to Human Cell Surface Antigens" by Stockinger *et al.*, eds: Coligan *et al.*, in *Current Protocols in Immunology* (2003 John Wiley & Sons, Inc) Online Posting Date: May, 2003; Print Publication Date: February, 2003; Penichet and Morrison, "CD Antibodies/molecules: Definition; Antibody Engineering" in *Wiley Encyclopedia of Molecular Medicine* Section: Chimeric, Humanized and Human Antibodies; posted online 15 January, 2002.

Further, see Looney "B cells as a therapeutic target in autoimmune diseases other than rheumatoid arthritis" *Rheumatology*, 44 Suppl 2: ii13-ii17 (2005); Chambers and Isenberg, "Anti-B cell therapy (rituximab) in the treatment of autoimmune diseases" *Lupus* 14(3): 210-214 (2005); Looney *et al.*, "B-cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalating trial of rituximab" *Arthritis Rheum.* 50: 2580-2589 (2004); Looney, "Treating human autoimmune disease by depleting B cells" *Ann Rheum. Dis.* 61: 863-866 (2002); Edelbauer *et al.*, "Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression Case report" *Pediatr. Nephrol.* 20(6): 811-813 (2005); D'Cruz and Hughes, "The treatment of lupus nephritis" *BMJ* 330(7488): 377-378 (2005); Looney, "B cell-targeted therapy in diseases other than rheumatoid arthritis" *J. Rheumatol. Suppl.* 73: 25-28; discussion 29-30 (2005); Sfikakis *et al.*, "Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial" *Arthritis Rheum.* 52(2): 501-513 (2005); Rastetter *et al.*, "Rituximab: expanding role in therapy for lymphomas and autoimmune diseases" *Annu. Rev. Med.* 55: 477-503 (2004); Silverman, "Anti-CD20 therapy in systemic lupus erythematosus: a step closer to the clinic" *Arthritis Rheum.* 52(2): 371-7 (2005), Erratum in: *Arthritis Rheum.* 52(4): 1342 (2005); Ahn *et al.*, "Long-term remission from life-threatening hypercoagulable state associated with lupus anticoagulant (LA) following rituximab therapy" *Am. J. Hematol.*

78(2): 127-129 (2005); Tahir *et al.*, "Humanized anti-CD20 monoclonal antibody in the treatment of severe resistant systemic lupus erythematosus in a patient with antibodies against rituximab" *Rheumatology*, 44(4): 561-562 (2005), Epub 2005 Jan 11; Looney *et al.*, "Treatment of SLE with anti-CD20 monoclonal antibody" *Curr. Dir. Autoimmun.* 8: 193-205 (2005); Cragg *et al.*, "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.* 8: 140-174 (2005); Gottenberg *et al.*, "Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases" *Ann. Rheum. Dis.* 64(6): 913-920 (2005) Epub 2004 Nov 18; Tokunaga *et al.*, "Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab" *Rheumatology* 44(2): 176-182 (2005), Epub 2004 Oct 19. See also Leandro *et al.*, "B cell repopulation occurs mainly from naïve B cells in patient with rheumatoid arthritis and systemic lupus erythematosus" *Arthritis Rheum.*, 48 (Suppl 9): S1160 (2003).

Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001) discloses successful use of four infusions of 375mg/m² of rituximab and high-dose glucocorticoids to treat Wegener's granulomatosis. The therapy was repeated after 11 months when the cANCA recurred, but therapy was without glucocorticoids. At 8 months after the second course of rituximab, the patients' disease remained in complete remission. Further, in another study, rituximab was found to be a well-tolerated, effective remission induction agent for severe ANCA-associated vasculitis, when used in a dose of 375 mg/m² x 4 along with oral prednisone 1 mg/kg/day, which was reduced by week 4 to 40 mg/day, and to complete discontinuation over the following 16 weeks. Four patients were re-treated with rituximab alone for recurring/rising ANCA titers. Other than glucocorticoids, no additional immunosuppressive agents seem to be necessary for remission induction and maintenance of sustained remission (6 months or longer). See online abstract submission and invitation Keogh *et al.*, "Rituximab for Remission Induction in Severe ANCA-Associated Vasculitis: Report of a Prospective Open-Label Pilot Trial in 10 Patients", American College of Rheumatology, Session Number: 28-100, Session Title: Vasculitis, Session Type: ACR Concurrent Session, Primary Category: 28 Vasculitis, Session 10/18/2004 (www.abstractsonline.com/viewer/SearchResults.asp). See also Keogh *et al.*, *Kidney Blood Press. Res.* 26:293 (2003), wherein it is reported that eleven patients with refractory ANCA-associated vasculitis were treated with four weekly doses of 375 mg/m² of rituximab and high-dose glucocorticoids, resulting in remission.

Patients with refractory ANCA-associated vasculitis were administered rituximab along with immunosuppressive medicaments such as intravenous cyclophosphamide, mycophenolate mofetil, azathioprine, or leflunomide, with apparent efficacy. Eriksson, "Short-term outcome and safety in 5 patients with ANCA-positive vasculitis treated with rituximab", *Kidney and Blood Pressure Research*, 26: 294 (2003) (five patients with ANCA-associated vasculitis treated with rituximab 375 mg/m² once a week for 4 weeks responded to the treatment); Jayne *et al.*, "B-cell depletion with rituximab for refractory vasculitis" *Kidney and Blood Pressure Research*, 26: 294-295 (2003) (six patients with refractory vasculitis receiving four weekly infusions of rituximab at 375 mg/m² with cyclophosphamide along with background immunosuppression and prednisolone experienced major falls in vasculitic activity). A further report of using rituximab along with intravenous cyclophosphamide at 375 mg/m² per dose in 4 doses for administering to

patients with refractory systemic vasculitis is provided in Jayne, poster 88 (11th International Vasculitis and ANCA workshop), 2003 American Society of Nephrology. See also Stone and Specks, "Rituximab Therapy for the Induction of Remission and Tolerance in ANCA-associated Vasculitis", in the Clinical Trial Research Summary of the 2002-2003 Immune Tolerance Network,

5 <http://www.immunetolerance.org/research/autoimmune/trials/stone.html>, in which a trial of rituximab in ANCA-associated vasculitis is proposed for a total length of 18 months. See also Eriksson, *J. Internal Med.*, 257: 540-548 (2005) regarding nine patients with ANCA-positive vasculitis who were successfully treated with two or four weekly doses of 500 mg of rituximab, as well as Keogh *et al.*, *Arthritis and Rheumatism*, 52: 262-268 (2005), who reported that in 11 patients with refractory ANCA-associated vasculitis, treatment or re-
10 treatment with four weekly doses of 375 mg/m² of rituximab induced remission by B lymphocyte depletion, the study being conducted between January 2000 and September 2002.

As to the activity of a humanized anti-CD20 antibody, see, for example, Vugmeyster *et al.*, "Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in *Macaca fascicularis*" *J. Immunother.* 28: 212-219 (2005). For discussion of a human monoclonal antibody, see Baker *et al.*, "Generation and
15 characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator" *Arthritis Rheum.* 48: 3253-3265 (2003)

There remains a need for approaches to treatment that reduce the frequency of infusions of active drug within a month's time. Further, there is a need to reduce the risk of toxic effects of currently used drugs such as steroids and chemotherapeutic agents, and to reduce the risk of disease flares, relapses, and
20 recurrences in patients with ANCA-associated vasculitis, and to sustain remission and maintain sustained remission for a prolonged period of time.

Summary of the Invention

The present invention involves administration of a CD20 antibody that provides a safe and active treatment regimen in subjects with ANCA-associated vasculitis, including selection of an efficacious dosing
25 regimen and scheduled or unscheduled re-treatment. This antagonist is effective both in initial therapy and in the management of refractory disease.

Accordingly, the invention is as claimed. In a first aspect, the present invention concerns treating ANCA-associated vasculitis in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

30 In a further aspect, the invention provides an article of manufacture comprising: a container comprising a CD20 antibody and a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the CD20 antibody of about 400 mg to 1.3 grams, at a frequency of one to three doses, is administered to the patient within a period of about one month.

In preferred embodiments of the above inventive aspects, the vasculitis is Wegener's granulomatosis
35 or microscopic polyangiitis, and/or a second medicament is administered in an effective amount to the patient, wherein the CD20 antibody is a first medicament. Such medicament may be one or more medicaments. More preferably, such second medicament is a chemotherapeutic agent, an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokines antagonist, a hormone, or a combination thereof.

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In still further aspects, the present invention relates to a method of treating ANCA-associated vasculitis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

5 In one preferred embodiment of this lattermost method involving multiple antibody exposures, the present invention relates to a method of treating ANCA-associated vasculitis in a subject comprising administering to the subject an effective amount of a CD20 antibody 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody

10 exposures is provided to the subject as about 1 to 4 doses of antibody, more preferably as a single dose or as two or three separate doses of antibody.

A specific preferred embodiment herein is a method of treating ANCA-associated vasculitis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until

15 from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably in such a method, the antibody exposures are of about 0.5 to 4 grams each.

In another preferred embodiment of these lattermost methods, a second medicament is administered with the initial exposure and/or later exposures, wherein the antibody is a first medicament. In a preferred

20 embodiment, the second medicament is one or more of those set forth above as preferred. In a more preferred embodiment, the second medicament is a steroid and/or an immunosuppressive agent. In a still preferred embodiment, a steroid is administered with the first exposure, but not with the second exposure, or is administered in lower amounts than are used with the initial exposure.

In still another preferred embodiment of these lattermost aspects, the subject has never been

25 previously treated with a CD20 antibody, and/or no other medicament than the CD20 antibody is administered to the subject to treat the vasculitis. In another preferred embodiment, the initial and second antibody exposures are with the same antibody, and more preferably all antibody exposures are with the same antibody. In another preferred embodiment, the subject is in remission after the initial or later antibody exposures, preferably when provided the second antibody exposure. More preferably, the subject is in remission when

30 provided all antibody exposures. Most preferably, such subject is in remission at least about six months after the last antibody exposure provided.

In yet another preferred embodiment of these lattermost aspects, the subject has an elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination of two or more

35 thereof.

Additionally, in further aspects, the invention provides an article of manufacture comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating ANCA-associated vasculitis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to

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provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

Preferably, such package insert is provided with instructions for treating ANCA-associated vasculitis 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as about one to four doses, preferably as a single dose or as two or three separate doses of antibody.

In a specific aspect, an article of manufacture is provided comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.

The treatments herein preferably reduce, minimize, or eliminate the need for co-, pre-, or post-administration of excessive amounts of second medicaments such as immunosuppressive agents and/or chemotherapeutic agents that are ordinarily standard treatment for such subjects, to avoid as much as possible the side effects of such standard treatment, as well as reduce costs and increase convenience to the subject, such as convenience of time and frequency of administration..

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 L chain (SEQ ID NO:13)

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

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

FIG. 5 is a sequence alignment comparing the light-chain amino acid sequences of the humanized 2H7.v16 variant (SEQ ID NO:2) and humanized 2H7.v138 variant (SEQ ID NO:28).

FIG. 6 is a sequence alignment comparing the heavy-chain amino acid sequences of the humanized 2H7.v16 variant (SEQ ID NO:8) and humanized 2H7.v138 variant (SEQ ID NO:29).

5 FIG. 7 shows an alignment of the mature 2H7.v16 and 2H7.v511 light chains (SEQ ID NOS: 13 and 30, respectively), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

FIG. 8 shows an alignment of the mature 2H7.v16 and 2H7.v511 heavy chains (SEQ ID NOS:14 and 31, respectively), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

10 FIG. 9A shows the sequence of the humanized 2H7.v114 variable light-chain domain (SEQ ID NO:32); FIG 9B shows the sequence of the humanized 2H7.v114 variable heavy-chain domain (SEQ ID NO:33); and FIG 9C shows the sequence of the humanized 2H7.v114 full-length heavy chain (SEQ ID NO:34), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

Detailed Description of the Preferred Embodiments

15 I. Definitions

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

20 A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antagonist that binds thereto. 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, 25 FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface markers herein are CD20 and CD22.

30 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.

35 The "CD22" antigen, or "CD22," also known as BL-CAM or Lyb8, is a type 1 integral membrane glycoprotein with molecular weight of about 130 (reduced) to 140kD (unreduced). It is expressed in both the cytoplasm and cell membrane of B-lymphocytes. CD22 antigen appears early in B-cell lymphocyte differentiation at approximately the same stage as the CD19 antigen. Unlike other B-cell markers, CD22 membrane expression is limited to the late differentiation stages comprised between mature B cells (CD22+) and plasma cells (CD22-). The CD22 antigen is described, for example, in Wilson *et al.*, *J. Exp. Med.* 40 173:137 (1991) and Wilson *et al.*, *J. Immunol.* 150:5013 (1993).

An "antagonist" is a molecule that, upon binding to CD20 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 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 as 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). Antagonists included within the scope of the present invention include antibodies, synthetic or native-sequence peptides, immunoadhesins, and small-molecule antagonists that bind to CD20, optionally conjugated with or fused to a cytotoxic agent. The preferred antagonist comprises an antibody.

An "antibody antagonist" herein is an 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 as 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).

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.

An "antibody that binds to a B-cell surface marker" is a molecule that, upon binding to a B-cell surface marker, 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 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 as 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). In one preferred embodiment, the antibody induces a major clinical response. In another preferred embodiment, the B-cell surface marker is CD20, so that the antibody that binds to a B-cell surface marker is an antibody that binds to CD20, or a "CD20 antibody." A particularly preferred embodiment is a CD20 antibody that induces a major clinical response. For purposes herein, a "major clinical response" is defined as achieving an American College of Rheumatology 70 response (ACR 70) for six consecutive months. ACR response scores are categorized as ACR 20, ACR 50 and ACR 70 with ACR 70 being the highest level of sign and symptom control in this evaluation system. ACR response scores measure improvement in rheumatoid arthritis disease activity, including joint swelling and tenderness, pain, level of

disability and overall patient and physician assessment. An example of a different type of antibody that induces a major clinical response as recognized by the FDA and as defined herein is etanercept (ENBREL®),

Examples of CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labelled 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 labelled with ¹³¹I to generate the "131I-B1" or "iodine 131 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 (WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (US Patent No. 5,677,180); a humanized 2H7 (WO 2004/056312 (Lowman *et al.*) and as set forth below); HUMAX-CD20™ fully human, high-affinity antibody targeted at the CD20 molecule in the cell membrane of B-cells (Genmab, Denmark; see, for example, Glennie and van de Winkel, *Drug Discovery Today* 8: 503-510 (2003) and Cragg *et al., Blood* 101: 1045-1052 (2003)); the human monoclonal antibodies set forth in WO04/035607 (Teeling *et al.*); AME-133™ antibodies (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: *Leukocyte Typing III* (McMichael, Ed., p. 440, Oxford University Press (1987)). The preferred CD20 antibodies herein are chimeric, humanized, or human CD20 antibodies, more preferably rituximab, a humanized 2H7, chimeric or humanized A20 antibody (Immunomedics), and HUMAX-CD20™ human CD20 antibody (Genmab).

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 which retain the ability to bind CD20.

Purely for the purposes herein and unless indicated otherwise, a "humanized 2H7" refers to a humanized CD20 antibody, 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 (V_LI), 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. See also WO 2004/056312 (Lowman *et al.*).

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). Another preferred embodiment is where the antibody is 2H7.v138 comprising the light- and heavy-chain amino acid sequences of SEQ ID NOS:28 and 29, respectively, as shown in Figs. 5 and 6, which are alignments of such sequences with the corresponding light- and heavy-chain amino acid sequences of 2H7.v16. Alternatively, such preferred intact humanized 2H7 antibody is 2H7.v477, which has the light- and heavy-chain sequences of 2H7.v138 except for the amino acid substitution of N434W. 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.*).

The most preferred humanized 2H7 variants are those having the variable light-chain domain of SEQ ID NO:2 and the variable heavy-chain domain of SEQ ID NO:8, *i.e.*, those with or without substitutions in the Fc region, and those having a variable heavy-chain domain with alteration N100A or D56A and N100A in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2, *i.e.*, those with or without substitutions in the Fc region. If substitutions are made in the Fc region, they are preferably one of those set forth in the table below.

In a summary of various preferred embodiments of the invention, the V 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 L chain as that of v16.

2H7 version	Heavy chain (V _H) changes	Light chain (V _L) changes	Fc changes
16			-
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

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

DIQMTQSPSSLSASVGDRVTITCRASSVSVMHWYQQKPGKAPKPLIYAPSNLASGVPSRPSGSGSGT
DFILTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and the variable heavy-chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYFDVWGQGTLLTVSS (SEQ ID
NO:8).

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQWFSNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
10 VTKSFNRGEC (SEQ ID NO:13);

and the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYFDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
15 GTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYFDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
20 GTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
25 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

In another preferred embodiment, the intact humanized 2H7 antibody comprises the light-chain
amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
30 VTKSFNRGEC (SEQ ID NO:28)

and the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASG
YTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL
RAEDTAVYYCARVYYSSASYWFYFDVWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAA
40 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC

VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKC
KVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO:29).

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"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 is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (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).

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"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 FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Doolan, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-341 (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)).

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

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"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).

"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, Rosenberg 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.

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 sequencer, 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.

"ANCA-associated vasculitis" or "anti-neutrophil cytoplasmic antibodies-associated vasculitis" or "AAV" as used herein is an autoimmune disease or disorder involving systemic vasculitis (or inflammation of blood-vessel walls) in which circulating anti-neutrophil cytoplasmic antibodies (ANCA) are normally present in the blood of the subject, or other clinical manifestations are present that define the vasculitis, as noted below. The term "ANCA-associated vasculitis" as used herein applies to ANCA-associated vasculitis no matter what the type and stage or severity, and no matter what symptoms are evident, provided the diagnosis is made. Examples of ANCA-associated vasculitis include microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome, renal-limited vasculitis (idiopathic necrotizing crescentic glomerulonephritis), and certain types of drug-induced vasculitis. Diagnoses for ANCA-associated vasculitis and its various manifestations include those set forth below.

Several diagnostic tests are commonly used in people suspected of having ANCA-associated vasculitis. Features that may aid in defining the specific type of vasculitic disorder include the type of organ involvement, presence and type of ANCA (myeloperoxidase-ANCA or proteinase 3-ANCA), presence of serum cryoglobulins, and the presence of evidence for granulomatous inflammation.

Exemplary auto-antibodies associated with ANCA-associated vasculitis include elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), such as autoantibodies directed against proteinase 3 (PR3) or against myeloperoxidase (MPO), or a combination thereof.

The ANCA antibodies can be detected using antigen-specific immunochemical assay to characterize PR3-ANCA and MPO-ANCA. Niles *et al.*, *supra*. Since an ELISA test for ANCA is associated with a substantially higher positive predictive value and likelihood ratios for ANCA-associated vasculitis, ELISA tests may be performed only on samples that are positive for ANCA by immunofluorescence. Stone *et al.*, *Arthritis Care and Research*, 13: 424-34 (2000); *Comment on Arthritis Care Res.* 13: 341-342 (2000); Russell *et al.*, *Clin. Immunol.*, 103: 196-203 (2002).

About 10 percent of patients with microscopic polyangiitis (the most common type of ANCA-associated vasculitis) and Wegener's granulomatosis have negative assays for ANCA; however, this finding does not completely rule out these diseases, and ANCA titers do not always correlate with disease activity. Jennette and Falk, *N. Engl J Med.*, *supra*. On the other hand, a positive ANCA assay result is not solely diagnostic of ANCA-associated vasculitis.

Table 1 summarizes the potential clinical manifestations of ANCA-associated vasculitis, which should be suspected in any patient who presents with a multisystem disease not caused by an infectious or malignant process (e.g., renal dysfunction, skin rashes, pulmonary manifestations, or neurologic manifestation). Constitutional symptoms are common. The frequency and combination of various system involvements vary among individual disease entities. See also Guillevin *et al.* *Arthritis Rheum.* 42:421-430 (1999); Pettersson *et al.*, *Clin. Nephrol.* 43:141-149 (1995); Savage *et al.*, *Lancet* 349:553-558 (1997); Guillevin *et al.*, *Br. J. Rheumatol.* 35:958-964 (1996).

Table 1
Clinical Manifestations of ANCA-Associated Vasculitis

System	Manifestations
Constitutional	Fever, weight loss, anorexia, general malaise
Musculoskeletal	Myalgia, arthralgia
Skin	Palpable purpura, urticaria
Kidneys	Proteinuria, hematuria, renal insufficiency, renal failure, necrotizing glomerulonephritis
Respiratory tract	Dyspnea, cough, hemoptysis; lung infiltrate, interstitial lung disease, pulmonary hemorrhage
Nervous system	Peripheral neuropathy, especially mononeuritis
Gastrointestinal tract	Fecal blood, elevated liver enzymes; diarrhea, nausea, vomiting, abdominal pain

The most common cutaneous lesion is palpable purpura—a slightly raised, non-blanching eruption that usually begins in the lower extremities. Occasionally, the rash is vesicular or slightly ulcerated. Urticaria can also be a manifestation of ANCA-associated vasculitis. Unlike non-vasculitic allergic urticaria, vasculitic urticaria lasts more than one day and may evolve into purpuric lesions. The presence of hypocomplementemia may indicate that the vasculitis is immune complex-mediated rather than ANCA-associated vasculitis.

Renal involvement in vasculitis may progress to renal failure. Results of biopsy of the kidney commonly reveal glomerulonephritis. Focal necrosis, crescentic formation and the absence or paucity of immunoglobulin deposits characterize glomerulonephritis in patients with ANCA-associated vasculitis. Pettersson *et al.*, *Clin. Nephrol.* 43:141-149 (1995). Lung involvement ranges from fleeting focal infiltrates or interstitial disease to massive pulmonary hemorrhagic alveolar capillaritis. The latter is the most life-threatening feature of small-vessel vasculitis.

It is important, however, to differentiate ANCA-associated vasculitis from other diseases that result in multi-system manifestations. Diseases with widespread embolization to different organs (e.g., atheroembolic disease, endocarditis, antiphospholipid syndrome, and atrial myxoma) can produce similar clinical presentations. Kelley, "Vasculitis and related disorders" In: *Textbook of rheumatology*. 5th ed. (Philadelphia: Saunders, 1997), pp.1079-1101. Persons with sepsis can also present with multisystem involvement. It is also important to realize that ANCA-associated vasculitis may be secondary to infections or

malignancy. Some viral, bacterial, and fungal infections may be complicated by vasculitis, which is predominantly a dermal vasculitis. The diagnosis is suggested by the clinical history. Malignancy, such as lymphomas, leukemia, myeloproliferative, and myelodysplastic syndromes, may be associated with ANCA-associated vasculitis; however, solid tumors are less commonly associated with such vasculitis. Underlying infectious or malignant causes should be thoroughly evaluated before the diagnosis of ANCA-associated vasculitis is made—even if the ANCA assay result is positive.

Table 2 depicts some of the clinical features that may help in the diagnosis of the specific type of vasculitis. Laboratory assessment should include a complete blood cell count and routine chemistry profile, urinalysis, fecal occult blood test, and chest radiography. There may be normocytic anemia, thrombocytosis, elevated erythrocyte sedimentation rate, increased liver function, or evidence of renal involvement. ANCA serum levels should also be measured. Other laboratory tests that should be performed to exclude ANCA-associated vasculitis include anti-nuclear antibody, rheumatoid factor, cryoglobulins, complement, antibodies to hepatitis B and C, and human immunodeficiency virus (HIV) testing. Chest and sinus computed tomographic scans may also be performed, if appropriate. Pathologic examination of the involved tissue (e.g., skin, nerve, lung, or kidney) may aid in documenting the type of ANCA-associated vasculitis. Biopsy should be obtained from symptomatic and accessible sites. Biopsies from asymptomatic sites have a low yield of positive results.

Table 2
Clinical Features That Favor Diagnosis
of a Specific Type of Vasculitis

<u>Clinical features</u>	<u>Probable type of vasculitis</u>
Pulmonary and renal symptoms	Wegener's granulomatosis; Microscopic polyangiitis
Pulmonary-dermal symptoms	Cryoglobulinemia; Henoch-Schonlein purpura
Asthma and eosinophilia	Churg-Strauss syndrome
Upper respiratory tract involvement	Wegener's granulomatosis (e.g., sinusitis and otitis media)

Information in Table 2 is from Jennette and Falk, *N. Engl. J. Med.*, *supra*, and Kelley, *supra*.

Wegener's granulomatosis is characterized by necrotizing granulomas of the upper and lower respiratory tract together with glomerulonephritis and systemic vasculitis, which involves usually the medium-sized vessels, with formation of granulomas and necrosis of the parenchyma. Kelley, *supra*. Upper respiratory tract signs and symptoms include sinusitis, nasal ulcers, otitis media, or hearing loss. Upper respiratory tract signs and symptoms are seen in 70 percent of patients and pulmonary infiltrates or nodules that may cavitate develop in 85 percent of patients. Kelley, *supra*. Serum antiprotease 3-ANCA (c-ANCA) is positive in 75 to 90 percent, although 20 percent may have positive p-ANCA. Open lung biopsy is the most definitive diagnostic test. Sinus biopsy is diagnostic in only 30 percent of cases because inflammatory findings are often nonspecific and renal biopsy is also relatively nonspecific. Radiographic findings are of mid and lower zone opacities, which are diffuse, and both alveolar and interstitial. Nodules, which may cavitate, are rare in children. CT scanning may show diffuse, ill-defined perivascular opacities. Wegener's

granulomatosis can affect patients at any age, with the peak incidence during the fourth decade of life and is slightly more common in men. Duna *et al.*, *Rheum. Dis. Clin. North Am.* 21:949-986 (1995). The most definite way to diagnose Wegener's granulomatosis is by performing a biopsy of an involved organ site (usually the sinuses, lung or kidney) to confirm the presence of vasculitis and granulomas, which together are diagnostic of the disease.

Microscopic polyangiitis is characterized by the presence of ANCA and few or no immune deposits in the involved vessels. Savage *et al.*, *Lancet* 349:553-558 (1997). The kidneys are the most commonly affected organs in 90 percent of patients who have this type of vasculitis. Kelley, *supra*. Patients present with variable combinations of renal manifestations, palpable purpura, abdominal pain, cough, and hemoptysis. Most patients have positive MPO-ANCA (p-ANCA), although PR3-ANCA (c-ANCA) may be also present in 40 percent of patients. The most common age of onset is 40 to 60 years and most common sex is men.

Churg-Strauss syndrome is a rare disease and has three phases: allergic rhinitis and asthma, eosinophilic infiltrative disease resembling pneumonia, and systemic small vessel vasculitis with granulomatous inflammation. Guillevin *et al.*, *Br. J. Rheumatol.*, 35:958-964 (1996). The vasculitic phase usually develops within three years of the onset of asthma. Almost all patients have more than 10 percent eosinophils in the blood. Coronary arteritis and myocarditis are the principal causes of morbidity and mortality. The age of onset varies from 15 to 70 years and is more common in men. Drug-induced vasculitis usually develops within seven to 21 days after a drug is started and may be confined to the skin. Jennette and Falk, *N. Engl. J. Med.*, *supra*. Skin lesions are identical to those seen in systemic small vessel vasculitis. Drugs cause approximately 10 percent of vasculitic skin lesions. Drugs that have been implicated include penicillin, aminopenicillins, sulfonamides, allopurinol, thiazides, quinolones, hydantoins, and propylthiouracil. Some drugs, such as propylthiouracil and hydralazine (APRESOLINE™), appear to cause vasculitis by inducing ANCA.

Another way to test for active disease and determine which patients/subjects are eligible for treatment is to determine the Birmingham Vasculitis Activity Score/Wegener's granulomatosis (BVAS/WG) value of the patient, whether major or minor. This score is an index of vasculitis activity and is designed to document clinical features that are directly due to active Wegener's granulomatosis. It has been found to be a valid and reliable disease-specific indicator for Wegener's granulomatosis. Stone *et al.*, *Arthritis & Rheumatism*, 44: 912-920 (2001). It can also be used for other ANCA-associated vasculitis diseases. The instrument separates the features that represent new or worse disease activity from those that represent persistent activity. Typically, the patient's BVAS/WG score is 3 or greater (or has been 3 or greater within 28 days of treatment). Each major item on the BVAS/WG evaluation form is scored 3 points. Each minor item is scored 1 point. However, another distinction used is that acute disease, either first presentation or relapse, shows a BVAS/WG of at least 10, whereas persistent disease shows a BVAS/WG of at least 4. Lymphopenia may also be a good marker for Wegener's granulomatosis. Izzedine *et al.*, *Nephron* 92:466-471 (2002).

A "subject" herein is a human subject, including a patient, eligible for treatment for ANCA-associated vasculitis who is experiencing or has experienced one or more signs, symptoms, or other indicators of ANCA-associated vasculitis, has been diagnosed with ANCA-associated vasculitis, whether, for example, newly diagnosed or previously diagnosed and now experiencing a recurrence or relapse, or is at risk for developing ANCA-associated vasculitis. The subject may have been previously treated with CD20 antibody

or not so treated. A subject eligible for treatment of ANCA-associated vasculitis may optionally be identified as one who has been screened, as in the blood, for elevated levels of infiltrating CD20 cells or is screened using an assay to detect auto-antibodies, wherein autoantibody production is assessed qualitatively, and preferably quantitatively.

5 A "patient" herein is a human subject eligible for treatment for ANCA-associated vasculitis who is experiencing or has experienced one or more signs, symptoms, or other indicators of ANCA-associated vasculitis, whether, for example, newly diagnosed or previously diagnosed and now experiencing a recurrence or relapse. The patient may have been previously treated with CD20 antibody or not so treated. A patient eligible for treatment of ANCA-associated vasculitis may optionally be identified as one who is screened
10 using an assay to detect auto-antibodies, such as those noted above, wherein autoantibody production is assessed qualitatively, and preferably quantitatively.

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

"Treatment" of a patient herein refers to therapeutic treatment. Those patients in need of treatment are those diagnosed with ANCA-associated vasculitis.

For purposes herein, a patient or subject is in "remission" if he/she has no symptoms of active
20 ANCA-associated vasculitis disease, such as those detectable by the methods disclosed herein, and has had no recurrence of ANCA titers or rising ANCA titers coinciding with or following reconstitution of B cells, since sustained or recurring ANCA levels have been found to be predictive of relapses in patients in clinical remissions from Wegener's granulomatosis. Boomsma *et al.*, *Arthritis Rheum.*, 43: 2025-2033 (2000). Those who are not in remission include, for example, those experiencing a disease flare after reconstitution of B
25 cells, those suffering organ damage such as kidney damage, or those who are asymptomatic but have had a recurrence of ANCA or an ANCA titer rise coinciding with or following reconstitution of B cells. Such subjects and patients experiencing a return of symptoms, including active disease and/or damage to organs, or exhibiting recurring or rising ANCA titers, are those who have "relapsed" or had a "recurrence."

A "symptom" of ANCA-associated vasculitis is any morbid phenomenon or departure from the
30 normal in structure, function, or sensation, experienced by the subject or patient and indicative of disease, such as those noted above.

The expression "effective amount" refers to an amount of the antibody or antagonist that is effective for treating ANCA-associated vasculitis.

"Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses
35 administered over a period of time of about 1 day to about 5 weeks. The doses may be given at one time or at a fixed or at irregular time intervals over this period of exposure, such as, for example, one dose weekly for four weeks or two doses separated by a time interval of about 13-17 days. Initial and later antibody exposures are separated in time from each other as described in detail herein.

An exposure not being administered or provided until a certain time "from the initial exposure" or
40 from any prior exposure means that the time for the second or later exposure is measured from the time any of

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the doses from the prior exposure were administered, if more than one dose was administered in that exposure. For example, when two doses are administered in an initial exposure, the second exposure is not given until at least about 16-54 weeks as measured from the time the first or the second dose was administered within that prior exposure. Similarly, when three doses are administered, the second exposure may be measured from the time of the first, second, or third dose within the prior exposure. Preferably, "from the initial exposure" or from any prior disclosure is measured from the time of the first dose.

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); non-steroidal 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, including SOLU-MEDROL[®] methylprednisolone sodium succinate, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); anti-malarial agents such as chloroquine and hydroxychloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antibodies including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor (TNF)-alpha antibodies (infliximab (REMICADE[®]) or adalimumab), anti-TNF-alpha immunoadhesin (etanercept), anti-TNF-beta antibodies, anti-interleukin-2 (IL-2) antibodies and anti-IL-2 receptor antibodies, and anti-interleukin-6 (IL-6) receptor antibodies and antagonists; 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; transforming growth factor-beta (TGF-beta); streptodornase; RNA or DNA from the host; FK506; RS-61443; chlorambucil; 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); BAFF antagonists such as BAFF antibodies and BR3 antibodies and zTNF4 antagonists (for review, see Mackay and Mackay, *Trends Immunol.*, 23:113-5 (2002) and see also definition below); biologic agents that interfere with T cell helper signals, such as anti-CD40 receptor or anti-CD40 ligand (CD154), including blocking antibodies to CD40-CD40 ligand (e.g., Durie *et al.*, *Science*, 261: 1328-30 (1993); Mohan *et al.*, *J. Immunol.*, 154: 1470-80 (1995)) and CTLA4-Ig (Finck *et al.*, *Science*, 265: 1225-7 (1994)); and T-cell receptor antibodies (EP 340,109) such as T10B9. Some preferred immunosuppressive agents herein include cyclophosphamide, chlorambucil, azathioprine, leflunomide, MMF, or methotrexate.

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 cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma I and calicheamicin omega I (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofof, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglutone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amasacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol;

pipobromun; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, *e.g.*, paclitaxel (TAXOL®), albumin-
 engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and doxorubicin (TAXOTERE®);
 chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and
 carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine
 (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin;
 5 aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids
 such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as
 combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of
 cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment
 10 regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Also included in this definition are anti-hormonal agents that act to regulate, reduce, block, or inhibit
 the effects of hormones that can promote the growth of cancer, and are often in the form of systemic, or
 whole-body treatment. They may be hormones themselves. Examples include anti-estrogens and selective
 estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®
 15 tamoxifen), raloxifene (EVISTA®), droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018,
 onapristone, and toremifene (FARESTON®); anti-progestagens; estrogen receptor down-regulators (ERDs);
 estrogen receptor antagonists such as fulvestrant (FASLODEX®); agents that function to suppress or shut
 down the ovaries, for example, leutinizing hormone-releasing hormone (LHRH) agonists such as leuprolide
 acetate (LUPRON® and ELIGARD®), goserelin acetate, buserelin acetate and triptorelin; anti-androgens
 20 such as flutamide, nilutamide and bicalutamide; and aromatase inhibitors that inhibit the enzyme aromatase,
 which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles,
 aminoglutethimide, megestrol acetate (MEGASE®), exemestane (AROMASIN®), formestane, fadrozole,
 vorozole (RIVISOR®), letrozole (FEMARA®), and anastrozole (ARIMIDEX®). In addition, such definition
 of chemotherapeutic agents includes bisphosphonates such as clodronate (for example, BONEPOS® or
 25 OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate
 (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risendronate (ACTONEL®); 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 aberrant cell proliferation, such as, for
 example, PKC- α , Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as
 30 THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN®
 vaccine, and VAXID® vaccine; topoisomerase I inhibitor (*e.g.*, LURTOTECAN®); mHRH (*e.g.*,
 ABARELIX®); lapatinib ditosylate (an ErbB-2 and EGFR dual tyrosine kinase small-molecule inhibitor also
 known as GW572016); and pharmaceutically acceptable salts, acids or derivatives of any of the above.

The term "cytokine" is a generic term for proteins released by one cell population that act on another
 35 cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs)
 such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15, including
 PROLEUKIN® rIL-2; 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.

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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; estradiol; hormone-replacement therapy; androgens such as calusterone, dromostanolone propionate, epitestosterone, mepitiostane, or testolactone; prolactin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); prolactin, placental lactogen, mouse gonadotropin-associated peptide, gonadotropin-releasing hormone; 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 *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.

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 hydroxychloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methotrexate).

azathioprine, D-penicillamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine including cyclosporine A and topical cyclosporine, *staphylococcal* protein A (Goodyear and Silverman, *J. Exp. Med.*, 197, (9), p1125-39 (2003)), including salts and derivatives thereof, *etc.*

Examples of "non-steroidal anti-inflammatory drugs" or "NSAIDs" include aspirin, acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, COX-2 inhibitors such as celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide and valdecoxib (BEXTRA®), and meloxicam (MOBIC®), including salts and derivatives thereof, *etc.* Preferably, they are aspirin, naproxen, ibuprofen, indomethacin, or tolmetin.

Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody, such as efalizumab (RAPTIVA®) commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab (ANTEGREN®) available from Biogen, or 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), ADAM disintegrin domain polypeptides (US2002/0042368), antibodies to alphavbeta3 integrin (EP 633945), *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, such as SOLU-MEDROL® methylprednisolone sodium succinate), dexamethasone or dexamethasone triamcinolone, hydrocortisone, and betamethasone. The preferred corticosteroids herein are prednisone, methylprednisolone, hydrocortisone, or dexamethasone.

The terms "BAFF," "BAFF polypeptide," "TALL-1" or "TALL-1 polypeptide," and "BLyS" when used herein encompass "native-sequence BAFF polypeptides" and "BAFF variants". "BAFF" is a designation given to those polypeptides that have any one of the amino acid sequences shown below:

Human BAFF sequence (SEQ ID NO:16):

1 MDDSTEREQSRLTSCLKKREEMKLKECVSILPRKESPSVRSSKDGKLLAATLLLALLSCC
61 LTVVSFYQVAALQGDLSLRAELQGHHAELKLPAGAGAPKAGLEEAPAVTAGLKIFEPPAP
121 GEGNSSQNSRNKRAVQGPEETVTQDCLQIADSETPTIQKGSYTFVPWLLSFKRGSALLEE
181 KENKLVKETGYFFTYGQVLYTDKTYAMGHLIQRKKVHVFGDELSLVTLFRCIQNMPETL
241 PNNSCYSAGIAKLEEGDELQLAIPRENAQISLDGDVTFPGALKLL

Mouse BAFF sequence (SEQ ID NO:17):

1 MDESAKTLPPPCLCFCSEKQEDMKVGYDPTFPQKEGAWFGICRDGRLLAATLLLALLSS
61 SPTAMSLYQLAALQADLMNLRMELQSYRGSATPAAAGAPELTAGVKLLTPAAPRPHNSSR
121 GHRNRRAPQGPEETEQDVLSAPPAPCLPGCRHSQHDDNGMNLRNIIQDCLQIADSDTP
181 TIRKGTYYTFVPWLLSFKRGNALBEEKENKIVVRQTGYFFTYSQVLYTDPIPAMGHVQRKK
241 VHVFGDELSLVTLFRCIQNMPKTL PNNSCYSAGIARLEEGDEIQLAIPRENAQISRNGDD
301 TFFGALKLL

and homologs and fragments and variants thereof, which have the biological activity of the native BAFF. A biological activity of BAFF can be selected from the group consisting of promoting B cell survival, promoting

B cell maturation and binding to BR3. Variants of BAFF will preferably have at least 80% or any successive integer up to 100% including, more preferably, at least 90%, and even more preferably, at least 95% amino acid sequence identity with a native sequence of a BAFF polypeptide.

5 A "native-sequence" BAFF polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding BAFF polypeptide derived from nature. For example, BAFF exists in a soluble form following cleavage from the cell surface by furin-type proteases. Such native-sequence BAFF polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means.

10 The term "native-sequence BAFF polypeptide" or "native BAFF" specifically encompasses naturally occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally occurring variant forms (e.g., alternatively spliced forms), and naturally occurring allelic variants of the polypeptide. The term "BAFF" includes those polypeptides described in Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); GenBank Accession No. AF136293; WO 1998/18921 published May 7, 1998; EP 869,180 published October 7, 1998; WO 1998/27114 published June 25, 1998; WO 1999/12964 published March 18, 1999; WO 1999/33980 published July 8, 1999; Moore *et al.*, *Science*, 285:260-263 (1999); Schneider *et al.*, *J. Exp. Med.*, 189:1747-1756 (1999) and Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274:15978-15981 (1999).

15 The term "BAFF antagonist" as used herein is used in the broadest sense, and includes any molecule that (1) binds a native-sequence BAFF polypeptide or binds a native-sequence of BR3 to partially or fully block BR3 interaction with BAFF polypeptide, and (2) partially or fully blocks, inhibits, or neutralizes native-sequence BAFF activity. In one preferred embodiment the BAFF receptor to be blocked is the BR3 receptor. Native BAFF activity promotes, among other things, B-cell survival and/or B-cell maturation. In one embodiment, the inhibition, blockage or neutralization of BAFF activity results in a reduction in the number of B cells. A BAFF antagonist according to this invention will partially or fully block, inhibit, or neutralize one or more biological activities of a BAFF polypeptide, *in vitro* and/or *in vivo*. In one embodiment, a biologically active BAFF potentiates any one or a combination of the following events *in vitro* and/or *in vivo*:
20 an increased survival of B cells, an increased level of IgG and/or IgM, an increased numbers of plasma cells, and processing of NF- κ B2/100 to p52 NF- κ B in splenic B cells (e.g., Batten *et al.*, *J. Exp. Med.* 192:1453-1465 (2000); Moore *et al.*, *Science* 285:260-263 (1999); Kayagaki *et al.* *Immunity* 17:515-524 (2002)).

As mentioned above, a BAFF antagonist can function in a direct or indirect manner to partially or fully block, inhibit or neutralize BAFF signaling, *in vitro* or *in vivo*. For instance, the BAFF antagonist can directly bind BAFF. For example, BAFF antibodies that bind within a region of human BAFF comprising residues 162-275 and/or a neighboring residue of a residue selected from the group consisting of 162, 163, 206, 211, 231, 233, 264 and 265 of human BAFF such that the antibody sterically hinders BAFF binding to BR3 are contemplated, where such residue numbers refer to SEQ ID NO:16. In another example, a direct binder is a polypeptide comprising any portion of a BAFF receptor that binds BAFF such as an extracellular domain of a BAFF receptor, or fragments and variants thereof that bind native BAFF. In another example, BAFF antagonists include the polypeptides having a sequence of a polypeptide comprising the sequence of
30 Formula I:

X_1 -C-X₃-D-X₅-L-X₇-X₈-X₉-X₁₀-X₁₁-X₁₂-C-X₁₄-X₁₅-X₁₆-X₁₇ (Formula I) (SEQ ID NO:18)

wherein X₁, X₃, X₅, X₇, X₈, X₉, X₁₀, X₁₁, X₁₂, X₁₄, X₁₅ and X₁₇ are any amino acid except cysteine; and

40 wherein X₁₆ is an amino acid selected from the group consisting of L, F, I and V; and

wherein the polypeptide does not comprise a cysteine within seven amino acid residues N-terminal to the most N-terminal cysteine C and C-terminal to the most C-terminal cysteine C of Formula I.

In one embodiment, a polypeptide comprising the sequence of Formula I has the two Cs joined by disulfide bonding; $X_3LX_7X_8$ forming the conformation of a type I beta turn structure with the center of the turn between L and X_7 ; and has a positive value for the dihedral angle phi of X_8 . In one embodiment, X_{10} is selected from the group consisting of W, F, V, L, I, Y, M and a non-polar amino acid. In another embodiment, X_{10} is W. In another embodiment, X_3 is an amino acid selected from the group consisting of M, V, L, I, Y, F, W and a non-polar amino acid. In another embodiment, X_3 is selected from the group consisting of V, L, P, S, I, A and R. In another embodiment, X_7 is selected from the group consisting of V, T, I and L. In another embodiment, X_8 is selected from the group consisting of R, K, G, N, H and a D-amino acid. In another embodiment, X_9 is selected from the group consisting of H, K, A, R and Q. In another embodiment, X_{11} is I or V. In another embodiment, X_{12} is selected from the group consisting of P, A, D, E and S. In another embodiment, X_{16} is L. In one specific embodiment, the sequence of Formula I is a sequence selected from the group consisting of ECFDLLVRAWVPCSVLK (SEQ ID NO:19), ECFDLLVRHWVPCGLLR (SEQ ID NO:20), ECFDLLVRRWVPCMLG (SEQ ID NO:21), ECFDLLVRSWVPCMLR (SEQ ID NO:22), ECFDLLVRHWVACGLLR (SEQ ID NO:23), and QCFDLNRAWVPCSVLK (SEQ ID NO:24). In a preferred embodiment, the BAFF antagonist comprises any one of the amino acid sequences selected from the group consisting of SEQ ID NO: 19, 20, 21, 22, and 23.

In still another example, BAFF antagonists include the polypeptides having a sequence of a polypeptide comprising the sequence of Formula II:

$X_1-C-X_2-D-X_3-L-V-X_4-X_5-W-V-P-C-X_{14}-X_{15}-L-X_{17}$ (Formula II) (SEQ ID NO:25)

wherein X_1 , X_3 , X_5 , X_8 , X_9 , X_{14} , X_{15} and X_{17} are any amino acid, except cysteine; and

wherein the polypeptide does not comprise a cysteine within seven amino acid residues N-terminal to the most N-terminal cysteine C and C-terminal to the most C-terminal cysteine C of Formula II.

In one embodiment, a polypeptide comprising the sequence of Formula II has a disulfide bond between the two Cs and has the conformation of $X_3LX_7X_8$ forming a type I beta turn structure with the center of the turn between L and X_7 ; and has a positive value for the dihedral angle phi of X_8 . In another embodiment of Formula II, X_3 is an amino acid selected from the group consisting of M, A, V, L, I, Y, F, W and a non-polar amino acid. In another embodiment of Formula II, X_3 is selected from the group consisting of V, L, P, S, I, A and R. In another embodiment of Formula II, X_8 is selected from the group consisting of R, K, G, N, H and D-amino acid. In another embodiment of Formula II, X_9 is selected from the group consisting of H, K, A, R and Q.

In a further embodiment, the BAFF receptor from which the extracellular domain or BAFF-binding fragment or BAFF-binding variant thereof is derived is TACI, BR3 or BCMA. Alternatively, the BAFF antagonist can bind an extracellular domain of a native-sequence BR3 at its BAFF binding region to partially or fully block, inhibit or neutralize BAFF binding to BR3 *in vitro*, *in situ*, or *in vivo*. For example, such indirect antagonist is an anti-BR3 antibody that binds in a region of BR3 comprising residues 23-38 of human BR3 as defined below (SEQ ID NO:26) or a neighboring region of those residues such that binding of human BR3 to BAFF is sterically hindered.

In some embodiments, a BAFF antagonist according to this invention includes BAFF antibodies and immunoadhesins comprising an extracellular domain of a BAFF receptor, or fragments and variants thereof that bind native BAFF. In a further embodiment, the BAFF receptor from which the extracellular domain or BAFF-binding fragment or BAFF-binding variant thereof is derived is TACI, BR3 or BCMA. In a still
5 another embodiment, the immunoadhesin comprises an amino acid sequence of that of Formula I or Formula II as set forth above, including an amino acid sequence selected from any one of the group consisting of SEQ ID NOS: 19, 20, 21, 22, 23, and 24.

According to one embodiment, the BAFF antagonist binds to a BAFF polypeptide or a BR3
10 polypeptide with a binding affinity of 100nM or less. According to another embodiment, the BAFF antagonist binds to a BAFF polypeptide or a BR3 polypeptide with a binding affinity of 10nM or less. According to yet another embodiment, the BAFF antagonist binds to a BAFF polypeptide or a BR3 polypeptide with a binding affinity of 1nM or less.

The terms "BR3", "BR3 polypeptide" or "BR3 receptor" when used herein encompass "native-
15 sequence BR3 polypeptides" and "BR3 variants" (which are further defined herein). "BR3" is a designation given to those polypeptides comprising the following amino acid sequence and homologs thereof, and variants or fragments thereof that bind native BAFF:

Human BR3 sequence (SEQ ID NO:26):

1 MRRGPRSLRGRDAPATPCVPAECFDLLVRHCVACGLLRTPRPKPAGASSPAPRTALQPQ
20 61 ESVGAGAGEAALPLPGLLPGAPALLGLALVLALVLVGLVSWRRRQRRLRGASSAEAPDGD
121 KDAPEPLDKVILSPGISDATAPAWPPPGEDPGTTPPGHSVPVPATELGSTELVTTKTAG
181 PEQQ.

The BR3 polypeptides of the invention can be isolated from a variety of sources, such as from human
25 tissue types or from another source, or prepared by recombinant and/or synthetic methods. The term BR3 includes the BR3 polypeptides described in WO 2002/24909 and WO 2003/14294.

A "native-sequence" BR3 polypeptide or "native BR3" comprises a polypeptide having the same
30 amino acid sequence as the corresponding BR3 polypeptide derived from nature. Such native-sequence BR3 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native-sequence BR3 polypeptide" specifically encompasses naturally occurring truncated, soluble or secreted forms (e.g., an extracellular domain sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally occurring allelic variants of the polypeptide. The BR3 polypeptides of the invention include the BR3 polypeptide comprising or consisting of the contiguous sequence of amino acid
35 residues 1 to 184 of a human BR3 (SEQ ID NO:26).

A BR3 "extracellular domain" or "ECD" refers to a form of the BR3 polypeptide that is essentially
40 free of the transmembrane and cytoplasmic domains. ECD forms of BR3 include a polypeptide comprising any one of the amino acid sequences selected from the group consisting of amino acids 1-77, 2-62, 2-71, 1-61, 7-71, 23-38 and 2-63 of human BR3. The invention contemplates BAFF antagonists that are polypeptides comprising any one of the above-mentioned ECD forms of human BR3 and variants and fragments thereof that bind a native BAFF.

Mini-BR3 is a 26-residue core region of the BAFF-binding domain of BR3, i.e., the amino acid
sequence: TPCVPAECFD LLVRHCVACG LLRTPR (SEQ ID NO:27)

"BR3 variant" means a BR3 polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a native-sequence, full-length BR3 or BR3 ECD and binds a native-sequence BAFF polypeptide. Optionally, the BR3 variant includes a single cysteine-rich domain. Such BR3 variant polypeptides include, for instance, BR3 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the BR3 ECD that bind a native sequence BAFF polypeptide are also contemplated. According to one embodiment, a BR3 variant polypeptide will have at least about 80% amino acid sequence identity, at least about 81% amino acid sequence identity, at least about 82% amino acid sequence identity, at least about 83% amino acid sequence identity, at least about 84% amino acid sequence identity, at least about 85% amino acid sequence identity, at least about 86% amino acid sequence identity, at least about 87% amino acid sequence identity, at least about 88% amino acid sequence identity, at least about 89% amino acid sequence identity, at least about 90% amino acid sequence identity, at least about 91% amino acid sequence identity, at least about 92% amino acid sequence identity, at least about 93% amino acid sequence identity, at least about 94% amino acid sequence identity, at least about 95% amino acid sequence identity, at least about 96% amino acid sequence identity, at least about 97% amino acid sequence identity, at least about 98% amino acid sequence identity or at least about 99% amino acid sequence identity with a human BR3 polypeptide or a specified fragment thereof (e.g., ECD). BR3 variant polypeptides do not encompass the native BR3 polypeptide sequence. According to another embodiment, BR3 variant polypeptides are at least about 10 amino acids in length, at least about 20 amino acids in length, at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, or at least about 70 amino acids in length.

In one preferred embodiment, the BAFF antagonists herein are immunoadhesins comprising a portion of BR3, TACI or BCMA that binds BAFF, or variants thereof that bind BAFF. In other embodiments, the BAFF antagonist is a BAFF antibody. A "BAFF antibody" is an antibody that binds BAFF, and preferably binds BAFF within a region of human BAFF comprising residues 162-275 of the human BAFF sequence disclosed herein under the "BAFF" definition (SEQ ID NO:16). In another embodiment, the BAFF antagonist is BR3 antibody. A "BR3 antibody" is an antibody that binds BR3, and is preferably one that binds BR3 within a region of human BR3 comprising residues 23-38 of the human BR3 sequence disclosed herein under the "BR3" definition (SEQ ID NO:26). In general, the amino acid positions of human BAFF and human BR3 referred to herein are according to the sequence numbering under human BAFF and human BR3, SEQ ID NOS: 16 and 26, respectively, disclosed herein under the "BAFF" and "BR3" definitions.

Other examples of BAFF-binding polypeptides or BAFF antibodies can be found in, e.g., WO 2002/092620, WO 2003/014294, Gordon *et al.*, *Biochemistry* 42(20):5977-5983 (2003), Kelley *et al.*, *J. Biol. Chem.*, 279(16):16727-16735 (2004), WO 1998/18921, WO 2001/12812, WO 2000/68378 and WO 2000/40716.

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.

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A "medicament" is an active drug to treat the ANCA-associated vasculitis or its symptoms or side effects.

II. Therapy

5 In one aspect, the present invention provides a method of treating ANCA-associated vasculitis in a patient comprising administering an antagonist, preferably an antibody, that binds to a B-cell surface marker (more preferably a CD20 antibody) to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

10 Thus, the invention contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering an antibody that binds to a B-cell surface marker to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

The invention also contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering an antagonist that binds to a B-cell surface marker to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

15 The invention also contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

20 In a preferred embodiment of each of these aspects, the dose is about 500 mg to 1.2 grams, more preferably about 750 mg to 1.1 grams. In another preferred embodiment, the antibody is administered in two to three doses, more preferably in two doses, but alternatively three doses. In a still preferred embodiment, the antibody is administered within a period of about 2 to 3 weeks, more preferably about two weeks, but alternatively three weeks.

25 In another embodiment, the present invention provides a method of treating ANCA-associated vasculitis in a subject eligible for treatment, comprising administering an effective amount of an antibody that binds to a B-cell surface marker (preferably a CD20 antibody) to the subject to provide an initial antibody exposure (of preferably about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, and still more preferably about 1.5 to 2.5 grams) followed by a second antibody exposure (of preferably about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, still more preferably about 1.5 to 2.5 grams), the second exposure not being provided until from about 16 to 54 weeks (preferably from about 20 to 30 weeks, more preferably from about 46 to 54 weeks) from the initial exposure.

30 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. Such re-treatment may be scheduled or unscheduled, but is preferably a scheduled redosing, particularly to protect organs such as kidneys from damage.

35 The method preferably comprises administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure (preferably of about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, still more preferably about 1.5 to 2.5 grams), the third exposure not being provided until from about 46 to 60 weeks (preferably about 46 to 55, more preferably about 46 to 52 weeks) from the initial exposure. Preferably, no further antibody exposure is provided until at least about 70-75 weeks from the

initial exposure, and still more preferably no further antibody exposure is provided until about 74 to 80 weeks from the initial exposure.

Any one or more of the antibody exposures herein may be provided to the subject as a single dose of antibody, or as separate doses, for example, about 1-4 separate doses of the antibody (e.g., constituting a first and second dose, or a first, second, and third dose, or a first, second, third, and fourth dose, etc). The particular number of doses (whether one, two or three or more) employed for each antibody exposure is dependent, for example, on the type of ANCA-associated vasculitis treated, the type of antibody employed, whether, what type, and how much and how many of a second medicament is employed as noted below, and the method and frequency of administration. Where separate doses are administered, the later dose (for example, second or third dose) is preferably administered from about 1 to 20 days, more preferably from about 6 to 16 days, and most preferably from about 14 to 16 days from the time the previous dose was administered. The separate doses are preferably administered within a total period of between about 1 day and 4 weeks, more preferably between about 1 and 20 days (e.g., within a period of 6-18 days). In one such aspect, the separate doses are administered about weekly, with the second dose being administered about one week from the first dose and any third or subsequent dose being administered about one week from the second dose. Each such separate dose of the antibody is preferably about 0.5 to 1.5 grams, more preferably about 0.75 to 1.3 grams.

In a most preferred embodiment, a method of treating ANCA-associated vasculitis in a subject is provided comprising administering an effective amount of an antibody that binds to a B-cell surface marker (e.g., a CD20 antibody) to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably in such a method, the antibody exposures are of about 0.5 to 4 grams each, and most preferably the amounts given above.

In one embodiment, the subject is provided at least about three 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 24 weeks. 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 separate doses of the antibody. However, not every antibody exposure need be provided as a single dose or as separate doses.

In one preferred embodiment, about 2-3 grams of the CD20 antibody is administered as the initial exposure. If about 3 grams are administered, then about 1 gram of the CD20 antibody is administered weekly for about three weeks as the initial exposure. If about 2 grams of the CD20 antibody is administered as the initial exposure, then about 1 gram of the CD20 antibody is administered followed in about two weeks by another about 1 gram of the antibody as the initial exposure. In a preferred aspect, the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams. In an alternative preferred aspect, the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.

In all the inventive methods set forth herein, the CD20 or B-cell surface marker 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 CD20 antibody herein is a chimeric, humanized, or human CD20 antibody, more preferably rituximab, a humanized 2H7 (e.g. comprising the variable domain sequences in SEQ ID Nos. 2 and 8, or comprising a variable heavy-chain domain with alteration N100A or D56A and N100A in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2), chimeric or humanized A20 antibody (Immunomedics), or HUMAX-CD20™ human CD20 antibody (Genmab). Still more preferred is rituximab or a humanized 2H7. Also, while the ANCA-associated vasculitis in all methods herein can be any such disease, in one preferred embodiment, it is Wegener's granulomatosis or microscopic polyangiitis.

In a further embodiment of all the methods herein, the subject or patient has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the ANCA-associated vasculitis and/or has never been previously treated with an antagonist (for example, antibody) to a B-cell surface marker (e.g. never been previously treated with a CD20 antibody). In a still further aspect, the subject or patient may have had a relapse with the ANCA-associated vasculitis or suffered organ damage such as kidney damage before being treated in any of the methods above, including after the initial or a later antibody exposure. However, preferably, the patient or subject has not relapsed with the vasculitis and more preferably has not had such a relapse before at least the initial treatment.

In another embodiment, the subject or patient has been previously treated with drug(s) to treat the vasculitis and/or has been previously treated with such antibody or antagonist. In another embodiment, the antagonist (for example, CD20 antibody) is the only medicament administered to the subject or patient to treat the vasculitis. In another embodiment, the antagonist (e.g., CD20 antibody) is one of the medicaments used to treat the vasculitis. In a further embodiment, the subject or patient does not have a malignancy. In a still further embodiment, the subject or patient does not have rheumatoid arthritis. In a still further embodiment, the subject or patient does not have multiple sclerosis. In a yet further embodiment, the subject or patient does not have lupus or Sjögren's syndrome. In yet another embodiment, the subject or patient does not have an autoimmune disease other than ANCA-associated vasculitis. In yet another aspect of the invention, the ANCA-associated vasculitis is not associated with a different autoimmune disease or with a risk of developing a different autoimmune disease. For purposes of these lattermost statements, an "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. Without being limited to any one theory, B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neolymphogenesis. Each of these pathways participates to different degrees in the pathology of autoimmune diseases.

In still another embodiment, the subject or patient has a BVAS/WG score of less than 3, more preferably less than about 2, still more preferably less than about 1, and most preferably 0 (complete remission) at about three months, preferably about six months, and most preferably about one year or greater, after administration of the antagonist or antibody. Specific embodiments of this BVAS response are

achieving a BVAS/WG score of less than 2 at three months after administration, or less than 1 (for example, 0.2 or 0.4) at 14 weeks or three months after administration, or less than 1 (for example, 0.6) at 6 months after administration, or most preferably, 0 at three or six months after administration. In another embodiment, the amount of steroid such as prednisone as compared to start of treatment is lessened without substantially affecting the lowered BVAS/WG score. Thus, for example, a subject or patient at a set interval after treatment (such as three months or six months after treatment) preferably has a lowered BVAS/WG score from baseline and is being administered less of a dose of a steroid from baseline (baseline being at the start of administration). In a still further embodiment, a step is included in the treatment method to test for the subject's or patient's response to treatment after the administration step to determine that the level of response is effective to treat the vasculitis. For example, a step is included to test the BVAS/WG score after administration and compare it to a baseline BVAS/WG score obtained before administration to determine if treatment is effective by measuring if, and by how much, it has been lowered. This test may be repeated at various scheduled or unscheduled time intervals after the administration to determine maintenance of any partial or complete remission. Alternatively, the methods herein comprise a step of testing the patient or subject, before administration, to see if one or more biomarkers are present for ANCA-associated vasculitis, such as one or more autoantibodies, a BVAS/WG score, or symptoms unique to ANCA-associated vasculitis, as set forth above. In another method, a step may be included to check the patient's or subject's clinical history, as detailed above, for example, to rule out infections or malignancy as causes, for example, primary causes, of the patient's or subject's condition, prior to administering the antibody or antagonist to the subject or patient. Preferably, the ANCA-associated vasculitis is primary (*i.e.*, the leading disease), and is not secondary, such as secondary to infection or malignancy, whether solid or liquid tumors.

In a preferred embodiment of the multi-exposure method herein, the subject is in remission after the initial or any later antibody exposures. More preferably, the multi-exposure method herein involves scheduled re-dosing or re-treating such that the patient is in remission when provided the second, and preferably all antibody exposures. Such re-dosing is scheduled to prevent any relapse, recurrence, or organ damage, rather than to treat it therapeutically. Most preferably, the subject is in remission for at least about six months, and still most preferably at least about nine months, and even still most preferably at least about a year since the last antibody exposure used in the re-treatment method.

In yet another embodiment, the subject is treated with the same CD20 antibody for at least two antibody exposures, and preferably for each antibody exposure. Thus, the initial and second antibody exposures are preferably with the same antibody, and more preferably all antibody exposures are with the same antibody, *i.e.*, treatment for the first two exposures, and preferably all exposures, is with one type of antibody that binds to a B-cell surface marker, such as CD20 antibody, *e.g.*, all with rituximab or all with the same humanized 2H7.

In any of the methods herein, one may administer to the subject or patient along with the antagonist or antibody that binds a B-cell surface marker an effective amount of a second medicament (where the antagonist or antibody that binds a B-cell surface marker (*e.g.*, the CD20 antibody) is a first medicament). The second medicament may be one or more medicaments, and include, for example, a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody, or any combination thereof. The type of such second

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medicament depends on various factors, including the type of vasculitis, the severity of the vasculitis, the condition and age of the patient, the type and dose of first medicament employed, *etc.*

5 Examples of such additional medicaments include a chemotherapeutic agent, an interferon class drug such as interferon-alpha (*e.g.*, from Amarillo Biosciences, Inc.), IFN-beta-1a (REBIF[®] and AVONEX[®]) or IFN-beta-1b (BETASERON[®]), an oligopeptide such as glatiramer acetate (COPAXONE[®]), an agent blocking CD40-CD40 ligand, a cytotoxic or immunosuppressive agent (such as mitoxantrone (NOVANTRONE[®]), methotrexate, cyclophosphamide, chlorambucil, leflunomide, and azathioprine), intravenous immunoglobulin (gamma globulin), lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, CAMPATH[™] antibodies, anti-CD4, cladribine, a polypeptide construct with at least two domains comprising a de-immunized, autoreactive antigen or its fragment that is specifically recognized by the Ig receptors of autoreactive B-cells (WO 2003/68822), total body irradiation, bone marrow transplantation), 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/ANTEGREN[®] available from Biogen, or others as noted above), drugs that treat symptoms secondary or related to ANCA-associated vasculitis 15 (*e.g.*, fungal and other infections) such as those noted herein, steroid such as corticosteroid (*e.g.*, prednisolone, methylprednisolone such as SOLU-MEDROL[™] methylprednisolone sodium succinate for injection, prednisone such as low-dose prednisone, dexamethasone, or glucocorticoid, *e.g.*, via joint injection, including systemic corticosteroid therapy), non-lymphocyte-depleting immunosuppressive therapy (*e.g.*, MMF or cyclosporine), cholesterol-lowering drug of the "statin" class (which includes cerivastatin (BAYCOL[™]), 20 fluvastatin (LESCOL[™]), atorvastatin (LIPITOR[™]), lovastatin (MEVACOR[™]), pravastatin (PRAVACHOL[™]), and simvastatin (ZOCOR[™])), estradiol, testosterone (optionally at elevated dosages; Stuve *et al. Neurology* 8:290-301 (2002)), androgen, hormone-replacement therapy, a TNF inhibitor such as an antibody to TNF-alpha, DMARD, NSAID, plasmapheresis or plasma exchange, trimethoprim-sulfamethoxazole (BACTRIM[™], SEPTRA[™]), mycophenolate mofetil, H2-blockers or proton-pump inhibitors (during the use of potentially ulcerogenic immunosuppressive therapy), levothyroxine, cyclosporin A (*e.g.* SANDIMMUNE[®]), somatostatin analogue, cytokine, anti-cytokine antagonist or antibody, anti-metabolite, immunosuppressive agent, rehabilitative surgery, radioiodine, thyroidectomy, BAFF antagonist 25 such as BAFF or BR3 antibodies or immunoadhesins, anti-CD40 receptor or anti-CD40 ligand (CD154), anti-IL-6 receptor antagonist/antibody, another B-cell surface antagonist or antibody such as a humanized 2H7 or other humanized or human CD20 antibody with rituximab, *etc.* 30

Preferred such medicaments are a chemotherapeutic agent, a cytotoxic agent, anti-integrin, gamma globulin, anti-CD4, cladribine, trimethoprim-sulfamethoxazole, an H2-blocker, a proton-pump inhibitor, a corticosteroid, cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, androgen, hormone-replacement drug, a TNF inhibitor, DMARD, NSAID (to treat, for example, musculoskeletal 35 symptoms), levothyroxine, cyclosporin A, somatostatin analogue, cytokine antagonist or cytokine-receptor antagonist, anti-metabolite, BAFF antagonist such as BAFF antibody or BR3 antibody, especially a BAFF antibody, immunosuppressive agent, and another B-cell surface marker antibody, such as a combination of rituximab and a humanized 2H7 or other humanized CD20 antibody.

The more preferred such medicaments are a chemotherapeutic agent, an immunosuppressive agent, 40 including an antibody against TNF-alpha, an antibody against CD40-CD40 ligand, and a BAFF antagonist

such as a BAFF or BR3 antibody, a DMARD, a cytotoxic agent, an integrin antagonist, a NSAID, a cytokine antagonist, or a hormone, or a combination thereof. Immunosuppressants may be required, for example, for very active disease with major organ involvement, and include such agents as cyclophosphamide (CYTOXAN®), chlorambucil, leflunomide, MMF, azathioprine (IMURAN®), and methotrexate. BAFF antagonists may be useful in combination with the first medicament for efficacy.

Still more preferred are a steroid, chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone, or a combination thereof, most preferably a steroid and/or an immunosuppressive agent, still most preferably, a corticosteroid and/or immunosuppressive agent.

In one particularly preferred embodiment, the second medicament is or comprises one or more steroids, for example, a corticosteroid, which is preferably prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone. Such steroid is preferably administered in lower amounts than are used if the first medicament, *e.g.*, CD20 antibody, is not administered to a patient treated with steroid. In a preferred aspect, the steroid is not administered with any second antibody exposure or is administered with the second exposure but in lower amounts than are used with the initial antibody exposure. Also preferred is wherein the steroid is not administered with third or later antibody exposures.

In a still further particularly preferred aspect, the second medicament is an immunosuppressive agent, more preferably cyclophosphamide, MMF, chlorambucil, azathioprine, leflunomide, or methotrexate, and preferably administered at least with the initial antibody exposure. In one embodiment, azathioprine, methotrexate, or MMF are preferably used instead of cyclophosphamide for the maintenance of remission.

In a yet further preferred aspect, the second medicament is a combination of one or more steroids and immunosuppressive agent.

Prophylactic treatment of the ANCA-associated vasculitis with fluconazole (DIFLUCAN™) orally for fungal infection may also be used, as well as trimethoprim-sulfamethoxazole (480 mg) three times weekly for prophylactic treatment of patients with *pneumocystis carinii*. Jayne and Rasmussen, *supra*.

All these second medicaments may be used in combination with each other or by themselves with the first medicament, so that the expression "second medicament" as used herein does not mean it is the only medicament besides the first medicament, respectively. Thus, the second medicament need not be one medicament, but may constitute or comprise more than one such drug.

These second medicaments as set forth herein 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. If such second medicaments are used at all, preferably, they are used in lower amounts than if the first medicament were not present, especially in subsequent dosings beyond the initial dosing with the first medicament, so as to eliminate or reduce side effects caused thereby.

For the re-treatment method herein, where a second medicament is administered in an effective amount with an antibody exposure, it may be administered with any exposure, for example, only with one exposure, or with more than one exposure. In one embodiment, the second medicament is administered with the initial exposure. In another embodiment, the second medicament is administered with the initial and second exposures. In a still further embodiment, the second medicament is administered with all exposures. It is preferred that after the initial exposure, such as of steroid, the amount of such second medicament is

reduced or eliminated so as to reduce the exposure of the subject to an agent with side effects such as prednisone, prednisolone, methylprednisolone, and cyclophosphamide.

As a specific example, treatment of patients with microscopic polyangiitis and Wegener's granulomatosis has three phases: (1) induction of remission, (2) maintenance of remission, and (3) treatment of relapse. Current induction therapy often consists of cyclophosphamide (CYTOXAN®) and corticosteroids. This includes a high dose of intravenous methylprednisolone for several days (e.g., one to five days), plus oral prednisone tapered over a period of time, such as 3-5 months. For aggressive disease, use of high-dose intravenous methylprednisolone for three days is recommended, combined with intravenous or oral cyclophosphamide. Tapering doses of prednisone preferably follows, along with cyclophosphamide maintenance for 12 to 18 months. When the first medicament is employed, such amount and frequency of dosing are preferably reduced further, since the lowest dosage of steroids that controls the disease should be used. Infection should be considered if the symptoms appear to exacerbate. For patients in sustained remission at 12 months, it is preferred that the use of all such second medicaments is discontinued at a faster rate with the first medicament herein administered than without it. Patients whose symptoms are under good control must, nevertheless, be closely followed at six-month intervals for signs and symptoms of relapse. During treatment with these agents, complete blood counts and liver function tests should be performed periodically.

The combined administration of a second medicament includes co-administration (concurrent administration), using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents (medicaments) simultaneously exert their biological activities.

The antibody or antagonist herein is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous (i.v.), intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, e.g., US 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody or antagonist may suitably be administered by pulse infusion, e.g., with declining doses of the antibody or antagonist. Preferably, the dosing is given intravenously or subcutaneously, and more preferably by intravenous infusion(s).

If multiple exposures of antibody are provided, each exposure may be provided using the same or a different administration means. In one embodiment, each exposure is by intravenous administration. In another embodiment, each exposure is given by subcutaneous administration. In yet another embodiment, the exposures are given by both intravenous and subcutaneous administration.

In one embodiment, the CD20 antibody is administered as a slow intravenous infusion rather than an intravenous push or bolus. For example, a steroid such as prednisolone or methylprednisolone (e.g., about 80-120 mg i.v., more specifically about 100 mg i.v.) is administered about 30 minutes prior to any infusion of the CD20 antibody. The CD20 antibody is, for example, infused through a dedicated line.

For the initial dose of a multi-dose exposure to CD20 antibody, or for the single dose if the exposure involves only one dose, such infusion is preferably commenced at a rate of about 50 mg/hour. This may be escalated, e.g., at a rate of about 50 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. However, if the subject is experiencing an infusion-related reaction, the infusion rate is preferably

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reduced, *e.g.*, to half the current rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such dose of CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed at about 255 minutes (4 hours 15 min.). Optionally, the subjects receive a prophylactic treatment of acetaminophen/paracetamol (*e.g.*, about 1 g) and diphenhydramine HCl (*e.g.*, about 50 mg or equivalent dose of similar agent) by mouth about 30 to 60 minutes prior to the start of an infusion.

If more than one infusion (dose) of CD20 antibody is given to achieve the total exposure, the second or subsequent CD20 antibody infusions in this infusion embodiment are preferably commenced at a higher rate than the initial infusion, *e.g.*, at about 100 mg/hour. This rate may be escalated, *e.g.*, at a rate of about 100 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. Subjects who experience an infusion-related reaction preferably have the infusion rate reduced to half that rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such second or subsequent dose of CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed by about 195 minutes (3 hours 15 minutes).

A discussion of methods of producing, modifying, and formulating such antibodies follows.

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.

CD20 antigen to be used for production of, or screening for, antibody(ies) may be, *e.g.*, a soluble form of CD20 or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing CD20 at their cell surface can be used to generate, or screen for, antibody(ies). Other forms of CD20 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 (*s.c.*) or intraperitoneal (*i.p.*) 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 $\text{R}^1\text{N}=\text{C}=\text{NR}$, 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 immunosorbent 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.*, *BioTechnology*, 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.

In addition, antibodies comprising a variant Fc region with high affinity for FcγR are useful for treating diseases where an enhanced efficacy of effector cell function is desired, such as autoimmune diseases,

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as set forth, for example, in US 2003/0037000 and WO 2004/63351 (MacroGenics, Inc. STAVENHAGEN *et al.*).

(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); Verhoeven *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*, 362:253-258 (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 *et al.*, *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.* 221:581-597 (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.*, *BioTechnology* 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 CD20 antigen. Other such antibodies may bind CD20 and further bind a second B-cell surface marker. Alternatively, an anti-CD20
5 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 CD20-binding arm and an arm that binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or
10 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 co-expression 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
15 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
20 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
25 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
30 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).

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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 (CD20) 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 is 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_1^1 , α_2^1 , α_3^1 , N-acetyl- γ_1^1 , PSAG and θ^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, *Aleurias fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAP1, PAP2, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria 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 Al^{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 glutaraldehyde), 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.

5 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).

10 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 convert it into its more active, cytotoxic form.

15 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
20 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
25 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
30 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 non-proteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody
35 fragments, such as Fab', linked to one or more PEG molecules are an especially preferred embodiment of the invention.

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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 WO 97/38731 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 protein or peptide antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

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

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include 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

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substitutional mutagenesis of antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 3 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 3, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 3

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
5 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;

10 (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
15 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
20 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
25 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
30 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
35 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.

5 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).

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

20 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 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621;
25 US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO
30 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)).

35 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. 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, *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).

WO 00/42072 (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., IgG₁, IgG₂, IgG₃, or IgG₄) 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 dextrans; 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).

5 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 10 mg/mL rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection, pH 6.5.

10 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.

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

The formulation herein may also contain more than one active compound (a second medicament as noted above) as necessary, preferably those with complementary activities that do not adversely affect each other. The type and effective amounts of such medicaments depend, for example, on the amount of antibody present in the formulation, and clinical parameters of the subjects. The preferred such medicaments are noted above.

20 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-(methylmethacrylate) 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).

25 Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable 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.

30 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, articles of manufacture containing materials useful for the treatment of ANCA-associated vasculitis described above are provided. In one aspect, the article of manufacture comprises (a) a container comprising an antagonist that binds to a B-cell surface marker (e.g., an antibody that so binds, including a CD20 antibody) (preferably the container comprises the antagonist or antibody and a pharmaceutically acceptable carrier or diluent within the container); and (b) a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the antagonist or antibody of about 400 mg to 1.3 grams at a frequency of one to three doses is administered to the patient within a period of about one month.

Thus, the invention provides an article of manufacture comprising: a container comprising a CD20 antibody, or an antibody or antagonist that binds to a B-cell surface marker; and a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the CD20 antibody, or the antibody or antagonist that binds to a B-cell surface marker, of about 400 mg to 1.3 grams, at a frequency of one to three doses, is administered to the patient within a period of about one month.

In a preferred embodiment, the article of manufacture herein further comprises a container comprising a second medicament, wherein the antagonist or antibody is a first medicament. This article further comprises instructions on the package insert for treating the patient with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone. The preferred second medicaments are those preferred as set forth above, and most preferred is a steroid or an immunosuppressive agent or both.

In another aspect, the invention provides an article of manufacture comprising: (a) a container comprising an antibody that binds to a B-cell surface marker (e.g., a CD20 antibody) (preferably the container comprises the antibody and a pharmaceutically acceptable carrier or diluent within the container); and (b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

Preferably, such package insert is provided with instructions for treating ANCA-associated vasculitis 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as about one to four doses, preferably as a single dose or as two or three separate doses of antibody.

In a specific aspect, an article of manufacture is provided comprising:

(a) a container comprising an antibody that binds to a B-cell surface marker (e.g., a CD20 antibody) (preferably the container comprises the antibody and a pharmaceutically acceptable carrier or diluent within the container); and

(b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably, the antibody exposures are of about 0.5 to 4 grams.

In a preferred embodiment of these inventive aspects, the article of manufacture herein further comprises a container comprising a second medicament, wherein the antibody is a first medicament, and which article further comprises instructions on the package insert for treating the subject with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone, most preferably a steroid or an immunosuppressive agent, or both.

In all of these aspects, the package insert is 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 ANCA-associated vasculitis 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 antagonist or antibody. The label or package insert indicates that the composition is used for treating ANCA-associated vasculitis in a patient or subject eligible for treatment with specific guidance regarding dosing amounts and intervals of antagonist or antibody and any other medicament being provided. The article of manufacture may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWI), phosphate-buffered saline, Ringer's solution, and/or 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.

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

Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study assesses the superiority of efficacy and safety of rituximab (MABTHERA®/RITUXAN®) in a certain dosing regimen compared to placebo for treatment of signs and symptoms in patients with Wegener's granulomatosis exhibiting one or more symptoms of systemic disease.

Rituximab (1000 mg i.v. x 2) is administered i.v. in two initial doses at days 1 and 15 with 1 mg/kg of oral prednisone daily (which is reduced to 40 mg/day at week 4, and tapered using a standardized tapering regimen resulting in complete discontinuation of prednisone over the following 3-5 months). This experimental regimen is compared to the same regimen except using rituximab placebo instead of rituximab, with 1:1 randomization between the two arms of the study, with about 48 patients per arm (total 96 patients).

Active disease is defined as a Birmingham Vasculitis Activity Score/Wegener's granulomatosis (BVAS/WG) score of greater than 0. For trial inclusion, the patient's BVAS/WG score must be 3 or greater (or have been 3 or greater within 28 days of randomization). Each major item on the BVAS/WG evaluation form is scored 3 points. Each minor item is scored 1 point. In determining the degree of disease activity, the investigator will distinguish between active vasculitis (new/worse BVAS/WG items as opposed to persistent items) and permanent organ damage (caused by previously active vasculitis).

A severe flare is a new occurrence of one or more major BVAS/WG items. (Major items have a * on the BVAS/WG scoring sheet.) Generally, such flares are treated by increases in prednisone dose or cyclophosphamide dose.

Severe Wegener's granulomatosis occurs in a patient whose disease is not classifiable as limited, by definition below.

A limited flare is a new occurrence of one or more minor BVAS/WG items. Generally, such flares are treated with increases in prednisone dose or an increase in methotrexate dose.

Limited Wegener's granulomatosis occurs in a patient who meets the modified American College of Rheumatology (ACR) criteria for a diagnosis of Wegener's granulomatosis but who does not have disease that poses an immediate threat to either a critical individual organ or to the patient's life. Specifically, this means that:

- The patient has no red blood cell casts in the urine.
- If hematuria (but no +RBC casts) is present, the serum creatinine must be less than or equal to 1.4 and there must be evidence of no rise of creatinine more than 25% above the patient's baseline.
- Pulmonary involvement must be circumscribed, such that the room air pO_2 is > 70 mmHg or the room air O_2 saturation by pulse oximetry is $> 92\%$. Pulmonary hemorrhage may be treated as limited disease provided there is no evidence of progression of the process. In the absence of data on progression, pulmonary hemorrhage may be treated as severe disease at the discretion of the physician.
- No disease may exist within any other critical organ (e.g., the gastrointestinal tract, eyes, central nervous system) that, without the immediate institution of maximal therapy (i.e., pulse methylprednisolone and daily oral cyclophosphamide), threatens the function of that organ and/or the patient's life.

A newly-diagnosed patient is a patient on his/her first treatment course of corticosteroids and/or a chemotherapeutic or immunosuppressive agent for Wegener's granulomatosis, with no history of increase in immunosuppressive therapy prior to entry into the study.

For the purpose of scoring BVAS/WG, persistent disease is defined as the presence of ongoing disease activity that was present at the previous trial evaluation (i.e., not new or worse activity).

A purified protein derivative skin test may be used to detect latent tuberculosis infection.

A refractory patient is a patient with a history of immunosuppressive therapy (corticosteroids and/or an immunosuppressive agent or chemotherapeutic agent) prior to the initiation of treatment for the Wegener's granulomatosis activity that makes the patient eligible for this study.

Patients will be considered to be in remission when their BVAS/WG score is 0.

This rituximab-based regimen challenges the current standard of care, and is intended to limit patient exposure to steroids and its known toxicities, and to demonstrate improved net clinical benefit. Patients are monitored for disease activity, use of additional immunosuppressants, steroid usage, and safety events over

the trial length of one year, with the primary efficacy endpoint of the trial measured at 3 months, with follow-up to 16.8 months. Safety follow-up is required until 12 months following the last dose of rituximab or return of ANCA into the normal range, whichever occurs later.

The primary objective is to determine the proportion of patients achieving a BVAS/WG score of 0 and successful prednisone taper at 6 months, and no pre-specified adverse events.

It is predicted and expected that rituximab (or a humanized 2H7 substituted for rituximab) is effective in inducing remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and to permit reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean prednisolone dose (mg/day) is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. If the mean BVAS/WG at entry is 3.6, at 6 months of treatment, it is expected to decrease to a statistically significant value of 0.6. Intermittently active disease is expected to be observed in fewer than 70% of the patients. In contrast, the control arm is expected to show much less decrease in BVAS/WG and C-reactive protein, and in steroid use, and fewer patients in remission.

Example 2

Study of Efficacy of Rituximab in Patients with Microscopic Polyangiitis

The protocol in Example 1 is followed except that the patients are treated for microscopic polyangiitis. It is expected that similar results will be observed as for Wegener's granulomatosis, i.e., that remission, as measured by the BVAS/WG score of 0, is expected to occur in at least 80% of the patients treated in the study arm and that steroid use is expected to decrease over the course of the study, which results are expected to be much better, in a statistically significant sense, than the control results.

Example 3

Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study assesses the superiority of efficacy and safety of re-treatment with rituximab (MABTHERA®/RITUXAN®) compared to placebo in adult subjects with Wegener's granulomatosis. Study I examines acute disease, either first presentation or relapse (BVAS ≥ 10 ; $n = 16$); study II examines persistent disease (BVAS ≥ 4 ; $n = 16$). Patients receive rituximab (1 g i.v.) in three initial doses at days 1, 8, and 15 for Studies I and II. Concomitant therapy in Study I includes 1 mg/kg/day oral prednisone tapered according to the regimen in Example 1 and cyclophosphamide (according to standard treatment). Study II patients receive rituximab and 1 mg/kg/day oral prednisone tapered according to the regimen in Example 1. All subjects receive a second rituximab/placebo infusion course of 1000 mg i.v. separated by 14 days at weeks 24 and 26, respectively, without steroids or cyclophosphamide, whether the patients exhibited symptoms or were in complete remission. Courses of rituximab treatment must be separated by a minimum interval of 16 weeks.

The experimental regimens are compared to rituximab placebo + the same doses of tapered oral prednisone and cyclophosphamide (Study I), or tapered oral prednisone (Study II).

Changes in immunosuppressive drugs are not permitted during the studies, unless mandated by toxicity, and requests to taper a drug other than the oral prednisone must be discussed in advance with the Medical Monitor. Study personnel will be trained on how to properly administer rituximab. Subjects may be hospitalized for observation, particularly for their first infusion, at the discretion of the investigator. Rituximab must be administered under close supervision, and full resuscitation facilities must be immediately available.

Patients are monitored each month for 12 months for disease activity, use of additional immunosuppressants, flares of disease, prednisone usage, and safety events over the 52 weeks of the study. The primary efficacy endpoint of the trial is at 52 weeks, and efficacy measures are assessed by a unique Examining Assessor who is not involved with patient treatment or other study procedures. The patients are assessed for their BVAS/WG scores and successful prednisone taper. At the end of 52 weeks, subjects who received rituximab placebo or rituximab but demonstrate a BVAS/WG score of 0 and successful prednisone taper at 6 months will complete study participation. Subjects who received rituximab but have not demonstrated such score at 52 weeks are observed for 6 months following the last course of rituximab or until a BVAS/WG score of 0, whichever occurs first. Sites will be informed as to whether a subject must continue in follow-up, but not whether the subject received placebo or rituximab. Safety follow-up is required until 12 months following the last dose of rituximab or a BVAS/WG score of 0, whichever occurs later.

These rituximab-based regimens challenge the current standard of care, and are expected to demonstrate improved net clinical benefit, with the primary objective to determine the proportion of patients achieving the primary endpoint of a BVAS/WG score of 0, and successful prednisone taper.

It is predicted and expected that administration of rituximab or a humanized 2H7 to the subject in the protocols of Studies I and II set forth above will induce remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and permit reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean steroid use for both Studies I and II is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. These results are expected to be significantly better than those of the control arms for Studies I and II.

It is also expected that at about week 48-54, another 2-g dose of the rituximab given all at once or spread out over about 14-16 days in 1-gram amounts would be effective to treat Wegener's granulomatosis for the entire second year (causing a BVAS/WG score of 0 in at least 80% of the enrolled patients), with or without the steroid(s) and/or other immunosuppressive agents, with a marked improvement over control patients receiving rituximab placebo rather than rituximab. Thus, the rituximab (or a humanized 2H7) would be administered initially within about the 2-week time period, followed by another treatment at about 4-8 months, followed by another treatment at about one year from initial treatment (measured from the time any one of the doses was given), followed by treatment at about two years from initial treatment, with expected success, in about one-gram x 2-4 dosing for each treatment, administered together, about weekly, or about

every other week over about two to four weeks. This re-treatment protocol is expected to be successfully used for several years with little or no adverse effects.

Example 4

Second Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study is the same as in Example 3 except that the initial dose of rituximab or rituximab placebo is given as 1000 mg i.v. x 2 (on day 0, with the second infusion occurring on Day 15 +/- 1 day), and the subsequent course of rituximab or placebo infusions administered at weeks 24 and 26 and consisting of 2 biweekly doses is administered only to those subjects in remission, e.g., those not exhibiting increasing disease activity, as by rising ANCA titers, sustained elevated ANCA titers, and other symptoms. All other criteria are the same.

It is predicted and expected that administration of rituximab or a humanized 2H7 to the subject in the scheduled redosing protocol set forth above will be effective in inducing remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and in permitting reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean prednisolone dose (mg/day) is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. These results are expected to be significantly better than those of the control.

It is also expected that at about week 48-54, another 2-g dose of the CD20 antibody (e.g., rituximab or a humanized 2H7) given all at once or spread out over about 14-16 days in 1-gram amounts would be effective to treat Wegener's granulomatosis for the entire second year (with at least 80% of the enrolled patients having a BVAS/WG score of 0), with or without the prednisone taper and i.v. methylprednisolone and/or other immunosuppressive agents. Thus, the CD20 antibody would be administered initially within about the 2-week time period, followed by another treatment at about 4-8 months, followed by another treatment at about one year from initial treatment (measured from the time any one of the doses was given), followed by treatment at about two years from initial treatment, with expected success, in about one-gram x 2-4 dosing for each treatment, administered together, about weekly, or about every other week over about two to four weeks. The results of this treatment would be expected to be much better than those of the control with placebo. This re-treatment protocol is expected to be successfully used for several years with little or no adverse effects.

Example 5

Third Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

It is expected that Example 4 results would be successful if the patients were initially treated with rituximab and then re-treated with rituximab one year after first being treated, using the same dosing

and other protocol of Example 4 except that rituximab is given at one-year intervals rather than six-month intervals.

Example 6

Study of Efficacy of Rituximab in Subjects with Generalized ANCA-Associated Vasculitis

5 A randomized, multi-center, double-masked, placebo-controlled trial is performed in patients with generalized ANCA-associated vasculitis using rituximab. Two-hundred patients are randomized to either (1) conventional treatment (cyclophosphamide and corticosteroids, followed by azathioprine); or (2) rituximab (plus corticosteroids, initially) for remission induction, using 1 gram of rituximab on day 1 and again on day 15.

10 The primary clinical comparison is the ability of rituximab in this dosing regimen and corticosteroids to induce disease remissions, as measured by the cumulative disease activity at six months. Consistent with the standard duration of treatment for ANCA-associated vasculitis, patients in the conventional therapy arm will receive cyclophosphamide for up to 6 months followed by azathioprine, to complete a total length of treatment of 18 months. To assess the ability of rituximab to restore B-cell tolerance, patients in both arms of the trial will be followed for a total of 18 months.

15 It is expected that rituximab (or a humanized 2H7 substituted for rituximab) will induce stable remissions in the patients with ANCA-associated vasculitis and will re-establish B-cell tolerance to the ANCA target antigens in at least two thirds of the patients. It is also expected that rituximab or other CD20 antibody will be at least as effective as the conventional treatment regimen for induction and maintenance of disease remission, offering substantial advantages over standard therapy by virtue of its superior side-effect profile, e.g., much less toxic than chemotherapeutics and steroids, and better at restoring tolerance.

Example 7

Re-treatment Study of Efficacy of Rituximab in Subjects with Severe Wegener's Granulomatosis or Severe Microscopic Polyangiitis

25 Twenty patients with active severe Wegener's granulomatosis or severe microscopic polyangiitis, positive ANCA test, and BVAS/WG score of at least 3, who are unresponsive to cyclophosphamide or have contraindications for cyclophosphamide use, are enrolled. See the definition of severe Wegener's granulomatosis in Example 1. The remission induction regimen consists of oral prednisone (1 mg/kg/day) and rituximab (1 gram at day 1 and 1 gram at day 15). By week 4, the prednisone is reduced to 40 mg/day. A standardized tapering regimen follows, resulting in complete discontinuation of prednisone over the following 16 weeks. This is compared with the same regimen, but with rituximab placebo rather than rituximab (control study). The protocol stipulates re-treatment with the same remission induction regimen at 6 months for all patients, whether they are experiencing a disease flare after reconstitution of B cells, whether they are asymptomatic with a recurrence of ANCA or ANCA titer rise coinciding or following reconstitution of B cells, or whether in complete remission. This re-treatment regimen includes the 1 g x 2 two weeks apart for

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rituximab and rituximab placebo. A clinical flare in the absence of B cells is considered treatment failure. The patients are assessed monthly for one year.

It is expected that the patients in the treatment arm will tolerate rituximab infusions well and that their B cells will be depleted swiftly and all will achieve complete remission (BVAS/WG of 0) by three months. All patients in the treatment arm are expected to complete the glucocorticoid taper by 6 months. It is expected that after 12 months, no patient in the treatment arm will experience a clinical flare, and that B cells will return in most, if not all, such patients in the 12 months. Other than glucocorticoids, no additional immunosuppressive agents are expected to be necessary for induction of remission and maintenance of sustained remission (6 months or longer) in the rituximab-treated patients.

Example 8

Humanized 2H7 Variants Useful Herein

Useful for purposes herein are humanized 2H7 antibodies comprising 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:35), 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:36), 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:37), for example, SEQ ID NO:11 (Fig. 1B), and

CDR H3 sequence of VVYYSSXXYWYFDV wherein the X at position 6 is N, A, Y, W, or D, and the X at position 7 is S or R (SEQ ID NO:38), for example, SEQ ID NO:12 (Fig. 1B).

The humanized 2H7 antibodies herein include those with heavy-chain amino acid sequences containing a C-terminal lysine and those without. 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_LXI), 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 non-native-sequence 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 sequence of SEQ ID NO:13 or 30, and heavy-chain amino acid sequence of SEQ ID NO:14, 15, 29, 31, 34, or 39, the sequence of SEQ ID NO:39 being given below.

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

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. 6,737,056, L. Presta.

5 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. 6,737,056, L. Presta.

10 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. 6,528,624, Idusogie *et al.*

15 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 in SEQ ID NO:8 of N100A; or D56A and N100A; or D56A, N100Y, and S100aR; and a variable light domain with alteration in SEQ ID NO:2 of M32L; or S92A; or M32L and S92A.

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.

20 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 Table 4 below. Unless otherwise indicated, the 2H7 variants will have the same light chain as that of v16.

Table 4
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, K322A, K334A,
138	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A,
477	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A, N434W
375	-	-	K334L
588	-	-	S298A, K326A, E333A, K334A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, K326A, E333A, K334A

25 One preferred humanized 2H7 comprises 2H7.v16 variable light-domain sequence:

DIQMTQSPSSLSASVGDRVTTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQWSFNPPFTFGQGTKVEIKR (SEQ ID NO:2);

and 2H7.v16 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAITYPGNGDTSYNQKFK
5 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYDVWGQGTLLTVSS (SEQ ID
NO:8).

Where the humanized 2H7.v16 antibody is an intact antibody, it may comprise the light-chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
10 DFTLTISSLQPEDFATYYCQQWSFNPPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO:13);

and the heavy-chain amino acid sequence of SEQ ID NO:14 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAITYPGNGDTSYNQKFK
15 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPELLEGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
20 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:15).

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

DIQMTQSPSSLSASVDRVTTCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISSLQPEDFATYYCQQWAFNPPFTFGQGTKVEIKR (SEQ ID NO:39)

and 2H7.v511 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAITYPGNGATSYNQKFK
25 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWFYDVWGQGTLLTVSS (SEQ ID
NO:40).

Where the humanized 2H7.v511 antibody is an intact antibody, it may comprise the light-chain amino acid sequence:

DIQMTQSPSSLSASVGDRVTTCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
30 DFTLTISSLQPEDFATYYCQQWAFNPPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO:30)

and the heavy-chain amino acid sequence of SEQ ID NO: 31 or:

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EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWYFDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
5 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
AALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:41).

See Figures 7 and 8, which align the mature light and heavy chains, respectively, of humanized
2H7.v511 with humanized 2H7.v16 using the C-terminal lysine sequence for the heavy chain..

10 Where the humanized 2H7.v31 antibody is an intact antibody, it may comprise the light-chain amino
acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPKGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSSLTLSKADYEKHKVYACEVTHQGLSSP
15 VTKSFNRGEC (SEQ ID NO:13)

and the heavy-chain amino acid sequence of SEQ ID NO:15 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYRYWYFDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
20 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:42) or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFK
25 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWYFDVWGQGTLLTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
AALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
30 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:43).

A preferred embodiment herein is where the antibody is humanized 2H7 comprising the variable
domain sequences in SEQ ID NOS:2 and 8 (version 16). Another preferred embodiment herein is where the
antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40 (version
511). Further preferred is where the antibody is humanized 2H7 comprising the variable domain sequences in
35 SEQ ID NOS:32 and 33 (see Figure 9 re version 114), such as one comprising the variable light-chain domain
in SEQ ID NO:32 and the heavy-chain amino acid sequence of SEQ ID NO:34. Further preferred is wherein
the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A
and N100A, or D56A, N100Y, and S100aR in SEQ ID NO:8 and a variable light-chain domain with alteration
M32L, or S92A, or M32L and S92A in SEQ ID NO:2.

WHAT IS CLAIMED IS:

1. A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.
2. The method of claim 1 wherein the dose is about 500 mg to 1.2 grams.
3. The method of claim 1 or 2 wherein the dose is about 750 mg to 1.1 grams.
4. The method of any one of claims 1-3 wherein the antibody is administered in two to three doses.
5. The method of any one of claims 1-4 wherein the antibody is administered in three doses.
6. The method of any one of claims 1-5 wherein the antibody is administered within a period of about 2 to 3 weeks.
7. The method of claim 6 wherein the period is about three weeks.
8. The method of any one of claims 1-7 wherein the ANCA-associated vasculitis is Wegener's granulomatosis.
9. The method of any one of claims 1-7 wherein the ANCA-associated vasculitis is microscopic polyangiitis.
10. The method of any one of claims 1-9 wherein a second medicament is administered in an effective amount, wherein the CD20 antibody is a first medicament.
11. The method of claim 10 wherein the second medicament is more than one medicament.
12. The method of claim 10 or 11 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a hormone, or a combination thereof.
13. The method of claim 12 wherein the second medicament is a steroid or an immunosuppressive agent or both.
14. The method of claim 13 wherein the second medicament is a steroid.
15. The method of claim 14 wherein the steroid is a corticosteroid.
16. The method of claim 15 wherein the steroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.
17. The method of any one of claims 14-16 wherein the steroid is administered in lower amounts than are used if the CD20 antibody is not administered to a patient treated with steroid.
18. The method of claim 13 wherein the second medicament is an immunosuppressive agent.
19. The method of claim 18 wherein the immunosuppressive agent is cyclophosphamide, chlorambucil, mycophenolate mofetil, leflunomide, azathioprine, or methotrexate.
20. The method of claim 19 wherein the immunosuppressive agent is cyclophosphamide.
21. The method of claim 13 wherein the second medicament is a steroid and an immunosuppressive agent.
22. The method of any one of claims 1-21 wherein the patient has never been previously treated with a CD20 antibody.
23. The method of any one of claims 1-22 wherein the patient has not relapsed with the vasculitis.
24. The method of any one of claims 1-22 wherein the antibody is a naked antibody.

25. The method of any one of claims 1-22 wherein the antibody is conjugated with another molecule.
26. The method of claim 25 wherein the other molecule is a cytotoxic agent.
27. The method of any one of claims 1-26 wherein the antibody is administered intravenously.
28. The method of any one of claims 1-26 wherein the antibody is administered subcutaneously.
- 5 29. The method of any one of claims 1-28 wherein no other medicament than the CD20 antibody is administered to the subject to treat the ANCA-associated vasculitis.
30. The method of any one of claims 1-29 wherein the antibody is rituximab.
31. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos. 2 and 8.
- 10 32. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40.
33. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:32 and 33.
- 15 34. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2.
35. The method of any one of claims 1-34 wherein the patient has a BVAS/WG score of 0 at six months after administration of the antibody.
- 20 36. The method of any one of claims 1-35 wherein the patient has an elevated level of a anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination thereof.
37. An article of manufacture comprising:
 - a. a container comprising a CD20 antibody; and
 - 25 b. a package insert with instructions for treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient, wherein the instructions indicate that a dose of the CD20 antibody of about 400 mg to 1.3 grams at a frequency of one to three doses is administered to the patient within a period of about one month.
- 30 38. The article of claim 37 further comprising a container comprising a second medicament, wherein the CD20 antibody is a first medicament, further comprising instructions on the package insert for treating the patient with the second medicament.
39. The article of claim 38 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.
- 35 40. The article of claim 38 or 39 wherein the second medicament is a steroid or an immunosuppressive agent or both.

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41. A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.
- 5 42. The method of claim 41 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.
43. The method of claim 41 or 42 wherein the second exposure is not provided until from about 20 to 30 weeks from the initial exposure.
- 10 44. The method of any one of claims 41-43 wherein the second exposure is not provided until from about 46 to 54 weeks from the initial exposure.
45. The method of any one of claims 41-44 wherein each of the initial and second antibody exposures is provided in amounts of about 0.5 to 4 grams.
46. The method of any one of claims 41-45 wherein each of the initial and second antibody exposures is provided in amounts of about 1.5 to 3.5 grams.
- 15 47. The method of any one of claims 41-46 wherein each of the initial and second antibody exposures is provided in amounts of about 1.5 to 2.5 grams.
48. The method of any one of claims 41-47 additionally comprising administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure, wherein the third exposure is not provided until from about 46 to 60 weeks from the initial exposure.
- 20 49. The method of claim 48 wherein the third antibody exposure is provided in an amount of about 0.5 to 4 grams.
50. The method of claim 48 or 49 wherein the third antibody exposure is provided in an amount of about 1.5 to 3.5 grams.
- 25 51. The method of any one of claims 48-50 wherein the third antibody exposure is provided in an amount of about 1.5 to 2.5 grams.
52. The method of any one of claims 48-51 wherein the third exposure is not provided until from about 46 to 55 weeks from the initial exposure.
53. The method of any one of claims 48-52 wherein no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.
- 30 54. The method of claim 53 wherein no further antibody exposure is provided until about 74 to 80 weeks from the initial exposure.
55. The method of any one of claims 41 or 43-54 wherein one or more of the antibody exposures is provided to the subject as a single dose of antibody.
- 35 56. The method of any one of claims 41-55 wherein each antibody exposure is provided to the subject as a single dose of antibody.

57. The method of any one of claims 41 or 43-54 wherein one or more of the antibody exposures is provided to the subject as separate doses of the antibody.
58. The method of any one of claims 41-54 or 57 wherein each antibody exposure is provided as separate doses of the antibody.
- 5 59. The method of claim 57 wherein the separate doses are from about 2 to 3 doses.
60. The method of any one of claims 57-59 wherein the separate doses constitute a first and second dose.
61. The method of any one of claims 57-59 wherein the separate doses constitute a first, second, and third dose.
- 10 62. The method of any one of claims 57-61 wherein a later dose is administered from about 1 to 20 days from the time the previous dose was administered.
63. The method of any one of claims 57-62 wherein a later dose is administered from about 6 to 16 days from the time the previous dose was administered.
64. The method of any one of claims 57-63 wherein a later dose is administered from about 14 to 16 days from the time the previous dose was administered.
- 15 65. The method of any one of claims 57-64 wherein the separate doses are administered within a total period of between about 1 day and 4 weeks.
66. The method of any one of claims 57-65 wherein the separate doses are administered within a total period of between about 1 and 25 days.
- 20 67. The method of any one of claims 57-66 wherein the separate doses are administered about weekly, with the second dose being administered about one week from the first dose and any later dose being administered about one week from the previous dose.
68. The method of any one of claims 57-67 wherein each separate dose of antibody is about 0.5 to 1.5 grams.
- 25 69. The method of any one of claims 57-68 wherein each separate dose of antibody is about 0.75 to 1.3 grams.
70. The method of any one of claims 41-69 wherein 4 to 20 antibody exposures are administered to the subject.
71. The method of any one of claims 41-70 wherein a second medicament is administered in an effective amount with an antibody exposure, wherein the CD20 antibody is a first medicament.
- 30 72. The method of claim 71 wherein the second medicament is administered with the initial exposure.
73. The method of claim 71 or 72 wherein the second medicament is administered with the initial and second exposures.
74. The method of any one of claims 71-73 wherein the second medicament is administered with all exposures.

75. The method of any one of claims 71-74 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a hormone, or a combination thereof.
- 5 76. The method of any one of claims 71-75 wherein the second medicament comprises a steroid or an immunosuppressive agent or both.
77. The method of any one of claims 71-76 wherein the second medicament is a steroid.
78. The method of claim 77 wherein the steroid is a corticosteroid.
79. The method of claim 78 wherein the steroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.
- 10 80. The method of any one of claims 77-79 wherein the steroid is administered in lower amounts than are used if the CD20 antibody is not administered to a subject treated with steroid.
81. The method of any one of claims 71-76 wherein the second medicament is an immunosuppressive agent.
- 15 82. The method of claim 81 wherein the immunosuppressive agent is cyclophosphamide, chlorambucil, leflunomide, mycophenolate mofetil, azathioprine, or methotrexate.
83. The method of claim 82 wherein the immunosuppressive agent is cyclophosphamide.
84. The method of any one of claims 71-76 wherein the second medicament comprises a steroid and an immunosuppressive agent.
- 20 85. The method of claim 72 wherein the second medicament is not administered with the second exposure, or is administered in lower amounts than are used with the initial exposure.
86. The method of any one of claims 41-85 wherein about 2-3 grams of the CD20 antibody is administered as the initial exposure.
- 25 87. The method of claim 86 wherein about 1 gram of the CD20 antibody is administered weekly for about three weeks as the initial exposure.
88. The method of claim 86 or 87 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.
89. The method of any one of claims 86-88 wherein the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.
- 30 90. The method of claim 86 wherein about 1 gram of the CD20 antibody is administered followed in about two weeks by another about 1 gram of the antibody as the initial exposure.
91. The method of claim 90 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.
- 35 92. The method of claim 90 or 91 wherein the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.

93. The method of any one of claims 86-92 wherein a steroid is administered to the subject before or with the initial exposure.
94. The method of claim 93 wherein the steroid is not administered with the second exposure or is administered with the second exposure but in lower amounts than are used with the initial exposure.
- 5 95. The method of claim 93 or 94 wherein the steroid is not administered with third or later exposures.
96. The method of any one of claims 41-95 wherein the subject has never been previously treated with a CD20 antibody.
97. The method of any one of claims 41-96 wherein the subject is in remission after the initial or a later antibody exposure.
- 10 98. The method of any one of claims 41-96 wherein the subject is in remission when provided the second antibody exposure.
99. The method of claim 98 wherein the subject is in remission when provided all antibody exposures.
100. The method of any one of claims 41-99 wherein the initial and second antibody exposures are with the same CD20 antibody.
- 15 101. The method of any one of claims 41-100 wherein all antibody exposures are with the same CD20 antibody.
102. The method of any one of claims 41-101 wherein the antibody is a naked antibody.
103. The method of any one of claims 41-101 wherein the antibody is conjugated with another molecule.
104. The method of claim 103 wherein the other molecule is a cytotoxic agent.
- 20 105. The method of any one of claims 41-104 wherein the antibody is administered intravenously.
106. The method of claim 105 wherein the antibody is administered intravenously for each antibody exposure.
107. The method of any one of claims 41-104 wherein the antibody is administered subcutaneously.
108. The method of claim 107 wherein the antibody is administered subcutaneously for each antibody exposure.
- 25 109. The method of any one of claims 41-108 wherein no other medicament than the CD20 antibody is administered to the subject to treat the ANCA-associated vasculitis.
110. The method of any one of claims 41-109 wherein the ANCA-associated vasculitis is Wegener's granulomatosis.
- 30 111. The method of any one of claims 41-109 wherein the ANCA-associated vasculitis is microscopic polyangiitis.
112. The method of any one of claims 41-111 wherein the antibody is rituximab.
113. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos. 2 and 8.

114. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40.

115. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:32 and 33.

5 116. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2.

10 117. The method of any one of claims 41-116 wherein the subject has a BVAS/WG score of 0 at six months after administration of the antibody.

118. The method of any one of claims 41-117 wherein the subject has an elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination thereof.

119. An article of manufacture comprising:

- 15 a. a container comprising a CD20 antibody; and
- b. a package insert with instructions for treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) 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 followed by a second antibody exposure, wherein the
- 20 second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

120. The article of claim 119 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.

121. The article of claim 119 or 120 wherein each of the initial and second antibody exposures is provided in an amount of 0.5 to 4 grams.

25 122. The article of any one of claims 119-121 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.

30 123. The article of claim 122 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

124. The article of claim 123 wherein the second medicament is a steroid or an immunosuppressive agent or both.

Sequence Alignment of Variable Light-Chain Domain

	FR1 10203040	CDR1	
2H7	QIVLSQSPAILSASPGEKVTMTC	[RASSSVS-YMH]	WYQQKP
	* * * *	* * * *	*
hu2H7.v16	DIQMTQSPSSLSASVGDRVITTC	[RASSSVS-YMH]	WYQQKP
		* * * *	
hum KI	DIQMTQSPSSLSASVGDRVITTC	[RASQISNYLA]	WYQQKP
	FR2 50607080	CDR2	FR3
2H7	GSSPKPNIY	[APSNLAS]	GVPARFSGSGSGTSYSLTISRVEA
	** *	*	*** ****
hu2H7.v16	GKAPKPLIY	[APSNLAS]	GVPSRFSGSGSGTDFTLTISLQP
	*	* * *	
hum KI	GKAPKLLIY	[AASSLES]	GVPSRFSGSGSGTDFTLTISLQP
	90	CDR3	FR4 100
2H7	EDAATYYC	[QQWSFNPPT]	FGAGTKLELKR
	*		* * *
hu2H7.v16	EDFATYYC	[QQWSFNPPT]	FGQGTKVEIKR
		*****	*
hum KI	EDFATYYC	[QQYNSLPWT]	FGQGTKVEIKR

FIG. 1A

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Sequence Alignment of Variable Heavy-Chain Domain

	FR1 10203040	CDR1	
2H7	QAYLQQSGAELVRPGASVKMSCKAS	[GYTFTSYNMH]	WVKQT
	*** ** *	*	*
hu2H7.v16	EVQLVESGGGLVQPGGSLRLSCAAS	[GYTFTSYNMH]	WVRQA
		* * *	
hum III	EVQLVESGGGLVQPGGSLRLSCAAS	[GFTFSSYAMS]	WVRQA
	FR2 50 a607080	CDR2	FR3
2H7	PRQGLEWIG	[AIYPGNGDTSYNQKFKG]	KATLTVDKSSSTAYM
	** *		** ** *
hu2H7.v16	PGKGLEWVG	[AIYPGNGDTSYNQKFKG]	RFTISVDKSKNTLYL
	* * *		*
hum III	PGKGLEWVA	[VISGDGGSTYYADSVKG]	RFTISADNSKNTLTL
	abc90100abcde110	CDR3	FR4
2H7	QLSSLTSEDSAVYFCAR	[VYYYSNSYWYFDV]	WGTGTLTVTVSS
	** * *		*
hu2H7.v16	QMNSLRAEDTAVYYCAR	[VYYYSNSYWYFDV]	WGQGLTVTVSS
		*****	*
hum III	QMNSLRAEDTAVYYCAR	[GRVGYSLY---DY]	WGQGLTVTVSS

FIG. 1B

Humanized 2H7.v16 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQWSFNPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG._2

Humanized 2H7.v16 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFYFDVWGQGTLLTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHAEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

FIG._3

Humanized 2H7.v31 Heavy Chain

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFYFDVWGQGTLLTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHAEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

FIG._4

Alignment of v16 and v138 Light Chains (Sequential Numbering):

	10	20	30	40	50
hu2H7.v16.light	DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP				

hu2H7.v138.light	DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP				
	10	20	30	40	50
	60	70	80	90	100
hu2H7.v16.light	SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG				

hu2H7.v138.light	SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG				
	60	70	80	90	100
	110	120	130	140	150
hu2H7.v16.light	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD				

hu2H7.v138.light	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD				
	110	120	130	140	150
	160	170	180	190	200
hu2H7.v16.light	NALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGL				

hu2H7.v138.light	NALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGL				
	160	170	180	190	200
	210				
hu2H7.v16.light	SSPVTKSFNRGEC				

hu2H7.v138.light	SSPVTKSFNRGEC				
	210				

FIG._5

Alignment of v16 and v138 Heavy Chains (Sequential Numbering):

	10	20	30	40	50
hu2H7.v16.heavy	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA				
hu2H7.v138.heavy	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA				
	10	20	30	40	50
	60	70	80	90	100
hu2H7.v16.heavy	IYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVV				
hu2H7.v138.heavy	IYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVV				
	60	70	80	90	100
	110	120	130	140	150
hu2H7.v16.heavy	YYSNSYWFVDVWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL				
hu2H7.v138.heavy	YYSASYWFVDVWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL				
	110	120	130	140	150
	160	170	180	190	200
hu2H7.v16.heavy	VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT				
hu2H7.v138.heavy	VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT				
	160	170	180	190	200
	210	220	230	240	250
hu2H7.v16.heavy	QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP				
hu2H7.v138.heavy	QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP				
	210	220	230	240	250
	260	270	280	290	300
hu2H7.v16.heavy	KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ				
hu2H7.v138.heavy	KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ				
	260	270	280	290	300
	310	320	330	340	350
hu2H7.v16.heavy	YNSTYRVVSVLTVHLHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE				
hu2H7.v138.heavy	YNATYRVVSVLTVHLHQLDNLNGKEYKCKVSNALPAPIAATISKAKGQPRE				
	310	320	330	340	350
	360	370	380	390	400
hu2H7.v16.heavy	PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP				
hu2H7.v138.heavy	PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP				
	360	370	380	390	400
	410	420	430	440	450
hu2H7.v16.heavy	PVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK				
hu2H7.v138.heavy	PVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK				
	410	420	430	440	450

FIG._6

Light Chain Alignment

	1	32
hu2H7.v16	DIQMTQSPSSLSASVGDRVTTTCRASSSVSYMHWYQQKPGKAPKPLIYAP	

hu2H7.v511	DIQMTQSPSSLSASVGDRVTTTCRASSSVSYLHWYQQKPGKAPKPLIYAP	
	52	
hu2H7.v16	SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQWSFNPPTFGQG	

hu2H7.v511	SNLASGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQWAFNPPTFGQG	
	102	
hu2H7.v16	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD	

hu2H7.v511	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD	
	152	
hu2H7.v16	NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL	

hu2H7.v511	NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGL	
	202	214
hu2H7.v16	SSPVTKSFNRGEC	

hu2H7.v511	SSPVTKSFNRGEC	

FIG. 7

Heavy Chain Alignment

hu2H7.v16	1	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
hu2H7.v511		EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHW
hu2H7.v16	37	52a82abc
hu2H7.v511		
hu2H7.v16	83	100abcde113
hu2H7.v511		
hu2H7.v16	118	
hu2H7.v511		
hu2H7.v16	132	
hu2H7.v511		
hu2H7.v16	182	
hu2H7.v511		
hu2H7.v16	232	
hu2H7.v511		
hu2H7.v16	282	
hu2H7.v511		
hu2H7.v16	332	
hu2H7.v511		
hu2H7.v16	382	
hu2H7.v511		
hu2H7.v16	432	447
hu2H7.v511		

FIG._8

Humanized 2H7.v114 Variable Light-Chain Domain:

DIQMTQSPSSLSASVGDRTTTCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSG
TDFTLTISLQPEDFATYYCQWAFNPPTFGQGTKVEIKR

FIG._9A

Humanized 2H7.v114 Variable Heavy-Chain Domain:

FEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAITYPGNGATSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSASYWYFDVWGQGTLTIVSS

FIG._9B

Humanized 2H7.v114 Full-Length Heavy Chain:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAITYPGNG
ATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSASYWYFDVWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTFPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPG

FIG._9C

Sequence Listing

<110> GENENTECH, INC. , BRUNETTA, PAUL G.

<120> METHOD FOR TREATING VASCULITIS

<130> P2177R1 PCT

<140> PCT/US2005/034647

<141> 2005-09-28

<150> US 60/616,104

<151> 2004-10-05

<160> 43

<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

cr

<210> 3
 <211> 108
 <212> PRT
 <213> Homo sapiens

<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> 26
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 4
 Arg Ala Ser Ser Ser Val Ser Tyr Met His Ala Pro Ser Asn Leu
 1 5 10 15
 Ala Ser Gln Gln Trp Ser Phe Asn Pro Pro Thr
 20 25

<210> 5
 <211> 26
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 5
 Arg Ala Ser Ser Ser Val Ser Tyr Met His Ala Pro Ser Asn Leu
 1 5 10 15
 Ala Ser Gln Gln Trp Ser Phe Asn Pro Pro Thr
 20 25

<210> 6
 <211> 27
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 6
 Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Ala Ala Ser Ser
 1 5 10 15
 Leu Glu Ser Gln Gln Tyr Asn Ser Leu Pro Trp Thr
 20 25

<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> Homo sapiens

<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

49
da

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> 40
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 10
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Ala Ile Tyr Pro Gly
1 5 10 15
Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Val Val Tyr
20 25 30
Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
35 40

<210> 11
<211> 40
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 11
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Ala Ile Tyr Pro Gly
1 5 10 15
Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Val Val Tyr
20 25 30
Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
35 40

<210> 12
<211> 37
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 12
Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Val Ile Ser Gly Asp
1 5 10 15
Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Gly Arg Val
20 25 30
Gly Tyr Ser Leu Tyr Asp Tyr
35

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

<220>

<223> Sequence is synthesized

<400> 13

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1          5          10          15
Gly Asp Arg Val Thr Ile Thr Cys Arg 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

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<210> 14

<211> 452

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 14

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1          5          10          15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
          20          25          30
Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
          35          40          45
Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly 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

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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> 285
 <212> PRT
 <213> Homo sapiens

<400> 16
 Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys
 1 5 10 15
 Leu Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile
 20 25 30
 Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly
 35 40 45
 Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys
 50 55 60
 Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp
 65 70 75
 Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu Lys
 80 85 90
 Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala
 95 100 105
 Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro
 110 115 120
 Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val
 125 130 135
 Gln Gly Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile
 140 145 150
 Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe
 155 160 165
 Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu
 170 175 180
 Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
 185 190 195
 Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
 200 205 210
 Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser
 215 220 225
 Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
 230 235 240
 Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu
 245 250 255
 Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile
 260 265 270

Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 275 280 285
 <210> 17
 <211> 309
 <212> PRT
 <213> Mus musculus
 <400> 17
 Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe
 1 5 10 15
 Cys Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile
 20 25 30
 Thr Pro Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp
 35 40 45
 Gly Arg Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser
 50 55 60
 Ser Phe Thr Ala Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala
 65 70 75
 Asp Leu Met Asn Leu Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser
 80 85 90
 Ala Thr Pro Ala Ala Ala Gly Ala Pro Glu Leu Thr Ala Gly Val
 95 100 105
 Lys Leu Leu Thr Pro Ala Ala Pro Arg Pro His Asn Ser Ser Arg
 110 115 120
 Gly His Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Glu
 125 130 135
 Gln Asp Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro Gly
 140 145 150
 Cys Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn
 155 160 165
 Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asp Thr Pro
 170 175 180
 Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser
 185 190 195
 Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val
 200 205 210
 Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr
 215 220 225
 Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
 230 235 240
 Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg
 245 250 255
 Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr
 260 265 270
 Ser Ala Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu
 275 280 285
 Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp
 290 295 300
 Thr Phe Phe Gly Ala Leu Lys Leu Leu
 305

<210> 18
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
<223> Sequence is synthesized

<220>
<221> Xaa
<222> 1
<223> X = Any Amino Acid except Cysteine

<220>
<221> Xaa
<222> 3
<223> X = Any Amino Acid except Cysteine

<220>
<221> Xaa
<222> 5
<223> X = Any Amino Acid except Cysteine

<220>
<221> Xaa
<222> 7-10
<223> X = Any Amino Acid except Cysteine

<220>
<221> Xaa
<222> 11, 12, 14, 15, 17
<223> X = Any Amino Acid except Cysteine

<220>
<221> Xaa
<222> 16
<223> X = L, F, I, or V

<400> 18
Xaa Cys Xaa Asp Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa
1 5 10 15
Xaa Xaa

<210> 19
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 19
Glu Cys Phe Asp Leu Leu Val Arg Ala Trp Val Pro Cys Ser Val
1 5 10 15
Leu Lys

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

<220>
<223> Sequence is synthesized

<400> 20
Glu Cys Phe Asp Leu Leu Val Arg His Trp Val Pro Cys Gly Leu
1 5 10 15
Leu Arg

<210> 21
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 21
 Glu Cys Phe Asp Leu Leu Val Arg Arg Trp Val Pro Cys Glu Met
 1 5 10 15

Leu Gly

<210> 22
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 22
 Glu Cys Phe Asp Leu Leu Val Arg Ser Trp Val Pro Cys His Met
 1 5 10 15

Leu Arg

<210> 23
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 23
 Glu Cys Phe Asp Leu Leu Val Arg His Trp Val Ala Cys Gly Leu
 1 5 10 15

Leu Arg

<210> 24
 <211> 16
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 24
 Gln Cys Phe Asp Arg Leu Asn Ala Trp Val Pro Cys Ser Val Leu
 1 5 10 15

Lys

<210> 25
 <211> 17
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<220>
 <221> Xaa
 <222> 1, 3, 5, 8, 9, 14, 15, 17
 <223> Xaa = Any Amino Acid Except Cysteine

<400> 25
 Xaa Cys Xaa Asp Xaa Leu Val Xaa Xaa Trp Val Pro Cys Xaa Xaa
 1 5 10 15

Leu Xaa

<210> 26
 <211> 184
 <212> PRT
 <213> Homo sapiens

<400> 26

Met Arg Arg Gly Pro Arg Ser Leu Arg Gly Arg Asp Ala Pro Ala
 1 5 10 15
 Pro Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg
 20 25 30
 His Cys Val Ala Cys Gly Leu Leu Arg Thr Pro Arg Pro Lys Pro
 35 40 45
 Ala Gly Ala Ser Ser Pro Ala Pro Arg Thr Ala Leu Gln Pro Gln
 50 55 60
 Glu Ser Val Gly Ala Gly Ala Gly Glu Ala Ala Leu Pro Leu Pro
 65 70 75
 Gly Leu Leu Phe Gly Ala Pro Ala Leu Leu Gly Leu Ala Leu Val
 80 85 90
 Leu Ala Leu Val Leu Val Gly Leu Val Ser Trp Arg Arg Arg Gln
 95 100 105
 Arg Arg Leu Arg Gly Ala Ser Ser Ala Glu Ala Pro Asp Gly Asp
 110 115 120
 Lys Asp Ala Pro Glu Pro Leu Asp Lys Val Ile Ile Leu Ser Pro
 125 130 135
 Gly Ile Ser Asp Ala Thr Ala Pro Ala Trp Pro Pro Pro Gly Glu
 140 145 150
 Asp Pro Gly Thr Thr Pro Pro Gly His Ser Val Pro Val Pro Ala
 155 160 165
 Thr Glu Leu Gly Ser Thr Glu Leu Val Thr Thr Lys Thr Ala Gly
 170 175 180
 Pro Glu Gln Gln

<210> 27
 <211> 26
 <212> PRT
 <213> Homo sapiens

<400> 27
 Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg His
 1 5 10 15
 Cys Val Ala Cys Gly Leu Leu Arg Thr Pro Arg
 20 25

<210> 28
 <211> 213
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 28
 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
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> 29
<211> 452
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
20 25 30
Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45
Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly 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 Ala 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
 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> 30
 <211> 213
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 30
 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> 31
 <211> 451
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 31
 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 Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr Asn
 50 55 60
 Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys
 65 70 75
 Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
 80 85 90
 Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg Tyr
 95 100 105
 Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
 110 115 120
 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
 125 130 135
 Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
 140 145 150
 Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 155 160 165
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 170 175 180
 Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
 185 190 195
 Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
 200 205 210

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
 215 220 225
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 230 235 240
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 245 250 255
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 260 265 270
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 275 280 285
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 290 295 300
 Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 305 310 315
 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala
 320 325 330
 Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly
 335 340 345
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
 350 355 360
 Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 365 370 375
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 380 385 390
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 395 400 405
 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
 410 415 420
 Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 425 430 435
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 440 445 450

Lys

<210> 32
 <211> 107
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 32
 Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 1 5 10 15
 Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser
 20 25 30
 Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
 35 40 45
 Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
 50 55 60
 Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 65 70 75
 Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
 80 85 90

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

Lys Arg

<210> 33

<211> 123

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 33

Phe Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
1 5 10 15

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

Thr Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
35 40 45

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

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

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

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Ala
95 100 105

Ser Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120

Val Ser Ser

<210> 34

<211> 451

<212> PRT

<213> Artificial sequence

<220>

<223> Sequence is synthesized

<400> 34

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15

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

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 Ala 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

<210> 35
 <211> 10
 <212> PRT
 <213> Artificial sequence

 <220>
 <223> Sequence is synthesized

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

641

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<400> 35
Arg Ala Ser Ser Ser Val Ser Tyr Xaa His
          5                      10

<210> 36
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

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

<400> 36
Gln Gln Trp Xaa Phe Asn Pro Pro Thr
          5

<210> 37
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

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

<400> 37
Ala Ile Tyr Pro Gly Asn Gly Xaa Thr Ser Tyr Asn Gln Lys Phe
  1          5                      10                      15

Lys Gly

<210> 38
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<220>
<221> Xaa
<222> 6
<223> X is N, A, Y, W, or D

<220>
<221> Xaa
<222> 7
<223> X is S or R

<400> 38
Val Val Tyr Tyr Ser Xaa Xaa Tyr Trp Tyr Phe Asp Val
          5                      10

<210> 39
<211> 107
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 39
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

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[illegible]

<210> 40
<211> 122
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400>	40														
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														

<210> 41
<211> 451
<212> PRT
<213> Artificial sequence

<220>
<223> Sequence is synthesized

<400> 41
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala 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 80 Leu Gln Met Asn Ser 85 Leu Arg Ala Glu Asp 90
 Thr Ala Val Tyr Tyr 95 Cys Ala Arg Val Val 100 Tyr Tyr Ser Tyr Arg 105
 Tyr Trp Tyr Phe Asp 110 Val Trp Gly Gln Gly 115 Thr Leu Val Thr Val 120
 Ser Ser Ala Ser Thr 125 Lys Gly Pro Ser Val 130 Phe Pro Leu Ala Pro 135
 Ser Ser Lys Ser Thr 140 Ser Gly Gly Thr Ala 145 Ala Leu Gly Cys Leu 150
 Val Lys Asp Tyr Phe 155 Pro Glu Pro Val Thr 160 Val Ser Trp Asn Ser 165
 Gly Ala Leu Thr Ser 170 Gly Val His Thr Phe 175 Pro Ala Val Leu Gln 180
 Ser Ser Gly Leu Tyr 185 Ser Leu Ser Ser Val 190 Val Thr Val Pro Ser 195
 Ser Ser Leu Gly Thr 200 Gln Thr Tyr Ile Cys 205 Asn Val Asn His Lys 210
 Pro Ser Asn Thr Lys 215 Val Asp Lys Lys Val 220 Glu Pro Lys Ser Cys 225
 Asp Lys Thr His Thr 230 Cys Pro Pro Cys Pro 235 Ala Pro Glu Leu Leu 240
 Gly Gly Pro Ser Val 245 Phe Leu Phe Pro Pro 250 Lys Pro Lys Asp Thr 255
 Leu Met Ile Ser Arg 260 Thr Pro Glu Val Thr 265 Cys Val Val Val Asp 270
 Val Ser His Glu Asp 275 Pro Glu Val Lys Phe 280 Asn Trp Tyr Val Asp 285
 Gly Val Glu Val His 290 Asn Ala Lys Thr Lys 295 Pro Arg Glu Glu Gln 300
 Tyr Asn Ala Thr Tyr 305 Arg Val Val Ser Val 310 Leu Thr Val Leu His 315
 Gln Asp Trp Leu Asn 320 Gly Lys Glu Tyr Lys 325 Cys Lys Val Ser Asn 330
 Ala Ala Leu Pro Ala 335 Pro Ile Ala Ala Thr 340 Ile Ser Lys Ala Lys 345
 Gly Gln Pro Arg Glu 350 Pro Gln Val Tyr Thr 355 Leu Pro Pro Ser Arg 360
 Glu Glu Met Thr Lys 365 Asn Gln Val Ser Leu 370 Thr Cys Leu Val Lys 375
 Gly Phe Tyr Pro Ser 380 Asp Ile Ala Val Glu 385 Trp Glu Ser Asn Gly 390
 Gln Pro Glu Asn Asn 395 Tyr Lys Thr Thr Pro 400 Pro Val Leu Asp Ser 405
 Asp Gly Ser Phe Phe 410 Leu Tyr Ser Lys Leu 415 Thr Val Asp Lys Ser 420
 Arg Trp Gln Gln Gly 425 Asn Val Phe Ser Cys 430 Ser Val Met His Glu 435
 Ala Leu His Asn His 440 Tyr Thr Gln Lys Ser 445 Leu Ser Leu Ser Pro 450
 Gly

<210> 42
 <211> 451
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Sequence is synthesized

<400> 42
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
 1 5 10 15
 Gly Ser Leu Arg Leu Ser Cys Ala 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
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 Asn Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser
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114

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[Continúa en la página siguiente]

(54) Título: MÉTODO PARA TRATAR VASCULITIS

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hu2H7.v511	VEVMAKTEPRKQVETATVSVL/VLQKMLKQKTECVSMALPAP
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hu2H7.v511	LNNYTGKSLSPK

(57) Extracto: Se provee un método de tratar vasculitis asociada con anticuerpos citoplásmicos anti-neutrófilos (vasculitis asociada con ANCA) en un paciente elegible para tratamiento, método que involucra administrar un antagonista, que se une a un marcador de superficie de linfocitos B, tal como un anticuerpo CD20, al paciente en una dosis de alrededor de 400 mg a 1.3 gramos con una frecuencia de una o más dosis, en un período de alrededor de un mes. Se provee otro método de tratar vasculitis asociada con ANCA en un sujeto elegible para tratamiento, método que involucra administrar una cantidad efectiva de un anticuerpo, que se une a un marcador de superficie de linfocitos B, al sujeto para proporcionar al sujeto una exposición inicial y una exposición subsiguiente al anticuerpo dentro de ciertos regímenes posológicos. Se proveen además artículos de fabricación útiles para tales métodos.

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BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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MÉTODO PARA TRATAR VASCULITIS

Solicitudes Relacionadas

Esta solicitud es una solicitud no provisional presentada bajo 37 CFR 1.53(b)(1), que reivindica prioridad bajo 35 USC 119(e) a la solicitud provisional número 60/616,104 presentada el 5 de octubre de 2004, cuyos contenidos se incorporan aquí por referencia.

Campo de la Invención

La presente invención concierne a métodos para tratar vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (ANCA) en un sujeto, y kits con instrucciones para tales usos.

Antecedentes de la Invención

Vasculitis

Las enfermedades autoinmunitarias, tales como artritis reumatoide, esclerosis múltiple, vasculitis, y lupus, entre otras, siguen siendo enfermedades clínicamente importantes en humanos. Como el nombre lo indica, las enfermedades autoinmunes causan sus estragos a través del propio sistema inmunitario del cuerpo. Aunque los mecanismos patológicos difieren entre tipos individuales de enfermedades autoinmunitarias, un mecanismo general mecanismo involucra la unión de ciertos anticuerpos (referidos aquí como anticuerpos auto-reactivos o autoanticuerpos) presentes.

La vasculitis se define por inflamación de la pared de un vaso sanguíneo y constituye el cimiento patológico de un grupo diverso de entidades de enfermedades individuales. La vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (ANCA), que es una vasculitis sistémica primaria común, incluye polivascúlitis microscópica, granulomatosis de Wegener, síndrome de Churg-Strauss, vasculitis limitada a los riñones (glomerulonefritis progresiva necrosante idiopática) (*Falk et*

al. N. Engl. J. Med., 318: 1651-1657 (1988)), y ciertos tipos de vasculitis inducida por medicamentos. Jennette *et al. Arthritis Rheum* 37:187-92 (1994); Jennette and Falk, *N. Engl. J. Med.* 337: 1512-1523 (1997). Las enfermedades ya mencionadas afecta a personas de todas la edades, pero son muy comunes en adultos mayores con edades entre 50 y 60 años, y afectan por igual a hombres y mujeres. Pettersson *et al, Clin. Nephrol.*, 43: 141-149 (1995); Falk *et al, Ann. Intern. Med.* 113: 656-663 (1990).

Los ANCA son anticuerpos específicos para antígenos en gránulos citoplásmicos de neutrófilos y lisosomas monocíticos, informados por primera vez en 1982. Niles *et al., Arch. Intern. Med.*, 156:440-445 (1996). Los ANCA se detectaron originalmente por inmunofluorescencia indirecta en neutrófilos fijados con etanol. Wiik, "Delineation of a standard procedure for indirect immunofluorescence detection of ANCA" *APMIS Suppl.* 6: 12-13 (1989). Por lo menos tres diferentes patrones de fluorescencia se han distinguido: un patrón citoplásmico/clásico (cANCA) con énfasis de la intensidad de fluorescencia en el área con los lóbulos nucleares, un patrón perinuclear (pANCA), y un patrón de tinción citoplásmica más difusa (ANCA atípicos). Aproximadamente 90% de los sueros que producen un patrón cANCA reaccionan con proteinasa 3 (PR3), una serina proteasa de los gránulos azurófilos de las células mieloides. Jennette and Falk, *N Engl. J. Med.*, *supra*. En pacientes con vasculitis sistémica primaria, que afecta predominantemente los vasos sanguíneos de tamaño medio y pequeño, aproximadamente 75% de los sueros que producen un patrón perinuclear (pANCA) reaccionan con mieloperoxidasa (MPO), una enzima lisosómica mieloide. Cohen Tervaert *et al., Am. J. Med.* 91: 59-66 (1991). En pacientes ANCA-positivos con otras enfermedades no vasculíticas, se reconocen a menudo especificidades antigénicas. El potencial diagnóstico de PR3-ANCA y MPO-ANCA está bastante bien establecido en la actualidad. En un paciente con signos y síntomas de vasculitis, ANCA con especificidad para PR3 (PR3-ANCA) sugiere una diagnosis de granulomatosis de Wegener, mientras que ANCA con una especificidad para MPO (MPO-ANCA) es sumamente sensible para

polivascularitis microscópica, glomerulonefritis creciente necrosante idiopática, o síndrome de Churg-Strauss activo. Cohen Tervaert *et al.*, *Sarcoidosis Vasc. Diffuse Lung Dis.* 13: 241 - 245 (1996). Véase también Xiao *et al.*, *J. Clin. Invest.*, 110: 955-963 (2002), quienes describen un modelo animal que ofrece un fuerte fundamento para un papel patogénico directo para ANCA IgG en glomerulonefritis y vasculitis humanas, y Popa *et al.*, *J. Allergy Clin. Immunol.*, 103: 885-894 (1999) muestran que en la granulomatosis de Wegener, la activación de linfocitos B se relaciona con la enfermedad activa, mientras que la activación de linfocitos T persiste durante la remisión de la enfermedad, lo cual apunta a un sistema inmunitario alterado intrínseco en esta enfermedad. Véase también Cupps *et al.*, *J. Immunol.*, 128: 2453-2457 (1982) con respecto al papel de la ciclofosfamida en suprimir la función de los linfocitos B humanos.

Dentro del espectro de los síndromes vasculíticos primarios, los síndromes relacionados con ANCA forman un grupo definido, con características que se sobreponen. La mayoría de los pacientes tienen una manifestación prodrómica parecida a la gripe, que consiste en indisposición, mialgias, artralgias, fiebre y pérdida de peso. Esta manifestación parecida a la gripe aparece en un término de días a semanas antes de la manifestación de enfermedad vasculítica o nefrítica explícita. La granulomatosis de Wegener se diferencia de las otras por la presencia de inflamación granulomatosa necrosante del tracto respiratorio superior e inferior, que se acompaña usualmente por vasculitis necrosante sistémica de los vasos pequeños y glomerulonefritis. El síndrome de Churg-Strauss se diferencia por la presencia de (una historia de) asma, rinitis alérgica, eosinofilia sistémica, además de la vasculitis sistémica con o sin glomerulonefritis. La polivascularitis microscópica se caracteriza por glomerulonefritis necrosante y/o progresiva y una vasculitis multi-sistema que compromete a los vasos pequeños. La polivascularitis microscópica comparte muchas de las características con la granulomatosis de Wegener y el síndrome de Churg-Strauss, pero carece de inflamación granulomatosa necrosante del tracto respiratorio y asma. Jennette *et al.*, *Arthritis Rheum.*, *supra*. En

glomerulonefritis necrosante y/o progresiva idiopática, el proceso vasculítico se limita a los riñones. Debido a que el tratamiento de pacientes con polivascularitis microscópica o granulomatosis de Wegener es esencialmente el mismo cuando hay una lesión de los órganos mayores, es innecesario distinguir concluyentemente entre estas variantes estrechamente relacionadas de vasculitis asociada con ANCA antes de iniciar el tratamiento. Jennette *et al.*, *Arthritis Rheum.*, *supra*.

Antes de aparecer el tratamiento, los pacientes con granulomatosis de Wegener generalizada tenían una supervivencia media de cinco meses. A comienzos de la década de 1970, Fauci y Wolff introdujeron un método que combinaba una terapia diaria con ciclofosfamida administrada durante un año luego de lograda la remisión con terapia con prednisona, iniciada con una dosis de 1 mg por kilogramo de peso corporal al día y disminuída progresivamente en una pauta posológica de día de por medio. Consistentemente se ha descubierto que este tratamiento induce la remisión en 80 a 100 por ciento de los pacientes y puede resultar en una supervivencia a largo plazo. En efecto, la terapia inmunosupresora prolongada (más de 1 año) con ciclofosfamida y esteroides es efectiva en inducir la remisión de la enfermedad y prevenir recaídas precoces en la mayoría de trastornos vasculíticos. Balow *et al.*, "Vasculitic diseases of the kidney, polyarteritis, Wegener's granulomatosis, necrotizing and crescentic glomerulonephritis, and other disorders." En: Schrier and Gottschalk (eds): *Diseases of the kidney*, 5th edition, (Little, Brown and Company, Boston, 1993), pp. 2095- 2117; Jayne *et al.*, *N. Engl. J. Med.*, 349: 36-44 (2003); Gaskin *et al.*, "Systemic vasculitis" En: Cameon *et al.*, (eds): *Oxford textbook of clinical nephrology*. (Oxford University Press, Oxford, 1992), pp. 612-636; Fauci *et al.*, *Ann. Intern. Med.* 89: 660-676 (1978); Fauci *et al.*, *Ann. Intern. Med.*, 98: 76-85 (1983); Hoffman *et al.*, *Ann. Int. Med.*, 116: 488-498 (1992); y Andrassy *et al.*, *Clin. Nephrol*, 35: 139- 147 (1991).

No obstante, cuando la terapia se reduce progresivamente y se discontinúa, las recaídas son comunes. En un estudio, en el cual se les hizo seguimiento a pacientes con granulomatosis de Wegener durante una media

de ocho años, la recaída ocurrió en 50 por ciento de los pacientes. Además, el uso continuo de ciclofosfamida para mantener la remisión no se recomienda, puesto que este régimen de tratamiento se asocia con efectos adversos graves y potencialmente letales, tales como la ocurrencia de infecciones oportunistas y el desarrollo de neoplasias malignas. Por ejemplo, ciclos repetidos de ciclofosfamida se asocian con supresión de la médula ósea, infección, cistitis, infertilidad, mielodisplasia, y carcinoma transicional de la vejiga. En ciertos casos, tales efectos tóxicos excluyen el uso posterior de la ciclofosfamida. Stillwell *et al.*, *Arthritis Rheum.*, 31: 465-470 (1988); Radis *et al.*, *Arthritis Rheum.* 38: 1120-1127 (1995). Por lo tanto, la ciclofosfamida se disminuye progresivamente o se suspende y se reemplaza por azatioprina una vez lograda la remisión para evitar efectos adversos, una política ensayada en un análisis clínico multi-centro riguroso y comprobada por ser igualmente efectiva en el seguimiento de durante 18 meses. Gaskin *et al.*, *supra*, 1992; Jayne, *Rheumatology* 39: 585-595 (2000). La azatioprina se considera menos efectiva en inducir la remisión que la ciclofosfamida, pero su toxicidad a largo plazo es mucho más baja. Bouroncle *et al.*, *Am. J. Med.*, 42: 314-318 (1967); Norton *et al.*, *Arch. Intern. Med.*, 121: 554-560 (1968).

Otros regímenes alternativos de terapia de mantenimiento incluyen metotrexato (de Groot *et al.*, *Arthritis Rheum.*, 39: 2052-2061 (1996)), ciclosporina A (Haubitz *et al.*, *Nephrol. Dial Transplant*, 13: 2074-2076 (1998)), micofenolato (Nowack *et al.*, *J. Am. Soc. Nephrol.*, 10: 1965-1971 (1999)), o trimetoprim-sulfametoxazol (Stegeman *et al.*, *N. Engl. J. Med.*, 335: 16-20 (1996)). Véase también Sanders, *et al.*, *N. Engl. J. Med.* 349: 2072-2073 (2003). Puesto que, sin embargo, frecuentemente se observan recaídas en vasculitis asociadas con ANCA, el tratamiento en tales casos tiene que intensificarse o reestablecerse. Hoffman *et al.*, *supra*; Gordon *et al.*, *Q J. Med.*, 86: 779-789 (1993); Nachman *et al.*, *J. Am. Soc. Nephrol.*, 7: 33-39 (1996); Guillevin *et al.*, *Medicine* 78: 26-37 (1999); Reinhold-Keller *et al.*, *Arthritis. Rheum.* 43: 1021-1032 (2000); Langford, *New Eng. J. Med.*, 349: 3-4 (July 2003).

El bloqueamiento del factor de necrosis tumoral α (TNF- α) con infliximab es una terapia potencial para la vasculitis asociada con ANCA, tanto para terapia inicial como en el manejo de enfermedad resistente al tratamiento. El infliximab fue efectivo en inducir la remisión en 88% de los pacientes con vasculitis asociada con ANCA y permitió la reducción en las dosis de esteroides. Booth *et al.*, *J. Am. Soc. Nephrol.* 15:717-721 (2004). Además, Stone *et al.*, *Arthritis and Rheumatism*, 44: 1149-1154 (2001) divulgaron que el inhibidor de TNF- α etanercept (ENBREL®), administrado 25 mg subcutáneamente dos veces a la semana en combinación con el tratamiento estándar para la granulomatosis de Wegener, fue bien tolerado en los pacientes con pocos efectos adversos, pero fue común la enfermedad intermitentemente activa (ocasionalmente grave).

Pacientes con el síndrome de Churg-Strauss responden usualmente a la terapia con altas dosis de cortico-esteroides sola, aunque algunos casos requieren la adición de medicamentos citotóxicos. Jayne and Rasmussen, *Mayo Clin. Proc.* 72-737-47 (1997). Condiciones de co-patológicas que aceleran la lesión vascular, por ejemplo, hipertensión, diabetes, hipercolesterolemia, y fumar, deben controlarse apropiadamente.

En la vasculitis inducida por medicamentos, el agente responsable debe suspenderse. Antihistaminas y fármacos anti-inflamatorios no esteroides ayudan a aliviar la incomodidad cutánea y reducen artralgias y mialgias asociadas. La enfermedad cutánea grave puede justificar la terapia corticosteroide oral. Jennette *et al.*, *Arthritis Rheum*, *supra*

La persistencia o reaparición de ANCA constituye un factor de riesgo para el desarrollo de una recaída de la actividad patológica, sugiriendo un papel patofisiológico *in vivo* para estos anticuerpos. Stegeman *et al.*, *Ann Intern Med*, 120 12-17 (1994), De'Oliviera *et al.*, *Am. J. Kidney Dis*, 25 380-389 (1995), Jayne *et al.*, *Q. J. Med.*, 88 127-133 (1995). Las recaídas de la granulomatosis de Wegener están precedidas con frecuencia por aumentos en la concentración de cANCA detectado por inmunofluorescencia indirecta (Cohen Tervaert *et al.*, *Arch Intern Med.*,

149:2461-2465 (1989)), y pueden prevenirse por tratamiento con inmunosupresores basados en aumentos en cANCA (Cohen Tervaert *et al.*, *Lancet*, 336 706-711 (1990)).

Para una discusión general sobre la vasculitis asociada con ANCA, véase Lhote and Guillevin, *Rheum Dis Clin North Am.* 21:911-947 (1995), "ANCA-associated vasculitis occurrence, prediction, prevention, and outcome of relapses" by Maarten Boomsma, PhD Thesis, Thesis University Groningen, ISBN 90-367-1451-6 (M.M. Boomsma, Groningen, 2001) (<http://www.ub.rug.nl/eldoc/dis/medicine/m.m.boomsma/thesis.pdf>), Kamesh *et al.*, *J. Am. Soc. Nephrol.* 13:1953-1960 (2002), and Jayne, *Kidney & Blood Pressure Research* 26:231-239 (2003)

Anticuerpos CD20 y Terapia con los mismos

Los linfocitos son uno de muchos tipos de leucocitos producidos en la médula ósea durante el proceso de hematopoyesis. Existen dos poblaciones principales de linfocitos B (células B) y linfocitos T (células T). Los linfocitos de interés particular en esta solicitud son los linfocitos B.

Los linfocitos B maduran dentro de la médula ósea y salen de la médula expresando un anticuerpo de unión a antígeno en su superficie celular. Cuando un linfocito B virgen encuentra por primera vez el antígeno para el cual es específico su anticuerpo unido a la membrana, la célula comienza a dividirse rápidamente y su progenie a diferenciarse en linfocitos B de memoria y células efectoras llamada "plasmocitos". Los linfocitos B de memoria tienen una vida útil más larga y continúan expresando anticuerpo unido a membrana con la misma especificidad que la célula progenitora original. Los plasmocitos no producen antígeno unido a membrana, pero en su lugar producen el anticuerpo en una forma que puede segregarse. Los anticuerpos secretados son las principales moléculas efectoras de la inmunidad humoral.

El antígeno CD20 (también llamado antígeno de diferenciación restringida a linfocito B humano, Bp35) es una proteína transmembrana hidrófoba con un peso molecular de aproximadamente 35 kD, localizada en

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linfocitos pre-B y linfocitos B maduros (Valentine et al. *J. Biol. Chem.* 264 (19):11282-11287 (1989); and Einfeld et al. *EMBO J.* 7(3):711-717 (1988)). El antígeno se expresa también en más del 90% de linfomas no hodgkinianos de linfocitos B (NHL) (Anderson et al. *Blood* 63 (6):1424-1433 (1984)), pero no se encuentra en células madre hematopoyéticas, linfocitos pre-B, plasmocitos normales u otros tejidos normales (Tedder et al. *J. Immunol.* 135 (2):973-979 (1985)). CD20 regula un paso(s) previo en el proceso de activación para la iniciación y diferenciación del ciclo celular (Tedder et al., *supra*) y, posiblemente, funciona como un canal de iones de calcio (Tedder et al. *J. Cell. Biochem.* 14D:195 (1990)).

Dada la expresión de CD20 en linfomas de linfocitos B, este antígeno puede servir como un candidato para el "reconocimiento" ("*targeting*") de tales linfomas. En esencia, tal reconocimiento puede generalizarse en la siguiente forma: anticuerpos específicos del antígeno de superficie CD20 de los linfocitos B se administran a un paciente. Estos anticuerpos anti-CD20 se unen específicamente al antígeno CD20 de (ostensiblemente) linfocitos B normales y malignos; el anticuerpo fijado al antígeno de superficie CD20 puede conducir a la destrucción y agotamiento de linfocitos B neoplásicos. Adicionalmente, agentes químicos o marcadores radioactivos que tienen el potencial de destruir el tumor pueden conjugarse con el anticuerpo anti-CD20 de manera que el agente se "administre" específicamente a los linfocitos B neoplásicos. Independientemente de la estrategia, un objetivo primario es destruir el tumor; la estrategia específica puede determinarse por el anticuerpo anti-CD20 particular que se utiliza y, por consiguiente, las estrategias disponibles para reconocer el antígeno CD20 pueden variar considerablemente.

El anticuerpo rituximab (RITUXAN®) es un anticuerpo monoclonal murino/humano modificado genéticamente, dirigido contra el antígeno CD20. Rituximab es el anticuerpo denominado "C2B8" en la Patente de los Estados Unidos No. 5,736,137 expedida el 7 de abril de 1998 (Anderson et al.). RITUXAN® es indicado para el tratamiento de pacientes con linfoma no hodgkiniano de linfocitos B, CD20 positivo, de bajo grado o folicular,

con recaída o resistente al tratamiento. Estudios del mecanismo de acción *in vitro* han demostrado que RITUXAN® une el complemento humano y disuelve líneas de linfocitos B linfoides mediante citotoxicidad dependiente de complemento (CDC). Reff *et al.*, *Blood* 83 (2) :435-445 (1994). Adicionalmente, posee una actividad significativa en análisis de citotoxicidad celular mediada por anticuerpo (ADCC). Más recientemente, se ha demostrado que el RITUXAN® posee efectos anti-proliferativos en los análisis de incorporación de timidina tritiada y que induce apoptosis directamente, mientras que otros anticuerpos anti-CD19 y CD20 no. Maloney *et al.*, *Blood* 88(10):637a (1996). De igual manera, se ha observado experimentalmente la sinergia entre el RITUXAN® y ciertas quimioterapias y toxinas. En particular, el RITUXAN® sensibiliza líneas de células de linfocitos B humanos, resistentes a los medicamentos, a los efectos citotóxicos de la doxorrubicina, CDDP, VP-16, la toxina de la difteria y la ricina (Demidem *et al.*, *Cancer Chemotherapy & Radiopharmaceuticals* 12 (3):177-186 (1997)). Estudios preclínicos *in vivo* han demostrado que el RITUXAN® agota los linfocitos B de la sangre periférica, nódulos linfáticos, y médula ósea de monos cynomolgus, presumiblemente a través de procesos mediados por célula y complemento. Reff *et al.*, *Blood* 83 (2) :435-445 (1994).

Rituximab se aprobó en los Estados Unidos en noviembre de 1997 para el tratamiento de pacientes con NHL de linfocitos B + CD20 folicular o de bajo grado, recurrente o resistente al tratamiento, con una posología de 375 mg/m² a la semana para cuatro dosis. En abril de 2001, la Administración de Drogas y Alimentos de los Estados Unidos (FDA) aprobó reivindicaciones adicionales para el tratamiento de NHL de grado bajo: re-tratamiento (semanal para cuatro dosis) y un régimen posológico adicional (semanal para ocho dosis). Ha habido más de 300.000 exposiciones de pacientes al rituximab, ya sea como monoterapia o en combinación con fármacos inmunosupresores o quimioterapéuticos. En igual forma, los pacientes han sido tratados con rituximab como terapia de mantenimiento durante hasta 2 años. Hainsworth *et al.*, *J. Clin. Oncol.* 21

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1746-1751 (2003), Hainsworth *et al.*, *J. Clin. Oncol.* 20:4261-4267 (2002). Asimismo, se ha usado el rituximab en el tratamiento de trastornos plasmocíticos malignos y no malignos. Treon and Anderson, *Semin. Oncol* 27 79-85 (2000).

Se ha estudiado asimismo el rituximab en una variedad de trastornos autoinmunitarios no malignos, en los cuales los linfocitos B y los autoanticuerpos parecen jugar un papel en la fisiopatología de enfermedades. Edwards *et al.*, *Biochem Soc. Trans.* 30:824-828 (2002). Se ha informado que el rituximab alivia potencialmente los signos y síntomas de, por ejemplo, la artritis reumatoide (RA) (Leandro *et al.*, *Ann. Rheum. Dis.* 61:883-888 (2002); Edwards *et al.*, *Arthritis Rheum.*, 46 (Suppl. 9): S46 (2002); Stahl *et al.*, *Ann Rheum. Dis.*, 62 (Suppl. 1): OP004 (2003), Shaw *et al.* *Ann. Rheum. Dis.* 62 Suppl 2:ii55-ii59 (2003), Weyand and Goronzy, *Ann N.Y. Acad Sci.* 987 140-149 (2003), Emery *et al.*, *Arthritis Rheum.* 48(9): S439 (2003)), lupus (Eisenberg, *Arthritis. Res. Ther.* 5:157-159 (2003), Anolik *et al.*, *Arthritis Rheum* 48 455-459 (2003), Leandro *et al.* *Arthritis Rheum.* 46:2673-2677 (2002); Gorman *et al.*, *Lupus*, 13: 312-316 (2004)), Tomietto *et al.*, *Thromb. Haemost.* 92 :1150-1153 (2004)), púrpura trombocitopénica inmunitaria (D'Arena *et al.*, *Leuk. Lymphoma* 44:561-562 (2003); Stasi *et al.*, *Blood*, 98: 952-957 (2001); Saleh *et al.*, *Semin. Oncol.*, 27 (Supp 12):99-103 (2000); Zaja *et al.*, *Haematologica*, 87: 189-195 (2002); Ratanatharathorn *et al.*, *Ann. Int. Med.*, 133:275-279 (2000)), aplasia pura de eritrocitos (Auner *et al.*, *Br. J. Haematol.*, 116: 725-728 (2002)); anemia autoinmunitaria (Zaja *et al.*, *Haematologica* 87:189-195 (2002) (errata aparece en *Haematologica* 87:336 (2002)); Raj *et al.*, *J. Pediatr. Hematol. Oncol.* 26: 312-314 (2004); Zecca *et al.*, *Blood* 101: 3857-3861 (2003); Quarter *et al.*, *Lancet* 358:1511-1513 (2001)), citopenias autoinmunitarias (Robak, *Eur. J. Haematol.* 72: 79-88 (2004)); enfermedad de crioaglutinina (Layios *et al.*, *Leukemia*, 15: 187-8 (2001); Berentsen *et al.*, *Blood*, 103:2925-2928 (2004); Berentsen *et al.*, *Br. J. Haematol.*, 115:79-83 (2001); Bauduer, *Br. J. Haematol.*, 112: 1083-1090 (2001); Damiani *et al.*, *Br. J. Haematol.*, 114: 229-234 (2001)); Lee and

Kueck, *Blood* 92: 3490-3491 (1998)), síndrome de tipo B de resistencia grave a la insulina (Coll *et al.*, *N. Engl. J. Med.*, 350: 310-311 (2004), crioglobulinemia mixta (DeVita *et al.*, *Arthritis Rheum.* 46 Suppl. 9:S206/S469 (2002)); Zaja *et al.*, *Haematologica* 84:1157-1158 (1999)), mastenia grave (Zaja *et al.*, *Neurology*, 55:1062-63 (2000); Wylam *et al.*, *J. Pediatr.*, 143: 674-677 (2003)), granulomatosis de Wegener (Specks *et al.*, *Arthritis & Rheumatism* 44: 2836-2840 (2001)), *pemphigus vulgaris* resistente a los medicamentos (Dupuy *et al.*, *Arch Dermatol.*, 140:91-96 (2004)), dermatomiositis (Levine, *Arthritis Rheum.*, 46 (Suppl.9):S1299 (2002)), síndrome de Sjögren (Somer *et al.*, *Arthritis & Rheumatism*, 49: 394-398 (2003)), crioglobulinemia mixta tipo II activa (Zaja *et al.*, *Blood*, 101:3827-3834 (2003)), *pemphigus vulgaris* (Dupay *et al.*, *Arch. Dermatol.*, 140: 91-95 (2004)), neuropatía autoinmunitaria (Pestronk *et al.*, *J. Neurol. Neurosurg. Psychiatry* 74:485-489 (2003)); Nobile-Orazio, *Curr. Opin. Neurol.* 17: 599-605 (2004); Rojas-Garcia *et al.*, *Neurology* 61:1814-1816 (2003); Renaud *et al.*, *Muscle Nerve* 27: 611-615 (2003)), síndrome de opsoclonia-mioclónica paraneoplásica (Pranzatelli *et al.* *Neurology* 60(Suppl. 1) PO5.128:A395 (2003)), inhibidores del factor VIII adquirido (Wiestner *et al.*, *Blood* 100: 3426-3428 (2002); y esclerosis múltiple recurrente-remitente (RRMS). Cross *et al.* (extracto) "Preliminary Results from a Phase II Trial of Rituximab in MS" Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis, 20-21 (2003).

Se condujo un estudio Fase II (WA16291) en pacientes con artritis reumatoide (RA), el cual proporcionó 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 se aleatorizaron uniformemente a cuatro ramas de tratamiento: metotrexato, rituximab solo, rituximab más metotrexato, y rituximab más ciclofosfamida (CTX). El régimen de tratamiento de rituximab consistió en un gramo administrado por vía intravenosa en los días 1 y 15. Infusiones de rituximab en la mayoría de los pacientes con RA

fueron bien toleradas por la mayor parte de los pacientes, con el 36% de los pacientes experimentando por lo menos un episodio adverso durante su primera infusión (comparado con el 30% de los pacientes que recibieron placebo). El general, se consideró que la mayoría de los episodios adversos era benigna a moderada en gravedad y que estaba bien balanceada a través de todos los grupos de tratamiento. Hubo un total de 19 episodios adversos serios a través de las cuatro ramas durante las 48 semanas, los cuales fueron ligeramente más frecuentes en el grupo rituximab/CTX. La incidencia de infecciones estuvo bien balanceada a través de todos los grupos. La tasa media de infección seria en esta población de pacientes con RA fue de 4.66 por 100 paciente-años, que es inferior a la tasa de infecciones que requieren ingreso hospitalario en pacientes con RA (9.57 por 100 paciente-años) relacionada en un estudio epidemiológico de base comunitaria. Doran *et al*, *Arthritis Rheum.* 46:2287-2293 (2002).

El perfil de seguridad publicado del rituximab en un pequeño número de pacientes con trastornos nerviosos, incluida neuropatía autoinmunitaria (Pestronk *et al*, *supra*), síndrome de opsoclonia-mioclonia (Pranzatelli *et al*, *supra*), y RRMS (Cross *et al*, *supra*), fue similar al descrito en oncología o RA. En un estudio clínico continuo patrocinado por el investigador (IST, *investigator-sponsored trial*) de rituximab en combinación con interferón-beta (IFN- β) o glatirámero acetato en pacientes con RRMS (Cross *et al*, *supra*), 1 de 10 pacientes tratados se admitieron al hospital para observación de un día para otro luego de experimentar fiebre moderada y escalofríos luego de la primera infusión de rituximab, mientras que los otros 9 pacientes completaron el régimen de cuatro infusiones sin que se informaran episodios adversos.

Patentes y publicaciones de patente concernientes a anticuerpos CD20 y moléculas de unión a CD20 incluyen las Patentes de los Estados Unidos No. 5,776,456, 5,736,137, 5,843,439, 6,399,061, y 6,682,734, así como US 2002/0197255, US 2003/0021781, US 2003/0082172, US 2003/0095963, US 2003/0147885 (Anderson *et al.*); Patente de los Estados Unidos No. 6,455,043 y WO 2000/09160 (Grillo-López, A.); WO

2000/27428 (Grillo-López y White); WO 2000/27433 (Grillo-López y Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO 2001/10461 (Rastetter y White); WO 2001/10460 (White y Grillo-López); 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, WO 2001/74388 y 6,896,885B5 (Hanna, N.), US 2002/0058029 (Hanna, N.), US 2003/0103971 (Hariharan and Hanna), US 2005/0123540 (Hanna *et al.*), US 2002/0009444 y WO 2001/80884 (Grillo-López, A), WO 2001/97858, US 2005/0112060, y Patente de los Estados Unidos No. 6,846,476 (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 and Davies), Patente de los Estados Unidos No. 6,171,586 y WO 1998/56418 (Lam *et al.*), WO 1998/58964 (Raju, S.), WO 1999/22764 (Raju, S.), WO 1999/51642, Patente de los Estados Unidos No. 6,194,551, Patente de los Estados Unidos No. 6,242,195, Patente de los Estados Unidos No. 6,528,624 y Patente de los Estados Unidos No. 6,538,124 (Idusogie *et al.*), WO 2000/42072 (Presta, L.), WO 2000/67796 (Curd *et al.*), WO 2001/03734 (Grillo-López *et al.*), US 2002/0004587 y WO 2001/77342 (Miller and Presta), US 2002/0197256 (Grewal, I.), US 2003/0157108 (Presta, L.), Patente de los Estados Unidos Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, y 5,595,721, (Kaminski *et al.*), Patente de los Estados Unidos Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, 6,652,852, 6,893,625 (Robinson *et al.*), Patente de los Estados Unidos No. 6,410,391 (Raubitschek *et al.*), Patente de los Estados Unidos No. 6,224,866 y WO 2000/20864 (Barbera-Guillem, E.), WO 2001/13945 (Barbera-Guillem, E.), WO 2000/67795 (Goldenberg), US 2003/0133930 y WO 2000/74718 (Goldenberg and Hansen), US 2003/0219433 y WO 2003/68821 (Hansen *et al.*), WO 2004/058298 (Goldenberg and Hansen), WO 2000/76542 (Golay *et al.*), WO 2001/72333 (Wolin and Rosenblatt), Patente de los Estados Unidos No. 6,368,596 (Ghetie *et al.*), Patente de los Estados Unidos No. 6,306,393 y US 2002/0041847 (Goldenberg, D.), US 2003/0026801

(Werner and Hartmann), WO 2002/102312 (Engleman, E.), US 2003/0068664 (Albitar *et al.*), WO 2003/002607 (Leung, S.), WO 2003/049694, US 2002/0009427, y US 2003/0185796 (Wolin *et al.*), WO 2003/061694 (Sing and Siegall), US 2003/0219818 (Bohen *et al.*), US 2003/0219433 y WO 2003/068821 (Hansen *et al.*), US 2003/0219818 (Bohen *et al.*), US 2002/0136719 (Shenoy *et al.*), WO 2004/032828 y US 2005/0180972 (Wahl *et al.*), y WO 2002/56910 (Hayden-Ledbetter). Véase también Patente de los Estados Unidos No. 5,849,898 y EP 330,191 (Seed *et al.*), EP332.865A2 (Meyer and Weiss), Patente de los Estados Unidos No. 4,861,579 (Meyer *et al.*), US 2001/0056066 (Bugelski *et al.*), WO 1995/03770 (Bhat *et al.*), US 2003/0219433 A1 (Hansen *et al.*), WO 2004/035607 (Teeling *et al.*), WO 2004/056312 (Lowman *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.*), WO 2005/016969 (Carr *et al.*), US 2005/0069545 (Carr *et al.*), WO 2005/014618 (Chang *et al.*); US 2005/0079174 (Barbera-Guillem and Nelson); US 2005/0106108 (Leung and Hansen); WO2005/044859 y US 2005/0123546 (Umana *et al.*); WO 2005/070963 (Allan *et al.*); US 2005/0186216 (Ledbetter and Hayden-Ledbetter); y Patente de los Estados Unidos No. 6,897,044 (Braslawski *et al.*).

Publicaciones concernientes a terapia con rituximab incluyen: Perotta y Abuel, "Response of chronic relapsing ITP of 10 years duration to rituximab" Abstract # 3360 *Blood* 10(1)(part 1-2): p. 88B (1998); Perotta *et al.*, "Rituxan in the treatment of chronic idiopathic thrombocytopaenic purpura (ITP)", *Blood*, 94:49 (abstract) (1999); 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*, *supra*; 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 and Rheumatism*, 46:2673-2677 (2002), en donde

durante un período de 2 semanas, cada paciente recibió dos infusiones de 500 mg de rituximab, dos infusiones de 750 mg de ciclofosfamida, y corticoesteroides orales en dosis altas, y en donde dos de los pacientes tratados tuvieron una recaída en los meses 7 y 8, respectivamente, y se volvieron a tratar, aunque con diferentes protocolos; "Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy" Weide *et al.*, *Lupus*, 12:779-782 (2003), en donde un paciente se trató con rituximab ($375 \text{ mg/m}^2 \times 4$, dosis repetida en intervalos semanales) y aplicaciones adicionales de rituximab se dispensaron cada 5-6 meses y luego se recibió terapia de mantenimiento con rituximab 375 mg/m^2 cada tres meses, y un segundo paciente con SLE (lupus eritematoso sistémico, por sus siglas en inglés) resistente a los medicamentos se trató exitosamente con rituximab y recibe terapia de mantenimiento cada tres meses, respondiendo bien ambos pacientes a la terapia con rituximab; Edwards and Cambridge, "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rheumatology* 40:205-211 (2001); Cambridge *et al.*, "B lymphocyte depletion in patients with rheumatoid arthritis: serial studies of immunological parameters" *Arthritis Rheum.*, 46 (Suppl. 9): S1350 (2002); Cambridge *et al.*, "Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis" *Arthritis Rheum.*, 48: 2146-2154 (2003); Edwards *et al.*, "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem Soc. Trans.*, *supra*; 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); Edwards *et al.*, "Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis" *N Engl. J. Med.* 350:2572-82 (2004); Pavelka *et al.*, *Ann. Rheum. Dis.* 63: (S1):289-90 (2004); Emery *et al.*, *Arthritis Rheum.* 50 (S9):S659 (2004); Levine and Pestronk, "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using rituximab" *Neurology* 52:1701-1704 (1999); Uchida *et al.*, "The innate mononuclear phagocyte network depletes B

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erythematosus: a step closer to the clinic" *Arthritis Rheum.* 52(2): 371-7 (2005), errata en: *Arthritis Rheum.* 52(4): 1342 (2005); Ahn *et al.*, "Long-term remission from life-threatening hypercoagulable state associated with lupus anticoagulant (LA) following rituximab therapy" *Am. J. Hematol.* 78(2): 127-129 (2005); Tahir *et al.*, "Humanized anti-CD20 monoclonal antibody in the treatment of severe resistant systemic lupus erythematosus in a patient with antibodies against rituximab" *Rheumatology*, 44(4): 561 - 562 (2005), Epub 2005 Jan 11; Looney *et al.*, "Treatment of SLE with anti-CD20 monoclonal antibody" *Curr. Dir. Autoimmun.* 8: 193-205 (2005); Cragg *et al.*, "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.* 8: 140-174 (2005); Gottenberg *et al.*, "Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases" *Ann. Rheum. Dis.* 64(6): 913-920 (2005) Epub 2004 Nov 18; Tokunaga *et al.*, "Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab" *Rheumatology* 44(2): 176-182 (2005), Epub 2004 Oct 19. Véase además Leandro *et al.*, "B cell repopulation occurs mainly from naive B cells in patient with rheumatoid arthritis and systemic lupus erythematosus" *Arthritis Rheum.*, 48 (Suppl 9): S1160 (2003).

Specks *et al.*, "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001) divulga el uso exitoso de cuatro infusiones de 375mg/m² de rituximab y glucocorticoides en dosis altas para tratar la granulomatosis de Wegener. La terapia se repitió después de 11 meses cuando recurrió cANCA, pero la terapia no incluyó glucocorticoides. A 8 meses después del segundo ciclo de rituximab, la enfermedad del paciente quedó en completa remisión. Además, en otro estudio, se descubrió que el rituximab era un agente de inducción de la remisión efectivo, bien tolerado, para vasculitis grave, asociada con ANCA, cuando se usaba en una dosis de 375 mg/m² x 4 junto con prednisona oral 1 mg/kg/día, la cual se redujo en la semana 4 a 40 mg/ día, y a discontinuación completa durante las

siguientes 16 semanas. Cuatro pacientes se volvieron a tratar con rituximab solo para concentraciones recurrentes/cada vez crecientes de ANCA. Aparte de glucocorticoides, no parecen ser necesarios agentes inmunosupresores adicionales para remisión de la inducción y mantenimiento de la remisión sostenida (6 meses o más). Véase la entrega del extracto en línea y la invitación Keogh *et al.*, "Rituximab for Remission Induction in Severe ANCA-Associated Vasculitis: Report of a Prospective Open-Label Pilot Trial in 10 Patients", American College of Rheumatology, Session Number: 28-100, Session Title: Vasculitis, Session Type: ACR Concurrent Session, Primary Category: 28 Vasculitis, Session 10/18/2004 (<www.abstractsonline.com/viewer/SearchResults.asp>). Véase asimismo Keogh *et al.*, *Kidney Blood Press. Res.* 26:293 (2003), en donde se informa que once pacientes con vasculitis asociada a ANCA, resistente al tratamiento, se trataron con cuatro dosis semanales de 375 mg/m² de rituximab y glucocorticoides en dosis altas, lo cual resultó en la remisión.

A pacientes con vasculitis asociada a ANCA, resistente al tratamiento, se les administró rituximab junto con medicamentos inmunosupresores tales como ciclofosfamida intravenosa, micofenolato mofetil, azatioprina, o leflunomida, con eficacia evidente. Eriksson, "Short-term outcome and safety in 5 patients with ANCA-positive vasculitis treated with rituximab", *Kidney and Blood Pressure Research*, 26: 294 (2003) (cinco pacientes con vasculitis asociada con ANCA, tratados con rituximab 375 mg/m² una vez por semana durante 4 semanas, respondieron al tratamiento); Jayne *et al.*, "B-cell depletion with rituximab for refractory vasculitis" *Kidney and Blood Pressure Research*, 26: 294-295 (2003) (seis pacientes con vasculitis resistente al tratamiento, que recibieron cuatro infusiones semanales de rituximab a 375 mg/m² con ciclofosfamida junto con inmunosupresión de fondo y prednisolona, experimentaron caídas significativas en la actividad vasculítica). Un informe adicional del uso de rituximab junto con ciclofosfamida intravenosa en 375 mg/m² por dosis en 4 dosis para administración a pacientes con vasculitis sistémica resistente al tratamiento, se suministra en Jayne, poster 88 (11th International Vasculitis

and ANCA workshop), 2003 American Society of Nephrology. Véase asimismo Stone and Specks, "Rituximab Therapy for the Induction of Remission and Tolerance in ANCA-associated Vasculitis", in the Clinical Trial Research Summary of the 2002-2003 Immune Tolerance Network, <http://www.immunetolerance.org/research/autoimmune/trials/stone.html>, en el cual se propone un estudio clínico de rituximab en vasculitis asociada con ANCA para una duración total de 18 meses. Véase igualmente Eriksson, *J. Internal. Med.*, 257:540-548 (2005) con respecto a nueve pacientes con vasculitis asociada con ANCA, quienes fueron tratados exitosamente con dos de cuatro dosis semanales de 500 mg de rituximab, así como Keogh *et al.*, *Arthritis and Rheumatism*, 52 262-268 (2005), quienes informaron que en 11 pacientes con vasculitis asociada con ANCA, resistente al tratamiento, el tratamiento o re-tratamiento con cuatro dosis semanales de 375 mg/m² de rituximab indujo la remisión por agotamiento de linfocitos B, estudio que se condujo entre enero de 2000 y septiembre de 2002.

En cuanto a la actividad de un anticuerpo anti-CD20 humanizado, véase, por ejemplo, Vugmeyster *et al.*, "Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in *Macaca fascicularis*" *J. Immunother* 28: 212-219 (2005). Para discusión de un anticuerpo monoclonal humano, véase Baker *et al.*, "Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator" *Arthritis Rheum* 48: 3253-3265 (2003).

Subsiste una necesidad de métodos de tratamiento que reduzcan la frecuencia de las infusiones de fármaco activo en un período de un mes. Además, existe una necesidad de reducir el riesgo de efectos tóxicos de los fármacos utilizados actualmente, tales como esteroides y agentes quimioterapéuticos, y de reducir el riesgo de exacerbación repentina de la enfermedad, recaídas, y recurrencias en pacientes con vasculitis asociada con ANCA, y de sostener la remisión y mantener la remisión sostenida durante un período de tiempo prolongado.

Sumario de la Invención

La presente invención involucra la administración de un anticuerpo CD20 que proporciona un régimen de tratamiento activo y seguro en sujetos con vasculitis asociada con ANCA, incluida la selección de un régimen posológico eficaz y re-tratamiento pautado o no pautado. Este antagonista es efectivo tanto en la terapia inicial como en el manejo de enfermedad resistente al tratamiento.

En consecuencia, la invención es como se reivindica. En un primer aspecto, la presente invención concierne al tratamiento de vasculitis asociada con ANCA en un paciente, que comprende administrar un anticuerpo CD20 al paciente en una dosis de alrededor de 400 mg a 13 gramos con una frecuencia de una a tres dosis dentro de un período de aproximadamente un mes.

En un aspecto, adicional invención provee un artículo de fabricación que comprende un envase que contiene un anticuerpo CD20 y un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un paciente, en donde las instrucciones indican que una dosis del anticuerpo CD20 de aproximadamente 400 mg a 13 gramos, con una frecuencia de una a tres dosis, se administra al paciente en un período de aproximadamente un mes.

En incorporaciones preferidas de los aspectos inventivos anteriores, la vasculitis es granulomatosis de Wegener o polivasculitis microscópica, y/o un Segundo medicamento se administra en una cantidad efectiva al paciente, en donde el anticuerpo CD20 es un primer medicamento. Tal medicamento puede ser uno o más medicamentos. Más preferiblemente, tal segundo medicamento es un agente quimioterapéutico, un agente inmunosupresor, un fármaco anti-reumático modificador de la enfermedad (DMARD), un agente citotóxico, un antagonista de integrina, un fármaco anti-inflamatorio no esteroide (AINE), un antagonista de citocina, una hormona, o una combinación de los mismos.

En todavía otros aspectos, la presente invención se relaciona con un método de tratar vasculitis asociada con ANCA en un sujeto, que

comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto para proporcionar una exposición inicial al anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se provee hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial.

En una incorporación preferida de este último método que involucra múltiples exposiciones a anticuerpo, la presente invención se refiere a un método de tratar vasculitis asociada con ANCA en a sujeto, que comprende administrar al sujeto una cantidad efectiva de un anticuerpo CD20 para proporcionar una primera exposición a anticuerpo de aproximadamente 0.5 a 4 gramos, seguida por una segunda exposición a anticuerpo de aproximadamente 0.5 a 4 gramos, en donde la segunda exposición a anticuerpo no se suministra hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial, y cada una de las exposiciones a anticuerpo se proporciona al sujeto como aproximadamente 1 a 4 dosis de anticuerpo, más preferiblemente como una dosis única o como dos o tres dosis separadas de anticuerpo.

Una incorporación preferida específica en esta solicitud es un método de tratar vasculitis asociada con ANCA en un sujeto, que comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto para proporcionar una exposición inicial al anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se suministra hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial y cada una de las exposiciones al anticuerpo se proporciona al sujeto como una dosis única o como dos o tres dosis separadas de anticuerpo. Preferiblemente en tal método, las exposiciones al anticuerpo son cada una de aproximadamente 0.5 a 4 gramos cada una.

En otra incorporación preferida de estos últimos métodos, un segundo medicamento se administra con la exposición inicial y/o las últimas exposiciones, en donde el anticuerpo es un primer medicamento. En una incorporación preferida, el segundo medicamento es uno o más de los divulgados más arriba, según se prefiera. En una incorporación más

preferida, el segundo medicamento es un esteroide y/o un agente inmunosupresor. En una incorporación todavía preferida, se administra un esteroide con la primera exposición, pero no con la segunda exposición, o se administra en cantidades menores que las que se utilizan con la exposición inicial.

En todavía otra incorporación preferida de estos últimos aspectos, el sujeto no ha sido nunca tratado previamente con un anticuerpo CD20, y/o ningún otro medicamento que el anticuerpo CD20 se administra al sujeto para tratar la vasculitis. En otra incorporación preferida, las exposiciones inicial y segunda a anticuerpo son con el mismo anticuerpo y, más preferiblemente, todas las exposiciones al anticuerpo son con el mismo anticuerpo. En otra incorporación preferida, el sujeto está en remisión después de las exposiciones inicial y última a anticuerpo, preferiblemente cuando se proporciona la segunda exposición a anticuerpo. Más preferiblemente, el sujeto está en remisión cuando se suministran todas las exposiciones a anticuerpo. Muy preferiblemente, tal sujeto está en remisión por lo menos aproximadamente seis meses después de haberse suministrado la última exposición a anticuerpo.

En todavía otra incorporación preferida de estos últimos aspectos, el sujeto tiene una concentración elevada de anticuerpos anti-nucleares (ANA), anticuerpos de factor anti-reumatoide (RF), creatinina, nitrógeno ureico en la sangre, anticuerpos anti-endoteliales, anticuerpos anticitoplasma de los neutrófilos (ANCA), o una combinación de dos o más de los mismos.

Adicionalmente, en aspectos adicionales, la invención proporciona un artículo de fabricación, que comprende:

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

(b) un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que se administra al sujeto una cantidad del anticuerpo que es efectiva para proporcionar una exposición inicial al anticuerpo, seguida por una segunda exposición al anticuerpo, en donde la segunda exposición no se proporciona

hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial.

Preferiblemente, tal prospecto de envase se provee con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que se administra al sujeto una cantidad del anticuerpo que es efectiva para proporcionar una primera exposición a anticuerpo de aproximadamente 0.5 a 4 gramos, seguida por una segunda exposición a anticuerpo de aproximadamente 0.5 a 4 gramos, en donde la segunda exposición a anticuerpo no se suministra hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial, y cada una de las exposiciones a anticuerpo se proporciona al sujeto como aproximadamente 1 a 4 dosis de anticuerpo, más preferiblemente como una dosis única o como dos o tres dosis separadas de anticuerpo.

En un aspecto específico, se provee un artículo de fabricación, que comprende:

(a) un envase que contiene un anticuerpo CD20, y

(b) un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que se administra al sujeto una cantidad del anticuerpo que es efectiva para proporcionar una exposición inicial al anticuerpo, seguida por una segunda exposición al anticuerpo, en donde la segunda exposición no se proporciona hasta entre aproximadamente 16 a 54 semanas a partir de la exposición inicial, y cada una de las exposiciones al anticuerpo se proporciona al sujeto como una dosis única o como dos o tres dosis separadas de anticuerpo.

Los tratamientos especificados en esta solicitud preferiblemente reducen, minimizan, o eliminan la necesidad de co-, pre-, o pos-administración de cantidades excesivas de segundos medicamentos tales como agentes inmunosupresores y/o agentes quimioterapéuticos que constituyen ordinariamente tratamiento estándar para tales sujetos, con el fin de evitar tanto como sea posible los efectos secundarios de tal tratamiento estándar, así como para reducir costos e incrementar la

conveniencia para el sujeto, tal como la conveniencia de tiempo y la frecuencia de administración.

Breve Descripción de los Dibujos

FIG. 1A es una alineación de secuencias que compara las secuencias de aminoácidos del dominio variable de cadena ligera (V_L) de cada variante 2H7 (SEQ ID NO:1) murina, 2H7.v16 (SEQ ID NO:2) humanizada y el subgrupo de cadena ligera kappa humano I (SEQ ID NO:3). Las CDR de V_L de 2H7 y hu2H7 v16 son como sigue: CDR1 (SEQ ID NO:4), CDR2 (SEQ ID NO:5), y CDR3 (SEQ ID NO:6).

FIG. 1B es una alineación de secuencias que compara las secuencias de aminoácidos del dominio variable de cadena pesada (V_H) de cada variante 2H7 (SEQ ID NO:7) murina, 2H7 v16 humanizada (SEQ ID NO:8), y la secuencia de consenso humana del subgrupo III de cadena pesada (SEQ ID NO:9). Las CDR de V_H de 2H7 y hu2H7 v16 son las siguientes: CDR1 (SEQ ID NO:10), CDR2 (SEQ ID NO:11), y CDR3 (SEQ ID NO:12).

En FIG. 1A y FIG. 1B, la CDR1, CDR2 y CDR3 en cada cadena se encierran entre paréntesis, flanqueadas por las regiones de estructura alterna del anticuerpo ("*the framework regions*"), FR1 - FR4, como se indica. 2H7 se refiere al anticuerpo 2H7 murino. Los asteriscos entre dos filas de secuencias indican las posiciones que son diferentes entre las dos secuencias. La numeración de los residuos es de acuerdo con Kabat *et al. Sequences of Immunological Interest*, 5th Ed Public Health Service, National Institutes of Health, Bethesda, Md (1991), con inserciones mostradas como a, b, c, d, y e.

FIG. 2 muestra la secuencia de aminoácidos de la cadena 2H7.v16 L madura (SEQ ID NO:13).

FIG. 3 muestra la secuencia de aminoácidos de la cadena 2H7.v16 H madura (SEQ ID NO:14).

FIG. 4 muestra la secuencia de aminoácidos de la cadena 2H7.v31 H madura (SEQ ID NO:15). La cadena L de 2H7.v31 es la misma que para 2H7.v16.

FIG. 5 es una alineación de secuencias que compara las secuencias de aminoácidos de cadena ligera de la variante 2H7.v16 humanizada (SEQ ID NO:2) y la variante 2H7.v138 humanizada (SEQ ID NO:28).

FIG. 6 es una alineación de secuencias que compara las secuencias de aminoácidos de cadena pesada de la variante 2H7.v16 humanizada (SEQ ID NO:8) y la variante 2H7.v138 humanizada (SEQ ID NO:29).

FIG. 7 muestra una alineación de las cadenas ligeras 2H7.v16 y 2H7.v511 maduras (SEQ ID NOS 13 y 30, respectivamente), con numeración de residuos de dominio variable de Kabat y numeración de residuos de dominio constante de Eu.

FIG. 8 muestra una alineación de las cadenas pesadas 2H7.v16 y 2H7.v511 maduras (SEQ ID NOS 14 y 31, respectivamente), con numeración de residuos de dominio variable de Kabat y numeración de residuos de dominio constante de Eu.

FIG. 9A muestra la secuencia del dominio de cadena ligera variable 2H7.v114 humanizada (SEQ ID NO:32); FIG. 9B muestra la secuencia del dominio de cadena pesada variable 2H7.v114 humanizada (SEQ ID NO:33), y FIG. 9C muestra la secuencia de la cadena pesada de longitud total 2H7.v114 humanizada (SEQ ID NO:34), con numeración de residuos de dominio variable de Kabat y numeración de residuos de dominio constante de Eu.

Descripción Detallada de las Incorporaciones Preferidas

I. Definiciones

Un "linfocito B" es un linfocito que madura en la médula ósea, e incluye un linfocito B virgen, un linfocito B de memoria, o linfocito B efector (plasmocitos).

Un "marcador de superficie de linfocito B" o "antígeno de superficie de linfocito B" en esta solicitud es un antígeno expresado en la superficie de un linfocito B que puede direccionarse con un antagonista que se une a él. Marcadores ejemplares de superficie de linfocito B incluyen los marcadores de superficie de leucocitos CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD37, CD40, CD53, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 y CD86. (Para descripciones, véase *The Leukocyte Antigen Facts Book*, 2nd Edition, 1997, ed. Barclay *et al.* Academic Press, Harcourt Brace & Co., New York). Otros marcadores de superficie de linfocitos B incluyen RP105, FcRH2, CR2 de linfocitos B, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, Bt1g, NAG14, SLGC16270, FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, y 239287. El marcador de superficie de linfocitos B de interés particular se expresa preferencialmente en linfocitos B comparado con otros tejidos no linfocitos B de un mamífero y puede expresarse tanto en linfocitos B precursores B como en linfocitos B maduros. Los marcadores preferidos de superficie de linfocitos B en este caso son CD20 y CD22.

El antígeno "CD20", o "CD20", es una fosfoproteína no glicosilada de alrededor de 35-kDa, hallada en la superficie de más del 90% de los linfocitos B provenientes de la sangre periférica u órganos linfoides. CD20 está presente tanto en los linfocitos B normales B, como también en los linfocitos B malignos, pero no se expresa en las células madre. Otras denominaciones para CD20 en la literatura incluyen "antígeno restringido a linfocitos B" y "Bp35". El antígeno CD20 se describe en Clark *et al. Proc. Natl. Acad. Sci. (USA)* 82: 1766 (1985), por ejemplo.

El antígeno "CD22", o "CD22", también conocido como BL-CAM ó Lyb8, es una glucoproteína de membrana integral tipo 1 con peso molecular de alrededor de 130 (reducido) a 140kD (no reducido). Se expresa tanto en el citoplasma como en la membrana celular de los linfocitos B. El antígeno CD22 aparece temprano en la diferenciación linfocitaria de las células B en aproximadamente la misma fase que el antígeno CD19. A diferencia de

otros marcadores de los linfocitos B, la expresión en la membrana de CD22 se limita a las etapas tardías de diferenciación comprendidas entre células B maduras (CD22+) y plasmocitos CD22-). El antígeno CD22 se describe, por ejemplo, en Wilson *et al.*, *J. Exp. Med.* 173: 137 (1991) y Wilson *et al.*, *J. Immunol.* 150:5013 (1993).

Un "antagonista" es una molécula que, luego de unirse a CD20 en los linfocitos B, destruye o agota linfocitos B en un mamífero y/o interfiere una o más funciones del linfocito B, *por ejemplo* reduciendo o impidiendo una respuesta humoral provocada por el linfocito B. Preferiblemente el antagonista es capaz de agotar linfocitos B (*es decir*, reducir niveles de linfocitos B en circulación) en un mamífero tratado con él. Tal agotamiento puede lograrse mediante diferentes mecanismos tales como citotoxicidad celular dependiente de anticuerpo (ADCC) y/o citotoxicidad dependiente de complemento (CDC), inhibición de la proliferación de linfocitos B, y/o inducción de la muerte de linfocitos B (*por ejemplo*, mediante apoptosis). Los antagonistas incluidos dentro del ámbito de la presente invención incluyen anticuerpos, péptidos de secuencia nativa o sintética, immunoadhesinas, y antagonistas de molécula pequeña que se unen a CD20, conjugados de manera opcional con o fusionados a un agente citotóxico. El antagonista preferido comprende un anticuerpo.

Un "antagonista de anticuerpo" en esta solicitud es un anticuerpo que, luego de unirse a un marcador de superficie de linfocito B en linfocitos B, destruye o agota linfocitos B en un mamífero e interfiere una o más de las funciones de los linfocitos B, *verbigracia*, reduciendo o previniendo una respuesta humoral provocada por el linfocito B. El antagonista de anticuerpo está preferiblemente en capacidad de agotar linfocitos B (*es decir*, reducir los niveles de linfocitos B en circulación) en mamíferos tratados con él. Tal agotamiento puede lograrse mediante varios mecanismos tales como citotoxicidad celular dependiente de anticuerpo (ADCC) y/o citotoxicidad dependiente de complemento (CDC), inhibición de la proliferación de linfocitos B y/o inducción de muerte de los linfocitos B (*verbigracia*, mediante apoptosis).

El término "anticuerpo" en esta solicitud se emplea en el sentido más amplio y cubre específicamente anticuerpos monoclonales, anticuerpos policlonales, anticuerpos multiespecíficos (*verbigracia*, anticuerpos bioespecíficos) formados de por lo menos dos anticuerpos intactos, y fragmentos de anticuerpo, siempre que exhiban la actividad biológica deseada.

"Fragmentos de anticuerpo" comprenden una porción de un anticuerpo intacto, que comprenda preferiblemente su región de unión de antígeno. Ejemplos de fragmentos de anticuerpo incluyen fragmentos Fab, Fab', F(ab')₂, Fv; diacuerpos; anticuerpos lineales; moléculas de anticuerpo de cadena única; y anticuerpos multiespecíficos formados de fragmentos de anticuerpo.

Para los propósitos de esta solicitud, un "anticuerpo intacto" es uno que comprende dominios variables de cadena ligera y pesada, así como una región Fc.

Un "anticuerpo que se une a un marcador de superficie de linfocito B" es una molécula que, luego de unirse a un marcador de superficie de linfocito B, destruye o agota linfocitos B en un mamífero e interfiere una o más de las funciones de los linfocitos B, *verbigracia*, reduciendo o previniendo una respuesta humoral provocada por el linfocito B. El anticuerpo está preferiblemente en capacidad de agotar linfocitos B (*es decir*, reducir los niveles de linfocitos B en circulación) en un mamífero tratado con él. Tal agotamiento puede lograrse mediante varios mecanismos tales como citotoxicidad celular dependiente de anticuerpo (ADCC) y/o citotoxicidad dependiente de complemento (CDC), inhibición de la proliferación de linfocitos B y/o inducción de muerte de los linfocitos B (*verbigracia*, mediante apoptosis). En una incorporación preferida, el anticuerpo induce una respuesta clínica importante. En otra incorporación preferida, el marcador de superficie de linfocito B es CD20, de modo que el anticuerpo que se une a un marcador de superficie de linfocito B es un anticuerpo que se une a CD20, o un "anticuerpo CD20". Una incorporación particularmente preferida es un anticuerpo CD20 que induce una respuesta

clínica importante. Para los efectos de esta solicitud, una "respuesta clínica importante" se define como la que logra una respuesta 70 del American College of Rheumatology (ACR 70) durante seis meses consecutivos. Los puntajes de respuesta ACR se clasifican como ACR 20, ACR 50 y ACR 70, siendo ACR 70 el nivel máximo de control de signos y síntomas en este sistema de evaluación. Los puntajes de respuesta ACR miden la mejora en la actividad patológica de la artritis reumatoide, incluida la hinchazón de las articulaciones y la sensibilidad a la palpación, dolor, nivel de incapacidad y evaluación general del paciente y el médico. Un ejemplo de un tipo diferente de anticuerpo que induce una respuesta clínica importante, tal como lo reconoce la FDA y se define en esta solicitud es etanercept (ENBREL®).

Ejemplos de anticuerpos CD20 incluyen "C2B8" que ahora se denomina "rituximab" ("RITUXAN®") (Patente de los Estados Unidos No. 5,736,137); el anticuerpo murino 2B8 marcado con itrio [90] designado "Y2B8" o "Ibritumomab Tiuxetan" (ZEVALIN®) disponible en el comercio de IDEC Pharmaceuticals, Inc (Patente de los Estados Unidos No 5,736,137, 2B8 depositada ante la ATCC bajo el número de acceso HB 1 1388 del 22 de junio de 1993), IgG2a murina "B1", denominada también "Tositumomab", marcado opcionalmente con ¹³¹I para generar el anticuerpo "¹³¹I-B1" o tositumoinab yodo I131, (BEXXAR™) disponible en el comercio de Corixa (véase, también, Patente de los Estados Unidos No. 5,595,721), anticuerpo monoclonal murino "1F5" (Press et al *Blood* 69(2) 584-591 (1987) y sus variantes, incluido 1F5 humanizado o "pegado en la estructura alterna" ("*framework patched*") (WO 2003/002607, Leung, S, ATCC depósito HB-96450), anticuerpo 2H7 murino y 2H7 quimérico (Patente de los Estados Unidos No 5,677,180), un 2H7 humanizado (WO 2004/056312 (Lowman *et al.*) como se expone más adelante), HUMAX-CD20™ anticuerpo de alta afinidad, completamente humano dirigido a la molécula CD20 de la membrana celular de linfocitos B (Genmab, Denmark, véase, por ejemplo, Glennie and van de Winkel, *Drug Discovery Today* 8:503-510 (2003) y Cragg *et al.*, *Blood* 101:1045-1052 (2003)), los

anticuerpos monoclonales humanos expuestos en WO04/035607 (Teeling *et al.*), los anticuerpos AME- 133™ (Applied Molecular Evolution), el anticuerpo A20 o sus variantes, tales como el anticuerpo A20 quimérico o humanizado (cA20, hA20, respectivamente) (US 2003/0219433, Immunomedics), y los anticuerpos monoclonales L27, G28-2, 93-1B3, B-C1 o, NU-B2 disponibles de the International Leukocyte Typing Workshop (Valentine *et al.*, In Leukocyte Typing III (McMichael, Ed., p 440, Oxford University Press (1987)). Los anticuerpos CD20 preferidos en esta solicitud son anticuerpos CD20 quiméricos, humanizados, o humanos, más preferiblemente rituximab, un anticuerpo CD20 2H7 humanizado, o un anticuerpo CD20 quimérico o humanizado (Immunomedics), y HUMAX-CD20™ un anticuerpo CD20 humano (Genmab).

Los términos "rituximab" y "RITUXAN®" en esta solicitud se refieren al anticuerpo monoclonal murino/humano quimérico genéticamente diseñado, dirigido contra el antígeno CD20 y designado "C2B8" en la Patente de los Estados Unidos No. 5,736,137, incluidos sus fragmentos, que conservan la capacidad de unir CD20.

Exclusivamente para los propósitos de esta solicitud y, a menos que se indique lo contrario, un "2H7 humanizado" se refiere a un anticuerpo CD20 humanizado, o su fragmento de unión de antígeno, en donde el anticuerpo es efectivo para agotar linfocitos B de primate *in vivo*, anticuerpo que comprende en la región variable de cadena H (V_H) por lo menos una secuencia CDR H3 de SEQ ID NO: 12 (Fig. 1B) de un anticuerpo CD20 anti-humano y sustancialmente los residuos de estructura alterna (FR) de consenso humanos del subgrupo III de cadena pesada humano (V_{HIII}). En una incorporación preferida, este anticuerpo comprende además la secuencia CDR H1 de cadena H de SEQ ID NO: 10 la secuencia CDR H2 de SEQ ID NO:11 y, más preferiblemente, comprende asimismo la secuencia CDR L1 de cadena L de SEQ ID NO:4, la secuencia CDR L2 de SEQ ID NO:5, la secuencia CDR L3 de SEQ ID NO:6 y sustancialmente los residuos de estructura alterna (FR) del subgrupo I humano de cadena ligera (V_L), en donde la región V_H puede unirse a una

región constante de cadena IgG humana, en donde la región puede ser, por ejemplo, IgG1 ó IgG3. Véase también WO 2004/056312 (Lowman *et al.*)

En una incorporación preferida, tal anticuerpo comprende la secuencia VH de SEQ ID NO:8 (vl 6, como se muestra en Fig. 1B), comprendiendo además de manera opcional la secuencia VL de SEQ ID NO:2 (vl 6, como se muestra en Fig. 1A), que puede tener las sustituciones de aminoácidos de D56A y N100A en la cadena H y S92A en la cadena L (v96). Preferiblemente, el anticuerpo es un anticuerpo intacto que comprende las secuencias de aminoácidos de cadena ligera y cadena pesada de SEQ ID NOS 13 y 14, respectivamente, como se muestra en Figs. 2 y 3. Otra incorporación preferida es donde el anticuerpo es 2H7 v31, que comprende las secuencias de aminoácidos de cadena ligera y cadena pesada de SEQ ID NOS 13 y 15, respectivamente, como se muestra en Figs. 2 y 4. El anticuerpo en esta solicitud puede comprender, además, por lo menos una sustitución de aminoácidos en la región Fc, que mejora la actividad ADCC y/o CDC, tal como una donde las sustituciones de aminoácidos son S298A/E333A/K334A, más preferiblemente 2H7 v31 que tiene la secuencia de aminoácidos de cadena pesada de SEQ ID NO:15 (como se muestra en Fig 4). Otra incorporación preferida es donde el anticuerpo es 2H7. v138 que comprende las secuencias de aminoácidos de cadena ligera y pesada de SEQ ID NOS: 28 y 29, respectivamente, como se muestra en Figs. 5 y 6, que son alineaciones de tales secuencias con las correspondientes secuencias de aminoácidos de cadena ligera y pesada de 2H7.v16. Alternativamente tal anticuerpo 2H7 humanizado intacto preferido es 2H7 v477, que tiene las secuencias de aminoácidos de cadena ligera y pesada de 2H7.v138, excepto por la sustitución de aminoácidos de N434W. Cualquiera de estos anticuerpos puede comprender además por lo menos una sustitución de aminoácidos en la región Fc, que disminuye la actividad CDC, por ejemplo, comprendiendo por lo menos la sustitución de K322A. Véase la Patente de los Estados Unidos No. 6,528,624B I (Idusogie *et al.*).

Las variantes de 2H7 humanizado más preferidas son aquellas que tienen el dominio de cadena ligera variable de SEQ ID NO:2 y el dominio

de cadena pesada variable de SEQ ID NO:8, *es decir*, aquellas con o sin substituciones en la región Fc, y aquellas que tienen un dominio de cadena pesada variable con alteración N100A o D56A y N100A en SEQ ID NO:8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO:2, *es decir*, aquellas con o sin substituciones en la región Fc. Si se hacen substituciones en la región Fc, éstas son preferiblemente una de las expuestas en la siguiente tabla.

En un resumen de varias incorporaciones preferidas de la invención, la región V de variantes basadas en la versión 16 de 2H7 tendrán las secuencias de aminoácidos de v16, excepto en las posiciones de substituciones de aminoácidos que se indican en la siguiente tabla. A menos que se indique lo contrario, las variantes 2H7 tendrán la misma cadena L que las de v16.

Versión 2H7	Cambios de cadena pesada (V _H)	Cambios de cadena ligera (V _L)	Cambios Fc
16			-
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

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

DIQMTQSPSSLSASVGDRVITTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFGSGSGTDFTLTISLQPEDFATYYCQQWSFNPPFTFGQGTKVEIKR (SEQ ID NO:2),

y la secuencia de cadena pesada variable:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFVDVWGQGTLT
VSS (SEQ ID NO:8).

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

DIQMTQSPSSLSASVGDRVITTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISLQPEDFATYYCQQWSFNPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13),

y la secuencia de aminoácidos de cadena pesada:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFVDVWGQGTLT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC
SVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

o la secuencia de aminoácidos de cadena pesada:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFVDVWGQGTLT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTTCPPCPAPELLGGPSV
LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRV
VSVLTVLHQDWLNGEYKCKVSNALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFC
SV MHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

En otra incorporación preferida, el anticuerpo 2H7 humanizado intacto comprende la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVITTCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISLQPEDFATYYCQQWAFNPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:28)

y la secuencia de aminoácidos de cadena pesada:

EVQLVESGGGLVQPGGSLRLSCAASG

YTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL
 RAEDTAVYYCARVYYYSASYWYFDVWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL
 GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH
 KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAL
 ALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
 NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPGK
 (SEQ ID NO:29).

"Citotoxicidad mediada por célula dependiente de anticuerpo" y "ADCC" se refiere a una reacción mediada por células en la cual células citotóxicas no específicas que expresan receptores Fc (FcR) (*por ejemplo*, linfocitos citolíticos naturales (NK), neutrófilos y macrófagos) reconocen anticuerpo unido en una célula diana y, posteriormente, ocasionan lisis de la célula diana. Las células primarias para mediar ADCC, linfocitos citolíticos naturales, expresan FcγRIII únicamente, mientras que los monocitos expresan FcγRI, FcγRII y FcγRIII. La expresión de FcR en las células hematopoyéticas se resume en la Tabla 3, página 464 de Ravetch y Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991). Para evaluar la actividad ADCC de una molécula de interés, puede llevarse a cabo un análisis ADCC *in vitro*, tal como el descrito en la Patente de los Estados Unidos No. 5,500,362 ó 5,821,337. Células efectoras útiles para tales análisis incluyen células mononucleares de sangre periférica (PBMC) y linfocitos citolíticos naturales (NK). Alternativa, o adicionalmente, la actividad ADCC de la molécula de interés puede evaluarse *in vivo*, *por ejemplo*, en un modelo animal tal como el divulgado en Clynes *et. al.*, *PNAS USA* 95:652-656 (1998).

"Células efectoras humanas" son leucocitos que expresan uno o más FcR y realizan funciones efectoras. Preferiblemente, las células expresan por lo menos FcγRIII y llevan a cabo la función efectora ADCC. Ejemplos de leucocitos humanos, que median ADCC, incluyen células mononucleares de sangre periférica (PBMC), linfocitos citolíticos naturales (NK), monocitos, linfocitos T citotóxicos y neutrófilos, prefiriéndose PBMC y linfocitos citolíticos naturales (NK).

Los términos "receptor Fc" o "FcR" se usan para describir un receptor que se une a la región Fc de un anticuerpo. El FcR preferido es un FcR humano de secuencia natural. Más aún, un FcR preferido es uno que une un anticuerpo IgG (un receptor gamma) e incluye receptores de las subclases FcγRI, FcγRII, y FcγRIII, incluidas variantes alélicas y, alternativamente, formas empalmadas de estos receptores. Los receptores FcγRII incluyen FcγRIIA (un "receptor activador") y FcγRIIB (un "receptor inhibidor"), los cuales poseen secuencias de aminoácidos similares que difieren principalmente en sus dominios citoplásmicos. El receptor activador FcγRIIA contiene un motivo de activación a base de tirosina de inmunorreceptor (ITAM) en su dominio citoplásmico. El receptor inhibidor FcγRIIB contiene un motivo de inhibición a base de tirosina de inmunorreceptor (ITIM) en su dominio citoplásmico, (véase Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). Los FcR se reseñan en Ravetch y Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et. al.*, *Immunomethods* 4: 25-34 (1994); y de Haas *et. al.*, *J. Lab. Clin. Med.* 126: 330-41 (1995). Otros FcR, incluidos aquellos por identificar en el futuro, se abarcan por el término "FcR" contenido aquí. El término comprende asimismo el receptor neonatal, FcRn, que es responsable de la transferencia de IgG maternas al feto. Guyer *et. al.*, *J. Immunol.* 117: 587 (1976) y Kim *et. al.*, *J. Immunol* 24: 249 (1994)).

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

Anticuerpos "inhibidores de crecimiento" son aquellos que previenen 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 linfocitos B *in vitro* y/o *in vivo*.

Anticuerpos que "inducen apoptosis" son aquellos que inducen muerte celular programada, *por ejemplo*, de un linfocito B, como se determina por análisis estándar de apoptosis, tales como unión de anexina V, fragmentación de ADN, encogimiento celular, dilatación del retículo endoplásmico, fragmentación celular, y/o formación de vesículas de la membrana (llamadas cuerpos apoptóticos).

"Anticuerpos naturales" son usualmente glucoproteínas heterotetraméricas de alrededor de 150.000 daltons, compuestas de dos cadenas ligeras (L) idénticas y dos cadenas pesadas (H) idénticas. Cada cadena ligera se enlaza a una cadena pesada por un enlace disulfuro covalente, mientras que el número de enlazamientos disulfuro varía entre las cadenas pesadas de diferentes isotipos de inmunoglobulina. Cada cadena ligera y pesada posee también puentes disulfuro intracadenas regularmente espaciados. Cada cadena pesada tiene en un extremo un dominio variable (V_H) seguido por un número de dominios constantes. Cada cadena ligera tiene un dominio variable en un extremo (V_L) y un dominio constante en su otro extremo; el dominio constante de la cadena ligera se alinea con el primer dominio constante de la cadena pesada, y el dominio variable de la cadena ligera se alinea con el dominio variable de la cadena pesada. Se cree que residuos de aminoácidos particulares forman una interfase entre los dominios variables de cadena ligera y cadena pesada.

El término "variable" se refiere al hecho de que ciertas porciones de los dominios variables difieren extensivamente en secuencia entre anticuerpos y se utilizan en la unión y especificidad de cada anticuerpo particular para su antígeno particular. No obstante, la variabilidad no está uniformemente distribuida a través de los dominios variables de los anticuerpos. Ésta se concentra en tres segmentos denominados regiones hipervariables, tanto en los dominios variables de cadena pesada como en los de cadena ligera. Las porciones más altamente conservadas de los dominios variables se llaman las regiones de estructura alterna (FR). Los

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

La digestión con papaína de anticuerpos produce dos fragmentos de unión de antígeno idénticos, llamados fragmentos "Fab", cada uno con un solo sitio de unión de antígeno, y un fragmento "Fc" residual, cuyo nombre refleja su capacidad de cristalizarse rápidamente. El tratamiento con pepsina proporciona un fragmento $F(ab')_2$ que posee dos sitios de unión de antígeno y es aún capaz de entrecruzar antígeno.

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

El fragmento Fab contiene igualmente el dominio constante de la cadena ligera y el primer dominio constante (CH1) de la cadena pesada. Los fragmentos Fab' difieren de los fragmentos Fab por la adición de unos pocos residuos en el término carboxi del dominio CH1 de cadena pesada, incluyendo una o más cisteínas de la región de unión flexible del anticuerpo. Fab'-SH es la designación en esta solicitud para Fab', en el cual el residuo(s) de cisteína de los dominios constantes lleva por lo menos un grupo tiol libre. Fragmentos F(ab')₂ de anticuerpo se produjeron originalmente como pares de fragmentos Fab', que tienen cisteínas de unión flexible entre ellos. Otros acoplamientos químicos de fragmentos de anticuerpo son también conocidos.

Las "cadenas livianas" de anticuerpos (inmunoglobulinas) de cualquier especie vertebrada pueden asignarse a uno de dos tipos claramente distintos, llamados kappa (κ) y lambda (λ), basados en las secuencias de aminoácidos de sus dominios constantes.

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

Fragmentos de anticuerpo "Fv de cadena única" o "scFv" comprenden los dominios V_H y V_L de anticuerpo, en donde estos dominios están presentes en una sola cadena polipeptídica. Preferiblemente, el polipéptido Fv comprende además un conector de polipéptido entre los dominios V_H y V_L, que permite que el scFv forme la estructura deseada para unión de antígeno. Para una reseña del scFv, véase Plückthun en *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, páginas 269-315(1994).

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

El término "anticuerpo monoclonal", como se emplea en esta solicitud, se refiere a un anticuerpo obtenido de una población de anticuerpos sustancialmente homogéneos, *es decir*, los anticuerpos individuales que componen la población son idénticos y/o unen el mismo epítipo, excepto por posibles variantes que pueden surgir durante la producción del anticuerpo monoclonal, estando presentes generalmente tales variantes en cantidades menores. En contraste con preparaciones convencionales de anticuerpo policlonal, que típicamente incluyen diferentes anticuerpos dirigidos contra diferentes determinantes (epítopos), cada anticuerpo monoclonal se dirige contra un solo determinante en el antígeno. Además de su especificidad, los anticuerpos monoclonales son ventajosos en el sentido de que no se contaminan por otras inmunoglobulinas. El calificativo "monoclonal" indica el carácter del anticuerpo como obtenido de una población sustancialmente homogénea de anticuerpos, y no tiene que interpretarse como que requiere la producción del anticuerpo por algún método particular. Por ejemplo, los anticuerpos monoclonales a utilizarse de acuerdo con la presente invención pueden elaborarse por el método del hibridoma, descrito por primera vez por Kohler *et al.*, *Nature*, 256:495 (1975), o pueden elaborarse por métodos de ADN recombinante (véase, *por ejemplo*, Patente de los Estados Unidos 4,816,567). Los "anticuerpos monoclonales" pueden aislarse también de bibliotecas de anticuerpos de fagos usando las técnicas descritas en

Clackson et al., *Nature*, 352:624-628 (1991); Marks et al., *J. Mol. Biol.*, 222:581-597 (1991), por ejemplo.

Los anticuerpos monoclonales en esta solicitud incluyen específicamente anticuerpos "quiméricos" (inmunoglobulinas), en los cuales una porción de la cadena pesada y/o ligera es idéntica con, u homóloga a, secuencias correspondientes en anticuerpos derivados de una especie particular, o pertenecientes a una clase o subclase de anticuerpos, mientras que el resto de la cadena(s) es idéntico con, u homólogo a, secuencias correspondientes en anticuerpos derivados de otra especie, o pertenecientes a otra clase o subclase de anticuerpos, así como fragmentos de tales anticuerpos, siempre que exhiban la actividad biológica deseada (Patente de los Estados Unidos 4,816,567; y Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Los anticuerpos quiméricos de interés indicados aquí incluyen anticuerpos "primatizados" que comprenden secuencias de unión de antígeno de dominio variable, derivadas de un primate no humano (*por ejemplo*, simio del viejo mundo, tales como babuino, mono rhesus o cynomolgus) y secuencias de región constante humana (Patente de los Estados Unidos No. 5,693,780).

Formas "humanizadas" de anticuerpos no humanos (*por ejemplo*, murinos) son anticuerpos quiméricos que contienen una secuencia mínima derivada de inmunoglobulina no humana. En su mayor parte, los anticuerpos humanizados son inmunoglobulinas humanas (anticuerpo destinatario), en las cuales residuos de una región hipervariable del destinatario se reemplazan por residuos de una región hipervariable de una especie no humana (anticuerpo donante) tal como ratón, rata, conejo o primate no humano, que tienen la especificidad, afinidad y capacidad deseadas. En algunos casos, residuos de la región de estructura alterna (FR) de la inmunoglobulina humana se reemplazan por residuos no humanos correspondientes. Más aún, los anticuerpos humanizados pueden comprender residuos, que no se encuentran en el anticuerpo destinatario ni en anticuerpo donante. Estas modificaciones se hacen para refinar aún más el desempeño del anticuerpo. En general, el anticuerpo humanizado

comprenderá sustancialmente la totalidad de por lo menos uno y, típicamente dos, dominios variables, en los cuales la totalidad o sustancialmente todos los lazos hipervariable corresponden a los de una inmunoglobulina no humana, y todas o sustancialmente todas las FR son las de una secuencia de inmunoglobulina humana. Opcionalmente, el anticuerpo humanizado comprenderá también por lo menos una porción de una región constante de inmunoglobulina (Fc), típicamente la de una inmunoglobulina humana. Para mayores detalles, véase Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); y Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992).

El término "región hipervariable", cuando se emplea aquí, se refiere a los residuos de aminoácidos de un anticuerpo que son responsables de la unión de antígeno. La región hipervariable comprende residuos de aminoácidos de una "región determinante de complementariedad" o "CDR" (*por ejemplo*, residuos 24-34 (L1), 50-56 (L2) y 89-97 (L3) en el dominio variable de cadena ligera y 31-35 (H1), 50-65 (H2) y 95-102 (H3) en el dominio variable de cadena pesada; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) y/o aquellos residuos de un "lazo hipervariable" (*por ejemplo*, residuos 26-32 (L1), 50-52 (L2) y 91-96 (L3) en el dominio variable de cadena ligera y 26-32 (H1), 53-55 (H2) y 96-101 (H3) en el dominio variable de cadena pesada; Chothia y Lesk *J. Mol. Biol.* 196:901-917 (1987)).

Residuos "de estructura alterna" o "FR" son aquellos residuos de dominio variable distintos de los residuos de región hipervariable, como se definen aquí.

Un "anticuerpo desnudo" es un anticuerpo (como se define en esta solicitud), que no se conjuga a una molécula heteróloga, tal como una fracción citotóxica o radiomarca.

Un anticuerpo "aislado" es uno que se ha identificado y separado y/o recuperado de un componente de su entorno natural. Componentes contaminantes de su entorno natural son materiales que interferirían los

usos diagnósticos o terapéuticos para el antagonista o anticuerpo, y pueden incluir enzimas, hormonas, y otros solutos proteináceos o no proteináceos. En incorporaciones preferidas, el anticuerpo se purificará (1) a más del 95% en peso del anticuerpo según se determine por el método de Lowry y, muy preferiblemente, más del 99% en peso, (2) a un grado suficiente para obtener por lo menos 15 residuos de secuencia de aminoácidos interna o N-terminal mediante el uso de un secuenciador de vaso, o (3) a homogeneidad por SDS-PAGE bajo condiciones reductoras o no reductoras usando azul Coomassie o, preferiblemente, tintura de plata. El anticuerpo aislado incluye el anticuerpo *in situ* en células recombinantes, puesto que por lo menos un componente del entorno natural del anticuerpo no estará presente. Por lo regular, sin embargo, el antagonista aislado se preparará por un paso de purificación, como mínimo.

"Vasculitis asociada con ANCA" o "vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (ANCA)" o "AAV" como se utiliza en esta solicitud es una enfermedad o trastorno autoinmunitario que involucra vasculitis sistémica (o inflamación de las paredes de un vaso sanguíneo), en los cuales los anticuerpos anticitoplasma de los neutrófilos en circulación (ANCA) están normalmente presentes en la sangre del sujeto, u están presentes otras manifestaciones clínicas que definen las vasculitis, como se anota más adelante. El término "vasculitis asociada con ANCA", tal como se emplea en esta solicitud, se aplica a vasculitis asociada con ANCA, sin importar qué tipo y etapa o gravedad, y sin importar qué síntomas son evidentes, siempre que se haga el diagnóstico. Ejemplos de vasculitis asociada con ANCA incluyen polivasculitis microscópica, granulomatosis de Wegener, síndrome de Churg-Strauss, vasculitis limitada a los riñones (glomerulonefritis progresiva necrosante idiopática), y ciertos tipos de vasculitis inducida por medicamentos. Los diagnósticos de vasculitis asociada con ANCA y sus diferentes manifestaciones incluyen los expuestos a continuación.

Varias pruebas de diagnóstico se utilizan comúnmente en personas sospechosas de tener vasculitis asociada con ANCA. Características que

pueden ayudar a definir el tipo específico de trastorno vasculítico incluyen el tipo de implicación del órgano, la presencia y tipo de ANCA (mieloperoxidasa-ANCA o proteinasa 3-ANCA), la presencia de crioglobulinas séricas, y la presencia de evidencia de inflamación granulomatosa. Auto-anticuerpos ejemplares relacionados con vasculitis asociada con ANCA incluyen una concentración elevada de anticuerpos anti-nucleares (ANA), anticuerpos de factor anti-reumatoide (RF), creatinina, nitrógeno ureico en la sangre, anticuerpos anti-endoteliales, anticuerpos anticitoplasma de los neutrófilos (ANCA), tales como autoanticuerpos dirigidos contra proteinasa 3 (PR3) o contra mieloperoxidasa (MPO), o una combinación de los mismos.

Los anticuerpos ANCA pueden detectarse usando análisis inmunoquímico específico de antígeno para caracterizar PR3-ANCA y MPO-ANCA. Niles *et al.*, *supra*. Puesto que una prueba ELISA de ANCA se asocia con un valor predictivo positivo sustancialmente más alto e índices de probabilidad para la vasculitis asociada con ANCA, las pruebas ELISA pueden realizarse únicamente en muestras que sean positivas para ANCA por inmunofluorescencia. Stone *et al.*, Arthritis Care and Research, 13 424-34 (2000), Comment on Arthritis Care Res 13 341-342 (2000), Russell *et al.*, Clin Immunol, 103 196-203 (2002).

Alrededor del 10 por ciento de pacientes con polivascúlitis microscópica (el tipo más común de vasculitis asociada con ANCA) y granulomatosis de Wegener tienen análisis negativos para ANCA; no obstante, este resultado no excluye completamente estas enfermedades, y las concentraciones de ANCA no siempre se correlacionan con esta actividad patológica. Jennette and Falk, *N. Engl J Med.*, *supra*. De otro lado, un resultado de análisis positivo para ANCA no es únicamente diagnóstico de vasculitis asociada con ANCA.

Tabla 1 recopila las potenciales manifestaciones clínicas de vasculitis asociada con ANCA, que deben sospecharse en cualquier paciente que se presente con una enfermedad multisistémica, no causada por un proceso infeccioso o maligno (*verbigracia*, disfunción renal, exantemas cutáneos,

manifestaciones pulmonares, o manifestación nerviosa). Síntomas constitutivos son comunes. La frecuencia y combinación de varios compromisos de un sistema difieren entre entidades patológicas individuales. Véase también Guillevin *et al.*, *Arthritis Rheum.* 42:421-430 (1999); Pettersson *et al.*, *Clin. Nephrol.* 43: 141-149 (1995); Savage *et al.*, *Lancet* 349:553-558 (1997); Guillevin *et al.*, *Br. J. Rheumatol* 35:958-964 (1996).

Tabla 1

Manifestaciones Clínicas de Vasculitis Asociada con ANCA

<u>Sistema</u>	<u>Manifestaciones</u>
Constitutivo	Fiebre, pérdida de peso, malestar general
Musculoesquelético	Mialgia, artralgia
Piel	Púrpura palpable, urticaria
Riñones	Proteinuria, hematuria, insuficiencia renal, deficiencia renal, glomerulonefritis necrosante
Tracto respiratorio	Disnea, tos, hemoptisis; infiltrado pulmonar, enfermedad pulmonar intersticial, hemorragia pulmonar
Sistema nervioso	Neuropatía periférica, especialmente mononeuritis
Tracto digestivo	Sangre fecal, enzimas hepáticas elevadas; diarrea, náuseas, vómito, dolor abdominal

La lesión cutánea más común es púrpura palpable – una erupción ligeramente levantada, sin blanqueamiento que comienza usualmente en las extremidades inferiores. Ocasionalmente, el exantema es vesicular o ligeramente ulcerado. La urticaria puede ser asimismo una manifestación de vasculitis asociada con ANCA. A diferencia de urticaria alérgica no vasculítica, la urticaria vasculítica dura más de un día y puede evolucionar en lesiones purpúreas. La presencia de hipocomplementemia puede indicar que la vasculitis es mediada por el complejo inmunitario en lugar de vasculitis asociada con ANCA.

El compromiso renal en la vasculitis puede evolucionar a deficiencia renal. Resultados de biopsia del riñón revelan comúnmente glomerulonefritis. Necrosis focal, formación progresiva y la ausencia o escasez de depósitos de inmunoglobulina caracterizan la glomerulonefritis en pacientes con vasculitis asociada con ANCA. Pettersson *et al.*, *Clin. Nephrol.* 43:141-149 (1995). El compromiso del pulmón varía desde infiltrados focales transitorios o enfermedad intersticial a capilaritis alveolar hemorrágica pulmonar masiva. La última es la característica más potencialmente mortal de la vasculitis de los vasos pequeños.

Es importante, sin embargo, diferenciar la vasculitis asociada con ANCA de otras enfermedades que resultan en manifestaciones multi-sistémicas. Enfermedades con embolización ampliamente difundida a diferentes órganos (*verbigracia*, enfermedad ateroembólica, endocarditis, síndrome antifosfolípido, y mixoma auricular) pueden producir presentaciones clínicas similares. Kelley, "Vasculitis and related disorders" In: *Textbook of rheumatology*. 5th ed. (Philadelphia: Saunders, 1997), pp.1079-1101. Personas con sepsis pueden presentarse asimismo compromiso multi-sistémico. Es igualmente importante tener en cuenta que la vasculitis asociada con ANCA puede ser secundaria a infecciones o neoplasia maligna. Algunas infecciones víricas, bacterianas y fúngicas pueden complicarse con vasculitis, las cual es predominantemente vasculitis dérmica. El diagnóstico es sugerido por la historia clínica. Neoplasia maligna, tal como linfomas, leucemia, síndromes mieloproliferativos y mielodisplásicos, pueden estar relacionados con vasculitis asociada con ANCA; no obstante, los tumores sólidos se asocian menos comúnmente con tal vasculitis. Causas infecciosas o malignas subyacentes deben evaluarse exhaustivamente antes de hacer el diagnóstico de vasculitis asociada con ANCA – incluso si el examen de ANCA resulta positivo.

Tabla 2 presenta algunas de las características clínicas que pueden ayudar al diagnóstico del tipo específico de vasculitis. La evaluación de laboratorio debe incluir un conteo completo de glóbulos blancos y perfil de química rutinario, examen de orina, prueba fecal de sangre oculta, y

radiografía del tórax. Puede haber anemia normocítica, trombocitosis, índice elevado de sedimentación de eritrocitos, función hepática incrementada, o evidencia de compromiso renal. Deben medirse también los niveles séricos de ANCA. Otras pruebas de laboratorio que deben realizarse para excluir la vasculitis asociada con ANCA incluyen exámenes de anticuerpos anti-nucleares, factor reumatoide, crioglobulinas, complemento, anticuerpos contra hepatitis B y C, virus de la inmunodeficiencia humana (VIH). De igual manera pueden practicarse radiografías tomográficas computarizadas del tórax y los senos, si se considera apropiado. El examen patológico del tejido comprometido (*verbigracia*, piel, nervio, pulmón, o riñón) puede ayudar en la documentación del tipo de vasculitis asociada con ANCA. Debe obtenerse una biopsia de los sitios sintomáticos y accesibles. Biopsias de sitios no sintomáticos tienen un bajo rendimiento de resultados positivos.

Tabla 2

Características Clínicas que Favorecen el Diagnóstico de un Tipo

Específico de Vasculitis	
<u>Características clínicas</u>	<u>Probable tipo de vasculitis</u>
Síntomas pulmonares y renales	Granulomatosis de Wegener; polivascularitis microscópica
Síntomas pulmonares- dérmicos	Crioglobulinemia; púrpura de Henoch-Schönlein
Asma y eosinofilia	Síndrome de Churg-Strauss
Compromiso del tracto respiratorio superior	Granulomatosis de Wegener (<i>verbigracia</i> , sinusitis y otitis media)

La información contenida en la Tabla 2 proviene de Jennette and Falk, *N Engl. J. Med, supra*, y Kelley, *supra*.

La granulomatosis de Wegener se caracteriza por granulomas necrosantes del tracto respiratorio superior e inferior, junto con glomerulonefritis y vasculitis sistémica, que compromete usualmente los

vasos de tamaño medio, con formación de granulomas y necrosis del parenquima. Kelley, *supra*. Los signos y síntomas del tracto respiratorio superior incluyen sinusitis, úlceras nasales, otitis media, o pérdida de la audición. Los signos y síntomas del tracto respiratorio superior se observan en 70 por ciento de los pacientes e infiltrados pulmonares o nódulos que pueden formar cavidades o cavernas se desarrollan en 85 por ciento de los pacientes. Kelley, *supra*. La antiproteasa 3-ANCA sérica (c-ANCA) es positiva en 75 a 90 por ciento, aunque el 20 por ciento puede tener p-ANCA positiva. La biopsia del pulmón abierto es la prueba diagnóstica más definitiva. La biopsia de los senos es diagnóstica únicamente en 30 por ciento de los casos porque los resultados inflamatorios son a menudo no específicos y la biopsia renal es también relativamente no específica. Los resultados radiográficos son de opacidades de la zona media e inferior, las cuales son difusas, y los dos nódulos alveolares e intersticiales, que pueden formar cavidades o cavernas, son raros en niños. La tomografía axial computarizada puede mostrar opacidades perivasculares mal definidas, difusas. La granulomatosis de Wegener puede afectar pacientes en cualquier edad, con la incidencia máxima durante la cuarta década de vida y es ligeramente más común en hombres. Duna *et al.*, *Rheum. Dis. Clin. North Am.* 21 949-986 (1995). El modo más definitivo de diagnosticar la granulomatosis de Wegener es practicando una biopsia de un sitio de un órgano comprometido (usualmente los senos, pulmón o riñón) para confirmar la presencia de vasculitis y granulomas, todos los cuales son diagnósticos de la enfermedad.

La polivascularitis microscópica se caracteriza por la presencia de ANCA y unos cuantos o ningunos depósitos inmunitarios en los vasos comprometidos. Savage *et al.*, *Lancet* 349 553-558 (1997). Los riñones son los órganos más comúnmente afectados en 90 por ciento de los pacientes que presentan este tipo de vasculitis. Kelley, *supra*. Los pacientes presentan combinaciones variables de manifestaciones renales, púrpura palpable, dolor abdominal, tos, y hemoptisis. La mayoría de los pacientes tienen MPO-ANCA positiva (p-ANCA), aunque PR3-ANCA (c-

ANCA) puede estar igualmente presente en 40 por ciento de los pacientes. La edad de aparición más común está entre los 40 y 60 años y el sexo más común es el masculino.

El síndrome de Churg-Strauss es una enfermedad rara y tiene tres fases: rinitis alérgica y asma, enfermedad infiltradora eosinofílica parecida a la neumonía, y vasculitis sistémica de los vasos pequeños con inflamación granulomatosa. Guillevin *et al.*, *Br. J. Rheumatol.*, 35 958 964 (1996). La fase vasculítica se desarrolla usualmente en el curso de tres años a partir de la manifestación del asma. Casi todos los pacientes tienen más del 10 por ciento de eosinófilos en la sangre. La arteritis y miocarditis coronaria son las causas principales de morimortalidad. La edad de aparición varía desde 15 a 70 años y es más común en los hombres. La vasculitis inducida por fármacos se desarrolla usualmente entre 7 a 21 días después de administrado un medicamento y puede limitarse a la piel. Jennette and Falk, *N. Engl. J. Med.*, *supra*. Las lesiones cutáneas son idénticas a las observadas en vasculitis sistémica de los vasos pequeños. Los medicamentos causan aproximadamente 10 por ciento de las lesiones cutáneas vasculíticas. Los medicamentos que se han visto implicados incluyen penicilina, aminopenicilinas, sulfonamidas, allopurinol, tiazidas, quinolonas, hidantoínas, y propiltiouracil. Algunas medicinas, tales como propiltiouracil e hidralazina (APRESOLINETM), parecen causar vasculitis induciendo ANCA.

Otra forma de comprobar la enfermedad activa y determinar qué pacientes/sujetos son elegibles para tratamiento es determinar el valor puntaje de actividad de vasculitis de Birmingham/ granulomatosis de Wegener (BVAS/WG) del paciente, si es mayor o menor. Este puntaje constituye un índice de la actividad de vasculitis y está diseñado para documentar características clínicas que se deben directamente a la granulomatosis activa de Wegener. Se ha descubierto que es un indicador válido y confiable, específico de la enfermedad, para la granulomatosis de Wegener. Stone *et al.*, *Arthritis & Rheumatism*, 44:912-920 (2001). Puede utilizarse asimismo para otras enfermedades de vasculitis asociada con

ANCA. El instrumento separa las características que representan actividad patológica nueva o peor de las que representan actividad persistente. Típicamente, el puntaje BVAS/WG del paciente es 3 o más (o ha sido 3 o más en el término de 28 días de tratamiento). Cada episodio mayor en la forma de evaluación BVAS/WG se califica con 3 puntos. Cada episodio menor se califica con 1 punto. No obstante, otra distinción empleada en esa enfermedad aguda, ya sea primera presentación o recaída, muestra un BVAS/WG de por lo menos 10, mientras que la enfermedad persistente muestra un BVAS/WG de por lo menos 4. La linfopenia puede ser también un buen indicador de la granulomatosis de Wegener. Izzedine *et al.*, *Nephron* 92 466-471 (2002).

Un "sujeto" en esta solicitud es un sujeto humano, incluido un paciente, elegible para tratamiento de vasculitis asociada con ANCA, que está experimentando o ha experimentado uno o más signos, síntomas, u otros indicadores de vasculitis asociada con ANCA, que ha sido diagnosticado con vasculitis asociada con ANCA, sin importar, por ejemplo, si ha sido diagnosticado recientemente o previamente y experimenta ahora una recurrencia o recaída, o está en riesgo de desarrollar vasculitis asociada con ANCA. El sujeto puede haber sido tratado previamente con anticuerpo CD20 o no haber sido tratado en esta forma. Un sujeto elegible para tratamiento de vasculitis asociada con ANCA puede identificarse de manera opcional como uno que ha sido cribado, como en la sangre, con respecto a concentraciones elevadas de células CD20 infiltradoras o se criba usando un análisis para detectar auto-anticuerpos, en donde la producción de auto-anticuerpos se evalúa cualitativamente, y preferiblemente, cuantitativamente.

Un "paciente" en esta solicitud es un sujeto humano elegible para tratamiento de vasculitis asociada con ANCA, que está experimentando o ha experimentado uno o más signos, síntomas, u otros indicadores de vasculitis asociada con ANCA, sin importar, por ejemplo, si diagnosticado recientemente o diagnosticado previamente y está experimentando ahora una recurrencia o recaída. El paciente puede haber sido tratado previamente

con anticuerpo CD20 o no haber sido tratado en esa forma. Un paciente elegible para tratamiento de vasculitis asociada con ANCA puede identificarse opcionalmente, como uno que ha sido sometido cribado usando un análisis para detectar auto-anticuerpos, tales como los anotados anteriormente, en donde la producción de auto-anticuerpos se evalúa cualitativamente y, de preferencia, cuantitativamente.

"Tratamiento" de un sujeto en esta solicitud se refiere tanto a tratamiento terapéutico como a medidas profilácticas o preventivas. Aquellas personas necesitadas de tratamiento incluyen las que ya tienen vasculitis asociada con ANCA, así como aquellas en las cuales tiene que prevenirse la vasculitis asociada con ANCA. Por consiguiente, el sujeto puede haber sido diagnosticado como teniendo la vasculitis asociada con ANCA o puede estar predispuesto o ser susceptible a la vasculitis asociada con ANCA. El tratamiento de un sujeto incluye el tratamiento de un paciente.

"Tratamiento" de un paciente se refiere en esta solicitud a tratamiento terapéutico. Aquellos pacientes necesitados de tratamiento son aquellos diagnosticados con vasculitis asociada con ANCA.

Para los efectos de esta solicitud, un paciente o sujeto está en "remisión" si él/ella no tiene síntomas de la enfermedad activa vasculitis asociada con ANCA, tales como los que se pueden detectar por los métodos divulgados en esta solicitud, y no ha tenido recurrencia de concentraciones de ANCA o concentraciones de ANCA cada vez mayores, coincidentes o luego de la reconstitución de linfocitos B, puesto que se ha descubierto que concentraciones sostenidas o recurrentes de ANCA son predictivas de recaídas en pacientes en remisiones clínicas de la granulomatosis de Wegener. Boomsma *et al.*, *Arthritis Rheum.*, 43 2025-2033 (2000). Aquellos que no están en remisión incluyen, por ejemplo, aquellos que experimentan una exacerbación repentina de la enfermedad luego de la reconstitución de linfocitos B, aquellos que sufren de lesiones de órganos tales como lesión renal, o aquellos que son asintomáticos pero han tenido una recurrencia de ANCA o un aumento en la concentración de ANCA

coincidente con, o luego de, la reconstitución de linfocitos B. Tales sujetos y pacientes que experimentan un retorno de síntomas, incluidos enfermedad activa y/o lesión a órganos, o que exhiben concentraciones de ANCA recurrentes o crecientes, son aquellos que han "recaído" o tuvieron una "recurrencia".

Un "síntoma" de vasculitis asociada con ANCA es cualquier fenómeno morboso o desviación de lo normal en estructura, función, o sensación, experimentado por un sujeto o paciente e indicativo de enfermedad, tal como las anotadas anteriormente.

La expresión "cantidad efectiva" se refiere a una cantidad del anticuerpo o antagonista que es efectiva para tratar vasculitis asociada con ANCA.

"Exposición a anticuerpo" se refiere a contacto con o exposición al anticuerpo, indicado en esta solicitud, en una o más dosis administradas durante un período de tiempo de alrededor de 1 día a alrededor de 5 semanas. Las dosis pueden administrarse de una sola vez o en intervalos fijos o irregulares de tiempo durante este período de exposición, tal como, por ejemplo, una dosis a la semana durante cuatro semanas o dos dosis separadas por un intervalo de tiempo de alrededor de 13-17 días. Exposiciones iniciales y posteriores se separan cronológicamente unas de otras, como se describe en detalle en esta solicitud.

Una exposición que no se administra o provee hasta un cierto tiempo "a partir de la exposición inicial" o a partir de cualquier exposición anterior significa que el tiempo para la segunda o posterior exposición se mide a partir del momento en que se administraron cualesquiera de las dosis de la exposición anterior, si se administraron más de una dosis en esa exposición. Por ejemplo, cuando se administraron dos dosis en una exposición inicial, la segunda exposición no se da hasta por lo menos alrededor de 16-54 semanas, contadas a partir del momento en que se administró la primera o segunda dosis dentro de esta exposición anterior. Análogamente, cuando se administran tres dosis, la segunda exposición puede medirse a partir del momento de la primera, segunda, o tercera dosis dentro de la exposición

previa. Preferiblemente, "a partir de la exposición inicial" o a partir de cualquier exposición previa se mide a partir del momento de la primera dosis.

El término "agente inmunodepresor", tal como se emplea aquí para terapia complementaria, se refiere a sustancias que actúan para suprimir o enmascarar el sistema inmunitario del mamífero que está siendo tratado aquí. Esto incluiría sustancias que suprimen la producción de citocinas, regulan por disminución o suprimen la expresión de auto-antígeno, o enmascaran los antígenos MHC. Ejemplos de tales agentes incluyen pirimidinas 2-amino-6-aril-5-sustituídas (véase Patente de los Estados Unidos No. 4,665,077); medicamentos anti-inflamatorios no esteroideos (AINE); ganciclovir, tacrolimus, glucocorticoides tales como cortisol o aldosterona, agentes anti-inflamatorios tales como inhibidor de ciclooxigenasa, un inhibidor de 5-lipooxigenasa, o un antagonista de receptor de leucotrieno, antagonistas de purina tales como azatioprina o micofenolato mofetil (MMF); agentes alquilantes tales como ciclofosfamida; bromocriptina; danazol; dapsona; glutaraldehído (que enmascara los antígenos MHC, como se describe en la Patente de los Estados Unidos No. 4,120,649); anticuerpos anti-idiotípicos para antígenos MHC y fragmentos MHC; ciclosporina A; esteroides tales como glucocorticoesteroides o análogos de glucocorticoide, *por ejemplo*, prednisona, metilprednisolona, incluido SOLUMEDROL® succinato sódico de metilprednisolona, y dexametasona; inhibidores de dihidrofolato reductasa tales como metotrexato (oral o subcutáneo); agentes anti-malaria tales como cloroquina e hidroxiclороquina; sulfasalazina; leflunomida; anticuerpos de citocina o receptor de citocina incluidos anti-interferón- γ , anticuerpos α o β , o, anticuerpos de factor de necrosis anti-tumoral (TNF)- α (infiximab REMICADE® o adalimumab), inmunoadhesina de anti-TNF α (etanercept), anticuerpos de anti-TNF- β , anticuerpos de anti-interleucina-2 (IL-2) y anticuerpos de receptor de anti-IL-2; y anticuerpos y antagonistas de receptor de interleucina-6 (IL-6); anticuerpos de anti-LFA-1, incluidos anticuerpos de anti-CD11a y anti-CD18; anticuerpos de anti-L3T4;

globulina anti-linfocito heterólogo; anticuerpos pan-T, preferiblemente anticuerpos anti-CD3 o anti-CD4/CD4a; péptido soluble que contiene un dominio de unión de LFA-3 (WO 90/08187 publicada el 7/26/90); estreptocinasa; factor de crecimiento transformante- β (TGF- β); estreptodornasa; ARN o ADN del hospedero; FK506; RS-61443; clorambucil; deoxiespergualina; rapamicina; receptor de linfocito T (Cohen *et al.*, Patente de los Estados Unidos 5,114,721); fragmentos de receptor de linfocitos T (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 90/11294; Ianeway, *Nature*, 341: 482 (1989); y WO 91/01133); antagonistas BAFF tales como anticuerpos BAFF y anticuerpos BR3 y antagonistas zTNF4 (para una reseña, véase Mackay and Mackay, *Trends Immunol.*, 23:113-5 (2002) y véase también la definición más adelante), agentes biológicos que interfieren las señales de linfocitos T cooperadores, tales como receptor anti-CD40 o ligando anti-CD40 (CD154), incluidos anticuerpos bloqueantes a ligando CD40-CD40 (*verbigracia*, Durie *et al.*, *Science*, 261:1328-30 (1993), Mohan *et al.*, *J. Immunol.*, 154:1470-80 (1995)) y CTLA4-Ig (Finck *et al.*, *Science*, 265:1225-7 (1994)), y anticuerpos de receptor de linfocitos T (EP 340,109) tales como T10B9. Algunos agentes inmunosupresores preferidos en esta solicitud incluyen ciclofosfamida, clorambucil, azatioprina, leflunomida, MMF, o metotrexato.

El término "agente citotóxico", tal como se usa en esta solicitud, se refiere a una sustancia que inhibe o previene la función de las células y/o causa destrucción de las células. El término tiene como finalidad incluir isótopos radioactivos (*por ejemplo*, At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² e isótopos radioactivos de Lu), agentes quimioterapéuticos, y toxinas tales como toxinas de molécula pequeña o toxinas enzimáticamente activas de origen bacteriano, fúngico, vegetal o animal, o sus fragmentos.

Un "agente quimioterapéutico" es un compuesto químico útil en el tratamiento del cáncer. Ejemplos de agentes quimioterapéuticos incluyen agentes alquilantes tales como tiotepa y ciclofosfamida CYTOXAN®; alquilosulfonatos tales como busulfán, improsulfán y piposulfán; aziridinas tales como benzodopa, carbocadona, meturedopa y uredopa; etileniminas y

metilamelaminas incluidas altretamina, trietilenmelamina, trietilenfosforamida, trietilentiofosforamida y trimetilolomelamina; acetogeninas (especialmente bulatacina y bulatacinona); delta-9-tetrahidrocanabinol (dronabinol, MARINOL®); beta-lapacona, lapacol, colquicinas, ácido betulínico, una camptotecina (incluido el análogo sintético topotecán (HYCAMTIN®), CPT-11 (irinotecán, CAMPTOSAR®), acetilcamptotecina, escoplectina, y 9-aminocamptotecina), briostatina; calistatina; CC-1065 (incluidos sus análogos sintéticos adozelesina, carzelesina y bizelesina); podofilotoxina; ácido podofílico; tenipósido; riptoficinas (particularmente criptoficina 1 y criptoficina 8); dolastatina; duocarmicina (incluidos los análogos sintéticos, KW-2189 y CB1-TM1); eleuterobina; pancratistatina; una sarcodictina; espongistatina; mostazas nitrogenadas tales como clorambucil, clornafazina, colofosfamida, estramustina, ifosfamida, mecloretamina, clorhidrato de óxido de mecloretamina, melfalán, novembichin, phenesterine, prednimustina, trofosfamida, mostaza de uracilo; nitrosureas tales como carmustina, clorozotocina, fotemustina, lomustina, nimustina, y ranimustina; antibióticos tales como los antibióticos de enediíno (*por ejemplo*, caliqueamicina, especialmente caliqueamicina gammall y caliqueamicina omegall (véase, *por ejemplo*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); dinemicina, incluida dinemicina A; una esperamicina; así como cromóforo de neocarzinostatina y cromóforos antibióticos relacionados de cromoproteína enediíno), aclacinomisinas, actinomicina, autramicina, azaserina, bleomicinas, cactinomicina, carabicina, carminomicina, carzinofilina, cromomicinas, dactinomicina, daunorubicina, detorubicina, 6-diazo-5-oxo-L-norleucina, doxorubicina (incluida ADRIAMYCIN®), morfolino-doxorubicina, cianomorfolino-doxorubicina, 2-pirrolino-doxorubicina, inyección de liposoma de clorhidrato de doxorubicina (DOXIL®), y deoxidoxorubicina), epirubicina, esorubicina, idarubicina, marcelomicina, mitomicinas tales como mitomicina C, ácido micofenólico, nogalamicina, olivomicinas, peplomicina, potfiromicina, puromicina, quelamicina, rodorubicina, estreptonigrina, estreptozocina,

tubercidina, ubenimex, zinostatina, zorubicina; anti-metabolitos tales como metotrexato, gemcitabina (GEMZAR®), tegadur (UFTORAL®), capecitabina (XELODA®), una epotilona, y 5-fluorouracilo (5-FU); análogos de ácido fólico tales como denopterina, metotrexato, pteropterina, trimetrexato; análogos de purina tales como fludarabina, 6-mercaptopurina, tiamiprina, tioguanina; análogos de pirimidina tales como ancitabina, azacitidina, 6-azauridina, carmofur, citarabina, dideoxiuridina, doxifluridina, enocitabina, floxuridina; anti-adrenales tales como aminoglutetimida, mitotano, trilostano; reabastecedor de ácido fólico tal como ácido frolínico; aceglatona; glucósido de aldofosfamida; ácido aminolevulínico; eniluracil; amsacrina; bestrabucil; bisantreno; edatraxato; defofaminae; demecolcine; diaziquone; elfornithine; acetato de eliptinio; una epotilona; etoglucid; nitrato de galio; hidroxurea; lentinan; lonidamine; maitansinoides tales como maitansina y ansamitocinas; mitoguazona; mitoxantrona; mopidamol; nitraerine; pentostatina; phenamet; pirarubicina; losoxantrona, 2-etilhidrazida; procarbazona; complejo polisacárido PSK® (JHS Natural Products, Eugene, OR); razoxano; rizoxina; sizofirano; espirogermanio; ácido tenuazónico; triazicuona; 2,2',2"- triclortrietilamina; tricotecenos (especialmente T-2 toxina, verracurin A, roridina A y anguidina); uretano; vindesina (ELDISINE®), FILDESIN®); dacarbazina; mannomustina; mitobronitol; mitolactol; pipobroman; gacitosina; arabinósido ("Ara-C"); tiotepa; taxoides, *por ejemplo*, paclitaxel (TAXOL®), formulación de nanopartículas manipuladas con albúmina de paclitaxel (ABRAXANE™), y doxetaxel (TAXOTERE®); clorambucil; 6-tioguanina; mercaptopurina; metotrexato; análogos de platino tales como cisplatino y carboplatino; vinblastina (VELBAN®); platino; etopósido (VP-16); ifosfamida; mitoxantrona; vincristina (ONCOVIN®); oxaliplatino; leucovovin; vinorelbina (NAVELBINE®); novantrona; edatrexato; daunomicina; aminopterina; xeloda; ibandronato; inhibidor de topoisomerasa RFS 2000; difluorometilornitina (DMFO); retinoides tales como ácido retinoico; sales, ácidos o derivados farmacéuticamente aceptables de cualquiera de los anteriores, así como combinaciones de dos o

más de los anteriores tales como CHOP, una abreviatura de una terapia combinada de ciclofosfamida, doxorubicina, vincristina y prednisolona, y FOLFOX, una abreviatura de un régimen de tratamiento con oxaliplatino (ELOXATIN™) combinado con 5-FU y leucovorina.

También incluidos en esta definición se encuentran agentes anti-hormonales que actúan para regular, reducir, bloquear o inhibir la acción hormonal en tumores, tales como anti-estrógenos y moduladores selectivos de receptor de estrógeno (SERMs), incluidos por ejemplo tamoxifeno (incluido NOLVADEX® tamoxifeno), raloxifeno (EVISTA®), droloxifeno, 4-hidroxitamoxifeno, trioxifeno, keoxifeno, LY117018, onapristona, y toremifeno (FARESTON®); anti-progesteronas, reguladores por disminución de receptor de estrógeno (ERDs), antagonistas de receptor de estrógeno tales como (FASLODEX®), agentes que funcionan para suprimir o suspender lo ovarios, por ejemplo, agonistas de luliberina (LHRH) tales como acetato de leuprolida (LUPRON® y ELIGARD®), acetato de goserelina, acetato de buserelina y triptorelina, anti-andrógenos tales como flutamida, nilutamida y bicalutamida; inhibidores de aromatasa que inhiben la enzima aromatasa, la cual regula la producción de estrógenos en las glándulas suprarrenales, tales como, por ejemplo, 4(5)-imidazoles, aminoglutetimida, acetato de megestrol MEGASE®, exemestano AROMASIN®, formestanie, fadrozol, vorozol RIVISOR®, letrozol FEMARA®, y anastrozol ARIMIDEX®. Además, tal definición de agentes quimioterapéuticos incluye bisfonatos tales como clodronato (por ejemplo, BONEFOS® u OSTAC®), etidronato (DIDROCAL®), NE-58095, ácido zoledrónico/zoledronato (ZOMETA®), alendronato (FOSAMAX®), pamidronato (AREDIA®), tiludronato (SKELID®), o risedronato (ACTONEL®), así como troxacitabina (un análogo de citosina 1,3-dioxolano nucleósido); oligonucleótidos complementarios, particularmente aquellos que inhiben la expresión de genes en vías de señalización implicadas en la proliferación celular aberrante, tal como, por ejemplo, PKC-alfa, Ralf y H-Ras; y receptor del factor de crecimiento epidérmico (EGF-R); vacunas tales como LA VACUNA THERATOPE® Y

vacunas de terapia génica, por ejemplo, la vacuna ALLOVECTIN®, la vacuna LEUVECTIN®, y la vacuna VAXID®; inhibidor de topoisomerasa 1 (*verbigracia*, LURTOTECAN®); mRH (*verbigracia*, ABARELIX®; lapatinib ditosilato (un inhibidor de molécula pequeña de tirosina cinasa dual ErbB-2 y EGFR también conocido como GW572016), y sus sales farmacéuticamente aceptables, ácidos o derivados de cualquiera de los anteriores.

El término "citocina" es un término genérico para proteínas liberadas por una población celular, que actúan sobre otras células como mediadores intercelulares. Ejemplos de tales citocinas son linfocinas, monocinas; interleucinas (ILs) tales como IL-1, IL-1 α , 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 polipeptídicos incluidos LIF y ligando kit (KL). Tal como se emplea aquí, el término citocina incluye proteínas de fuentes naturales o de cultivo de células recombinantes y equivalentes biológicamente activos de las citocinas de secuencia nativa, incluidas entidades de pequeña molécula producidas sintéticamente y sus derivados y sales farmacéuticamente aceptables.

El término "hormona" se refiere a hormonas polipeptídicas, que generalmente se secretan por órganos glandulares con ductos. Incluidas entre las hormonas se encuentran, por ejemplo, somatotropina tal como somatotropina humana, somatotropina humana N-metionilo, y somatotropina bovina; hormona paratiroide; tiroxina; insulina; proinsulina; relaxina; estradiol, terapia de sustitución de hormonas, andrógenos tales como calusterona, dromostanolona propionato, epitioestanol, mepitioestano, o testolactona prorelaxina; hormonas de glucoproteína tales como folitropina (FSH), tirotropina (TSH), y lutropina (LH); prolactina; lactógeno placentario; péptido asociado con gonadotropina de ratón; inhibina; activina; sustancia inhibidora de Müller; y trombopoietina. Tal como se emplea en esta solicitud, el término hormona incluye proteínas de fuentes naturales o de cultivo de células recombinantes y equivalentes biológicamente activos de la hormona de secuencia nativa, incluidas

entidades de pequeña molécula producidas sintéticamente y sus derivados y sales farmacéuticamente aceptables.

El término "factor de crecimiento" se refiere a proteínas que promueven el crecimiento, e incluye, por ejemplo, el factor de crecimiento hepático; el factor de crecimiento de fibroblastos; el factor de crecimiento endotelial vascular; factores de crecimiento neural tales como NGF- β ; el factor de crecimiento derivado de plaquetas; los factores de crecimiento transformantes (TGFs) tales como TGF- α y TGF- β ; factor de crecimiento insulinoide tipo I y II; eritropoietina (EPO); factores osteoinductores; interferones tales como interferón - α , - β , y - γ ; y factores estimulantes de colonias (CSFs) tales como CSF de macrófagos (M-CSF), CSF de granulocitos-macrófagos (GM-CSF), y CSF de granulocitos (G-CSF). Tal como se emplea en esta solicitud, el término somatotropina incluye proteínas de fuentes naturales o de cultivo de células recombinantes y equivalentes biológicamente activos de la somatotropina de secuencia nativa, incluidas entidades de pequeña molécula producidas sintéticamente y sus derivados y sales farmacéuticamente aceptables.

El término "integrina" se refiere a una proteína de receptor que permite a las células unirse y responder a la matriz extracelular y se encuentra involucrada en una variedad de funciones celulares tales como curación de heridas, diferenciación celular, migración de células tumorales y apoptosis. Son parte de una gran familia de receptores de adhesión celular que se hallan involucrados en la matriz celular-extracelular e interacciones célula-célula. Las integrinas funcionales constan de dos subunidades de glucoproteína transmembrana, llamadas α y β , que no se enlazan covalentemente. Todas las subunidades α comparten cierta homología entre sí, como las subunidades β . Los receptores contienen siempre una cadena α y una cadena β . Los ejemplos incluyen Alfa6beta1, Alfa3beta1 y Alfa7beta1, *etc.* Tal como se emplea en esta solicitud, el término "integrina" incluye proteínas de fuentes naturales o de cultivo de células recombinantes y equivalentes biológicamente activos de la integrina

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de secuencia nativa, incluidas entidades de pequeña molécula producidas sintéticamente y sus derivados y sales farmacéuticamente aceptables.

Para los propósitos contemplados aquí, "factor alfa de necrosis tumoral (TNF α)" se refiere a una molécula TNF α humana que comprende la secuencia de aminoácidos como se describe en Pennica *et al.*, *Nature*, 312:721 (1984) o Aggarwal *et al.*, *JBC*, 260:2345 (1985). Un "inhibidor de TNF α " contemplado aquí es un agente que inhibe, hasta cierto punto, una función biológica de TNF α , generalmente mediante la unión a TNF α y neutralizando su actividad. Ejemplos de inhibidores de TNF contemplados específicamente aquí son etanercept (ENBREL®), Infliximab (REMICADE®) y Adalimumab (HUMIRA™).

Ejemplos de "fármacos anti-reumáticos modificadores de la enfermedad" o "DMARD" incluyen hidroxicloroquina, sulfasalazina, metotrexato, leflunomida, etanercept, infliximab (más metotrexato oral y subcutáneo), azatioprina, D-penicilamina, sales de oro (oral), sales de oro (intramuscular), minociclina, ciclosporina incluida ciclosporina y ciclosporina tópica, proteína A estafilocócica (Goodyear and Silverman, *J. Exp. Med.*, 197, (9), p 1125-39 (2003)), incluidas sus sales y derivados, etc.

Ejemplos de "fármacos anti-inflamatorios no esteroides" o "NSAIDs" incluyen aspirina, ácido acetilsalicílico, ibuprofeno, naproxeno, indometacina, sulindac, tolmetina, inhibidores de COX-2 tales como celecoxib (CELEBREX®, 4-(5-(4-metilfenil)-3-(trifluorometil)-1H-pirazol-1-il)bencenosulfonamida y valdecoxib (BEXTRA®), y meloxicam (MOBIC®), incluidas sus sales y derivados, etc. Preferiblemente, éstos son aspirina, naproxeno, ibuprofeno, indometacina, o tolmetina.

Ejemplos de "antagonistas o anticuerpos de integrina" en esta solicitud incluyen un anticuerpo LFA-1, tal como efalizumab (RAPTIVA®), disponible comercialmente de Genentech, o un anticuerpo de integrina $\alpha 4$ tal como natalizumab (ANTEGREN®), obtenible de Biogen, o derivados de fenilalanina diazacíclica (WO 2003/89410), derivados de fenilalanina (WO 2003/70709, WO 2002/28830, WO

2002/16329 y WO 2003/53926), derivados de ácido fenilpropiónico (WO 2003/10135), derivados de enamina (WO 2001/79173), derivados de de ácido propanoico (WO 2000/37444), derivados de de ácido alcanoico (WO 2000/32575), derivados de fenilo sustituidos (Patentes de los Estados Unidos Nos. 6,677,339 y 6,348,463), derivados de amina aromática (Patente de los Estados Unidos No. 6,369,229), polipéptidos del dominio desintegrina ADAM (US2002/0042368), anticuerpos contra integrina alphavbeta3 (EP 633945), derivados de aminoácidos bicíclicos con puente aza (WO 2002/02556), *etc.*

"Corticosteroide" se refiere a una cualquiera de varias sustancias sintéticas o de origen natural con la estructura química general de esteroides, que imitan o aumentan los efectos de corticoesteroides de origen natural. Ejemplos de corticoesteroides sintéticos incluyen prednisona, prednisolona (incluida metilprednisolona, tal como SOLU-MEDROL® succinato sódico de metilprednisolona), dexametasona o dexametasona triamcinolona, hidrocortisona, y betametasona. Los corticoesteroides preferidos en esta solicitud son prednisona, metilprednisolona, hidrocortisona, o dexametasona.

Los términos "BAFF" "polipéptido BAFF," "TALL-1" o "polipéptido TALL-1" y "BLyS", cuando se usan en esta solicitud, comprenden "polipéptidos BAFF de secuencia nativa" y "variantes BAFF". "BAFF" es una designación dada a aquellos polipéptidos que tienen una cualquiera de las secuencias de aminoácidos mostradas a continuación.

Secuencia BAFF humana (SEQ ID NO:16)

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1 DDSTEREQSRLTSCLKKREEMKLKECVSILPRKESPSVRSSKDGLLAATLLLALLSCC
61 LTVVSFYQVAALQGD LASRAELQGHHA EKLPAGAGAPKAGLEEAPAVTAGLKIFEPPAP
121 GEGNSSQNSRNKRAVQGPEETVTQDCLQLIADSETPTIQKGSYTFVPWLLSFKRGSAL EE
181 KENKILVKETGYFFIYGQVLYTDKTYAMGH LIQRKKVHVFGDELSLVTLFRCIQNMPETL
241 PNNSCYSAGIAKLEEGDELQLAIPRENAQISLDG DVTFFGALKLL

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Secuencia BAFF de ratón (SEQ ID NO:17)

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1 MDESAKTLPPPCLCFCSEKGEDMKVGYDPITPQKEEGAWFGICRDGRLLAATLLLALLSS

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61 SFTAMSLYQLAALQADLMNLRMELQSYRGSATPAAAGAPELTAGVKLLTPAAPRPHNSSR
 121 GHRNRRAFQGPEETEQDVDLS APPAPCLPGCRHSQHDDNGMNLRNIIQDCLQLIADSDTP
 181 TIRKGTYTTFVPWLLSFKRGNALEEKENKIVVRQTGYFFIYSQVLYTDPFAMGHVIQRKK
 241 VHVFGDELSLVTLFRCIQNMPKTLPNNSCYSAGIARLEEGDEIQLAIPRENAQISRNGDD
 301 TFFGALKLL

y sus homólogos y fragmentos y variantes, que poseen la actividad biológica del BAFF nativo. Una actividad biológica de BAFF puede seleccionarse del grupo conformado por promover la supervivencia de linfocitos B, promover la maduración de linfocitos B y unirse a BR3. Las variantes de BAFF tendrán preferiblemente por lo menos 80% o cualquier entero sucesivo hasta 100% inclusive, más preferiblemente, por lo menos 90% y, aún más preferiblemente, por lo menos 95% de identidad de secuencia de aminoácidos con una secuencia nativa de un polipéptido BAFF.

Un polipéptido BAFF de "secuencia nativa" comprende un polipéptido que tiene la misma secuencia de aminoácidos que el polipéptido correspondiente BAFF derivado de la naturaleza. Por ejemplo, BAFF existe en una forma soluble luego de la segmentación de la superficie celular por proteasas tipo furina. Tales polipéptidos BAFF de secuencia nativa pueden aislarse de la naturaleza o pueden producirse por medios recombinantes y/o sintéticos.

El término "polipéptido BAFF de secuencia nativa" o "BAFF nativo" comprende específicamente formas segregadas o truncadas de origen natural (por ejemplo, una secuencia de dominio extracelular), formas variantes de origen natural (por ejemplo, formas cortadas y empalmadas alternativamente), y variantes alélicas de origen natural del polipéptido. El término "BAFF" incluye aquellos polipéptidos descritos en Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); GenBank Accession No. AF136293; WO 1998/18921 publicada el 7 de mayo de 1998; EP 869,180 publicada el 7 de octubre de 1998; WO 1998/27114 publicada el 25 de junio de 25, 1998; WO 1999/12964 publicada el 18 de marzo de 1999; WO 1999/33980 publicada el 8 de julio de 1999; Moore *et al.*, *Science*, 285:260-263 (1999);

Schneider *et al.*, *J. Exp. Med.*, 189: 1747- 1756 (1999) y Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274: 15978-15981 (1999).

Los términos "antagonista BAFF", tal como se emplea en esta solicitud, se emplea en el sentido más amplio, e incluye cualquier molécula que (1) une un polipéptido BAFF de secuencia nativa o una secuencia nativa de BR3 para bloquear parcial o completamente la interacción de BR3 con el polipéptido BAFF, y que (2) bloquea parcial o completamente, inhibe, o neutraliza la actividad BAFF de secuencia nativa. En una incorporación preferida el receptor BAFF a bloquearse es el receptor BR3. La actividad BAFF nativa promueve, entre otras cosas, la supervivencia de los linfocitos B y/o la maduración de los linfocitos B. En una incorporación, la inhibición, bloqueo o neutralización de la actividad BAFF resulta en una reducción en el número de linfocitos B. Un antagonista BAFF de acuerdo con esta invención bloqueará parcial o completamente, inhibirá, o neutralizará una o más actividades biológicas de un polipéptido BAFF, *in vitro* y/o *in vivo*. En una incorporación, un BAFF biológicamente activo potencia una cualquiera o una combinación de los siguientes episodios *in vitro* y/o *in vivo*: una supervivencia incrementada de linfocitos B, un mayor nivel de IgG y/o IgM, un número cada vez mayor de plasmocitos, y procesamiento de NF- κ B2/100 en p52 NF- κ B en linfocitos B esplénicos (por ejemplo, Batten *et al.*, *J. Exp. Med.* 192: 1453-1465 (2000); Moore *et al.*, *Science* 285:260-263 (1999); Kayagaki *et al.*, *Immunity* 17:515-524 (2002)).

Como ya se mencionó, un antagonista BAFF puede funcionar en una manera directa o indirecta para bloquear parcial o completamente, inhibir o neutralizar la señalización BAFF, *in vitro* o *in vivo*. Por ejemplo, el antagonista BAFF puede unir a BAFF directamente. Por ejemplo, se contemplan anticuerpos BAFF que se unen en una región de un BAFF humano, que comprende los residuos 162-275 y/o un residuo contiguo de un residuo seleccionado del grupo conformado por 162, 163, 206, 211, 231, 233, 264 y 265 de BAFF humano, de modo que el anticuerpo impide estéricamente la unión de BAFF a BR3, donde tales números de residuo se

13.0
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refieren a SEQ ID NO: 16. En otro ejemplo, un ligador directo es un polipéptido que comprende cualquier porción de un receptor BAFF que se une a BAFF tal como un dominio extracelular de un receptor BAFF, o sus fragmentos y variantes que se unen a BAFF nativo. En otro ejemplo, los antagonistas BAFF incluyen los polipéptidos que tienen una secuencia de un polipéptido, que comprende la secuencia de Fórmula I:

X_1 -C- X_3 -D- X_5 -L- X_7 - X_8 - X_9 - X_{10} - X_{11} - X_{12} -C- X_{14} - X_{15} - X_{16} - X_{17} (Fórmula I)
(SEQ ID NO: 18)

en donde X_1 , X_3 , X_5 , X_7 , X_8 , X_9 , X_{10} , X_{11} , X_{12} , X_{14} , X_{15} y X_{17} son cualquier aminoácido excepto cisteína; y en donde X_{16} es un aminoácido seleccionado del grupo conformado por L, F, I y V; y

en donde el polipéptido no comprende una cisteína en siete residuos de aminoácido N-terminales a la cisteína más N-terminal C y C-terminal a la cisteína más C-terminal de Fórmula I.

En una incorporación, un polipéptido que comprende la secuencia de Fórmula I tiene las dos Cs unidas por enlace disulfuro; X_5 L X_7 X_8 que forma la conformación de una estructura de giro tipo I β con el centro del giro entre L y X_7 , y tiene un valor positivo para el ángulo diédrico ϕ de X_8 . En una incorporación, X_{10} se selecciona del grupo conformado por W, F, V, L, I, Y, M y un aminoácido no polar. En otra incorporación, X_{10} es W. En otra incorporación, X_3 es un aminoácido seleccionado del grupo conformado por M, V, L, I, Y, F, W y un aminoácido no polar. En otra incorporación, X_5 se selecciona del grupo conformado por V, L, P, S, I, A y R. En otra incorporación, X_7 se selecciona del grupo conformado por V, T, I y L. En otra incorporación, X_8 selecciona del grupo conformado por R, K, G, N, H y un D-aminoácido. En otra incorporación, X_9 se selecciona del grupo conformado por H, K, A, R y Q. En otra incorporación, X_{11} es I o V. En otra incorporación, X_{12} se selecciona del grupo conformado por P, A, D, E y S. En otra incorporación, X_{16} es L. En una incorporación específica, la secuencia de Fórmula I es una secuencia seleccionada del grupo conformado por ECFDLLVRAWVPCSVLK (SEQ ID NO:19), ECFDLLVRHWVPCGLLR (SEQ ID NO:20), ECFDLLVRRWVPCSEMLG

(SEQ ID NO:21), ECFDLLVRSWVPCHMLR (SEQ ID NO 22), ECFDLLVRHWVACGLLR (SEQ ID NO:23), y QCFDRLNAWVPCSVLK (SEQ ID NO:24). En una incorporación preferida, el antagonista BAFF comprende una cualquiera de las secuencias de aminoácidos seleccionadas del grupo conformado por SEQ ID NO:19, 20, 21, 22, y 23.

En todavía otro ejemplo, los antagonistas BAFF incluyen los polipéptidos con una secuencia de un polipéptido que comprende la secuencia de Fórmula II:

X_1 -C- X_3 -D- X_5 -L-V- X_8 - X_9 -W-V-P-C- X_{14} - X_{15} -L- X_{17} (Formula II) (SEQ ID NO:25)

en donde X_1 , X_3 , X_5 , X_8 , X_9 , X_{14} , X_{15} y X_{17} son cualquier aminoácido, excepto cisteína, y

en donde el polipéptido no comprende una cisteína en siete residuos de aminoácido N-terminales a la cisteína más N-terminal C y C-terminal a la cisteína más C-terminal de Fórmula II.

En una incorporación, un polipéptido que comprende la secuencia de Fórmula II tiene un enlace disulfuro entre las dos Cs y tiene la conformación de X_5 L X_7 X_8 que forma una estructura de giro tipo I β con el centro del giro entre L y X_7 , y tiene un valor positivo para el ángulo dihédrico ϕ de X_8 . En otra incorporación de Fórmula II, X_3 es un aminoácido seleccionado del grupo conformado por M, A, V, L, I, Y, F, W y un aminoácido no polar. En otra incorporación de Fórmula II, X_5 se selecciona del grupo conformado por V, L, P, S, I, A y R. En otra incorporación de Fórmula II, X_8 se selecciona del grupo conformado por R, K, G, N, H y un D-aminoácido. En otra incorporación de Fórmula II, X_9 se selecciona del grupo conformado por H, K, A, R y Q.

En una incorporación adicional, el receptor BAFF del cual se deriva el dominio extracelular o fragmento de unión BAFF o su variante de unión BAFF es TACI, BR3 o BCMA. Alternativamente, el antagonista BAFF puede unir un dominio extracelular de una secuencia nativa BR3 en su región de unión BAFF para bloquear parcial o completamente, inhibir o

C2
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neutralizar la unión de BAFF a BR3 *in vitro*, *in situ*, o *in vivo*. Por ejemplo, tal antagonista indirecto es un anticuerpo anti-BR3 que se une en una región de BR3, que comprende los residuos 23-38 de BR3 humano como se define más adelante (SEQ ID NO:26) o una región contigua de esos residuos de modo que se impida la unión de BR3 humano a BAFF.

En algunas incorporaciones, un antagonista BAFF de acuerdo con esta invención incluye anticuerpos BAFF e inmunoadhesinas que comprenden un dominio extracelular de un receptor BAFF, o sus fragmentos y variantes que unen BAFF nativo. En una incorporación adicional, el receptor BAFF del cual se deriva el dominio extracelular o fragmento de unión BAFF o su variante de unión BAFF es TACI, BR3 o BCMA. En todavía otra incorporación, la inmunoadhesina comprende una secuencia de aminoácidos de la de Fórmula I o Fórmula II, como se expone más arriba, incluida una secuencia de aminoácidos seleccionada de una cualquiera del grupo conformado por SEQ ID NOS: 19, 20, 21, 22, 23, y 24.

De acuerdo con una incorporación, el antagonista BAFF se une a un polipéptido BAFF o un polipéptido BR3 con una finidad de unión de 100nM o menos. De acuerdo con otra incorporación, el antagonista BAFF se une a un polipéptido BAFF o un polipéptido BR3 con una afinidad de unión de 10nM o menos. De acuerdo con todavía otra incorporación, el antagonista BAFF se une a un polipéptido BAFF o un polipéptido BR3 con una afinidad de 1 nM o menos.

Los términos "BR3", "polipéptido BR3" o "receptor BR3", cuando se utilizan en esta solicitud, comprenden "polipéptidos BR3 de secuencia nativa" y "variantes BR3" (que se definen posteriormente en esta solicitud). "BR3" es una designación dada a aquellos polipéptidos que comprenden la siguiente secuencia de aminoácidos y sus homólogos, y sus variantes o fragmentos que unen BAFF nativo:

Secuencia BR3 humana (SEQ ID NO:26)

I MRRGPRSLRGRDAPAPTPCVPAECFDLLVRHCVACGLLRTPRPKPAGASSPAPRTALQPQ

61 ESVGAGAGEAALPLPGLLFGAPALLGLALVLALVLVGLVSWRRRQRRLRGASSAEAPDGD
121 KDAPEPLDKVIILSPGISDATAPAWPPPGEDPGTTPPGHSPVPATELGSTELVTTKTAG
181 PEQQ

Los polipéptidos BR3 de la invención pueden aislarse de una variedad de fuentes, tales como de tipos de tejido humano o de otra fuente, o se preparan por métodos recombinantes y/o sintéticos. El término BR3 incluye los polipéptidos BR3 descritos en WO 2002/24909 y WO 2003/14294.

Un polipéptido BR3 de "secuencia nativa" o "BR3 nativo" comprende un polipéptido que tiene la misma secuencia de aminoácidos que el polipéptido BR3 correspondiente derivado de la naturaleza. Tales polipéptidos BR3 de secuencia nativa pueden aislarse de la naturaleza o pueden producirse por medios recombinantes y/o sintéticos. El término "polipéptido BR3 de secuencia nativa" comprende específicamente formas segregadas o truncadas de origen natural (*por ejemplo*, una secuencia de dominio extracelular), formas variantes de origen natural (*por ejemplo*, formas cortadas y empalmadas alternativamente) y variantes alélicas de origen natural del polipéptido. Los polipéptidos BR3 de la invención incluyen el polipéptido BR3, que comprende o está conformado por la secuencia contigua de residuos de aminoácido 1 a 184 de un BR3 humano (SEQ ID NO:26).

Un "dominio extracelular" de BR3 o "ECD" se refiere a una forma del polipéptido BR3 que está esencialmente libre de transmembrana y dominios citoplásmicos. Formas ECD de BR3 incluyen un polipéptido, que comprende una cualquiera de las secuencias de aminoácidos seleccionada del grupo conformado por los aminoácidos 1-77, 2-62, 2-71, 1-61, 7-71, 23-38 y 2-63 de BR3 humano. La invención contempla antagonistas BAFF que son polipéptidos que comprenden una cualquiera de las formas ECD ya mencionadas de BR3 humano y sus variantes y fragmentos que se unen a un BAFF nativo.

Mini-BR3 es una región central de 26 residuos del dominio de unión BAFF de BR3, *es decir*, la secuencia de aminoácidos TPCVPAECFD LLVRHCVACG LLRTPR (SEQ ID NO:27)

"Variante BR3" significa un polipéptido BR3 que tiene por lo menos 80% de identidad de secuencia de aminoácidos con la secuencia de aminoácidos de una secuencia nativa, BR3 de longitud total o ECD BR3 y un polipéptido BAFF de secuencia nativa. Opcionalmente, la variante BR3 incluye un dominio único rico en cisteína. Tales polipéptidos de variante BR3 incluyen, por ejemplo, polipéptidos BR3 en donde uno o más residuos de aminoácido se agregan, o eliminan, en el N- y/o C-término, así como en uno o más dominios internos, de la secuencia de aminoácidos de longitud total. También se contemplan fragmentos de la ECD BR3, que unen un polipéptido BAFF de secuencia nativa. De acuerdo con una incorporación, un polipéptido de variante BR3 tendrá por lo menos alrededor de 80% de identidad de secuencia de aminoácidos, por lo menos alrededor de 81 % de identidad de secuencia de aminoácidos, por lo menos alrededor de 82% de identidad de secuencia de aminoácidos, por lo menos alrededor de 83% de identidad de secuencia de aminoácidos, por lo menos alrededor de 84% de identidad de secuencia de aminoácidos, por lo menos alrededor de 85% de identidad de secuencia de aminoácidos, por lo menos alrededor de 86% de identidad de secuencia de aminoácidos, por lo menos alrededor de 87% de identidad de secuencia de aminoácidos, por lo menos alrededor de 88% de identidad de secuencia de aminoácidos, por lo menos alrededor de 89% de identidad de secuencia de aminoácidos, por lo menos alrededor de 90% de identidad de secuencia de aminoácidos, por lo menos alrededor de 91 % de identidad de secuencia de aminoácidos, por lo menos alrededor de 92% de identidad de secuencia de aminoácidos, por lo menos alrededor de 93% de identidad de secuencia de aminoácidos, por lo menos alrededor de 94% de identidad de secuencia de aminoácidos, por lo menos alrededor de 95% de identidad de secuencia de aminoácidos, por lo menos alrededor de 96% de identidad de secuencia de aminoácidos, por lo menos alrededor de 97% de identidad de secuencia de aminoácidos, por lo menos

alrededor de 98% de identidad de secuencia de aminoácidos o por lo menos alrededor de 99% de identidad de secuencia de aminoácidos con un polipéptido de BR3 humano o su fragmento especificado (*por ejemplo*, ECD). Polipéptidos de variante BR3 no comprenden la secuencia polipeptídica BR3 nativa. De acuerdo con otra incorporación, polipéptidos de la variante BR3 son por lo menos alrededor de 10 aminoácidos de longitud, por lo menos alrededor de 20 aminoácidos de longitud, por lo menos alrededor de 30 aminoácidos de longitud, por lo menos alrededor de 40 aminoácidos de longitud, por lo menos alrededor de 50 aminoácidos de longitud, por lo menos alrededor de 60 aminoácidos de longitud, o por lo menos alrededor de 70 aminoácidos de longitud.

En una incorporación preferida, los antagonistas BAFF en esta solicitud son inmunoadhesinas que comprenden una porción de BR3, TACI o BCMA que une BAFF, o sus variantes que unen BAFF. En otras incorporaciones, el antagonista BAFF es un anticuerpo BAFF. Un "anticuerpo BAFF" es un anticuerpo que une BAFF y, preferiblemente, une BAFF en una región de BAFF humano que comprende los residuos 162-275 de la secuencia BAFF humana divulgada en esta solicitud bajo la definición "BAFF" (SEQ ID NO:16). En otra incorporación, el antagonista BAFF es el anticuerpo BR3. Un "anticuerpo BR3" es un anticuerpo que une BR3, y es preferiblemente uno que une BR3 en una región de BR3 humano, que comprende los residuos 23-38 de la secuencia BR3 humana divulgada en esta solicitud bajo la definición "BR3" (SEQ ID NO:26). En general, las posiciones de los aminoácidos de BAFF humano y BR3 humano, mencionados en esta solicitud, están de acuerdo con la numeración de secuencias bajo BAFF humano y BR3 humano, SEQ ID NOS: 16 y 26, respectivamente, divulgadas en esta solicitud bajo las definiciones "BAFF" y "BR3".

Otros ejemplos de polipéptidos de unión a BAFF o anticuerpos BAFF pueden hallarse en, *por ejemplo*, WO 2002/092620, WO 2003/014294, Gordon *et al.*, *Biochemistry* 42(20) 5977-5983 (2003), Kelley *et al.*, *J. Biol.*

Chem., 279(16):16727-16735 (2004), WO 1998/18921, WO 2001/12812, WO 2000/68378 y WO 2000/40716.

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

Un "medicamento" es un fármaco activo para tratar la vasculitis asociada con ANCA o sus síntomas o efectos secundarios.

II. Terapia

En un aspecto, la presente invención provee un método de tratar vasculitis asociada con ANCA en un paciente, que comprende administrar un antagonista, preferiblemente un anticuerpo que se une a un marcador de superficie de linfocito B (más preferiblemente un anticuerpo CD20) al paciente en una dosis de alrededor de 400 mg a 1.3 gramos con una frecuencia de una a tres dosis en un período de alrededor de un mes.

En consecuencia, la invención contempla un método de tratar vasculitis asociada con ANCA en un paciente, que comprende administrar un anticuerpo que se une a un marcador de linfocitos B, al paciente en una dosis de alrededor de 400 mg a 1.3 gramos, con una frecuencia de una a tres dosis en un período de alrededor de un mes.

La invención contempla asimismo un método de tratar vasculitis asociada con ANCA en un paciente, que comprende un antagonista que se une a un marcador de superficie de linfocitos B, al paciente en una dosis de alrededor de 400 mg a 1.3 gramos, con una frecuencia de una a tres dosis en un período de alrededor de un mes.

La invención contempla asimismo un método de tratar vasculitis asociada con ANCA en un paciente, que comprende administrar un anticuerpo CD20 al paciente en una dosis de alrededor de 400 mg a 1.3

gramos con una frecuencia de una a tres dosis en un período de alrededor de un mes.

En una incorporación preferida de cada uno de estos aspectos, la dosis es de alrededor de 500 mg a 1.2 gramos, más preferiblemente alrededor de 750 mg a 1.1 gramos. En otra incorporación preferida, el anticuerpo se administra en dos a tres dosis, más preferiblemente en dos dosis, pero alternativamente tres dosis. En una incorporación todavía preferida, el anticuerpo se administra en un período de alrededor de 2 a 3 semanas, más preferiblemente alrededor de dos semanas, pero alternativamente tres semanas.

En otra incorporación, la presente invención provee un método de tratar vasculitis asociada con ANCA en un sujeto elegible para tratamiento, que comprende administrar una cantidad efectiva de un anticuerpo que se une a un marcador de superficie de linfocito B (preferiblemente un anticuerpo CD20) al sujeto para proporcionar una exposición inicial a anticuerpo (de preferiblemente alrededor de 0.5 a 4 gramos, más preferiblemente alrededor de 1.5 a 3.5 gramos, y aún más preferiblemente alrededor de 1.5 a 2.5 gramos), seguida por una segunda exposición a anticuerpo (de preferiblemente alrededor de 0.5 a 4 gramos, más preferiblemente alrededor de 1.5 a 3.5 gramos, todavía más preferiblemente alrededor de 1.5 a 2.5 gramos), no proporcionándose la segunda exposición hasta desde alrededor de 16 a 54 semanas (preferiblemente desde alrededor de 20 a 30 semanas, más preferiblemente desde alrededor de 46 a 54 semanas) a partir de la exposición inicial.

Para los propósitos de esta invención, la segunda exposición a anticuerpo es la próxima vez que el sujeto se trata con el anticuerpo CD20 después de la exposición inicial a anticuerpo, no habiendo tratamiento con anticuerpo CD20 interpuesto, o exposición entre las exposiciones inicial y segunda. Tal re-tratamiento puede pautarse o no pautarse, pero es preferiblemente una re-dosificación pautada, particularmente para proteger de lesión órganos tales como los riñones.

El método preferiblemente comprende administrar al sujeto una cantidad efectiva del anticuerpo CD20 para proporcionar una tercera exposición a anticuerpo (preferiblemente de alrededor de 0.5 a 4 gramos, más preferiblemente alrededor de 1.5 a 3.5 gramos, todavía más preferiblemente alrededor de 1.5 a 2.5 gramos), no proporcionándose la tercera exposición hasta desde alrededor de 46 a 60 semanas (preferiblemente alrededor de 46 a 55, más preferiblemente alrededor de 46 a 52 semanas), a partir de la exposición inicial. Preferiblemente, no se proporciona exposición adicional a anticuerpo hasta por lo menos alrededor de 70-75 semanas a partir de la exposición inicial, y todavía más preferiblemente no se proporciona exposición adicional a anticuerpo hasta por lo menos alrededor de 74 a 80 semanas a partir de la exposición inicial.

Una cualquiera o más de las exposiciones a anticuerpo de esta solicitud puede proporcionarse al sujeto como una dosis única de anticuerpo, o como dosis separadas, por ejemplo, alrededor de 1-4 dosis separadas del anticuerpo (*por ejemplo*, constituyendo una primera y segunda dosis, o una primera, segunda, y tercera dosis, o una primera, segunda, tercera, y cuarta, etc). El número particular de dosis (ya sea una, dos o tres o más) empleadas para cada exposición a anticuerpo depende, por ejemplo, del tipo de vasculitis asociada con ANCA tratada, el tipo de anticuerpo empleado, si, qué tipo, y qué cantidad o cuánto de un segundo medicamento se emplea, como se anota más adelante, y el método y frecuencia de administración. Donde se administran dosis separadas, la última dosis (por ejemplo, segunda o tercera dosis) se administra preferiblemente desde alrededor de 1 a 20 días, más preferiblemente desde alrededor de 6 a 16 días y, muy preferiblemente desde alrededor de 14 a 16 días a partir del momento en que se administró la dosis previa. Las dosis separadas se administran preferiblemente en un período total de entre alrededor de 1 día y 4 semanas, más preferiblemente entre alrededor de 1 y 20 días (*por ejemplo*, en un período de 6-18 días). En uno de tales aspectos, las dosis separadas se administran alrededor de semanalmente, administrándose la segunda dosis alrededor de una semana a partir de la

primera dosis y administrándose cualquier tercera dosis o dosis subsiguiente alrededor de una semana a partir de la segunda dosis. Cada dosis separada similar del anticuerpo es preferiblemente alrededor de 0.5 a 1.5 gramos, más preferiblemente alrededor de 0.75 a 1.3 gramos.

En una incorporación muy preferida, se provee un método de tratar vasculitis asociada con ANCA en un sujeto, que comprende administrar una cantidad efectiva de un anticuerpo que se une a un marcador de superficie de linfocito B (*por ejemplo*, un anticuerpo CD20) al sujeto para proporcionar una exposición inicial a anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se proporciona hasta desde alrededor de 16 a 54 semanas a partir de la exposición inicial, y cada una de las exposiciones a anticuerpo se proporciona al sujeto como una dosis única o como dos o tres dosis separadas de anticuerpo. Preferiblemente en tal método, las exposiciones a anticuerpo son de alrededor de 0.5 a 4 gramos cada una y, muy preferiblemente, las cantidades indicadas más arriba.

En una incorporación, se proporcionan al sujeto por lo menos alrededor de tres exposiciones del anticuerpo, por ejemplo, desde alrededor de 3 a 60 exposiciones y, más particularmente alrededor de 3 a 40 exposiciones, muy particularmente, alrededor de 3 a 20 exposiciones. Preferiblemente, tales exposiciones se administran en intervalos cada uno de 24 semanas. En una incorporación, cada exposición a anticuerpo se proporciona como una dosis única del anticuerpo. En una incorporación alternativa, cada exposición a anticuerpo se proporciona como dosis separadas del anticuerpo. No obstante, no toda exposición a anticuerpo necesita proporcionarse como una dosis única o como dosis separadas.

En una incorporación preferida, se administran alrededor de 2-3 gramos del anticuerpo CD20 como la exposición inicial. Si se administran alrededor de 3 gramos, entonces alrededor de 1 gramo del anticuerpo CD20 se administra semanalmente durante alrededor de tres semanas como la exposición inicial. Si se administran alrededor de 2 gramos del anticuerpo CD20 como la exposición inicial, entonces se administra alrededor de 1

gramo del anticuerpo CD20, seguido en alrededor de dos semanas por otro alrededor de 1 gramo del anticuerpo como la exposición inicial. En un aspecto preferido, la segunda exposición es en alrededor de seis meses a partir de la exposición inicial y se administra en una cantidad de alrededor de 2 gramos. En un aspecto preferido alternativo, la segunda exposición es en alrededor de seis meses a partir de la exposición inicial y se administra como alrededor de 1 gramo del anticuerpo, seguido en alrededor de dos semanas por otro alrededor de 1 gramo del anticuerpo.

En todos los métodos inventivos expuestos en esta solicitud, el anticuerpo CD20 o de marcador de superficie de linfocito B puede ser un anticuerpo desnudo o puede conjugarse con otra molécula tal como un agente citotóxico, tal como un compuesto radioactivo. El anticuerpo CD20 preferido en esta solicitud es un anticuerpo CD20 quimérico, humanizado, o humano, más preferiblemente rituximab, un 2H7 humanizado (*por ejemplo*, que comprende secuencias de dominio variable en SEQ ID NOs. 2 y 8, o que comprende un dominio de cadena pesada variable con alteración N100A o D56A y N100A en SEQ ID NO:8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO:2), un anticuerpo A20 quimérico o humanizado (Immunomedics), o un anticuerpo CD20 humano HUMAX-CD20™ (Genmab). Todavía más preferido es rituximab o 2H7 humanizado. Además, la vasculitis asociada con ANCA en todos los métodos en esta solicitud puede ser cualquier enfermedad similar, en una incorporación preferida es granulomatosis de Wegener o polivascularitis microscópica.

En una incorporación adicional de todos los métodos contemplados en esta solicitud, el sujeto o paciente nunca ha sido previamente tratado con fármaco(s), tales como un agente(s) inmunosupresor, para tratar vasculitis asociada con ANCA y/o nunca ha sido previamente tratado con un antagonista (*por ejemplo*, anticuerpo) contra un marcador de superficie de linfocito B (*por ejemplo*, nunca ha sido previamente tratado con un anticuerpo CD20). En todavía otro aspecto, el sujeto o paciente puede haber tenido una recaída con la vasculitis asociada con ANCA o sufrido

lesión de un órgano tal como lesión de riñón, antes de ser tratado en cualquiera de los métodos ya mencionados, inclusive después de la exposición inicial o última. No obstante, preferiblemente, el paciente o sujeto no ha recaído con la vasculitis y, más preferiblemente, no ha tenido tal recaída antes de por lo menos el tratamiento inicial.

En otra incorporación, el sujeto o paciente ha sido tratado previamente con fármaco(s) para tratar la vasculitis y/o ha sido previamente tratado con tal anticuerpo o antagonista. En otra incorporación, el antagonista (por ejemplo, anticuerpo CD20) es el único medicamento administrado al sujeto o paciente para tratar la vasculitis. En otra incorporación, el antagonista (*por ejemplo*, el anticuerpo CD20) es uno de los medicamentos utilizados para tratar la vasculitis. En una incorporación adicional, el sujeto o paciente no tiene una neoplasia maligna. En todavía otra incorporación, el sujeto o paciente no tiene artritis reumatoide. En todavía una incorporación adicional, el sujeto o paciente no tiene esclerosis múltiple. En aún otra incorporación, el sujeto o paciente no tiene lupus o síntoma de Sjögren. En todavía otra incorporación, el sujeto o paciente no tiene una enfermedad autoinmunitaria distinta de la vasculitis asociada con ANCA. En todavía otro aspecto de la invención, la vasculitis asociada con ANCA no está relacionada con una enfermedad autoinmunitaria diferente o con un riesgo de desarrollar una enfermedad autoinmunitaria diferente. Para efectos de estas últimas menciones, una "enfermedad autoinmunitaria", en esta solicitud, es una enfermedad o trastorno proveniente de, y dirigida contra, los propios tejidos u órganos de un individuo, o un co-segregado o manifestación de los mismos o condición resultante de ellos. Sin entrar en limitaciones teóricas de ningún tipo, los linfocitos B demuestran un efecto patógeno en las enfermedades autoinmunitarias a revés de una multitud de rutas mecánicas, incluidas producción de anticuerpos, formación de complejo inmunitario, activación de linfocitos T y células dendríticas, síntesis de citocinas, liberación directa de quimiocinas, y provisión de un foco de infección para neo-linfogénesis ectópica. Cada una de estas rutas

participa en diferentes grados en la patología de las enfermedades autoinmunitarias.

El todavía otra incorporación, el sujeto o paciente tiene un puntaje BVAS/WG de menos de 3, más preferiblemente menos de alrededor de 2, aún más preferiblemente menos de alrededor de 1 y, muy preferiblemente, 0 (remisión completa) en alrededor de tres meses, preferiblemente alrededor de seis meses y, muy preferiblemente, alrededor de un año o más luego de la administración del antagonista o anticuerpo. Incorporaciones específicas de la respuesta BVAS son lograr un puntaje BVAS/WG de menos de 2 en tres meses luego de la administración, o menos de 1 (por ejemplo, 0.2 ó 0.4) en 14 semanas o tres meses luego de la administración, o menos de 1 (por ejemplo, 0.6) en 6 meses luego de la administración, o muy preferiblemente, 0 en tres o seis meses luego de la administración. En otra incorporación, la cantidad de esteroide tal como prednisona, comparada con la iniciación del tratamiento se reduce sin afectar sustancialmente el nivel BVAS/WG disminuido. En consecuencia, por ejemplo, un sujeto o paciente en un intervalo establecido luego del tratamiento (tal como tres meses o seis meses después del tratamiento) tiene preferiblemente un puntaje BVAS/WG disminuido a partir de la línea de referencia y se le está administrando menos de una de un esteroide a partir de la línea de referencia (estando la línea de referencia en el inicio de la administración). En todavía otra incorporación, se incluye un paso en el método de tratamiento para sondear la respuesta del sujeto o el paciente al tratamiento luego del paso de administración, con el fin de determinar que el nivel de respuesta es efectivo para tratar la vasculitis. Por ejemplo, se incluye un paso para examinar el puntaje BVAS/WG luego de la administración y compararlo con un puntaje BVAS/WG de línea de referencia, obtenido antes de la administración, con el fin de determinar si el tratamiento es efectivo cuando midiendo si, y en cuánto, se ha reducido. Esta prueba puede repetirse en diferentes intervalos de tiempo pautados o no pautados luego de la administración para determinar el mantenimiento de cualquier remisión parcial o completa. Alternativamente, los métodos contemplados

en esta solicitud comprenden un paso de examinar a un paciente o sujeto, antes de la administración, para ver si están presentes uno o más biomarcadores para vasculitis asociada con ANCA, tales como uno o más autoanticuerpos, un puntaje BVAS/WG, o síntomas exclusivos de vasculitis asociada con ANCA, como se expuso anteriormente. En otro método, puede incluirse un paso para verificar la historia del paciente o sujeto, como ya se detalló, por ejemplo, para excluir infecciones o neoplasia maligna como causas, por ejemplo, causas primarias, de la condición del paciente o sujeto, antes de administrar el anticuerpo o antagonista al sujeto o paciente. Preferiblemente, la vasculitis asociada con ANCA es primaria (*es decir*, la enfermedad dominante), y no es secundaria, tal como la secundaria a infección o neoplasia maligna, ya se trate de tumores sólidos o líquidos.

En una incorporación preferida del método multi-exposición contemplado en esta solicitud, el sujeto está en remisión luego de las exposiciones, inicial o última a anticuerpo. Más preferiblemente, el método multi-exposición incluido en esta solicitud involucra una re-dosificación pautada o re-tratamiento de modo que el paciente esté en remisión cuando se proporciona la segunda y, preferiblemente, todas las exposiciones a anticuerpo. Tal re-dosificación se pauta para prevenir cualquier recaída, recurrencia, o lesión a órganos, más que tratarla terapéuticamente. Muy preferiblemente, el sujeto está en remisión durante por lo menos alrededor de seis meses y, aún muy preferiblemente, por lo menos alrededor de nueve meses y, todavía muy preferiblemente, por lo menos alrededor de un año desde la última exposición a anticuerpo utilizado en el método de re-tratamiento.

En todavía otra incorporación, el sujeto se trata con el mismo anticuerpo CD20 durante por lo menos dos exposiciones a anticuerpo y, preferiblemente, durante cada exposición a anticuerpo. Por consiguiente, las exposiciones inicial y segunda a anticuerpo son preferiblemente con el mismo anticuerpo y, más preferiblemente, todas las exposiciones a anticuerpo son con el mismo anticuerpo, *es decir*, el tratamiento durante las primeras dos exposiciones y, preferiblemente todas las exposiciones, es con

un tipo de anticuerpo que se une a un marcador de superficie de linfocito B, tal como un anticuerpo CD20, *por ejemplo*, todas con rituximab o todas con el mismo 2H7 humanizado.

En cualquiera de los métodos incluidos en esta solicitud, se puede administrar al sujeto o paciente, junto con el antagonista o anticuerpo, que se une al marcador de superficie de linfocito B, una cantidad efectiva de un segundo medicamento (en donde el antagonista o anticuerpo que se une a un marcador de superficie de linfocito B (*por ejemplo*, el anticuerpo CD20) es un primer medicamento). El segundo medicamento puede ser uno o más medicamentos, e incluyen, *por ejemplo*, un agente citotóxico, un agente quimioterapéutico, un agente inmunosupresor, citocina, antagonista de citocina o anticuerpo, factor de crecimiento, hormona, integrina, antagonista o anticuerpo de integrina, o cualquier combinación de los mismos. El tipo de tal segundo medicamento depende de diferentes factores, incluido el tipo de vasculitis, la gravedad de la vasculitis, la condición y edad del paciente, el tipo y dosis del primer medicamento empleado, *etc.*

Ejemplos de tales medicamentos adicionales incluyen un agente quimioterapéutico, un fármaco de la clase de interferón tal como interferón- α (*por ejemplo*, de Amarillo Biosciences, Inc), IFN-beta-1a (REBIF[®] y AVONEX[®]) o IFN-beta-1b (BETASERON[®]), un oligopéptido tal como glatiramer acetato (COPAXONE[®]), un agente que bloquea el CD40-ligando CD40, un agente citotóxico o inmunosupresor (tal como mitoxantrona (NOVANTRONE[®]), metotrexato, ciclofosfamida, clorambucil, leflunomida, y azatioprina), inmunoglobulina intravenosa (gamma globulina), terapia agotadora de linfocitos (*por ejemplo*, mitoxantrona, ciclofosfamida, anticuerpos CAMPATH[™], anti-CD4, cladribina, un constructo polipeptídico con por lo menos dos dominios que comprende un antígeno auto-reactivo des-inmunizado o su fragmento, que es reconocido específicamente por los receptores de Ig de linfocitos B auto-reactivos (WO 2003/68822), irradiación total del cuerpo, trasplante de médula ósea), antagonista o anticuerpo de integrina (*por ejemplo*, un anticuerpo LFA-1 tal como efalizumab/RAPTIVA[®], disponibles

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comercialmente de Genentech, o un anticuerpo de integrina α -4 tal como natahzumab/ANTEGREN[®], obtenible de Biogen, u otros, como se anota anteriormente), fármacos que tratan síntomas secundarios o relacionados con vasculitis asociada con ANCA (*por ejemplo*, infecciones fúngicas y otras infecciones), tales como los anotados en esta solicitud, esteroides tales como corticosteroides (*por ejemplo*, prednisolona, metilprednisolona tal como SOLU-MEDROL[™] succinato sódico de metilprednisolona para inyección, prednisona tal como prednisona en dosis bajas, dexametasona, o glucocorticoides, *por ejemplo*, mediante inyección en las articulaciones, incluida terapia sistémica con corticoesteroides), terapia inmunosupresora no agotadora de linfocitos (*por ejemplo*, MMF o ciclosporina), fármaco reductor del colesterol de la clase de "estatinas" (que incluye cerivastatina (BAYCOL[™]), fluvastatina (LESCOL[™]), atorvastatina (LIPITOR[™]), lovastatina (MEVACOR[™]), pravastatina (PRAVACHOL[™]), y simvastatina (ZOCOR[™])), estradiol, testosterona (opcionalmente en dosis elevadas, Stuve *et al. Neurology* 8:290-301 (2002)), andrógeno, terapia de sustitución hormonal, un inhibidor de TNF tal como el anticuerpo contra TNF- α , DMARD, NSAID, plasmaféresis o cambio de plasma, trimetoprim-sulfametoxazol (BACTRIM[™], SEPTRA[™]), micofenolato mofetil, bloqueadores H2 o inhibidores de bomba de protones (durante el uso de terapia inmunosupresora potencialmente ulcerógena), levotiroxina, ciclosporina A (*por ejemplo*, SANDIMMUNE[®]), análogo de somatastatina, citocina, antagonista o anticuerpo anti-citocina, anti-metabolito, agente inmunosupresor, cirugía rehabilitante, radioyoduro, tiroidectomía, antagonista BAFF tal como anticuerpos BAFF o BR3 o inmunoadhesinas, recepto de anti-CD40 o ligando anti-CD40 (CD154), antagonista/anticuerpo de receptor anti-IL-6, otro antagonista o anticuerpo de superficie de linfocito B, tal como 2H7 humanizado u otro anticuerpo CD20 humano o humanizado con rituximab, *etc.*

Medicamentos similares preferidos son un agente quimioterapéutico, un agente citotóxico, anti-integrina, gamma globulina, anti-CD4, cladibrina, trimetoprim-sulfametoxazol, un bloqueador H2, un inhibidor de bomba de

protones, un corticosteroide, ciclosporina, fármaco reductor del colesterol de la clase de estatinas, estradiol, testosterona, andrógeno, fármaco de sustitución hormonal, un inhibidor de TNF, DMARD, NSAID (para tratar, por ejemplo, síntomas musculoesqueléticos), levotiroxina, ciclosporina A, análogo de somatostatina, antagonista de citocina o antagonista de receptor de citocina, anti-metabolito, antagonista BAFF tal como anticuerpo BAFF o anticuerpo BR3, especialmente un anticuerpo BAFF, un agente inmunosupresor, y otro anticuerpo de marcador de superficie de linfocito B, tal como una combinación de rituximab y un 2H7 humanizado u otro anticuerpo CD20 humanizado.

Los medicamentos semejantes más preferidos son un agente quimioterapéutico, un agente inmunosupresor, incluido un anticuerpo contra TNF- α , un anticuerpo contra CD40-ligando CD40, y un antagonista BAFF tal como un anticuerpo BAFT o BR3, un DMARD, un agente citotóxico, un antagonista de integrina, un NSAID, un antagonista de citocina, o una hormona, o una combinación de los mismos. Pueden requerirse inmunosupresores, por ejemplo, para cada enfermedad activa con compromiso serio de órganos, e incluyen agentes tales como ciclofosfamida (CYTOXAN®), clorambucil, leflunomida, MMF, azatioprina (IMURAN®), y metotrexato. Los antagonistas BAFF pueden ser útiles en combinación con el primer medicamento para eficacia.

Todavía más preferidos son un esteroide, un agente quimioterapéutico, un agente inmunosupresor, un agente citotóxico, un antagonista de integrina, un antagonista de citocina, o una hormona, o una combinación de los mismos, muy preferiblemente un esteroide y/o un agente inmunosupresor, aún muy preferiblemente, un corticoesteroide y/o un agente inmunosupresor.

En una incorporación particularmente preferida, el segundo medicamento es o comprende uno o más esteroides, por ejemplo, un corticoesteroide, el cual es preferiblemente prednisona, prednisolona, metilprednisolona, hidrocortisona, o dexametasona. Tal esteroide se administra preferiblemente en cantidades más bajas que las utilizadas si el

primer medicamento, *por ejemplo*, anticuerpo CD20, no se administra al paciente tratado con esteroide. En un aspecto preferido, el esteroide no se administra con cualquier segunda exposición a anticuerpo o se administra con la segunda exposición pero en cantidades más bajas que las utilizadas con la exposición inicial a anticuerpo. Además se prefiere en el caso en que esteroide no se administra con las exposiciones tercera o última a anticuerpo.

En un aspecto adicional todavía particularmente preferido, el segundo medicamento es un agente inmunosupresor, más preferiblemente ciclofosfamida, MMF, clorambucil, azatioprina, leflunomida, o metotrexato, y se administra preferiblemente por lo menos con la exposición inicial a anticuerpo. En una incorporación, azatioprina, metotrexato, o MMF se usan preferiblemente en lugar de ciclofosfamida para el mantenimiento de la remisión.

En todavía un aspecto adicional preferido, el segundo medicamento es una combinación de uno o más esteroides y un agente inmunosupresor.

Para infección fúngica puede usarse también el tratamiento profiláctico con fluconazol (DIFLUCAN™), por vía oral, de la vasculitis asociada con ANCA, así como trimetoprim-sulfametoxazol (480 mg) tres veces semanalmente para tratamiento profiláctico de pacientes con *pneumocystis carinii*. Jayne and Rasmussen, *supra*.

Todos estos segundos medicamentos pueden usarse en combinación unos con otros, o en sí mismos, con el primer medicamento, de modo que la expresión "segundo medicamento", tal como emplea aquí, no significa que es el único medicamento aparte del primer medicamento, respectivamente. En consecuencia, el segundo medicamento no necesita ser un medicamento, sino que puede constituir o comprender más de un fármaco semejante.

Estos segundos medicamentos, tal como se expone en esta solicitud, se usan generalmente en las mismas posologías y con vías de administración, como se emplea más arriba en el texto, o alrededor de desde 1 a 99% de las posologías utilizadas hasta la fecha. Si tales segundos medicamentos sí se utilizan, preferiblemente se los emplea en cantidades

más bajas que si el primer medicamento no estuviera presente, en especial en posologías subsiguientes más allá de la posología inicial con el primer medicamento, con el fin de eliminar o reducir efectos secundarios causados por él.

Para el método de re-tratamiento contemplado en esta solicitud, donde un segundo medicamento se administra en una cantidad efectiva con exposición a anticuerpo, éste puede administrarse con cualquier exposición, por ejemplo, sólo con una exposición, o con más de una exposición. En una incorporación, el segundo medicamento se administra con la exposición inicial. En otra incorporación, el segundo medicamento se administra con las exposiciones inicial y segunda. En todavía una incorporación adicional, el segundo medicamento se administra con todas las exposiciones. Se prefiere que después de la exposición inicial, tal como de esteroide, la cantidad de tal segundo medicamento se reduzca o elimine, con el fin de disminuir la exposición del sujeto a un agente con efectos secundarios, tal como prednisona, prednisolona, metilprednisolona, y ciclofosfamida.

Como un ejemplo específico, el tratamiento de pacientes con polivasculitis microscópica y granulomatosis de Wegener tiene tres fases (1) inducción de la remisión, (2) mantenimiento de la remisión, y (3) tratamiento de la recaída. La terapia actual de inducción consiste a menudo de ciclofosfamida (CYTOXAN®) y corticoesteroides. Esto incluye una dosis elevada de metilprednisolona intravenosa durante varios días (*por ejemplo*, uno a cinco días), más prednisona oral disminuida progresivamente durante un período de tiempo, tal como 3-5 meses. Para una enfermedad crónica activa, se recomienda el uso de metilprednisolona intravenosa en dosis altas durante tres días, combinada con ciclofosfamida intravenosa u oral. Preferiblemente siguen dosis disminuidas progresivamente de prednisona, junto con mantenimiento de ciclofosfamida durante 12 a 18 meses. Cuando se emplea el primer medicamento, tal cantidad y frecuencia de dosificación se reducen preferiblemente todavía más, puesto que debe emplearse la dosificación mínima de esteroides que controla la enfermedad. La infección debe considerarse si los síntomas

parecen exacerbarse. Para pacientes en remisión sostenida en 12 meses, se prefiere que el uso de tales segundos medicamentos se suspenda a una velocidad mayor con el primer medicamento administrado en este caso que sin él. No obstante, pacientes cuyos síntomas están bajo control tienen que ser monitoreados muy de cerca en intervalos de seis meses con respecto a signos y síntomas de recaída. Durante el tratamiento con estos agentes, deben realizarse periódicamente recuentos de células sanguíneas y exámenes de función hepática.

La administración combinada de un segundo medicamento incluye la co-administración (administración concurrente), usando formulaciones separadas o una formulación farmacéutica única, y administración consecutiva en cualquier orden, en donde, preferiblemente, hay un período de tiempo mientras ambos (o todos los) agentes activos (medicamentos) ejercen simultáneamente sus actividades biológicas.

El anticuerpo o antagonista contemplado en esta solicitud se administra por cualquier medio apropiado, incluida la administración parenteral, tópica, subcutánea, intraperitoneal, intrapulmonar, intranasal, y/o intralesional. Las infusiones parenterales incluyen la administración intramuscular, intravenosa (i.v.), intraarterial, intraperitoneal, o subcutánea. Se contempla asimismo la administración intratecal (véase, *por ejemplo*, US 2002/0009444, Grillo-López, A concerning intrathecal delivery of a CD20 antibody). Además, el anticuerpo o antagonista puede administrarse convenientemente por infusión de pulsación, *por ejemplo*, con dosis decrecientes del anticuerpo o antagonista. Preferiblemente, la dosificación se administra por vía intravenosa o subcutánea y, más preferiblemente, por infusión (infusiones) intravenosa(s).

Si se proporcionan múltiples exposiciones de anticuerpo, cada exposición puede proveerse usando el mismo o un diferente medio de administración. En una incorporación, cada exposición es por administración intravenosa. En otra incorporación, cada exposición se aplica por administración subcutánea. En todavía otra incorporación, las exposiciones se aplican por administración intravenosa y subcutánea.

En una incorporación, el anticuerpo CD20 se administra como una infusión intravenosa lenta en lugar de una inyección intravenosa rápida o súbita. Por ejemplo, un esteroide tal como prednisolona o metilprednisolona (*por ejemplo*, alrededor de 80-120 mg i.v, más específicamente alrededor de 100 mg i.v.) se administra alrededor de 30 minutos antes de cualquier infusión del anticuerpo CD20. El anticuerpo CD20, *por ejemplo*, se administra por infusión a través de una línea dedicada.

Para la dosis inicial de una exposición multi-dosis al anticuerpo CD20, o para la dosis única, si la exposición implica sólo una dosis, tal infusión se comienza preferiblemente a una tasa de alrededor de 50 mg/hora. Ésta puede ser escalonada, *por ejemplo*, a una tasa de incrementos de alrededor de 50 mg/hora alrededor de cada 30 minutos a un máximo alrededor de 400 mg/hora. No obstante, si el sujeto está experimentando una reacción relacionada con la infusión, preferiblemente se reduce la tasa de infusión, *por ejemplo*, a la mitad de la tasa corriente, *por ejemplo*, desde 100 mg/hora a 50 mg/hora. Preferiblemente, la infusión de tal dosis de anticuerpo CD20 (*por ejemplo*, una dosis total de alrededor de 1.000 mg) se completa a alrededor de 255 minutos (4 horas 15 mm). Opcionalmente, los sujetos reciben un tratamiento profiláctico de acetaminofén/paracetamol (*por ejemplo*, alrededor de 1 g) y difenhidramina HCl (*por ejemplo*, alrededor de 50 mg o dosis equivalente de agente similar) por vía oral alrededor de 30 a 60 minutos antes del inicio de una infusión.

Si se aplica más de una infusión (dosis) de anticuerpo CD20 para lograr la exposición total, la segunda infusión o las infusiones subsiguientes de anticuerpo CD20 en esta incorporación de infusiones se comienzan preferiblemente a una tasa más alta que la infusión inicial, *por ejemplo*, a alrededor de 100 mg/hora. Esta tasa puede escalonarse, *por ejemplo*, a una tasa de incrementos de alrededor de 100 mg/hora alrededor de cada 30 minutos a un máximo de alrededor de 400 mg/hora. Sujetos que experimentan una reacción relacionada con la infusión tienen

preferiblemente una tasa de infusión reducida a la mitad de la tasa, *por ejemplo*, desde 100 mg/hora a 50 mg/hora. Preferiblemente, la infusión de tal segunda dosis o dosis subsiguiente de anticuerpo CD20 (*por ejemplo*, una dosis total de alrededor de 1.000 mg) se completa en alrededor de 195 minutos (3 horas 15 minutos).

Sigue a continuación una discusión de los métodos de producir, modificar, y formular tales anticuerpos.

III. Producción de Anticuerpos

Los métodos y artículos de fabricación de la presente invención utilizan, o incorporan, un anticuerpo que se une a un marcador de superficie de linfocito B, especialmente uno que se une a CD20. En consecuencia, se describirán aquí métodos de generar tales anticuerpos.

El antígeno CD20 a usarse para la producción de, o tamizaje con respecto a, anticuerpo(s) puede ser, *por ejemplo*, una forma soluble de CD20 o una porción del mismo, que contenga el epítipo deseado. Alternativa, o adicionalmente, células que expresan CD20 en su superficie celular pueden usarse para generar, o tamizar con respecto a, anticuerpo(s). Otras formas de CD20 útiles para generar anticuerpos serán evidentes a los expertos en el arte.

Sigue a continuación una descripción con respecto a técnicas ejemplares para la producción de los anticuerpos utilizados de acuerdo con la presente invención

(i) *Anticuerpos policlonales*

Los anticuerpos policlonales se crían preferiblemente en animales mediante inyecciones múltiples subcutáneas (sc) o intraperitoneales (ip) del antígeno relevante y un coadyuvante. Puede ser útil conjugar el antígeno relevante con una proteína que sea inmunógena en la especie a inmunizarse, *por ejemplo*, hemocianina de lapa, albúmina sérica, tiroglobulina bovina, o inhibidor de tripsina de soya, usando un agente bifuncional o derivatizante, *por ejemplo*, éster de maleimidobenzoyl sulfosuccinimida (conjugación a

través de residuos de cisteína), N-hidroxisuccinimida (a través de residuos de lisina), glutaraldehído, anhídrido succínico, SOCl_2 , ó $\text{R}^1\text{N}=\text{C}=\text{NR}$, donde R y R^1 son grupos alquilo diferentes.

Los animales se inmunizan contra el antígeno, conjugados inmunógenos, o derivados combinando, *por ejemplo*, 100 μg ó 5 μg de la proteína o conjugado (para conejos o ratones, respectivamente) con 3 volúmenes de coadyuvante completo de Freund e inyectando la solución intradérmicamente en sitios múltiples. Un mes después los animales se refuerzan con 1/5 a 1/10 de la cantidad original de péptido o conjugado en coadyuvante completo de Freund mediante inyección subcutánea en múltiples sitios. Siete a 14 días después, los animales se desangran y se analiza el suero para detectar la volumetría de anticuerpos. Los animales se refuerzan hasta que la volumetría se estabiliza. Preferiblemente, el animal se refuerza con el conjugado del mismo antígeno, pero conjugado con una proteína diferente y/o a través de un reactivo diferente de enlazamiento cruzado. Los conjugados pueden prepararse también en cultivo de células recombinantes como fusiones de proteína. Asimismo, agentes agregantes tales como alumbre se emplean adecuadamente para intensificar la respuesta inmunitaria.

(ii) *Anticuerpos monoclonales*

Los anticuerpos monoclonales se obtienen de una población de anticuerpos sustancialmente homogéneos, *es decir*, los anticuerpos individuales que conforman la población son idénticos y/o unen el mismo epítipo, excepto por posibles variantes que surgen durante la producción del anticuerpo monoclonal, estando tales variantes presentes por lo general en cantidades menores. Por lo tanto, el calificativo "monoclonal" indica el carácter del anticuerpo como no siendo una mezcla de anticuerpos discretos o policlonales.

Por ejemplo, los anticuerpos monoclonales pueden elaborarse usando el método de hibridoma descrito por primera vez por Kohler *et al.*, *Nature*,

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256: 495 (1975), o pueden elaborarse por métodos de ADN recombinante (Patente de los Estados Unidos No. 4,816,567).

En el método de hibridoma, un ratón u otro animal hospedero apropiado, tal como un hámster, se inmuniza, como se describe más arriba en el texto, para provocar linfocitos que produzcan, o sean capaces de producir, anticuerpos que se fijarán específicamente a la proteína utilizada para inmunización. Alternativamente, los linfocitos pueden inmunizarse *in vitro*. Seguidamente los linfocitos se fusionan con células de mieloma usando un agente apropiado de fusión, tal como polietilen glicol, para formar una célula de hibridoma (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

Las células de hibridoma así preparadas se siembran y cultivan en un medio de cultivo adecuado, el cual contiene preferiblemente una o más sustancias que inhiben el crecimiento o supervivencia de las células de mieloma progenitoras, no fusionadas. Por ejemplo, si las células de mieloma progenitoras carecen de la enzima hipoxantina guanina fosforibosil transferasa (HGPRT o HPRT), el medio de cultivo para los hibridomas incluirá típicamente hipoxantina, aminopterina, y timidina (medio HAT), sustancias que previenen el crecimiento de células deficientes en HGPRT.

Células preferidas de mieloma son aquellas que se fusionan eficientemente, favorecen la producción estable, de alto nivel, de anticuerpo por las células seleccionadas productoras de anticuerpos, y son sensibles a un medio tal como el medio HAT. Entre éstas, estirpes celulares preferidas de mieloma se encuentran estirpes de mieloma murino, tales como las derivadas de tumores de ratón MOPC-21 y MPC-11, disponibles del Salk Institute Cell Distribution Center, San Diego, California USA, y células SP-2 ó X63-Ag8-653 disponibles de la American Type Culture Collection, Maryland USA. Se han descrito igualmente estirpes celulares de mieloma humano y heteromieloma ratón-humano para la producción de anticuerpos monoclonales humanos (Kozbor, *J. Immunol.*, 133: 3001 (1984); Brodeur *et*

al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

El medio de cultivo en el cual crecen las células de hibridoma se analiza con respecto a la producción de anticuerpos monoclonales dirigidos contra el antígeno. Preferiblemente, la especificidad de unión de los anticuerpos monoclonales, producidos por células de hibridoma, se determina por inmunoprecipitación o por un análisis de unión *in vitro*, tal como radioinmunoanálisis (RIA) o enzimoimmunoanálisis absorbente (ELISA).

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

Una vez identificadas las células de hibridoma que producen anticuerpos de la especificidad, afinidad, y/o actividad deseada, los clones pueden subclonarse por procedimientos limitantes de dilución y cultivarse por métodos estándar (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)). Medios de cultivo apropiados para este propósito incluyen, por ejemplo, medio D-MEM o RPMI-1640. Adicionalmente, las células de hibridoma pueden cultivarse *in vivo* como tumores ascíticos en un animal.

Los anticuerpos monoclonales secretados por los subclones se separan adecuadamente del medio de cultivo, fluido ascítico, o suero mediante procedimientos convencionales de purificación de inmunoglobulina tales como, por ejemplo, proteína A-SEPHAROSE™, cromatografía de hidroxilapatita, electroforesis en gel, diálisis, o cromatografía de afinidad.

ADN que codifica los anticuerpos monoclonales se aísla fácilmente y se secuencia usando procedimientos convencionales (*por ejemplo*, usando sondas de oligonucleótidos que son capaces de fijarse específicamente a genes que codifican las cadenas ligera y pesada de anticuerpos murinos). Las células de hibridoma sirven como fuente preferida de tal ADN. Una vez aislado, el ADN puede ponerse en vectores de expresión, los cuales se

transfectan luego en células hospederas tales como células de *E. coli*, células COS de simios, células de ovario de hámster de la China (CHO), o células de mieloma que, de otra forma, no producen proteína inmunoglobulina, para obtener la síntesis de anticuerpos monoclonales en las células hospederas recombinantes. Artículos de reseña sobre la expresión recombinante en bacterias de ADN que codifica el anticuerpo incluyen Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) y Plückthun, *Immunol. Revs.*, 130: 151-188 (1992).

En una incorporación adicional, anticuerpos o fragmentos de anticuerpo pueden aislarse de bibliotecas de fagos de anticuerpos generados usando las técnicas descritas en McCafferty *et al.*, *Nature*, 348: 552-554 (1990). Clackson *et al.*, *Nature*, 352: 624-628 (1991) y Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describen el aislamiento de anticuerpos humanos y murinos, respectivamente, utilizando bibliotecas de fagos. Publicaciones subsiguientes describen la producción de anticuerpos humanos de alta afinidad (rango nM) por barajado de cadenas ("*chain shuffling*") (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), así como infección combinatoria y recombinación *in vivo* como estrategia para construir bibliotecas muy grandes de fagos (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21: 2265-2266 (1993)). Por lo tanto, éstas técnicas son alternativas viables a las técnicas tradicionales de hibridoma de anticuerpos monoclonales para el aislamiento de anticuerpos monoclonales.

Del mismo modo puede modificarse el ADN, por ejemplo, sustituyendo la secuencia codificante para los dominios constantes humanos de cadena ligera y pesada en lugar de las secuencias murinas homólogas (Patente de los Estados Unidos No. 4,816,567; Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81: 6851 (1984)), o juntando covalentemente a la secuencia codificante de inmunoglobulina la totalidad o parte de la secuencia codificante para un polipéptido no-inmunoglobulina.

Típicamente tales polipéptidos no-inmunoglobulina se sustituyen por los dominios constantes de un anticuerpo, o se sustituyen por los dominios variables de un sitio combinante de antígeno de un anticuerpo para crear un

anticuerpo bivalente quimérico, que comprende un sitio combinante de antígeno con especificidad para un antígeno, y otro sitio combinante de antígeno con especificidad para un antígeno diferente.

Además, anticuerpos que comprenden una región Fc variante con alta afinidad para FcγR son útiles para tratar enfermedades en donde se desea una eficacia mejorada o de la función de células efectoras, tal como enfermedades autoinmunitarias, como se expone, por ejemplo, en US 2005/0037000 y WO 2004/63351 (Macrogenics, Inc. STA VENHAGEN *et al.*).

(iii) Anticuerpos humanizados

Métodos para humanizar anticuerpos no humanos se han descrito en el arte. Preferiblemente, un anticuerpo humanizado tiene uno o más residuos de aminoácidos introducidos en él de una fuente que es no humana. Estos residuos de aminoácidos no humanos se denominan a menudo residuos de "importación", los cuales se toman típicamente de un dominio variable de "importación". La humanización puede realizarse esencialmente siguiendo el método de Winter *et al.*, (Jones *et al.*, (1986) Nature 321:522-525; Riechmann *et al.*, Nature 332:323-327 (1988); Verhoeven *et al.*, Science 239:1534-1536 (1988), sustituyendo secuencias de región hipervariable por las secuencias correspondientes de un anticuerpo humano. En consecuencia, tales anticuerpos "humanizados" son anticuerpos quiméricos (Patente de los Estados Unidos No. 4,816,567), en donde sustancialmente menos de un dominio variable humano intacto se ha sustituido por la secuencia correspondiente de una especie no humana. En la práctica, anticuerpos humanizados son típicamente anticuerpos humanos en los cuales algunos residuos de región hipervariable y, posiblemente, algunos residuos FR se sustituyen con residuos de sitios análogos en anticuerpos de roedor.

La elección de dominios variables humanos, livianos y pesados, para usarlos en elaborar los anticuerpos humanizados es muy importante para reducir la antigenicidad. De acuerdo con el denominado método de "ajuste

óptimo", se criba la secuencia del dominio variable de un anticuerpo de roedor contra toda la biblioteca de secuencias de dominio variable conocidas. La secuencia humana que es más próxima a la del roedor se acepta entonces como la región de estructura alterna (FR) humana para el anticuerpo humanizado Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987). Otro método usa una región de estructura alterna particular derivada de la secuencia de consenso de todos los anticuerpos humanos de un subgrupo particular de cadenas ligeras y pesadas. La misma estructura alterna puede utilizarse para varios anticuerpos humanizados diferentes (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993)).

Es además importante que los anticuerpos se humanicen conservando una alta afinidad para el antígeno y otras propiedades biológicas favorables. Para lograr este objetivo, de acuerdo con un método preferido, anticuerpos humanizados se preparan por un proceso de análisis de las secuencias progenitoras y varios productos humanizados conceptuales usando modelos tridimensionales de las secuencias progenitoras y humanizadas. Comúnmente se dispone de modelos tridimensionales de inmunoglobulina y son familiares a los expertos en el arte. Se dispone de programas de computador, los cuales ilustran y visualizan estructuras probables de conformación tridimensional de secuencias seleccionadas de inmunoglobulina candidata. La inspección de estas visualizaciones permite el análisis del papel probable de los residuos en el funcionamiento de la secuencia de inmunoglobulina candidata, es decir, el análisis de residuos que influyen en la capacidad de la inmunoglobulina candidata de fijar su antígeno. De esta forma, pueden seleccionarse residuos FR y combinarse de las secuencias receptoras y de importación, de modo que se logre la característica del anticuerpo deseado, tal como creciente afinidad para el antígeno(s) diana. En general, los residuos de región hipervariable se hallan directa y, la mayoría de las veces, sustancialmente implicados en influir en la unión del antígeno.

(iv) *Anticuerpos humanos*

Como una alternativa a la humanización, pueden generarse anticuerpos humanos. Por ejemplo, actualmente es posible producir animales transgénicos (por ejemplo, ratones) que son capaces, luego de la inmunización, de producir un completo repertorio de anticuerpos humanos en la ausencia de producción de inmunoglobulina endógena. Por ejemplo, se ha descrito que la eliminación homocigótica del gen de la región de unión a la cadena pesada (JH) del anticuerpo en ratones mutantes quiméricos y de línea germinativa resulta en la completa inhibición de la producción de anticuerpos endógenos. La transferencia del arreglo de genes de inmunoglobulina de línea germinativa humana en tales ratones mutantes de línea germinativa resultará en la producción de anticuerpos humanos a la exposición al antígeno. Véase, por ejemplo, Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immuno.*, 7:33 (1993); Patentes de los Estados Unidos No. 5,591,669, 5,589,369 y 5,545,807.

Alternativamente, tecnología muestrario de fagos (McCafferty et al., *Nature* 348:552-553 (1990)) puede usarse para producir anticuerpos humanos y fragmentos de anticuerpo in vitro, a partir de repertorios de genes de dominio variable (V) de inmunoglobulina de donantes no inmunizados. De acuerdo con esta técnica, genes de dominio V de anticuerpo se clonan sin cambio en la fase de lectura ("*are cloned in-frame*") en un gen de cubierta proteínica mayor o menor de un bacteriófago filamentoso, tal como M13 ó fd, y se exponen como fragmentos funcionales de anticuerpo en la superficie de la partícula del fago. Debido a que la partícula filamentosa contiene una copia de ADN monocatenario del genoma del fago, las selecciones basadas en las propiedades funcionales del anticuerpo resultan igualmente en la selección del gen codificante del anticuerpo que exhibe estas propiedades. Por consiguiente, el fago imita algunas de las propiedades del linfocito B. La exposición en fago puede realizarse en una variedad de formatos; para su reseña véase, por ejemplo, Johnson et al., *Current Opinion in Structural Biology* 3 564-571 (1993). Para el muestrario de fagos pueden emplearse varias fuentes de segmentos

de gen V. Clackson *et al.*, *Nature* 352:624-628 (1991) aislaron un arreglo diverso de anticuerpos de anti-oxazolona de una pequeña biblioteca combinatoria aleatoria de genes V derivados de los bazo de ratones inmunizados. Puede construirse un repertorio de genes V de donantes no inmunizados humanos, y anticuerpos a un arreglo diverso de antígenos (incluidos auto-antígenos) pueden aislarse siguiendo esencialmente las técnicas descritas por Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), o Griffith *et al.*, *EMBO J.* 12:725-734 (1993). Véase también, Patentes de los Estados Unidos No. 5,565,332 y 5,573,905.

Anticuerpos humanos pueden generarse igualmente in vitro por linfocitos B activados (véase Patentes de los Estados Unidos No. 5,567,610 y 5,229,275).

(v) *Fragmentos de anticuerpos*

Se han desarrollado varias técnicas para la producción de fragmentos de anticuerpo. Tradicionalmente, estos fragmentos se derivaron por digestión proteolítica de anticuerpos intactos (véase, *por ejemplo*, Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); y Brennan *et al.*, *Science* 229:81 (1985)). Sin embargo, estos fragmentos pueden producirse ahora directamente por células hospederas recombinantes. Por ejemplo, los fragmentos de anticuerpo pueden aislarse de bibliotecas de fagos de anticuerpos discutidas anteriormente. Alternativamente, fragmentos Fab'-SH pueden recuperarse directamente de *E. coli* y acoplarse químicamente para formar fragmentos F(ab')₂ (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). De acuerdo con otra metodología, fragmentos F(ab')₂ pueden aislarse directamente de cultivo de células hospederas recombinantes. Otras técnicas para la producción de fragmentos de anticuerpos serán evidentes al profesional experto. En otras incorporaciones, el anticuerpo de elección es un fragmento Fv de cadena única (scFv). Véase WO 93/16185; Patente de los Estados Unidos No. 5,571,894 y Patente de los Estados Unidos No. 5,587,458. El fragmento de anticuerpo puede ser igualmente un "anticuerpo lineal", *por ejemplo*, como

se describe en la Patente de los Estados Unidos 5,641,870. Tales fragmentos de anticuerpo lineal pueden ser monoespecíficos o bioespecíficos.

(vi) Anticuerpos bioespecíficos

Anticuerpos bioespecíficos son anticuerpos que tienen especificidades de unión para por lo menos dos epítopos diferentes. Anticuerpos bioespecíficos ejemplares pueden fijarse a dos diferentes epítopos del antígeno CD20. Otros anticuerpos semejantes pueden unir CD20 y fijar además un segundo marcador de superficie de linfocito B. Alternativamente, un brazo de unión anti-CD20 puede combinarse con un brazo que se fije a una molécula desencadenante en un leucocito tal como una molécula de receptor de leucocito T (*por ejemplo*, CD2 ó CD3), o receptores Fc para IgG (FcγR), tales como FcγRI (CD64), FcγRII (CD32) y FcγRIII (CD 16), de modo que se enfoquen mecanismos de defensa celular al linfocito B. Anticuerpos bioespecíficos pueden usarse además para localizar agentes citotóxicos al linfocito B. Estos anticuerpos poseen un brazo de unión CD20 y un brazo que fija el agente citotóxico (*por ejemplo*, saporina, anti-interferón-α, vincristina/vinblastina ("*vinca alkaloid*"), cadena de ricina A, metotrexato o hapteno de isótopo radioactivo). Anticuerpos bioespecíficos pueden prepararse como anticuerpos de longitud total o fragmentos de anticuerpo (*por ejemplo*, anticuerpos bioespecíficos F(ab')₂).

Métodos para elaborar anticuerpos bioespecíficos son conocidos en el arte. La producción tradicional de anticuerpos bioespecíficos de longitud total se basa en la co-expresión de dos parejas de cadena ligera-cadena pesada de inmunoglobulina, donde las dos cadenas tienen especificidades diferentes (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Debido a la clasificación aleatoria de cadenas ligeras y pesadas de inmunoglobulina, estos hibridomas (cuadromas) producen una mezcla potencial de 10 diferentes moléculas de anticuerpo, de las cuales sólo una posee la estructura bioespecífica correcta. La purificación de la molécula correcta, lo

cual se hace usualmente por los pasos de cromatografía de afinidad, es más bien difícil, y los rendimientos del producto son bajos. Procedimientos similares se divulgan en WO 93/08829 y en Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

De acuerdo con un enfoque diferente, dominios variables de anticuerpo con las especificidades de unión deseadas (sitios combinantes de anticuerpo-antígeno) se fusionan a secuencias de dominio constante de inmunoglobulina. La fusión es preferiblemente con un dominio constante de cadena pesada de inmunoglobulina, que comprende por lo menos parte de las regiones de unión flexible, CH2 y CH3. Se prefiere tener la primera región constante de cadena pesada (CH1) conteniendo el sitio necesario para unión de la cadena ligera, presente en por lo menos una de las fusiones. ADN que codifican las fusiones de cadena pesada de inmunoglobulina y, si se desea, la cadena ligera de inmunoglobulina, se insertan en vectores de expresión separados, y se co-transfectan en un organismo hospedero apropiado. Esto facilita una gran flexibilidad en ajustar las proporciones mutuas de los tres fragmentos del polipéptido en incorporaciones, cuando proporciones desiguales de las tres cadenas de polipéptido usadas en la construcción proporcionan los rendimientos óptimos. No obstante, es posible insertar las secuencias codificantes para las dos o todas las tres cadenas de polipéptido en un vector de expresión, cuando la expresión de por lo menos dos cadenas de polipéptido en proporciones iguales resulta en altos rendimientos, o cuando las proporciones no son de significancia particular.

En una incorporación preferida de este enfoque, los anticuerpos bioespecíficos se componen de una cadena pesada de inmunoglobulina híbrida con una primera especificidad de unión en un brazo, y una pareja cadena ligera-cadena pesada de inmunoglobulina híbrida (que proporciona una segunda especificidad de unión) en el otro brazo. Se encontró que esta estructura asimétrica facilita la separación del compuesto bioespecífico deseado de combinaciones no deseadas de cadena de inmunoglobulina, en la medida en que la presencia de una cadena ligera de inmunoglobulina en

sólo una mitad de las moléculas biespecíficas ofrece un modo fácil de separación. Esta metodología se divulga en WO 94/04690. Para mayores detalles de generar anticuerpos bioespecíficos, véase, por ejemplo, Suresh *et al.*, *Methods in Enzymology* 121: 210 (1986).

De acuerdo con otro enfoque descrito en Patente de los Estados Unidos 5,731,168, la interfase entre una pareja de moléculas de anticuerpo puede diseñarse para maximizar el porcentaje de heterodímeros que se recuperan del cultivo de células recombinantes. La interfase preferida comprende por lo menos una parte del dominio C_H3 de un dominio constante de anticuerpo. En este método, una o más cadenas laterales pequeñas de aminoácidos de la interfase de la primera molécula de anticuerpo se reemplazan con cadenas laterales más grandes (*por ejemplo*, tirosina o triptófano). "Cavidades" compensatorias de tamaño idéntico o similar a la cadena(s) lateral grande se crean en la interfase de la segunda molécula de anticuerpo reemplazando cadenas laterales de aminoácido grandes con más pequeñas (*por ejemplo*, alanina o treonina). Esto proporciona un mecanismo para aumentar el rendimiento del heterodímero sobre otros productos finales indeseados tales como homodímeros.

Los anticuerpos bioespecíficos incluyen anticuerpos enlazados cruzadamente o de "heteroconjugado". Por ejemplo, uno de los anticuerpos en el heteroconjugado puede acoplarse a avidina, el otro a biotina. Tales anticuerpos, por ejemplo, han sido propuestos para dirigir células del sistema inmunitario a células indeseadas, Patente de los Estados Unidos 4,676,980, y para tratamiento de la infección VIH (WO 1991/00360, WO 1992/200373 y EP 0308936). Anticuerpos de heteroconjugado pueden elaborarse usando métodos cualesquiera de enlazamiento cruzado. Agentes apropiados de enlazamiento cruzado son bien conocidos en el arte, y se divulgan, por ejemplo, en la Patente de los Estados Unidos No. 4,676,980, junto con un número de técnicas de enlazamiento cruzado.

Se han descrito asimismo en la literatura técnicas para generar anticuerpos bioespecíficos de fragmentos de anticuerpo. Por ejemplo, anticuerpos bioespecíficos pueden prepararse usando enlazamiento

químico. Brennan *et al. Science*, 229:81 (1985) describe un procedimiento en donde anticuerpos intactos se segmentan proteolíticamente para generar fragmentos $F(ab')_2$. Estos fragmentos se reducen en la presencia arsenita sódica del agente acomplejante ditiol para estabilizar ditioles vecinos y evitar la formación de disulfuro intermolecular. Los fragmentos Fab' generados se convierten entonces en derivados de tionitrobenzoato (TNB). Uno de los derivados TNB- Fab' se reconvierte luego en el tiol- Fab' mediante reducción con mercaptoetilamina y se mezcla con una cantidad equimolar del otro derivado TNB- Fab' para formar el anticuerpo bioespecífico. Los anticuerpos bioespecíficos producidos pueden usarse como agentes para la inmovilización selectiva de enzimas.

Se han descrito igualmente varias técnicas para elaborar y aislar fragmentos de anticuerpo bioespecífico directamente de cultivo de células recombinantes. Por ejemplo, se han producido anticuerpos bioespecíficos usando cierres de leucina ("*leucine zippers*"). Kostelny *et al. J. Immunol.*, 148(5):1547-1553 (1992). Los péptidos del cierre de leucina de las proteínas Fos y Jun se enlazaron a las porciones Fab' de dos anticuerpos diferentes mediante fusión génica. Los homodímeros del anticuerpo se redujeron en la región flexible para formar monómeros y luego se re-oxidaron para formar los heterodímeros del anticuerpo. Este método puede utilizarse también para la producción de homodímeros del anticuerpo. La tecnología de "diacuerpo" descrita por Hollinger *et al., Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) ha proporcionado un mecanismo alternativo para elaborar fragmentos de anticuerpo bioespecífico. Los fragmentos comprenden un dominio variable de cadena pesada (V_H) conectado a un dominio variable de cadena ligera (V_L) por un conector que es demasiado corto para permitir el apareamiento entre los dos dominios en la misma cadena. Por consiguiente, los dominios V_H y V_L de un fragmento son forzados a aparearse con los dominios V_L y V_H complementarios de otro fragmento, formando así dos sitios de unión de antígeno. Se ha descrito también otra estrategia para elaborar fragmentos de anticuerpos

bioespecíficos mediante el uso de dímeros Fv de cadena única (sFv). Véase Gruber *et al.*, *J. Immunol.*, 152:5368 (1994).

Se contemplan anticuerpos con más de dos valencias. Por ejemplo, pueden prepararse anticuerpos trispecíficos. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

IV. Conjugados y Otras Modificaciones del Anticuerpo

El anticuerpo utilizado en los métodos o incluidos en los artículos de fabricación en esta solicitud se conjuga opcionalmente con un agente citotóxico. Por ejemplo, el anticuerpo (CD20) puede conjugarse con un fármaco, como se describe en WO2004/032828.

Agentes quimioterapéuticos útiles en la generación de tales conjugados anticuerpo-agente citotóxico se han descrito más arriba.

Conjugados de un anticuerpo y uno o más toxinas de molécula pequeña, tales como una caliqueamicina, una maitansina (Patente de los Estados Unidos No. 5,208,020), un tricoteno, y CC1065, se contemplan también en esta solicitud. En una incorporación de la invención, el anticuerpo se conjuga con una o más moléculas de maitansina (*por ejemplo*, alrededor de 1 a alrededor de 10 moléculas de maitansina por molécula de anticuerpo). La maitansina puede convertirse, por ejemplo, en May-SS-Me, la cual puede reducirse a May-SH3 y hacerse reaccionar con el anticuerpo modificado (Chari *et al.*, *Cancer Research* 52: 127-131 (1992)) para generar un conjugado maitansinoide-anticuerpo.

Alternativamente, el anticuerpo se conjuga con una o más moléculas de caliqueamicina. La familia caliqueamicina de antibióticos es capaz de producir fragmentos de ADN bicatenario en concentraciones sub-picomolar. Análogos estructurales de caliqueamicina que pueden emplearse incluyen, pero no se limitan a, γ_1^I , α_2^I , α_3^I , N-acetil-, γ_1^I , PSAG y θ^I (Hinman *et al.*, *Cancer Research* 53:3336-3342 (1993) y Lode *et al.*, *Cancer Research* 58: 2925- 2928 (1998)).

Toxinas enzimáticamente activas y sus fragmentos que pueden usarse incluyen cadena de difteria A, fragmentos activos no fijadores de toxina

difteria, cadena de exotoxina A (de *Pseudomonas aeruginosa*), cadena de ricina A, cadena de abrina A, cadena de modeccina A, alfa-sarcina, proteínas de *Aleurites fordii*, proteínas de diantina, proteínas de *Phytolaca americana* (PAPI, PAPII, y PAP-S), inhibidor de momordica charantia, curcina, crotina, inhibidor de sapaonaria officinalis, gelonina, mitogelina, restrictocina, fenomicina, enomicina y los tricotecenos. Véase, por ejemplo, WO 1993/21232 publicada el 28 de octubre de 1993.

La presente invención contempla igualmente un anticuerpo conjugado con un compuesto con actividad nucleolítica (*por ejemplo*, una ribonucleasa o una ADN endonucleasa tal como una deoxiribonucleasa; ADNasa).

Una variedad de isótopos radioactivos se encuentran disponibles para la producción de anticuerpos radioconjugados. Los ejemplos incluyen At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} e isótopos radioactivos de Lu.

Conjugados del anticuerpo y agente citotóxico pueden prepararse usando una variedad de agentes de acoplamiento de proteína bifuncionales tales como N-succinimidil-3-(2-piridilditiol)propionato (SPDP), succinimidil-4-(N-maleimidometil)ciclohexano-1-carboxilato, iminotiolano (IT), derivados bifuncionales de imidoésteres (tales como dimetil adipimidato HCL), ésteres activos (tales como disuccinimidil suberato), aldehídos (tales como glutareldehído), compuestos bis-azido (tales como bis (p-azidobenzoil)hexanidiamina), derivados de bis-diazonio (tales como bis-(p-diazoniobenzoil)-etilendiamina), diisocianatos (tales como tolieno 2,6-diisocianato), y compuestos de flúor bis-activo (tales como 1,5-difluoro-2,4-dinitrobenceno). Por ejemplo, una inmunotoxina de ricina puede prepararse como se describe en Vitetta *et al.*, *Science* 238: 1098 (1987). Ácido 1-isotiocianatobencil-3-metildietileno triaminapentaacético marcado con carbono¹⁴ (MX-DTPA) es un agente quelante ejemplar para conjugación de radionucleótido al anticuerpo. Véase WO 1994/11026. El conector puede ser un "conector segmentable" que facilite la liberación del medicamento citotóxico en la célula. Por ejemplo, puede emplearse un

conector lábil a los ácido, un conector sensible a peptidasa, un conector de dimetilo o un conector que contenga disulfuro (Chari *et al. Cancer Research* 52: 127-131 (1992)).

Alternativamente, una proteína de fusión que comprende el anticuerpo y el agente citotóxico puede elaborarse, *por ejemplo*, mediante técnicas recombinantes o síntesis peptídica.

En todavía otra incorporación, el anticuerpo puede conjugarse con un "receptor" (tal como estreptavidina) para utilización en el direccionamiento previo del tumor, en donde el conjugado anticuerpo-receptor se administra al sujeto, seguido por el retiro del conjugado no fijado de la circulación, usando un agente de depuración y, luego, por la administración de un "ligando" (*por ejemplo*, avidina) que se conjuga con un agente citotóxico (*por ejemplo*, un radionucleótido).

Los anticuerpos de la presente invención pueden conjugarse además con una enzima activante de profármaco, que convierte un profármaco (*por ejemplo*, un agente quimioterapéutico peptídico, véase WO 1981/01145) en un medicamento anti-neoplásico activo. Véase, por ejemplo, WO 1988/07378 y Patente de los Estados Unidos No. 4,975,278.

El componente enzimático de tales conjugados incluye cualquier enzima capaz de actuar sobre un profármaco de tal manera que lo convierte en su forma citotóxica más activa.

Las enzimas que son útiles en el método de esta invención incluyen, pero no se limitan a, fosfatasa alcalina útil para convertir profármacos que contienen fosfatasa en medicamentos libres; arilsulfatasa útil para convertir profármacos que contienen sulfato en medicamentos libres; citosina deaminasa útil para convertir 5-fluorocitosina no tóxica en el medicamento antineoplásico 5-fluorouracilo; proteasas, tales como serratia proteasa, termolisina, subtilisina, carboxipeptidasas (*por ejemplo*, carboxipeptidasas y catepsinas (tales como catepsinas B y L), que son útiles para convertir profármacos que contienen péptidos en medicamentos libres; D-alanilcarboxipeptidasas, útiles para convertir profármacos que contienen sustituyentes D-aminoácidos; enzimas de segmentación de carbohidratos

tales como β -galactosidasa y neuraminidasa útiles para convertir profármacos glucosilados en medicamentos libres; β -lactamasa útil para convertir medicamentos derivados con β -lactamas en medicamentos libres; y penicilina amidasas, tales como penicilina V amidasa o penicilina G amidasa, útiles para convertir medicamentos derivados en sus nitrógenos de amina con grupos fenoxiacetilo o fenilacetilo, respectivamente, en medicamentos libres. Alternativamente, anticuerpos con actividad enzimática, también conocidos en el arte como "abzimas" pueden emplearse para convertir los profármacos de la invención en medicamentos libres activos (véase, *por ejemplo*, Massey, *Nature* 328: 457-458 (1987)). Conjugados anticuerpo-abzima pueden prepararse como se describe aquí para entrega de la abzima a una población de células neoplásicas.

Las enzimas de esta invención pueden unirse covalentemente al anticuerpo mediante técnicas bien conocidas en el arte, tales como el uso de reactivos de entrecruzamiento heterobifuncional, ya discutidos. Alternativamente, proteínas de fusión que comprenden por lo menos la región de unión de antígeno de un anticuerpo de la invención, conectada a por lo menos una porción funcionalmente activa de una enzima de la invención, pueden construirse utilizando técnicas de ADN recombinante bien conocidas en el arte (véase, *por ejemplo*, Neuberger *et al.*, *Nature* 312: 604-608 (1984)).

Otras modificaciones del anticuerpo se contemplan en esta solicitud. Por ejemplo, el anticuerpo puede conectarse a uno de una variedad de polímeros no proteináceos, *por ejemplo*, polietilen glicol (PEG), polipropilen glicol, polioxialquilenos, o copolímeros de polietelen glicol y polipropilen glicol. Fragmentos de anticuerpo, tales como Fab', enlazados a una o más moléculas de PEG constituyen una incorporación especialmente preferida de la invención.

Los anticuerpos divulgados en esta solicitud pueden formularse además como liposomas. Los liposomas que contienen el anticuerpo se preparan por métodos conocidos en el arte, tales como los descritos en Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*,

Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); Patente de los Estados Unidos No. 4,485,045 y 4,544,545; y WO 1997/38731 publicada el 23 de octubre de 1997. Liposomas con tiempo de circulación mejorado se divulgan en la Patente de los Estados Unidos No. 5,013,556.

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

Se contempla la proteína de modificación (modificaciones) de secuencia de aminoácidos, o anticuerpos de péptido, descritas en esta solicitud. Por ejemplo, puede ser deseable mejorar la afinidad de unión y/o otras propiedades biológicas del anticuerpo. Variantes de secuencia de aminoácidos del anticuerpo se preparan introduciendo cambios apropiados de nucleótidos en el ácido nucleico del anticuerpo, o por síntesis peptídica. Tales modificaciones incluyen, por ejemplo, eliminaciones de, y/o inserciones en y/o sustituciones de, residuos dentro de las secuencias de aminoácidos del anticuerpo. Se realiza cualquier combinación de eliminación, inserción y sustitución para llegar al constructo final, siempre que el constructo final posea las características deseadas. Los cambios de aminoácidos pueden alterar asimismo procesos pos-traduccionales del anticuerpo, tales como cambiar el número o posición de sitios de glucosilación.

Un método útil para la identificación de ciertos residuos o regiones del anticuerpo que son sitios preferidos para la mutagénesis se denomina "mutagénesis dirigida de rastreo de alanina" [*"alanine-scanning mutagenesis"*] como se describe por Cunningham y Wells *Science*,

244:1081-1085 (1989). Aquí, se identifica un residuo o grupo de residuos diana (*por ejemplo*, residuos cargados tales como arg, asp, his, lys, y glu) y se reemplazan por un aminoácido neutro o cargado negativamente (muy preferiblemente alanina o polialanina) para afectar la interacción de los aminoácidos con antígeno. Aquellas localizaciones de aminoácidos, que demuestren sensibilidad funcional a las sustituciones, se refinan entonces introduciendo variantes adicionales u otras en, o para, los sitios de sustitución. Por lo tanto, aunque se predetermina el sitio para introducir una variación de secuencia de aminoácidos, la naturaleza de la mutación *per se* no necesita predeterminarse. Por ejemplo, para analizar el desempeño de una mutación en un sitio dado, la mutagénesis de rastreo *ala* o aleatoria se conduce en el codón o región diana, y las variantes del anticuerpo expresado se evalúan selectivamente con respecto a la actividad deseada.

Inserciones de secuencia de aminoácidos incluyen fusiones de terminal amino- y/o carboxilo, que varían en longitud desde un residuo hasta polipéptidos que contienen cien o más residuos, así como inserciones intrasecuencia de residuos de aminoácidos únicos o múltiples. Ejemplos de inserciones de terminal incluyen un anticuerpo con un residuo metionilo N-terminal o el anticuerpo fusionado a un polipéptido citotóxico. Otras variantes de inserción de la molécula del anticuerpo incluyen la fusión al término N o C del anticuerpo de una enzima, o un polipéptido que incrementa la semivida del anticuerpo.

Otro tipo de variante es una variante de sustitución de aminoácidos. Estas variantes tienen por lo menos un residuo de aminoácidos en la molécula del anticuerpo reemplazada por un residuo diferente. Los sitios de máximo interés para la mutagénesis de sustitución de anticuerpos de anticuerpo incluyen las regiones hipervariables, aunque se contemplan también alteraciones FR. Sustituciones conservadoras se muestran en la Tabla 3 bajo el encabezamiento de "sustituciones preferidas". Si tales sustituciones resultan en un cambio en la actividad biológica, entonces cambios más sustanciales, denominados "sustituciones ejemplares" en Tabla 3, o como se describe con mayor detalle más adelante con referencia

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a clases de aminoácidos, pueden introducirse y evaluarse selectivamente los productos.

Tabla 3

Residuo Original	Sustituciones Ejemplares	Sustituciones Preferidas
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucina	Leu
Leu (L)	Norleucina; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; 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; Norleucina	Leu

Modificaciones sustanciales en las propiedades biológicas del anticuerpo se llevan a cabo seleccionando sustituciones que difieren

significativamente en su efecto sobre el mantenimiento de (a) la estructura del esqueleto polipeptídico en el área de la substitución, por ejemplo, como una hoja o conformación helicoidal, (b) la carga de hidrofobicidad de la molécula en el sitio diana, o (c) la parte principal de la cadena lateral. Los aminoácidos pueden agruparse de acuerdo con similitudes en las propiedades de sus cadenas laterales (en: A.L. Lehninger, in *Biochemistry*, second ed., pp 73-75, Worth Publishers, New York (1975))

(1) no polares Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) polares sin carga: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y). Asn (N), Gln (Q)

(3) ácidos: Asp (D), Glu (E)

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

Alternativamente, residuos de ocurrencia natural se dividen en grupos basados en propiedades comunes de cadena lateral:

(1) hidrófobo: Norleucina, Met, Ala, Val, Leu, Ile;

(2) hidrófilo neutro: Cys, Ser, Thr, Asn, Gln;

(3) ácido: Asp, Glu;

(4) básico: His, Lys, Arg;

(5) residuos que influyen en la orientación de la cadena: Gly, Pro;

y

(6) aromático: Trp, Tyr, Phe.

Substituciones no conservadoras incluirán intercambiar un miembro de una de estas clases por otra clase.

Puede sustituirse además cualquier residuo de cisteína no involucrado en mantener la conformación apropiada del anticuerpo, generalmente con serina, para mejorar la estabilidad oxidativa de la molécula y prevenir entrelazamiento cruzado aberrante. Inversamente, enlace(s) de cisteína puede(n) agregarse al anticuerpo para mejorar sus estabilidad (particularmente donde el anticuerpo es un fragmento de anticuerpo tal como un fragmento Fv).

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Un tipo particularmente preferido de variante de sustitución involucra sustituir uno o más residuos de región hipervariable de un anticuerpo progenitor. Generalmente, la variante(s) resultante para el desarrollo posterior tendrá propiedades biológicas mejoradas relativas al anticuerpo progenitor, del cual se generan. Un modo conveniente para generar tales variantes de sustitución es maduración de afinidad usando la técnica exposición en fago. En síntesis, varios sitios de región hipervariable (*por ejemplo*, 6-7 sitios) se mutan para generar todas las sustituciones posibles de aminoácidos en cada sitio. Las variantes de anticuerpo así generadas se exponen en una manera monovalente de partículas de fago filamentoso como fusiones al producto del gen III de M13 empaquetado con cada partícula. Las variantes expuestas en fago se evalúan entonces selectivamente con respecto a su actividad biológica (*por ejemplo*, afinidad de unión), tal como se divulga en esta solicitud. Con el fin de identificar sitios candidatos de región hipervariable para modificación, puede realizarse mutagénesis dirigida de rastreo de alanina para identificar residuos de región hipervariable que contribuyen significativamente a la unión de antígeno. Alternativa, o adicionalmente, puede ser benéfico analizar una estructura cristalina del complejo antígeno-anticuerpo para identificar puntos de contacto entre el anticuerpo y el antígeno. Tales residuos de contacto y residuos contiguos son candidatos para sustitución de acuerdo con las técnicas elaboradas en esta solicitud. Una vez se generan tales variantes, el panel de variantes se somete a cribado ("*screening*"), como se describe en esta solicitud, y pueden seleccionarse para desarrollo posterior anticuerpos con propiedades superiores en uno o más análisis relevantes.

Otro tipo de variante de aminoácidos del anticuerpo altera el patrón de glucosilación original del anticuerpo. Tal alteración incluye delección de una o más fracciones carbohidrato, halladas en el anticuerpo, y/o agregar uno o más sitios de glucosilación que no están presentes en el anticuerpo.

La glucosilación de polipéptidos es típicamente N u O enlazada. N enlazada se refiere a la unión de la fracción carbohidrato a la cadena lateral

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de un residuo de asparagina. Las secuencias de tripéptido asparagina-X-serina y asparagina-X-treonina, donde X es cualquier aminoácido excepto prolina, son las secuencias de reconocimiento para unión enzimática de la fracción carbohidrato a la cadena lateral de asparagina. Por consiguiente, la presencia de cualquiera de estas secuencias de tripéptido en un polipéptido crea un sitio potencial de glucosilación. Glucosilación O enlazada se refiere a la unión de uno de los azúcares N-acetilgalactosamina, galactosa, o xilosa a un hidroxiaminoácido, muy comúnmente serina o treonina, si bien pueden usarse igualmente 5-hidroxiprolina o 5-hidroxilisina.

La adición de sitios de glucosilación al anticuerpo se lleva convenientemente a cabo alterando la secuencia de aminoácidos, de manera que contenga una o más de las secuencias de tripéptidos ya descritas (para sitios de glucosilación N enlazados). La alteración puede hacerse además por la adición de, o sustitución por, uno o más residuos de serina o treonina a la secuencia del anticuerpo original (para sitios de glucosilación O enlazados).

Donde el anticuerpo comprende una región Fc, el carbohidrato unido a ella puede alterarse. Por ejemplo, anticuerpos con una estructura carbohidrato madura que carece de fucosa unida a una región Fc del anticuerpo se describen en la Solicitud de Patente de los Estados Unidos No. US 2003/0157108 (Presta, L). Véase también US 2004/0093621 (Kyowa Hakko Kogyo Co, Ltd). Anticuerpos con una N-acetilglucosamina bisecante (GlcNAc) en el carbohidrato unido a una región Fc del anticuerpo se referencian en WO 2003/01 1878, Jean-Mairet *et al.*, y Patente de los Estados Unidos No. 6,602,684, Umana *et al.* Anticuerpos con por lo menos un residuo de galactosa en el oligosacárido unido a una región Fc del anticuerpo se divulgan en WO 1997/30087, Patel *et al.* Véase, además, WO 1998/58964 (Raju, S.) y WO 1999/22764 (Raju, S.) en lo concerniente a anticuerpos con un carbohidrato alterado a la región Fc del mismo. Véase también US 2005/0123546 (Umana *et al.*) con respecto a moléculas de unión a antígeno con glucosilación modificada.

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La variante de glucosilación preferida en esta solicitud comprende una región Fc, en donde una estructura carbohidrato unida a la región Fc carece de fucosa. Tales variantes han mejorado la función ADCC. Opcionalmente, la región Fc comprende además una o más sustituciones de aminoácidos en ella, las cuales mejoran aún más la ADCC, por ejemplo, sustituciones en las posiciones 298, 333, y/o 334 de la región Fc (numeración Eu de residuos). Ejemplos de publicaciones relacionadas con anticuerpos "defucosilados" o "deficientes en fucosa" incluyen: US 2003/0157108, WO 2000/61739, WO 2001/29246, US 2003/0115614, US 2002/0164328, US 2004/0093621, US 2004/0132140, US 2004/0110704, US 2004/0110282, US 2004/0109865, WO 2003/085119, WO 2003/084570, WO 2005/035586, WO 2005/035778, WO2005/053742, Okazaki *et al.* *J. Mol. Biol.* 336:1239-1249 (2004), Yamane-Ohnuki *et al.* *Biotech Bioeng* 87:614 (2004). Ejemplos de líneas de células que producen anticuerpos defucosilados incluyen células CHO Led 3 deficientes en fucosilación de proteína (Ripka *et al.* *Arch. Biochem. Biophys.* 249:533-545 (1986); Solicitud de Patente de los Estados Unidos No. US 2003/0157108 A1, Presta, L, y WO 2004/056312 A1, Adams *et al.*, especialmente en el Ejemplo 11), y líneas celulares con genes específicos inactivados ("*knockout cell lines*"), tales como el gen de α -1,6-fucosiltransferasa, *FUT8*, células CHO con genes específicos inactivados (Yamane-Ohnuki *et al.* *Biotech Bioeng* 87:614 (2004)).

Moléculas de ácido nucleico que codifican variantes de secuencia de aminoácidos del anticuerpo se preparan por una variedad de métodos conocidos en el arte. Estos métodos incluyen, pero no se limitan a, aislamiento de una fuente natural (en el caso de variantes de secuencia de aminoácidos de ocurrencia natural) o preparación por mutagénesis mediada por oligonucleótido (o dirigida al sitio), mutagénesis de PCR, y mutagénesis de casete de una variante previamente preparada o una versión no-variante del anticuerpo.

Puede ser deseable modificar el anticuerpo de la invención con respecto a la función efectora, *por ejemplo*, con el fin de intensificar la

citotoxicidad celular dependiente de antígeno (ADCC) y/o citotoxicidad dependiente de complemento (CDC) del anticuerpo. Esto puede lograrse introduciendo una o más sustituciones de aminoácidos en la región Fc de un anticuerpo. Alternativa o adicionalmente, residuo(s) de cisteína pueden introducirse en la región Fc, permitiendo así la formación de enlaces disulfuro intercadena en esta región. El anticuerpo homodimérico así generado puede tener una capacidad de internalización mejorada y/o creciente eliminación de células mediada por complemento y citotoxicidad celular dependiente de anticuerpo (ADCC). Véase Caron *et al.*, *J. Exp. Med.* 176:1191-1195 (1992) y Shopes, *J. Immunol.* 148: 2918-2922 (1992). Anticuerpos homodiméricos con actividad antineoplásica mejorada pueden prepararse también usando conectores cruzadores heterobifuncionales como se describe en Wolff *et al.*, *Cancer Research* 53: 2560-2565 (1993). Alternativamente, puede diseñarse un anticuerpo que tenga regiones Fc duales y, por ello, pueda tener lisis de complemento mejorada y capacidades ADCC. Véase Stevenson *et al.*, *Anti-Cancer Drug Design* 3:219-230 (1989).

WO 00/42072 (Presta, L) describe anticuerpos con función ADCC mejorada en la presencia de células efectoras humanas, donde los anticuerpos comprenden sustituciones de aminoácidos en la región Fc de los mismos. Preferiblemente, el anticuerpo con ADCC mejorada comprende sustituciones en las posiciones 298, 333, y/o 334 de la región Fc. Preferiblemente la región Fc alterada es una región Fc de la IgG1 humana que comprende o consta de sustituciones en una, dos o tres de estas posiciones.

Anticuerpos con unión C1q alterada y/o citotoxicidad dependiente de complemento (CDC) se describen en WO99/51642, Patente de los Estados Unidos No. 6,194,551B1, Patente de los Estados Unidos No. 6,242,195B1, Patente de los Estados Unidos No. 6,528,624B1 y Patente de los Estados Unidos No. 6,538,124 (Idusogie *et al.*). Los anticuerpos comprenden una sustitución de aminoácido en una o más posiciones de aminoácidos 270, 322, 326, 327, 329, 313, 333 y/o 334 de la región Fc de los mismos.

Para incrementar la semivida sérica del anticuerpo, se puede incorporar un epítipo de unión a receptor de salvamento al anticuerpo (especialmente un fragmento de anticuerpo) como se describe en la Patente de los Estados Unidos 5,739,277, por ejemplo. Tal como se emplea en esta solicitud, el término "epítipo de unión a receptor de salvamento" se refiere a un epítipo de la región Fc de una molécula IgG (*por ejemplo*, IgG₁, IgG₂, IgG₃, o IgG₄) que es responsable de aumentar la semivida sérica *in vivo* de la molécula IgG. Anticuerpos con sustituciones en una región Fc de los mismos y semividas incrementadas se describen también en WO00/42072 (Presta, L.).

También se contemplan anticuerpos genéticamente manipulados con tres o más (preferiblemente, cuatro) sitios de unión funcional a antígeno (Solicitud de los Estados Unidos No. US2002/0004587 A1, Miller *et al.*).

V. Formulaciones Farmacéuticas

Formulaciones terapéuticas de los anticuerpos usados de acuerdo con la presente invención se preparan para almacenamiento mezclando un anticuerpo que tenga el grado deseado de pureza, con vehículos, excipientes o estabilizantes opcionales, farmacéuticamente aceptables (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), en la forma de formulaciones liofilizadas o soluciones acuosas. Vehículos, excipientes, o estabilizantes aceptables son no tóxicos a los destinatarios en las posologías y concentraciones empleadas, e incluyen tampones tales como fosfato, citrato, y otros ácidos orgánicos; antioxidantes incluidos ácido ascórbico y metionina; preservantes (tales como cloruro de octadecildimetilbencil amonio; cloruro de hexametonio; cloruro de benzalconio, cloruro de benzetonio; fenol, alcohol butílico o bencílico; alquil parabenos tales como metil o propil parabeno; catecol; resorcinol; ciclohexanol; 3-pentanol; y m-cresol); polipéptidos de bajo peso molecular (menos de alrededor de 10 residuos); proteínas, tales como albúmina sérica, gelatina, o inmunoglobulinas; polímeros hidrófilos tales como polivinilpirrolidona; aminoácidos tales como glicina, glutamina, asparagina, histidina, arginina,

o lisina; monosacáridos, disacáridos, y otros carbohidratos incluidas glucosa, mannososa, o dextrinas; agentes quelantes tales como EDTA; azúcares tales como sacarosa, manitol, trehalosa o sorbitol; iones contrarios formadores de sal tales como sodio; complejos de metal (*por ejemplo*, complejos zinc-proteína); y/o surfactantes no iónicos tales como TWEEN™, PLURONICS™, o polietilen glicol (PEG).

Formulaciones ejemplares de anticuerpo anti-CD20 se describen en WO 1998/56418. Esta publicación describe una formulación líquida multidosis que comprende 40 mg/mL rituximab, 25 mM acetato, 150 mM trehalosa, 0.9% alcohol bencílico, y 0.02% polisorbato 20 con un pH 5.0 que tiene una vida útil mínima de dos años de almacenamiento a 2-8°C. Otra formulación anti-CD20 de interés comprende 10mg/mL rituximab en 9.0 mg/mL en cloruro de sodio, 7.35 mg/mL dihidrato de citrato sódico, 0.7mg/mL polysorbate™ 80, y agua esterilizada para inyección, pH 6.5.

Formulaciones liofilizadas adaptadas para administración subcutánea se describen en Patente de los Estados Unidos No. 6,267,958 (Andya *et al.*). Tales formulaciones liofilizadas pueden reconstituirse con diluyente apropiado a una concentración con contenido elevado de proteína, y la formulación reconstituida puede administrarse subcutáneamente al mamífero a tratarse en esta solicitud.

También se contemplan formas cristalizadas del anticuerpo. Véase, por ejemplo, US 2002/0136719A1 (Shenoy *et al.*).

La formulación en esta solicitud puede contener asimismo más de un compuesto activo (un segundo medicamento, como se anota anteriormente), según sea necesario, preferiblemente aquellos con actividades complementarias que no se afectan negativamente entre sí. El tipo y cantidades efectivas de tales medicamentos dependen, por ejemplo, de la cantidad de anticuerpo presente en la formulación, así como de parámetros clínicos de los sujetos. Los medicamentos preferidos semejantes se anotan más arriba.

En igual forma los ingredientes activos pueden atraparse en microcápsulas preparadas, por ejemplo, por técnicas de aglomeración o por

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polimerización interfacial, por ejemplo, hidroximetilcelulosa o microcápsulas de gelatina y microcápsulas de poli-(metilmetacilato), respectivamente, en sistemas coloidales de liberación de medicamentos (por ejemplo, liposomas, microesferas de albúmina, microemulsiones, nanopartículas y nanocápsulas) o en macroemulsiones. Tales técnicas se divulgan en *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Pueden prepararse preparaciones de liberación sostenida. Ejemplos apropiados de preparaciones de liberación sostenida incluyen matrices semipermeables de polímeros hidrófobos sólidos que contienen el anticuerpo, matrices que están en la forma de artículos moldeados, *por ejemplo*, películas, o microcápsulas. Ejemplos de matrices de liberación sostenida incluyen poliésteres, hidrogeles (por ejemplo, poli(2-hidroxietilmetacrilato), o poli(vinilalcohol)), poliláctidos (Patente de los Estados Unidos No. 3,773,919), copolímeros de ácido L-glutámico y γ -etil-L-glutamato, etilen vinil acetato, no degradable, copolímeros degradables de ácido láctico-ácido glicólico tales como los LUPRONDEPOT™ (microesferas inyectables compuestas de copolímero de ácido láctico-ácido glicólico y acetato de leuprolida), y ácido poli-D-(-)-3-hidroxibutírico.

Las formulaciones a utilizarse para administración *in vivo* tienen que ser estériles. Esto se logra fácilmente por filtración a través de membranas de filtración estériles.

VI. Artículos de Fabricación

En otra incorporación de la invención, se provee un artículo de fabricación que contiene materiales útiles para el tratamiento de vasculitis asociada con ANCA, descrita anteriormente. En un aspecto, el artículo de fabricación comprende (a) un envase que contiene un antagonista que se une a un marcador de superficie de linfocito B (*por ejemplo*, un anticuerpo se une en esa forma, inclusive un anticuerpo CD20) (preferiblemente el envase comprende el antagonista o anticuerpo y un excipiente o diluyente farmacéuticamente aceptables en el envase), y (b) un prospecto de envase

con instrucciones para tratar vasculitis asociada con ANCA en un paciente, en donde las instrucciones indican que una dosis del antagonista o anticuerpo de alrededor de 400 mg a 1.3 gramos, con una frecuencia de una a tres dosis, se administra al paciente en un período de alrededor de un mes.

Por consiguiente, la invención proporciona un artículo de fabricación que comprende un envase, el cual contiene un anticuerpo CD20, o un anticuerpo o antagonista que se fija a un marcador de superficie de linfocito B, y un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un paciente, en donde las instrucciones indican que una dosis del anticuerpo CD20, o el antagonista o anticuerpo, que se une al marcador de superficie de linfocito B, de alrededor de 400 mg a 1.3 gramos, con una frecuencia de una a tres dosis, se administra al paciente en un período de alrededor de un mes.

En una incorporación preferida el artículo de fabricación, contemplado en esta solicitud, comprende además un envase que contiene un segundo medicamento, en donde el antagonista o anticuerpo es un primer medicamento. Este artículo comprende además instrucciones en el prospecto de envase para tratar al paciente con el segundo medicamento, en una cantidad efectiva. El segundo medicamento puede ser cualquiera de los expuestos anteriormente, siendo el segundo medicamento ejemplar un agente quimioterapéutico, un agente inmunosupresor, un agente citotóxico, un antagonista de integrina, un antagonista de citocina, o una hormona. Los segundos medicamentos preferidos son aquellos expuestos más arriba y, muy preferido es un esteroide o un agente inmunosupresor o ambos.

En otro aspecto, la invención proporciona un artículo de fabricación que comprende, (a) un envase que contiene un anticuerpo que se une a un marcador de superficie de linfocito B (*por ejemplo*, un anticuerpo CD20) (preferiblemente el envase contiene el anticuerpo y un excipiente o diluyente farmacéuticamente aceptables en el envase); y (b) un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que una cantidad del anticuerpo se administra al sujeto que es efectiva para proporcionar una exposición

inicial a anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se provee hasta entre alrededor de 16 a 54 semanas a partir de la exposición inicial.

Preferiblemente, tal prospecto de envase se proporciona con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que una cantidad del anticuerpo se administra al sujeto, la cual es efectiva para proporcionar una exposición inicial a anticuerpo de alrededor de 0.5 a 4 gramos, seguida por una segunda exposición a anticuerpo de alrededor de 0.5 a 4 gramos, en donde la segunda exposición no se proporciona hasta entre alrededor de 16 a 54 semanas a partir de la exposición inicial y cada una de las exposiciones a anticuerpo se provee al sujeto como alrededor de una a cuatro dosis, preferiblemente como una dosis única o como dos o tres dosis separadas de anticuerpo.

En un aspecto específico, se provee un artículo de fabricación que comprende:

(a) un envase que contiene un anticuerpo que se une a un marcador de superficie de linfocito B (*por ejemplo*, un anticuerpo CD20) (preferiblemente el envase contiene el anticuerpo y un excipiente o diluyente farmacéuticamente aceptables en el envase), y

(b) un prospecto de envase con instrucciones para tratar vasculitis asociada con ANCA en un sujeto, en donde las instrucciones indican que una cantidad del anticuerpo se administra al sujeto, la cual es efectiva para proporcionar una exposición inicial a anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se proporciona hasta entre alrededor de 16 a 54 semanas a partir de la exposición inicial y cada una de las exposiciones a anticuerpo se provee al sujeto como alrededor de una a cuatro dosis, preferiblemente como una dosis única o como dos o tres dosis separadas de anticuerpo. Preferiblemente, las exposiciones a anticuerpo son de alrededor de 0.5 a 4 gramos.

En una incorporación preferida de estos aspectos inventivos, el artículo de fabricación, contemplado en esta solicitud, comprende además

un envase que contiene un segundo medicamento, en donde el anticuerpo es un primer medicamento, y el cual artículo comprende además instrucciones en el prospecto de envase para tratar al sujeto con el segundo medicamento, en una cantidad efectiva. El segundo medicamento puede ser cualquiera de los expuestos anteriormente, siendo un segundo medicamento ejemplar un agente quimioterapéutico, un agente inmunosupresor, un agente citotóxico, un antagonista de integrina, un antagonista de citocina, o una hormona, muy preferiblemente un esteroide o un agente inmunosupresor, o ambos.

En la totalidad de estos aspectos, el prospecto de envase está sobre, o asociado, el envase. Envases apropiados incluyen, por ejemplo, frascos, ampollitas, jeringas, etc. Los envases pueden formarse de una variedad de materiales tales como vidrio o plástico. El envase aloja o contiene una composición que es efectiva para tratar la vasculitis asociada con ANCA y puede tener un puerto de acceso estéril (por ejemplo, el envase puede ser una bolsa de solución intravenosa o un frasquito con un tapón perforable por una aguja hipodérmica). Por lo menos un agente activo en la composición es el antagonista o anticuerpo. La etiqueta o prospecto de envase indica que la composición se emplea para tratar vasculitis asociada con ANCA en un paciente o sujeto elegible para tratamiento con instrucciones específicas respecto a las cantidades de dosificación e intervalos de antagonista o anticuerpo y cualquier otro medicamento que se esté proporcionando. El artículo de fabricación puede comprender además un envase adicional que comprende un diluyente amortiguador farmacéuticamente aceptable, tal como agua bacteriostática para inyección (BWFI), solución salina amortiguada con fosfato, solución salina de Ringer, y/o solución de dextrosa. El artículo de fabricación puede incluir además otros materiales deseables desde un punto de vista comercial y del consumidor, incluidos otros amortiguadores, diluyentes, filtros, agujas y jeringas.

Otros detalles de la invención se ilustran por los siguientes Ejemplos no limitantes. Las divulgaciones de todas las citas en la especificación se incorporan expresamente en esta solicitud por referencia.

Ejemplo 1

Estudio de la Eficacia de Rituximab en Pacientes con Granulomatosis de Wegener

Este estudio evalúa la superioridad de la eficacia y seguridad de rituximab (MABTHERA®/RITUXAN®) en un cierto régimen posológico, comparado con placebo para el tratamiento de signos y síntomas en pacientes con granulomatosis de Wegener, que exhiben uno o más síntomas de enfermedad sistémica.

Rituximab (1000 mg i.v. x 2) se administra i.v. en dos dosis iniciales los días 1 y 15 con 1 mg/kg de prednisona oral diariamente (que se reduce a 40 mg/día en la semana 4, y se reduce progresivamente usando un régimen estandarizado de reducción gradual, resultante en una discontinuación completa de la prednisona durante los siguientes 3-5 meses). Este régimen experimental se compara con el mismo régimen excepto que se usa placebo de rituximab en lugar de rituximab, con aleatorización 1:1 entre las dos ramas del estudio, con alrededor de 48 pacientes por rama (total 96 pacientes). La enfermedad activa se define como un Puntaje de Actividad de Vasculitis Birmingham/Granulomatosis de Wegener (BVAS/WG) de más de 0. Para inclusión en el estudio clínico, el puntaje BVAS/WG del paciente tiene que ser 3 o mayor (o haber sido 3 o mayor en 28 días de aleatorización). Cada episodio principal en la forma de evaluación BVAS/WG se califica con 3 puntos. Cada episodio menor se califica con 1 punto. Al determinar el grado de actividad patológica, el investigador distinguirá entre vasculitis activa (episodios BVAS/WG nuevos/peores, por oposición a episodios persistentes) y lesión permanente a un órgano (causada por vasculitis previamente activa).

Una exacerbación repentina intensa es una nueva ocurrencia de uno o más de los episodios BVAS/WG principales. (Los episodios importantes tienen un * en la hoja de puntaje BVAS/WG). Generalmente, tales

exacerbaciones repentinas se tratan por aumentos en la dosis de prednisona o la dosis de ciclofosfamida.

La granulomatosis grave de Wegener ocurre en un paciente cuya enfermedad no es clasificable como limitada, por la siguiente definición.

Una exacerbación repentina limitada es una nueva ocurrencia de uno o más episodios BVAS/WG menores. Generalmente, tales exacerbaciones repentinas se tratan con incrementos en la dosis de prednisona o una dosis de metotrexato.

La granulomatosis limitada de Wegener ocurre en un paciente que satisface los criterios modificados del American College of Rheumatology (ACR) para el diagnóstico de la granulomatosis de Wegener, pero que no tiene una enfermedad que represente una amenaza inmediata a un órgano individual crítico a o la vida del paciente. Específicamente, esto significa que:

- El paciente no tiene cilindros eritrocíticos ("*red blood cell casts*") en la orina.
- Si hay presencia de hematuria (pero no de + cilindros RBC), la creatinina sérica tiene que ser menos de o igual a 1.4 y tiene que haber evidencia de no aumento de creatinina superior al 25% por encima de la línea de referencia del paciente.
- El compromiso pulmonar tiene que estar circunscrito, de modo que el pO_2 del aire de la sala sea > 70 mmHg o la saturación de O_2 del aire de la sala por oxitometría de pulsación sea $> 92\%$. La hemorragia pulmonar puede tratarse como enfermedad limitada, siempre que no haya evidencia de la evolución del proceso. En ausencia de datos sobre la evolución, la hemorragia pulmonar puede tratarse como enfermedad grave a discreción del médico.
- No puede existir enfermedad en cualquier otro órgano crítico (*por ejemplo*, el tubo digestivo, ojos, sistema nerviosos central) que, sin la institución inmediata de una terapia máxima (*es decir*, metilprednisolona con pulsación y ciclofosfamida oral diaria), que amenace la función de ese órgano y/o la vida del paciente.

Un paciente recién diagnosticado es un paciente en su primer ciclo de tratamiento de corticoesteroides y/o un agente quimioterapéutico o inmunosupresor para la granulomatosis de Wegener, sin historia de aumento en la terapia inmunosupresora antes del ingreso al estudio.

Para el propósito de calificar el BVAS/WG, la enfermedad persistente se define como la presencia de actividad patológica continua, que estuvo presente en la evaluación del estudio clínico previo (*es decir*, actividad no nueva o peor).

Una prueba cutánea derivada con proteína purificada puede emplearse para detectar infección por tuberculosis latente.

Un paciente resistente a tratamiento es un paciente con una historia de terapia inmunosupresora (corticoesteroides y/o un agente inmunosupresor o agente quimioterapéutico) antes de la iniciación del tratamiento para la actividad de granulomatosis de Wegener, que hace al paciente elegible para este estudio.

Se considerará que los pacientes están en remisión cuando su puntaje BVAS/WG es 0.

El régimen a base de rituximab desafía el estándar corriente de atención médica, y tiene como fin limitar la exposición del paciente a esteroides y sus toxicidades conocidas, y demostrar el beneficio clínico neto mejorado. Se monitorea a los pacientes con respecto a la actividad patológica, uso de inmunosupresores adicionales, utilización de esteroides, y acontecimientos de seguridad en la duración del estudio clínico de un año, midiéndose el punto final de eficacia primaria del estudio clínico en 3 meses, con seguimiento hasta 16.8 meses. Se requiere el monitoreo de la seguridad hasta 12 meses después de la última dosis de rituximab o retorno de ANCA al rango normal, lo que ocurra más tarde.

El objetivo primario es determinar la proporción de pacientes que logran un puntaje BVAS/WG de 0 y disminución gradual exitosa de la prednisona en 6 meses, y sin efectos adversos especificados previamente.

Se predice y se espera que el rituximab (o un 2H7 humanizado en sustitución de rituximab) es efectivo en inducir la remisión (alcanzar

puntajes BVAS/WG de 0) en por lo menos 80% de los pacientes inscritos con granulomatosis de Wegener y permitir una reducción en las dosis de esteroides sobre la rama de control. Se espera que el BVAS/WG disminuya desde el punta al momento del ingreso a alrededor de 0.2 a 0.4 en la semana 14. Se espera que la proteína C-reactiva (mg/L) disminuya desde el nivel al momento de ingreso a un rango de alrededor de 3 a 1.1 en la semana 14. Se espera que la dosis media de prednisolona (mg/día) disminuya desde el valor al momento del ingreso a un valor más bajo, estadísticamente significativo, en la semana 14. Se espera que ocurra una recaída en poco menos de cinco pacientes después de una media de 27 semanas. Si el BVAS/WG medio en el momento del ingreso es 3.6, a 6 meses del tratamiento, se espera que disminuya a un valor estadísticamente significativo de 0.6. Se espera que la enfermedad de actividad intermitente se observe en poco menos de 70% de los pacientes. En contraste, se espera que la rama de control muestre mucho menos disminución en BVAS/WG y proteína C-reactiva, y en el uso de esteroides, y menos pacientes en remisión.

Ejemplo 2

Estudio de la Eficacia del Rituximab en Pacientes con Polivascularitis Microscópica

Se sigue el protocolo en el Ejemplo 1, excepto que los pacientes se tratan para polivascularitis microscópica. Se espera que se observarán resultados similares a los de la granulomatosis de Wegener, *por ejemplo*, se espera que la remisión, medida por un puntaje BVAS/WG de 0, ocurra en por lo menos 80% de los pacientes tratados en la rama del estudio, y se espera que el uso de esteroides disminuya durante el curso del estudio, resultados que se espera que sean mucho mejores, en un sentido estadísticamente significativo, que los resultados del control.

Ejemplo 3

Estudio con Re-tratamiento de la Eficacia del Rituximab en Pacientes con Granulomatosis de Wegener

Este estudio evalúa la superioridad de la eficacia y la seguridad del re-tratamiento con rituximab (MABTHERA®/RITUXAN®), comparado con placebo en sujetos adultos con granulomatosis de Wegener. El estudio I examina la enfermedad aguda, ya sea primera presentación o recaída (BVAS ≥ 4 ; $n = 16$); estudio II examina la enfermedad persistente (BVAS ≥ 4 ; $n = 16$). Los pacientes recibieron rituximab (1 g i.v.) en tres dosis iniciales los días 1, 8, y 15 para los estudios I y II. La terapia concomitante en estudio I incluye 1 mg/kg/día de prednisona oral reducida gradualmente de acuerdo con el régimen en Ejemplo 1 y ciclofosfamida (de acuerdo con el tratamiento estándar). Los pacientes del estudio II recibieron rituximab y 1 mg/kg/día de prednisona oral reducida gradualmente de acuerdo con el régimen en Ejemplo 1. Todos los sujetos recibieron un segundo curso de infusión de placebo de rituximab de 1.000 mg i.v. separados por 14 días en las semanas 24 y 26, respectivamente, sin esteroides o ciclofosfamida, ya sea que los pacientes exhibieran síntomas o estuvieran en remisión completa. Los ciclos de tratamiento con rituximab tienen que estar separados por un intervalo mínimo de 16 semanas.

Los regímenes experimentales se comparan con placebo de rituximab + las mismas dosis de prednisona oral reducida gradualmente y ciclofosfamida (estudio I), o prednisona oral reducida gradualmente (estudio II).

Durante los estudios no se permiten cambios en los medicamentos inmunosupresores, a menos que lo exija la citotoxicidad, y las solicitudes de reducir gradualmente un fármaco distinto de la prednisona oral tienen que discutirse por adelantado con el monitor médico. El personal del estudio tiene que estar entrenado sobre la forma de administrar adecuadamente el rituximab. Los sujetos pueden hospitalizarse para observación, particularmente para su primera infusión, a la discreción del investigador.

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El rituximab tiene que administrarse bajo estricta supervisión, y tienen que estar disponibles equipos completos de resucitación.

Los pacientes se monitorean cada mes durante 12 meses con respecto a actividad patológica, uso de inmunosupresores adicional, exacerbaciones repentinas de la enfermedad, uso de prednisona, y acontecimientos de seguridad durante las 52 semanas del estudio. El punto final de eficacia primaria del estudio clínico es en 52 semanas, y las mediciones de eficacia se evalúan por un solo asesor de examen, quien no tiene nada que ver con el tratamiento de pacientes u otros procedimientos del estudio. Los pacientes se examinan con respecto a sus puntajes BVAS/WG y reducción gradual exitosa de prednisona. Al final de las 52 semanas, los sujetos que recibieron placebo de rituximab o rituximab, pero demostraron un puntaje BVAS/WG de 0 y reducción gradual exitosa de prednisona a los 6 meses completarán la participación en el estudio. Los sujetos que recibieron rituximab, pero no habían demostrado tal puntaje a las 52 semanas, se observaron durante 6 meses luego del último ciclo de rituximab o hasta un puntaje BVAS/WG de 0, lo que ocurra primero. Se informará a los centros en cuanto a si un sujeto tiene que continuar en monitoreo, pero no si el sujeto recibió placebo o rituximab. Se requiere el monitoreo de seguridad hasta 12 meses, luego de la última dosis de rituximab o un puntaje BVAS/WG de 0, el que ocurra más tarde.

Los regímenes a base de rituximab desafían el estándar corriente de atención médica, y se espera que demuestren un beneficio clínico neto mejorado, con el objetivo primario de determinar la proporción de pacientes que logran el punto final primario de un puntaje BVAS/WG de 0, y reducción gradual exitosa de prednisona.

Se predice y se espera que la administración de rituximab o un 2H7 humanizado al sujeto en los protocolos de estudios I y II ya expuestos inducirá la remisión (alcanzando puntajes BVAS/WG de 0) en por lo menos 80% de los pacientes inscritos con granulomatosis de Wegener y permitirá la reducción de dosis de esteroides en la rama de control. Se espera que BVAS/WG disminuya desde el puntaje en el momento del ingreso a

alrededor de 0.2 a 0.4 en la semana 14. Se espera que la proteína C-reactiva (mg/L) disminuya desde el nivel en el momento del ingreso a un rango de alrededor de 3 a 11 en la semana 14. Se espera que el uso medio de esteroides para los dos estudios I y II disminuya desde el valor en el momento del ingreso a un valor más bajo estadísticamente significativo en la semana 14. Se entiende que la recaída ocurra en menos de cinco pacientes luego de una media de 27 semanas. Se espera que estos resultados sean significativamente mejores que los de las ramas de control para los estudios I y II.

Se espera igualmente que alrededor de la semana 48-54, otra dosis de 2 g del rituximab administrado de una sola vez o repartido durante alrededor de 14-16 días en cantidades de 1 gramo sea efectiva para tratar la granulomatosis de Wegener durante el segundo año completo (causando un puntaje BVAS/WG de 0 en por lo menos 80% de los pacientes inscritos), con o sin el esteroide(s) y/u otros agentes inmunosupresores, con una mejora marcada sobre los pacientes de control que recibían placebo de rituximab en lugar de rituximab. En consecuencia, el rituximab (o un 2H7 humanizado) se administraría inicialmente en alrededor del período de 2 semanas, seguido por otro tratamiento en alrededor de 4-8 meses, seguido por otro tratamiento en alrededor de un año, a partir del tratamiento inicial (medios desde el momento en que se aplicó una cualquiera de las dosis), seguido por el tratamiento en alrededor de dos años desde el tratamiento inicial, con éxito esperado, en alrededor de 1 gramo x 2-4 dosis para cada tratamiento, administradas juntas, alrededor de semanalmente, o alrededor de cada otra semana durante alrededor de dos a cuatro semanas. Se espera que este protocolo de re-tratamiento se emplee exitosamente durante varios años con pocos o ningún efecto adverso.

Ejemplo 4

Segundo Estudio con Re-tratamiento de la Eficacia de Rituximab en Pacientes con Granulomatosis de Wegener

Este estudio es el mismo que en Ejemplo 3, excepto que la dosis inicial de rituximab o placebo de rituximab se administra como 1.000 mg i.v. x 2 (el día 0, ocurriendo la segunda infusión el día 15 +/- 1 día), y el ciclo subsiguiente de infusiones de rituximab o placebo, administradas en las semanas 24 y 26 y constando de 2 dosis bisemanales, se administra únicamente a aquellos sujetos en remisión, *por ejemplo*, aquellos que no exhiben una actividad patológica cada vez mayor, como concentraciones ANCA crecientes, concentraciones ANCA elevadas sostenidas, y otros síntomas. Todos los demás criterios son los mismos.

Se predice y se espera que la administración de rituximab o un 2H7 humanizado al sujeto en el protocolo de redosificación programada ya expuesto será efectivo en inducir la remisión (alcanzando puntajes BVAS/WG de 0) en por lo menos 80% de los pacientes inscritos con granulomatosis de Wegener en permitir la reducción en dosis de esteroides en el la rama de control. Se espera que el BVAS/WG disminuya desde el puntaje en el momento del ingreso a alrededor de 0.2 a 0.4 en la semana 14. Se espera que la proteína C-reactiva (mg/L) disminuya desde el nivel al momento del ingreso a un rango de alrededor de 3 a 11 en la semana 14. Se espera que la dosis media de prednisolona (mg/día) disminuya desde el valor en el momento del ingreso a un valor más bajo, estadísticamente significativo, en la semana 14. Se espera que ocurra recaída en poco menos de cinco pacientes luego de una media de 27 semanas. Se espera que estos resultados sean significativamente mejores que los del control.

Se espera asimismo que a alrededor de las semanas 48-54, otra dosis de 2 g del anticuerpo CD20 (*por ejemplo*, rituximab o un 2H7 humanizado), aplicada de una sola vez o repartida durante alrededor de 14-16 días en cantidades de 1 gramo, será efectiva para tratar granulomatosis de Wegener durante todo el segundo año (teniendo por lo menos 80% de los pacientes inscritos un puntaje BVAS/WG de 0), con o sin la reducción gradual de prednisona y metilprednisolona i.v. y/u otros agentes inmunosupresoras. Por consiguiente, el anticuerpo CD20 se administrará inicialmente en el período de alrededor de 2 semanas, seguido por otro tratamiento en

alrededor de 4-8 meses, seguido por otro tratamiento de alrededor de un año desde el tratamiento inicial (medido desde el momento en que se aplicó una cualquiera de las dosis), seguido por tratamiento en alrededor de dos años desde el tratamiento inicial, con éxito esperado, en una dosificación de alrededor de dosis de 1 gramo x 2-4 para cada tratamiento, administrada conjuntamente, alrededor de semanalmente, o alrededor una semana de por medio durante alrededor de dos a cuatro semanas. Se espera que los resultados de este tratamiento sean mucho mejores que aquellos de los del control con placebo. Se espera que este protocolo de re-tratamiento se utilice exitosamente durante varios años con pocos o ningún efecto adverso.

Ejemplo 5

Tercer Estudio con Re-tratamiento de la Eficacia de Rituximab en Pacientes con Granulomatosis de Wegener

Se espera que los resultados de Ejemplo 4 serán exitosos si los pacientes se tratan inicialmente con rituximab y luego de volvieren a tratar con rituximab un año después de ser tratados por primera vez, usando la misma posología y el otro protocolo de Ejemplo 4, excepto que el rituximab se administra en intervalos de un año, en lugar de intervalos de seis meses.

Ejemplo 6

Estudio de la Eficacia del Rituximab en Sujetos con Vasculitis Generalizada Asociada con ANCA

Se realizó un estudio clínico aleatorizado, multi-centro, doblemente enmascarado, controlado con placebo, en pacientes con vasculitis generalizada asociada con ANCA, usando rituximab. Doscientos pacientes se reparten aleatoriamente a (1) tratamiento convencional (ciclofosfamida y corticoesteroides, seguidos por azatioprina), o (2) rituximab (más corticoesteroides, inicialmente) para inducción de la remisión, usando 1 gramo de rituximab el día 1 y nuevamente el día 15.

La comparación clínica primaria es la capacidad del rituximab en este régimen posológico y los corticoesteroides de inducir remisiones de la enfermedad, medida por la actividad patológica acumulada en seis meses. Consistente con la duración estándar de tratamiento para vasculitis asociada con ANCA, los pacientes en la rama de terapia convencional recibirán ciclofosfamida hasta durante 6 meses, seguida por azatioprina, para completar una duración total de tratamiento de 18 meses. Para evaluar la capacidad del rituximab de restaurar la tolerancia de los linfocitos B, los pacientes en ambas ramas del estudio clínico serán monitoreados durante un total de 18 meses.

Se espera que el rituximab (o un 2H7 humanizado sustituido en lugar del rituximab) induzca remisiones estables en los pacientes con vasculitis asociada con ANCA y restablezca la tolerancia de los linfocitos B a antígenos diana ANCA en por lo menos dos terceras partes de los pacientes. Se espera asimismo que el rituximab u otro anticuerpo CD20 sea por lo menos tan efectivo como el régimen de tratamiento convencional para la inducción y el mantenimiento de la remisión de la enfermedad, ofreciendo ventajas sustanciales sobre la terapia estándar en virtud de su perfil superior de efectos secundarios, *por ejemplo*, mucho menos tóxico que los quimioterapéuticos y esteroides, y mejor en tolerancia restauradora.

Ejemplo 7

Estudio con Re-tratamiento de la Eficacia de Rituximab en Sujetos con Granulomatosis Grave de Wegener o Polivasculitis Microscópica Grave

Se inscriben 20 pacientes con granulomatosis de Wegener grave y activa o polivasculitis microscópica grave, prueba de ANCA positiva, y puntaje BVAS/WG de por lo menos 3, que no son sensibles a ciclofosfamida o tienen contraindicaciones para el uso de ciclofosfamida. Véase la definición de granulomatosis de Wegener grave en Ejemplo 1. El régimen de inducción de la remisión consta de prednisona oral (1

mg/kg/día) y rituximab (1 gramo el día 1 y 1 gramo el día 15). En la semana 4, la prednisona se reduce a 40 mg/día. Sigue un régimen estandarizado de disminución gradual, resultante en la completa discontinuación de la prednisona en las siguientes 16 semanas. Esto se compara con el mismo régimen, pero con placebo de rituximab en lugar de rituximab (estudio de control). El protocolo estipula re-tratamiento con el mismo régimen de inducción de la remisión a los 6 meses para todos los pacientes, sea que estén experimentando una exacerbación repentina de la enfermedad luego de la reconstitución de linfocitos B, ya sea que sean asintomáticos con una recurrencia de ANCA o aumento en la concentración de ANCA coincidente con, o subsiguiente a, la reconstitución de linfocitos B, o que se encuentren en completa remisión. Este régimen de re-tratamiento incluye la dosis de 1 g x 2 dos semanas separadas para rituximab y placebo de rituximab. Una exacerbación repentina clínica en la ausencia de linfocitos B se considera fracaso del tratamiento. Los pacientes se evalúan mensualmente durante un año.

Se espera que los pacientes en la rama de tratamiento tolerarán bien las infusiones de rituximab y que sus linfocitos B se agotarán rápidamente y todos lograrán una remisión completa (BVAS/WG de 0) en tres meses. Se espera que todos los pacientes en la rama de tratamiento completen la reducción gradual de glucocorticoide en 6 meses. Se espera que después de 12 meses, ningún paciente en la rama de tratamiento experimente una exacerbación repentina clínica, y que los linfocitos B regresen en la mayoría de, si no en todos, los pacientes en los 12 meses. A diferencia de los glucocorticoides, se espera que no sean necesarios agentes inmunosupresores adicionales para la inducción de la remisión y el mantenimiento de la remisión sostenida (6 meses o más) en los pacientes tratados con rituximab.

Ejemplo 8

Variantes de 2H7 Humanizado Útiles en esta Solicitud

Útiles para los propósitos contemplados aquí son los anticuerpos 2H7 humanizados que comprenden una, dos, tres, cuatro, cinco, o seis de las siguientes secuencias de CDR.

Secuencia CDR L1 RASSSVSYXH en donde X es M o L (SEQ ID NO:35), por ejemplo, SEQ ID NO:4 (Fig 1A),
secuencia CDR L2 de SEQ ID NO:5 (Fig 1A),
secuencia CDR L3 QQWXFNPPT en donde X es S o A (SEQ ID NO:36), por ejemplo, SEQ ID NO:6 (Fig 1A),
secuencia CDR H1 de SEQ ID NO:10 (Fig 1B),
secuencia CDR H2 de AIYPGNGXTSYNQKFKG en donde X es D o A (SEQ ID NO:37), por ejemplo, SEQ ID NO:11 (Fig 1B), y
secuencia CDR H3 de VVYYSXXYWFYFDV en donde la X en la posición 6 es N, A, Y, W, o D, y la X en la posición 7 es S o R (SEQ ID NO:38), por ejemplo, SEQ ID NO:12 (Fig 1B).

Los anticuerpos 2H7 humanizados contemplados en esta solicitud incluyen aquellos con secuencias de aminoácidos de de cadena pesada, que contienen una lisina C-terminal, y aquellos sin ella. Las anteriores secuencias CDR están generalmente presentes en secuencias de estructura alterna humanas pesadas variables y ligeras variables, tales como sustancialmente los residuos FR de consenso humano del subgrupo I kappa humano de cadena ligera (V_LKI), y sustancialmente los residuos FR de consenso humano del subgrupo III humano de cadena pesada (V_HIII). Véase también WO 2004/056312 (Lowman *et al.*).

La región pesada variable puede unirse a una región constante de cadena IgG humana, en donde la región puede ser, por ejemplo, IgG1 o IgG3, incluidas regiones constantes de secuencia nativa y secuencia no nativa.

En una incorporación preferida, tal anticuerpo comprende la secuencia de dominio pesado variable de SEQ ID NO:8 (v16, como se muestra en Fig 1B), que comprende opcionalmente también la secuencia de dominio ligero variable de SEQ ID NO:2 (v16, como se muestra en Fig 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 ligero variable. Preferiblemente, el anticuerpo es un anticuerpo intacto que comprende la secuencia de aminoácidos de cadena ligera de SEQ ID NO:13 ó 30, y la secuencia de aminoácidos de cadena pesada de SEQ ID NO:14, 15, 29, 31, 34, ó 39, dándose a continuación la secuencia de SEQ ID NO:39.

Un anticuerpo 2H7 humanizado preferido es ocrelizumab (Genentech, Inc).

El anticuerpo contemplado en esta solicitud puede comprender además por lo 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, usando la numeración Eu de residuos de cadena pesada. Véase también la Patente de los Estados Unidos No 6,737,056, L Presta.

Cualquiera de estos anticuerpos puede comprender por lo menos una sustitución en la región Fc que mejore la unión FcRn o la semivida sérica, *por ejemplo*, una sustitución en la posición de cadena pesada 434, tal como N434W. Véase también la Patente de los Estados Unidos No 6,737,056, L Presta.

Cualquiera de estos anticuerpos puede comprender además por lo menos una sustitución de aminoácido en la región Fc, que incrementa la actividad CDC, *por ejemplo*, que comprende por lo menos una sustitución en la posición 326, preferiblemente K326A o K326W. Véase igualmente la Patente de los Estados Unidos No 6,528,624, Idusogie *et al*.

Algunas variantes preferidas de 2H7 humanizado son aquellas que comprenden el dominio ligero variable de SEQ ID NO:2 y el dominio pesado variable de SEQ ID NO:8, incluidas aquellas con o sin sustituciones en una región Fc (si está presente), y aquellas que comprenden un dominio pesado variable con alteración en SEQ ID NO:8 de N100A, o D56A y

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N100A, o D56A, N100Y, y S100aR, y un dominio ligero variable con alteración en SEQ ID NO:2 de M32L, o S92A, o M32L y S92A.

M34 en el dominio pesado variable de 2H7.v16 ha sido identificado como una fuente potencial de estabilidad de anticuerpo y es otro posible candidato para sustitución.

En un resumen de varias incorporaciones preferidas de la invención, la región variable de las variantes basadas en 2H7.v16 comprenden las secuencias de aminoácidos de v16, excepto en las posiciones de las sustituciones de aminoácido que se indican en la siguiente Tabla 4. A menos que se indique otra cosa, las variantes de 2H7 tendrán la misma cadena ligera que la de v16.

Tabla 4
Variantes Ejemplares de Anticuerpo 2H7 Humanizado

Versión 2H7	Cambios de cadena pesada (V _H)	Cambios de cadena liviana (V _L)	Cambios Fc
16 para referencia			
31	-	-	S298A, E333A, K334A
73	N100A	M32L	
75	N100A	M32L	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N100A	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L
116	D56A, N100A	M32L, S92A	S298A, K322A, K334A,
138	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A,
477	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A, N434W
375	-	-	K334L
588	-	-	S298A, K326A, E333A, K334A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, K326A, E333A, K334A

Un 2H7 humanizado preferido comprende la secuencia de dominio ligero variable:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFLTITSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2),

y la secuencia de dominio pesado variable 2H7.v16:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFYFDVWGQGTLLVT
VSS (SEQ ID NO: 8)

Donde el anticuerpo 2H7.v16 humanizado es un anticuerpo intacto,
éste puede comprender la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13),

y la secuencia de aminoácidos de cadena pesada de SEQ ID NO:14 o

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSY
NQKFVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFYFDVWGQGTLLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFPYSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:15)

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:39)

y la secuencia de dominio pesado variable 2H7 v511:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSY
NQKFVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWFYFDVWGQGTLLVT
VSS (SEQ ID NO:40)

Donde el anticuerpo 2H7.v511 humanizado es un anticuerpo intacto,
éste puede comprender la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:30)

y la secuencia de aminoácidos de cadena pesada de SEQ ID NO:31 o:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSY
NQKFVKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWFYFDVWGQGTLLVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR

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VVSVLTVLHQDWLNGKEYKCKVSNALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:41).

Véanse Figuras 7 y 8, que alínean las cadenas maduras pesada y ligera, respectivamente, de 2H7.v511 humanizado con 2H7.v16 humanizado usando la secuencia de lisina C-terminal para la cadena pesada.

Donde el anticuerpo 2H7.v31 humanizado es un anticuerpo intacto, éste puede comprender la secuencia de aminoácidos de cadena ligera:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFLTITSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

y la secuencia de aminoácidos de cadena pesada de SEQ ID NO:15 o
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA IYPGNGDTSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARV VYYSNSYWFYFDVWGQGT LVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPA VLQSS
GLYSLSV VTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:42) or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA IYPGNGATSY
NQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARV VYYSYRYWFYFDVWGQGT LVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPA VLQSS
GLYSLSV VTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYR
VVSVLTVLHQDWLNGKEYKCKVSNALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:43)

Una incorporación preferida en esta solicitud es donde el anticuerpo es 2H7 humanizado, que comprende las secuencias de dominio variable en SEQ ID NOS: 2 y 8 (versión 16). Otra incorporación preferida en esta solicitud es donde el anticuerpo es 2H7 humanizado, que comprende las secuencias de dominio variable en SEQ ID NOS:39 y 40 (versión 511). Se prefiere además donde el anticuerpo es 2H7 humanizado, que comprende las secuencias de dominio variable en SEQ ID NOS:32 y 33 (véase Figura 9, referencia versión 114), tal como uno que comprende el dominio de

cadena ligera variable en SEQ ID NO:32 y la secuencia de aminoácidos de cadena pesada de SEQ ID NO:34. Se prefiere también donde el anticuerpo es 2H7 humanizado, que comprende un dominio de cadena pesada variable con alteración N100A, o D56A y N100A, o D56A, N100Y, y S100aR en SEQ ID NO:8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO:2.

LO QUE SE REIVINDICA ES:

1. Un método de tratar vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (vasculitis asociada con ANCA) en un paciente, que comprende administrar un anticuerpo CD20 al paciente en una dosis de alrededor de 400 mg 1.3 gramos con una frecuencia de una a tres dosis en un período de alrededor de un mes.
2. El método de la reivindicación 1 en donde la dosis es alrededor de 500 mg a 1.2 gramos.
3. El método de la reivindicación 1 ó 2 en donde la dosis es alrededor de 750 mg a 1.1 gramos.
4. El método de una cualquiera de las reivindicaciones 1-3 en donde el anticuerpo se administra en dos a tres dosis.
5. El método de una cualquiera de las reivindicaciones 1-4 en donde el anticuerpo se administra en tres dosis.
6. El método de una cualquiera de las reivindicaciones 1-5 en donde el anticuerpo se administra en un período de alrededor de 2 a 3 semanas.
7. El método de la reivindicación 6 en donde el período es alrededor de tres semanas.
8. El método de una cualquiera de las reivindicaciones 1-7 en donde la vasculitis asociada con ANCA es granulomatosis de Wegener.
9. El método de una cualquiera de las reivindicaciones 1-7 en donde la vasculitis asociada con ANCA es polivascularitis microscópica.

10. El método de una cualquiera de las reivindicaciones 1-9 en donde un segundo medicamento se administra en una cantidad efectiva, en donde el anticuerpo CD20 es un primer medicamento.
11. El método de la reivindicación 10 en donde el segundo medicamento es más de un medicamento.
12. El método de la reivindicación 10 u 11 en donde el segundo medicamento es un agente quimioterapéutico, un agente inmunosupresor, un fármaco anti-reumático modificador de la enfermedad (DMARD), un agente citotóxico, un antagonista de integrina, un medicamento anti-inflamatorio no esteroide (AINE), un antagonista de citocina, o una hormona, o una combinación de los mismos.
13. El método de la reivindicación 12 en donde el segundo medicamento es un esteroide o un agente inmunosupresor, o ambos.
14. El método de la reivindicación 13 en donde el segundo medicamento es un esteroide.
15. El método de la reivindicación 14 en donde el esteroide es un corticosteroide.
16. El método de la reivindicación 15 en donde el esteroide es prednisona, prednisolona, metilprednisolona, hidrocortisona, o dexametasona.
17. El método de una cualquiera de las reivindicaciones 14-16 en donde el esteroide se administra en cantidades menores que las utilizadas si el anticuerpo CD20 no se administra a un paciente tratado con esteroide.

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18. El método de la reivindicación 13 en donde el segundo medicamento es un agente inmunosupresor.
19. El método de la reivindicación 18 en donde el agente inmunosupresor es ciclofosfamida, clorambucil, micofenolato mofetil, leflunomida, azatioprina, o metotrexato.
20. El método de la reivindicación 19 en donde el agente inmunosupresor es ciclofosfamida.
21. El método de la reivindicación 13 en donde el segundo medicamento es un esteroide y un agente inmunosupresor.
22. El método de una cualquiera de las reivindicaciones 1-21 en donde el paciente no ha sido nunca previamente tratado con un anticuerpo CD20.
23. El método de una cualquiera de las reivindicaciones 1-22 en donde el paciente no ha recaído con la vasculitis.
24. El método de una cualquiera de las reivindicaciones 1-22 en donde el anticuerpo es un anticuerpo desnudo.
25. El método de una cualquiera de las reivindicaciones 1-22 en donde el anticuerpo se conjuga con otra molécula.
26. El método de la reivindicación 25 en donde la otra molécula es un agente citotóxico.
27. El método de una cualquiera de las reivindicaciones 1-26 en donde el anticuerpo se administra intravenosamente.

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28. El método de una cualquiera de las reivindicaciones 1-26 en donde el anticuerpo se administra subcutáneamente.
29. El método de una cualquiera de las reivindicaciones 1-28 en donde ningún otro medicamento que el anticuerpo CD20 se administra al sujeto para tratar la vasculitis asociada con ANCA.
30. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo es rituximab.
31. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 2 y 8.
32. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 39 y 40.
33. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 32 y 33.
34. El método de una cualquiera de las reivindicaciones 1-29 en donde el anticuerpo es 2H7 humanizado que comprende un dominio de cadena pesada variable con alteración N100A, o D56A y N100A, o D56A, N100Y, y S100aR en SEQ ID NO: 8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO: 2.
35. El método de una cualquiera de las reivindicaciones 1-34 en donde el paciente tiene un puntaje BVAS/WG de 0 seis meses después de

la administración del anticuerpo.

36. El método de una cualquiera de las reivindicaciones 1-35 en donde el paciente tiene un nivel elevado de anticuerpos anti-nucleares (ANA), anticuerpos de factor anti-reumatoide (RF), creatinina, nitrógeno ureico en la sangre, anticuerpos anti- endoteliales, anticuerpos anticitoplasma de los neutrófilos (ANCA), o una combinación de los mismos.
37. Un artículo de fabricación, que comprende
- a. un envase que contiene un anticuerpo CD20, y
 - b. un prospecto de envase con instrucciones para tratar vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (vasculitis asociada con ANCA) en un paciente, en donde las instrucciones indican que una dosis del anticuerpo CD20 de alrededor de 400 mg a 1.3 gramos con una frecuencia de una a tres dosis se administra al paciente en un período de alrededor de un mes.
38. El artículo de la reivindicación 37 que comprende además un envase que contiene un segundo medicamento, en donde el anticuerpo CD20 es un primer medicamento, incluyendo además instrucciones en el prospecto de envase para tratar al paciente con el segundo medicamento.
39. El artículo de la reivindicación 38 en donde el segundo medicamento es un agente quimioterapéutico, un agente inmunosupresor, un agente citotóxico, un antagonista de integrina, un antagonista de citocina, o una hormona.
40. El artículo de la reivindicación 38 ó 39 en donde el segundo

medicamento es un esteroide o un agente inmunosupresor, o ambos.

41. Un método de tratar vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (vasculitis asociada con ANCA) en un sujeto, que comprende administrar una cantidad efectiva de un anticuerpo CD20 al sujeto, para proporcionar una exposición inicial a anticuerpo, seguida por una segunda exposición a anticuerpo, en donde la segunda exposición no se proporciona hasta entre alrededor de 16 a 54 semanas a partir de la exposición inicial.
42. El método de la reivindicación 41 en donde cada una de las exposiciones a anticuerpo se proporciona al sujeto como dosis única o como dos o tres dosis separadas de anticuerpo.
43. El método de la reivindicación 41 ó 42 en donde la segunda exposición no se proporciona hasta entre alrededor de 20 a 30 semanas a partir de la exposición inicial.
44. El método de una cualquiera de las reivindicaciones 41-43 en donde la segunda exposición no se proporciona hasta entre alrededor de 46 a 54 semanas a partir de la exposición inicial.
45. El método de una cualquiera de las reivindicaciones 41-44 en donde cada una de las exposiciones inicial y segunda a anticuerpo se proporciona en cantidades de alrededor de 0.5 a 4 gramos.
46. El método de una cualquiera de las reivindicaciones 41-45 en donde cada una de las exposiciones inicial y segunda a anticuerpo se proporciona en cantidades de alrededor de 1.5 a 3.5 gramos.
47. El método de una cualquiera de las reivindicaciones 41-46 en donde cada una de las exposiciones inicial y segunda a anticuerpo se

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proporciona en cantidades de alrededor de 1.5 a 2.5 gramos.

48. El método de una cualquiera de las reivindicaciones 41-47 que comprende adicionalmente administrar al sujeto una cantidad efectiva del anticuerpo CD20 para proporcionar una tercera exposición a anticuerpo, en donde la tercera exposición no se proporciona hasta entre alrededor de 46 a 60 semanas a partir de la exposición inicial.
49. El método de la reivindicación 48 en donde la tercera exposición a anticuerpo se proporciona en una cantidad de alrededor de 0.5 a 4 gramos
50. El método de la reivindicación 48 ó 49 en donde la tercera exposición a anticuerpo se proporciona en una cantidad de alrededor de 1.5 a 3.5 gramos.
51. El método de una cualquiera de las reivindicaciones 48-50 en donde la tercera exposición a anticuerpo se proporciona en una cantidad de alrededor de 1.5 a 2.5 gramos.
52. El método de una cualquiera de las reivindicaciones 48-51 en donde la tercera exposición no se proporciona hasta entre alrededor de 46 a 55 semanas a partir de la exposición inicial
53. El método de una cualquiera de las reivindicaciones 48-52 en donde no se proporciona otra exposición a anticuerpo hasta por lo menos alrededor de 70-75 semanas a partir de la exposición inicial.
54. El método de la reivindicación 53 en donde no se proporciona otra exposición a anticuerpo hasta alrededor de 74 a 80 semanas a partir de la exposición inicial.

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55. El método de una cualquiera de las reivindicaciones 41 ó 43-54 en donde una o más de las exposiciones a anticuerpo se proporciona al sujeto como una dosis única de anticuerpo.
56. El método de una cualquiera de las reivindicaciones 41-55 en donde cada exposición a anticuerpo se proporciona al sujeto como una dosis única de anticuerpo.
57. El método de una cualquiera de las reivindicaciones 41 ó 43-54 en donde una o más de las exposiciones a anticuerpo se proporciona al sujeto como dosis separadas del anticuerpo.
58. El método de una cualquiera de las reivindicaciones 41-54 ó 57 en donde cada exposición a anticuerpo se proporciona como dosis separadas del anticuerpo.
59. El método de la reivindicación 57 en donde las dosis separadas son desde alrededor de 2 a 3 dosis.
60. El método de una cualquiera de las reivindicaciones 57-59 en donde las dosis separadas constituyen una primera y segunda dosis.
61. El método de una cualquiera de las reivindicaciones 57-59 en donde las dosis separadas constituyen una dosis primera, segunda y tercera.
62. El método de una cualquiera de las reivindicaciones 57-61 en donde una última dosis se administra desde alrededor de 1 a 20 días a partir del momento en que se administró la dosis previa.
63. El método de una cualquiera de las reivindicaciones 57-62 en donde

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una última dosis se administra desde alrededor de 6 a 16 días a partir del momento en que se administró la dosis previa.

64. El método de una cualquiera de las reivindicaciones 57-63 en donde una última dosis se administra desde alrededor de 14 a 16 días a partir del momento en que se administró la dosis previa.
65. El método de una cualquiera de las reivindicaciones 57-64 en donde las dosis separadas se administran en un período total de entre alrededor de 1 día y 4 semanas.
66. El método de una cualquiera de las reivindicaciones 57-65 en donde las dosis separadas se administran en un período total de entre alrededor de 1 y 25 días.
67. El método de una cualquiera de las reivindicaciones 57-66 en donde las dosis separadas se administran alrededor de semanalmente, administrándose la segunda dosis alrededor de una semana a partir de la primera dosis, y administrándose la última dosis alrededor de una semana a partir de la dosis previa.
68. El método de una cualquiera de las reivindicaciones 57-67 en donde cada dosis separada de anticuerpo es alrededor de 0.5 a 1.5 gramos.
69. El método de una cualquiera de las reivindicaciones 57-68 en donde cada dosis separada de anticuerpo es alrededor de 0.75 a 1.3 gramos.
70. El método de una cualquiera de las reivindicaciones 41-69 en donde 4 a 20 exposiciones a anticuerpo se administran al sujeto.
71. El método de una cualquiera de las reivindicaciones 41-70 en donde a segundo medicamento se administra en una cantidad efectiva con

una exposición a anticuerpo, en donde el anticuerpo CD20 es un primer medicamento.

72. El método de la reivindicación 71 en donde el segundo medicamento se administra con la exposición inicial.
73. El método de la reivindicación 71 ó 72 en donde el segundo medicamento se administra con las exposiciones inicial y segunda.
74. El método de una cualquiera de las reivindicaciones 71-73 en donde el segundo medicamento se administra con todas las exposiciones.
75. El método de una cualquiera de las reivindicaciones 71-74 en donde el segundo medicamento es un agente quimioterapéutico, un agente inmunosupresor, un fármaco anti-reumático modificador de la enfermedad (DMARD), un agente citotóxico, un antagonista de integrina, un medicamento anti-inflamatorio no esteroide (AINE), un antagonista de citocina, o una hormona, o una combinación de los mismos.
76. El método de una cualquiera de las reivindicaciones 71-75 en donde el segundo medicamento comprende un esteroide o un agente inmunosupresor, o ambos.
77. El método de una cualquiera de las reivindicaciones 71-76 en donde el segundo medicamento es un esteroide.
78. El método de la reivindicación 77 en donde el esteroide es un corticosteroide.
79. El método de la reivindicación 78 en donde el esteroide es prednisona, prednisolona, metilprednisolona, hidrocortisona, o

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dexametasona.

80. El método de una cualquiera de las reivindicaciones 77-79 en donde el esteroide se administra en cantidades más bajas que las utilizadas si el anticuerpo CD20 no se administra a un sujeto tratado con esteroide.
81. El método de una cualquiera de las reivindicaciones 71-76 en donde el segundo medicamento es un agente inmunosupresor.
82. El método de la reivindicación 81 en donde el agente inmunosupresor es ciclofosfamidás, clorambucil, leflunomida, micofenolato mofetil, azatioprina, o metotrexato.
83. El método de la reivindicación 82 en donde el agente inmunosupresor es ciclofosfamida.
84. El método de una cualquiera de las reivindicaciones 71-76 en donde el segundo medicamento comprende un esteroide y un agente inmunosupresor.
85. El método de la reivindicación 72 en donde el segundo medicamento no se administra con la segunda exposición, o se administra en cantidades más bajas que las utilizadas con la exposición inicial.
86. El método de una cualquiera de las reivindicaciones 41-85 en donde alrededor de 2-3 gramos del anticuerpo CD20 se administran como la exposición inicial.
87. El método de la reivindicación 86 en donde alrededor de 1 gramo del anticuerpo CD20 se administra semanalmente durante alrededor

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de tres semanas como la exposición inicial.

88. El método de la reivindicación 86 u 87 en donde la segunda exposición es alrededor de seis meses a partir de exposición inicial y se administra en una cantidad de alrededor de 2 gramos.
89. El método de una cualquiera de las reivindicaciones 86-88 en donde la segunda exposición es a alrededor de seis meses a partir de la exposición inicial y se administra como alrededor de 1 gramo del anticuerpo, seguido en alrededor de dos semanas por otro alrededor de 1 gramo del anticuerpo.
90. El método de la reivindicación 86 en donde alrededor de 1 gramo del anticuerpo CD20 se administra seguido en alrededor de dos semanas por otro alrededor de 1 gramo del anticuerpo como la exposición inicial.
91. El método de la reivindicación 90 en donde la segunda exposición es a alrededor de seis meses a partir de la exposición inicial y se administra en una cantidad de alrededor de 2 gramos.
92. El método de la reivindicación 90 ó 91 en donde la segunda exposición es a alrededor de seis meses a partir de la exposición inicial y se administra como alrededor de 1 gramo del anticuerpo seguido en alrededor de dos semanas por otro alrededor de 1 gramo del anticuerpo.
93. El método de una cualquiera de las reivindicaciones 86-92 en donde un esteroide se administra al sujeto antes o con la exposición inicial.
94. El método de la reivindicación 93 en donde el esteroide no se administra con la segunda exposición o se administra con la

segunda exposición, pero en cantidades menores que las utilizadas con la exposición inicial.

95. El método de la reivindicación 93 ó 94 en donde el esteroide no se administra con exposiciones tercera o última.
96. El método de una cualquiera de las reivindicaciones 41-95 en donde el sujeto nunca ha sido tratado previamente con un anticuerpo CD20.
97. El método de una cualquiera de las reivindicaciones 41-96 en donde el sujeto está en remisión después de la exposición inicial o última a anticuerpo.
98. El método de una cualquiera de las reivindicaciones 41-96 en donde el sujeto está en remisión cuando se proporciona la segunda exposición a anticuerpo.
99. El método de la reivindicación 98 en donde el sujeto está en remisión cuando se proporcionan todas las exposiciones a anticuerpo.
100. El método de una cualquiera de las reivindicaciones 41-99 en donde las exposiciones inicial y segunda a anticuerpo son con el mismo anticuerpo CD20.
101. El método de una cualquiera de las reivindicaciones 41-100 en donde todas las exposiciones a anticuerpo son con el mismo anticuerpo CD20.
102. El método de una cualquiera de las reivindicaciones 41-101 en donde el anticuerpo es un anticuerpo desnudo.

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103. El método de una cualquiera de las reivindicaciones 41-101 en donde el anticuerpo se conjuga con otra molécula.
104. El método de la reivindicación 103 en donde la otra molécula es un agente citotóxico.
105. El método de una cualquiera de las reivindicaciones 41-104 en donde el anticuerpo se administra intravenosamente.
106. El método de la reivindicación 105 en donde el anticuerpo se administra intravenosamente para cada exposición a anticuerpo.
107. El método de una cualquiera de las reivindicaciones 41-104 en donde el anticuerpo se administra subcutáneamente.
108. El método de la reivindicación 107 en donde el anticuerpo se administra subcutáneamente para cada exposición a anticuerpo.
109. El método de una cualquiera de las reivindicaciones 41-108 en donde ningún otro medicamento que el anticuerpo CD20 se administra al sujeto para tratar la vasculitis asociada con ANCA.
110. El método de una cualquiera de las reivindicaciones 41-109 en donde la vasculitis asociada con ANCA es granulomatosis de Wegener.
111. El método de una cualquiera de las reivindicaciones 41-109 en donde la vasculitis asociada con ANCA es polivascúlitis microscópica.
112. El método de una cualquiera de las reivindicaciones 41-111 en

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donde el anticuerpo es rituximab.

113. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 2 y 8.
114. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 39 y 40.
115. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 32 y 33.
116. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado, que comprende un dominio de cadena pesada variable con alteración N100A, o D56A y N100A, o D56A, N100Y, y S100aR en SEQ ID NO: 8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO: 2.
117. El método de una cualquiera de las reivindicaciones 41-116 en donde el sujeto tiene un puntaje BVAS/WG de 0 seis meses después de la administración del anticuerpo.
118. El método de una cualquiera de las reivindicaciones 41-117 en donde el paciente tiene un nivel elevado de anticuerpos anti-nucleares (ANA), anticuerpos de factor anti-reumatoide. (RF), creatinina, nitrógeno ureico en la sangre, anticuerpos anti-endoteliales, anticuerpos anticitoplasma de los neutrófilos (ANCA), o una combinación de los mismos.

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donde el anticuerpo es rituximab.

113. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 2 y 8.
114. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 39 y 40.
115. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado que comprende las secuencias de dominio variable en SEQ ID NOS: 32 y 33.
116. El método de una cualquiera de las reivindicaciones 41-111 en donde el anticuerpo es 2H7 humanizado, que comprende un dominio de cadena pesada variable con alteración N100A, o D56A y N100A, o D56A, N100Y, y S100aR en SEQ ID NO: 8 y un dominio de cadena ligera variable con alteración M32L, o S92A, o M32L y S92A en SEQ ID NO: 2.
117. El método de una cualquiera de las reivindicaciones 41-116 en donde el sujeto tiene un puntaje BVAS/WG de 0 seis meses después de la administración del anticuerpo.
118. El método de una cualquiera de las reivindicaciones 41-117 en donde el paciente tiene un nivel elevado de anticuerpos anti-nucleares (ANA), anticuerpos de factor anti-reumatoide (RF), creatinina, nitrógeno ureico en la sangre, anticuerpos anti-endoteliales, anticuerpos anticitoplasma de los neutrófilos (ANCA), o una combinación de los mismos.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

PATENTE ☒ PCT/US2005/034647 MODELO DE UTILIDAD ☐ DISEÑO INDUSTRIAL ☐

SOLICITANTES, RAZON SOCIAL O APELLIDOS Y NOMBRES GENENTECH, INC.

DOMICILIO SOUTH SAN FRANCISCO, CALIFORNIA, ESTADOS UNIDOS DE AMERICA

APODERADO JUAN PABLO CADENA SARMIENTO

TITULO METODO PARA TRATAR VASCULITIS

CLASIFICACION _____

EXPEDIENTE No. _____ FECHA DE SOLICITUD _____ ROLLO MICROFILM _____

CERTIFICADO No. _____ FECHA DE CONCESION _____ VIGENCIA DESDE _____ HASTA _____

PRORROGA _____ CAMBIO DE NOMBRE _____

TRASPASO A _____

INVENTOR (ES) PAUL G. BRUNETTA

OTROS _____

OFICINA DE COMUNICACIONES

119. Un artículo de fabricación que comprende

- a. un envase que contiene un anticuerpo CD20, y**
- b. un prospecto de envase con instrucciones para tratar vasculitis asociada con anticuerpos anticitoplasma de los neutrófilos (vasculitis asociada con ANCA) en un sujeto, en donde las instrucciones indican que una cantidad del anticuerpo se administra al sujeto que es efectiva para proporcionar una exposición inicial a anticuerpo seguido por una segunda exposición a anticuerpo, en donde la segunda exposición no se proporciona hasta entre alrededor de 16 a 54 semanas a partir de la exposición inicial.**

120. El artículo de la reivindicación 119 en donde cada una de las exposiciones a anticuerpo se proporciona al sujeto como una dosis única o como dos o tres dosis separadas de anticuerpo.

121. El artículo de la reivindicación 119 ó 120 en donde cada una de las exposiciones inicial y segunda se proporciona en una cantidad de 0.5 a 4 gramos.

122. El artículo de una cualquiera de las reivindicaciones 119-121 que comprende además un envase que contiene un segundo medicamento, en donde el anticuerpo CD20 es un primer medicamento, y que contiene además instrucciones en el prospecto de envase para tratar al sujeto con el segundo medicamento.

123. El artículo de la reivindicación 122 en donde el segundo medicamento es un agente quimioterapéutico, un agente inmunosupresor, un agente citotóxico, un antagonista de integrina, un antagonista de citocina, o una hormona.

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124. El artículo de la reivindicación 123 en donde el segundo medicamento es un esteroide o un agente inmunosupresor, o ambos.

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Alineación de Secuencias de Dominio de Cadena Ligera Variable

	FR1 1020	CDR1 3040	
2H7	QIVLSQSPAILSASPGEKVTMTC	[RASSSVS-YMH] WYQQK	
	* * * *	* * *	
hu2H7.v16	DIQMTQSPSSLSASVGDRVITTC	[RASSSVS-YMH] WYQQK	
		* * * *	
hum KI	DIQMTQSPSSLSASVGDRVITTC	[RASQISNYLA] WYQQK	
	FR2 50	CDR2 60	FR3 7080
2H7	GSSPKPWIY	[APSNLAS]	GVPARFSGSGSGTSYSLTISRVEA
	** *		* ** *
hu2H7.v16	GKAPKPLIY	[APSNLAS]	GVPSRFSGSGSGTDFTLTISLQP
	*	* * *	
hum KI	GKAPKLLIY	[AASSLES]	GVPSRFSGSGSGTDFTLTISLQP
	CDR3 90	FR4 100	
2H7	EDAATYYC	[QQWSFNPPT]	FGAGTKLELKR
	*		* * *
hu2H7.v16	EDFATYYC	[QQWSFNPPT]	FGQGTVKVEIKR

hum KI	EDFATYYC	[QQYNSLPWT]	FGQGTVKVEIKR

FIG._1A

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Alineación de Secuencias de Dominio de Cadena Pesada Variable

	FR1 10203040	CDR1	FR3
2H7	QAYLQQSGAELVRPGASVKMSCKAS	[GYTFTSYNMH]	WVKQT
	*** ** * * * *		* *
hu2H7.v16	EVQLVESGGGLVQPGGSLRLSCAAS	[GYTFTSYNMH]	WVRQA
		* * * *	
hum III	EVQLVESGGGLVQPGGSLRLSCAAS	[GFTFSSYAMS]	WVRQA
	FR2 50 a 60	CDR2	FR3 7080
2H7	PRQGLEWIG	[AIYPGNGDTSYNQKPKG]	KATLTVDKSSSTAYM
	** *		** ** * *
hu2H7.v16	PGKGLEWVG	[AIYPGNGDTSYNQKPKG]	RFTISVDKSKNTLYL
	* * * * *		* *
hum III	PGKGLEWVA	[VISGDGGSTYYADSVKG]	RFTISRDNKNTLTL
	FR4 abc90100abcde110	CDR3	
2H7	QLSSLTSEDSAVYFCAR	[VVYYSNSYWYFDV]	WGTGTLTVTVSS
	** ** *		* *
hu2H7.v16	QMNSLRAEDTAVYYCAR	[VVYYSNSYWYFDV]	WGQGLTVTVSS
		***** * *	
hum III	QMNSLRAEDTAVYYCAR	[GRVGYSLY---DY]	WGQGLTVTVSS

FIG._1B

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Cadena Ligera de 2H7.v16 Humanizado

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHYQOKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFLTITISLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG._2**Cadena Pesada de 2H7.v16 Humanizado**

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFVDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFPSCSVMEALHNHY
TQKSLSLSPGK (SEQ ID NO:14)

FIG._3**Cadena Pesada de 2H7.v31 Humanizado**

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWFVDVWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFPSCSVMEALHNHY
TQKSLSLSPGK (SEQ ID NO:15)

FIG._4

265
270

Alineación de Cadena Ligera de V16 y v138 (Numeración Secuencial)

	10	20	30	40	50
hu2H7.v16.light	DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP				
hu2H7.v138.light	DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP				
	10	20	30	40	50
	60	70	80	90	100
hu2H7.v16.light	SNLASGVPSRPSGSGSGTDFTLTISSLQPEDFATYYCQWSPNPPTFGQG				
hu2H7.v138.light	SNLASGVPSRPSGSGSGTDFTLTISSLQPEDFATYYCQWAFNPPTFGQG				
	60	70	80	90	100
	110	120	130	140	150
hu2H7.v16.light	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD				
hu2H7.v138.light	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD				
	110	120	130	140	150
	160	170	180	190	200
hu2H7.v16.light	NALQSGNSQESVTEQDSKDSSTLTLSKADYEKHKVYACEVTHQGL				
hu2H7.v138.light	NALQSGNSQESVTEQDSKDSSTLTLSKADYEKHKVYACEVTHQGL				
	160	170	180	190	200
	210				
hu2H7.v16.light	SSPVTKSFNRGEC				
hu2H7.v138.light	SSPVTKSFNRGEC				
	210				

FIG. 5

267
231

Alineación de Cadena Pesada de V16 y v138 (Numeración Secuencial)

	10	20	30	40	50
hu2H7.v16.heavy	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA				
hu2H7.v138.heavy	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGA				
	10	20	30	40	50
	60	70	80	90	100
hu2H7.v16.heavy	IYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVV				
hu2H7.v138.heavy	IYPGNGDTSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVV				
	60	70	80	90	100
	110	120	130	140	150
hu2H7.v16.heavy	YYSNSYWFYFDVWGQGTLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCL				
hu2H7.v138.heavy	YYSASYWFYFDVWGQGTLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCL				
	110	120	130	140	150
	160	170	180	190	200
hu2H7.v16.heavy	VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT				
hu2H7.v138.heavy	VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT				
	160	170	180	190	200
	210	220	230	240	250
hu2H7.v16.heavy	QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP				
hu2H7.v138.heavy	QTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP				
	210	220	230	240	250
	260	270	280	290	300
hu2H7.v16.heavy	KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ				
hu2H7.v138.heavy	KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ				
	260	270	280	290	300
	310	320	330	340	350
hu2H7.v16.heavy	YNSTYRVVSVLTVTLHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE				
hu2H7.v138.heavy	YNATYRVVSVLTVTLHQLDNLNGKEYKCKVSNKALPAPIAATISKAKGQPRE				
	310	320	330	340	350
	360	370	380	390	400
hu2H7.v16.heavy	PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP				
hu2H7.v138.heavy	PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP				
	360	370	380	390	400
	410	420	430	440	450
hu2H7.v16.heavy	FVLDSGSGFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLSPGK				
hu2H7.v138.heavy	FVLDSGSGFFLYSKLTVDKSRWQQGNVFSQSVMHREALHNHYTQKSLSLSPGK				
	410	420	430	440	450

FIG._6

268
22

Alineación de Cadena Ligera

	1	32
hu2H7.v16	DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAP	
hu2H7.v511	DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAP	
	52	
hu2H7.v16	SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQG	
hu2H7.v511	SNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWAFNPPTFGQG	
	102	
hu2H7.v16	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD	
hu2H7.v511	TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD	
	152	
hu2H7.v16	NALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGL	
hu2H7.v511	NALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYACEVTHQGL	
	202	214
hu2H7.v16	SSPVTKSFNRGEC	
hu2H7.v511	SSPVTKSFNRGEC	

FIG. 7

269
273

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	RAEDTAVYYCARVVYYISNSYWFYFDVWGQGTLLVTVSS	

hu2H7.v511	RAEDTAVYYCARVVYYISYRYWFYFDVWGQGTLLVTVSS	
	118	
hu2H7.v16	ASTKGPSVFPLAPS	

hu2H7.v511	ASTKGPSVFPLAPS	
	132	
hu2H7.v16	SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS	

hu2H7.v511	SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS	
	182	
hu2H7.v16	LSSVVTVPSSSLGTQTYICNVNHKPENTKVDKKVEPKSCDKTHTCPPCPA	

hu2H7.v511	LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA	
	232	
hu2H7.v16	PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG	

hu2H7.v511	PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG	
	282	
hu2H7.v16	VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP	

hu2H7.v511	VEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAAALPAP	
	332	
hu2H7.v16	IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW	
	* *****	
hu2H7.v511	IAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW	
	382	
hu2H7.v16	ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEA	

hu2H7.v511	ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEA	
	432	447
hu2H7.v16	LHNHYTQKSLSLSPGK	

hu2H7.v511	LHNHYTQKSLSLSPGK	

FIG._8

270
27k

8 / 8

Dominio de Cadena Ligera Variable de 2H7.v114 Humanizado:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSG
TDFTLTISSLQPEDFATYYCQNAFNPPTFGQGTKVEIKR

FIG._9A**Dominio de Cadena Pesada Variable de 2H7.v114 Humanizado:**

FEVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQK
FKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYASASYWYFDVWGQGTLLTVSS

FIG._9B**Cadena de Longitud Total de 2H7.v114 Humanizado:**

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNG
ATSYNQKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYASASYWYFDVWGQ
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPGCP
APPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNATYRVSVLTVLTQDNLNGKEYKCKVSNKALPAPIAATISKAKGQPREPQVYTL
PPSRREEMTKNQVSLTCLVKGFYPSDIAVENESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFPSCSVMHREALHNHYTQKSLSLSPG

FIG._9C

271.
225

Listado de Secuencias

<110> GENENTECH, INC. , BRUNETTA, PAUL G. :

<120> MÉTODO PARA TRATAR VASCULITIS

<130> P2177R1 PCT

<140> PCT/US2005/034647

<141> 2005-09-28

<150> US 60/616,104

<151> 2004-10-05

<160> 43

<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> Secuencia artificial

<220>

<223> Se sintetiza la secuencia

<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

372
276

<210> 3
<211> 108
<212> PRT
<213> Homo sapiens

<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> 26
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 4
Arg Ala Ser Ser Ser Val Ser Tyr Met His Ala Pro Ser Asn Leu
1 5 10 15
Ala Ser Gln Gln Trp Ser Phe Asn Pro Pro Thr
20 25

<210> 5
<211> 26
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 5
Arg Ala Ser Ser Ser Val Ser Tyr Met His Ala Pro Ser Asn Leu
1 5 10 15
Ala Ser Gln Gln Trp Ser Phe Asn Pro Pro Thr
20 25

<210> 6
<211> 27
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 6
Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala Ala Ala Ser Ser
1 5 10 15
Leu Glu Ser Gln Gln Tyr Asn Ser Leu Pro Trp Thr
20 25

<210> 7
<211> 122

223
227

<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> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<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> Homo sapiens

<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

274
228

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> 40
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 10
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Ala Ile Tyr Pro Gly
1 5 10 15
Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Val Val Tyr
20 25 30
Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
35 40

<210> 11
<211> 40
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 11
Gly Tyr Thr Phe Thr Ser Tyr Asn Met His Ala Ile Tyr Pro Gly
1 5 10 15
Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys Gly Val Val Tyr
20 25 30
Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val
35 40

<210> 12
<211> 37
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 12
Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Val Ile Ser Gly Asp
1 5 10 15
Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Gly Arg Val
20 25 30
Gly Tyr Ser Leu Tyr Asp Tyr
35

<210> 13
<211> 213
<212> PRT
<213> Secuencia artificial

<220>

<223> Se sintetiza la secuencia

<400> 13

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1 5 10 15
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser 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> Secuencia artificial

<220>

<223> Se sintetiza la secuencia

<400> 14

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly 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

276
280

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> Secuencia artificial

227
282

<220>

<223> Se sintetiza la secuencia

<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

228
282

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> 285
<212> PRT
<213> Homo sapiens

<400> 16
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys
1 5 10 15
Leu Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile
20 25 30
Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly
35 40 45
Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys
50 55 60
Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp
65 70 75
Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu Lys
80 85 90
Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala
95 100 105
Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro
110 115 120
Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val
125 130 135
Gln Gly Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile
140 145 150
Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe
155 160 165
Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu
170 175 180
Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
185 190 195
Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
200 205 210
Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser
215 220 225
Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
230 235 240
Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu
245 250 255
Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile
260 265 270

279.
283

Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
275 280 285

<210> 17
<211> 309
<212> PRT
<213> Mus musculus

<400> 17
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe
1 5 10 15
Cys Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile
20 25 30
Thr Pro Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp
35 40 45
Gly Arg Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser
50 55 60
Ser Phe Thr Ala Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala
65 70 75
Asp Leu Met Asn Leu Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser
80 85 90
Ala Thr Pro Ala Ala Ala Gly Ala Pro Glu Leu Thr Ala Gly Val
95 100 105
Lys Leu Leu Thr Pro Ala Ala Pro Arg Pro His Asn Ser Ser Arg
110 115 120
Gly His Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu Thr Glu
125 130 135
Gln Asp Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro Gly
140 145 150
Cys Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn
155 160 165
Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asp Thr Pro
170 175 180
Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser
185 190 195
Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val
200 205 210
Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr
215 220 225
Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
230 235 240
Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg
245 250 255
Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr
260 265 270
Ser Ala Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu
275 280 285
Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp
290 295 300
Thr Phe Phe Gly Ala Leu Lys Leu Leu
305

<210> 18
<211> 17
<212> PRT
<213> Secuencia artificial

280
284

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 1
<223> X = Cualquier aminoácido excepto cisteína

<220>
<221> Xaa
<222> 3
<223> X = Cualquier aminoácido excepto cisteína

<220>
<221> Xaa
<222> 5
<223> X = Cualquier aminoácido excepto cisteína

<220>
<221> Xaa
<222> 7-10
<223> X = Cualquier aminoácido excepto cisteína

<220>
<221> Xaa
<222> 11, 12, 14, 15, 17
<223> X = Cualquier aminoácido excepto cisteína

<220>
<221> Xaa
<222> 16
<223> X = L, F, I, o V

<400> 18
Xaa Cys Xaa Asp Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa
1 5 10 15

Xaa Xaa

<210> 19
<211> 17
<212> PRT.
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 19
Glu Cys Phe Asp Leu Leu Val Arg Ala Trp Val Pro Cys Ser Val
1 5 10 15

Leu Lys

<210> 20
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 20
Glu Cys Phe Asp Leu Leu Val Arg His Trp Val Pro Cys Gly Leu
1 5 10 15

Leu Arg

<210> 21
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

281
285

<400> 21
Glu Cys Phe Asp Leu Leu Val Arg Arg Trp Val Pro Cys Glu Met
1 5 10 15

Leu Gly

<210> 22
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 22
Glu Cys Phe Asp Leu Leu Val Arg Ser Trp Val Pro Cys His Met
1 5 10 15

Leu Arg

<210> 23
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 23
Glu Cys Phe Asp Leu Leu Val Arg His Trp Val Ala Cys Gly Leu
1 5 10 15

Leu Arg

<210> 24
<211> 16
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 24
Gln Cys Phe Asp Arg Leu Asn Ala Trp Val Pro Cys Ser Val Leu
1 5 10 15

Lys

<210> 25
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 1, 3, 5, 8, 9, 14, 15, 17
<223> Xaa = Cualquier aminoácido excepto aistaina

<400> 25
Xaa Cys Xaa Asp Xaa Leu Val Xaa Xaa Trp Val Pro Cys Xaa Xaa
1 5 10 15

Leu Xaa

<210> 26
<211> 184
<212> PRT
<213> Homo sapiens

<400> 26

282
286

Met Arg Arg Gly Pro Arg Ser Leu Arg Gly Arg Asp Ala Pro Ala
1 5 10 15
Pro Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg
20 25 30
His Cys Val Ala Cys Gly Leu Leu Arg Thr Pro Arg Pro Lys Pro
35 40 45
Ala Gly Ala Ser Ser Pro Ala Pro Arg Thr Ala Leu Gln Pro Gln
50 55 60
Glu Ser Val Gly Ala Gly Ala Gly Glu Ala Ala Leu Pro Leu Pro
65 70 75
Gly Leu Leu Phe Gly Ala Pro Ala Leu Leu Gly Leu Ala Leu Val
80 85 90
Leu Ala Leu Val Leu Val Gly Leu Val Ser Trp Arg Arg Arg Gln
95 100 105
Arg Arg Leu Arg Gly Ala Ser Ser Ala Glu Ala Pro Asp Gly Asp
110 115 120
Lys Asp Ala Pro Glu Pro Leu Asp Lys Val Ile Ile Leu Ser Pro
125 130 135
Gly Ile Ser Asp Ala Thr Ala Pro Ala Trp Pro Pro Pro Gly Glu
140 145 150
Asp Pro Gly Thr Thr Pro Pro Gly His Ser Val Pro Val Pro Ala
155 160 165
Thr Glu Leu Gly Ser Thr Glu Leu Val Thr Thr Lys Thr Ala Gly
170 175 180
Pro Glu Gln Gln

<210> 27
<211> 26
<212> PRT
<213> Homo sapiens

<400> 27
Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg His
1 5 10 15
Cys Val Ala Cys Gly Leu Leu Arg Thr Pro Arg
20 25

<210> 28
<211> 213
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 28
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

283
28A

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> 29
<211> 452
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 29
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
20 25 30
Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45
Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly 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 Ala 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

234
288

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> 30
<211> 213
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 30
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

285
289

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> 31
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 31
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 Ile Tyr Pro Gly Asn Gly Ala Thr Ser Tyr Asn
50 55 60
Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser Lys
65 70 75
Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
80 85 90
Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Tyr Arg Tyr
95 100 105
Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
110 115 120
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
125 130 135
Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
140 145 150
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
155 160 165
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
170 175 180
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
185 190 195
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
200 205 210

286
290

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
215 220 225
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
230 235 240
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
245 250 255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
260 265 270
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
275 280 285
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
290 295 300
Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
305 310 315
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Ala
320 325 330
Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys Gly
335 340 345
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
350 355 360
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
365 370 375
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
380 385 390
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
395 400 405
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
410 415 420
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
425 430 435
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
440 445 450

Lys

- <210> 32
- <211> 107
- <212> PRT
- <213> Secuencia artificial

- <220>
- <223> Se sintetiza la secuencia

<400> 32
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

287
291

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

Lys Arg

<210> 33
<211> 123
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 33
Phe Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro
1 5 10 15
Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe
20 25 30
Thr Ser Tyr Asn Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
35 40 45
Leu Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Ala Thr Ser
50 55 60
Tyr Asn Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys
65 70 75
Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu
80 85 90
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Ala
95 100 105
Ser Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr
110 115 120
Val Ser Ser

<210> 34
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 34
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr
20 25 30
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 Ala 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

283
292

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

- <210> 35
- <211> 10
- <212> PRT
- <213> Secuencia artificial
- <220>
- <223> Se sintetiza la secuencia
- <220>
- <221> xaa
- <222> 9
- <223> X es M o L

<400> 35
Arg Ala Ser Ser Ser Val Ser Tyr Xaa His
5 10

<210> 36
<211> 9
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 4
<223> X es S o A

<400> 36
Gln Gln Trp Xaa Phe Asn Pro Pro Thr
5

<210> 37
<211> 17
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 8
<223> X es D o A

<400> 37
Ala Ile Tyr Pro Gly Asn Gly Xaa Thr Ser Tyr Asn Gln Lys Phe
1 5 10 15

Lys Gly

<210> 38
<211> 13
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<220>
<221> Xaa
<222> 6
<223> X es N, A, Y, W, o D

<220>
<221> Xaa
<222> 7
<223> X es S o R

<400> 38
Val Val Tyr Tyr Ser Xaa Xaa Tyr Trp Tyr Phe Asp Val
5 10

<210> 39
<211> 107
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 39
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

290
294

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Pro
35 40 45
Leu Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro Ser Arg
50 55 60
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
80 85 90
Ala Phe Asn Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
95 100 105
Lys Arg

<210> 40
<211> 122
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 40
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

<210> 41
<211> 451
<212> PRT
<213> Secuencia artificial

<220>
<223> Se sintetiza la secuencia

<400> 41
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly
1 5 10 15
Gly Ser Leu Arg Leu Ser Cys Ala 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

291
295

Lys Asn Thr Leu Tyr 80 Leu Gln Met Asn Ser 85 Leu Arg Ala Glu Asp 90
 Thr Ala Val Tyr Tyr 95 Cys Ala Arg Val Val 100 Tyr Tyr Ser Tyr Arg 105
 Tyr Trp Tyr Phe Asp 110 Val Trp Gly Gln Gly 115 Thr Leu Val Thr Val 120
 Ser Ser Ala Ser Thr 125 Lys Gly Pro Ser Val 130 Phe Pro Leu Ala Pro 135
 Ser Ser Lys Ser Thr 140 Ser Gly Gly Thr Ala 145 Ala Leu Gly Cys Leu 150
 Val Lys Asp Tyr Phe 155 Pro Glu Pro Val Thr 160 Val Ser Trp Asn Ser 165
 Gly Ala Leu Thr Ser 170 Gly Val His Thr Phe 175 Pro Ala Val Leu Gln 180
 Ser Ser Gly Leu Tyr 185 Ser Leu Ser Ser Val 190 Val Thr Val Pro Ser 195
 Ser Ser Leu Gly Thr 200 Gln Thr Tyr Ile Cys 205 Asn Val Asn His Lys 210
 Pro Ser Asn Thr Lys 215 Val Asp Lys Lys Val 220 Glu Pro Lys Ser Cys 225
 Asp Lys Thr His Thr 230 Cys Pro Pro Cys Pro 235 Ala Pro Glu Leu Leu 240
 Gly Gly Pro Ser Val 245 Phe Leu Phe Pro Pro 250 Lys Pro Lys Asp Thr 255
 Leu Met Ile Ser Arg 260 Thr Pro Glu Val Thr 265 Cys Val Val Val Asp 270
 Val Ser His Glu Asp 275 Pro Glu Val Lys Phe 280 Asn Trp Tyr Val Asp 285
 Gly Val Glu Val His 290 Asn Ala Lys Thr Lys 295 Pro Arg Glu Glu Gln 300
 Tyr Asn Ala Thr Tyr 305 Arg Val Val Ser Val 310 Leu Thr Val Leu His 315
 Gln Asp Trp Leu Asn 320 Gly Lys Glu Tyr Lys 325 Cys Lys Val Ser Asn 330
 Ala Ala Leu Pro Ala 335 Pro Ile Ala Ala Thr 340 Ile Ser Lys Ala Lys 345
 Gly Gln Pro Arg Glu 350 Pro Gln Val Tyr Thr 355 Leu Pro Pro Ser Arg 360
 Glu Glu Met Thr Lys 365 Asn Gln Val Ser Leu 370 Thr Cys Leu Val Lys 375
 Gly Phe Tyr Pro Ser 380 Asp Ile Ala Val Glu 385 Trp Glu Ser Asn Gly 390
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 Asp Gly Ser Phe Phe 410 Leu Tyr Ser Lys Leu 415 Thr Val Asp Lys Ser 420
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292
296

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<220>
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1 5 10
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr 30
20 25 30
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35 40 45
Glu Trp Val Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr 60
50 55 60
Asn Gln Lys Phe Lys Gly Arg Phe Thr Ile Ser Val Asp Lys Ser 75
65 70 75
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp 90
80 85 90
Thr Ala Val Tyr Tyr Cys Ala Arg Val Val Tyr Tyr Ser Asn Ser 105
95 100 105
Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val 120
110 115 120
Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro 135
125 130 135
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140 145 150
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser 165
155 160 165
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln 180
170 175 180
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200 205 210
Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys 225
215 220 225
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230 235 240
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr 255
245 250 255
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260 265 270
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275 280 285
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 300
290 295 300
Tyr Asn Ala Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His 315
305 310 315
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn 330
320 325 330
Lys Ala Leu Pro Ala Pro Ile Ala Ala Thr Ile Ser Lys Ala Lys 345
335 340 345

293
297

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

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 35 40 45
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 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

294
298

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

PATENT COOPERATION TREATY

RECEIVED

MAR 02 2007

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

GENENTECH, INC.
LEGAL DEPT.

To:

GENENTECH, INC.
Attn. Hasak, Janet E.
1 DNA Way
South San Francisco, CA 94080-4990
ETATS-UNIS D'AMERIQUENOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year)

23/02/2007

Applicant's or agent's file reference

P2177R1

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/US2005/034647

International filing date
(day/month/year)

28/09/2005

Applicant

GENENTECH, INC.

Calendared/LRS

Respond to ISR, WO,
Chapter 2 Deadline - 5 Apr 07

Due Date

Cal Conf - national phase

1. ☒ 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 44.1).
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.

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 **30 months** (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority

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

Authorized officer

Francesca Reginelli

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.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

If a demand for international preliminary examination is made, the written opinion of the International Searching Authority will, except in certain cases where the International Preliminary Examining Authority did not act as International Searching Authority and where it has notified the International Bureau under Rule 66.1 bis(b), be considered to be a written opinion of the International Preliminary Examining Authority. If a demand is made, the applicant may submit to the International Preliminary Examining Authority a reply to the written opinion together, where appropriate, with amendments before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later (Rule 43bis.1(c)).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see the *PCT Applicant's Guide*, Volume II.

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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 P2177R1	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2005/034647	International filing date (day/month/year) 28/09/2005	(Earliest) Priority Date (day/month/year) 05/10/2004
Applicant GENENTECH, INC.		

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

This international search report consists of a total of 8 sheets.

☐ 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:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (See Box No. II)

3. ☒ **Unity of invention is lacking** (see Box No III)

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ 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. 8
☒ 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/US2005/034647

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

☒

a sequence listing

☐

table(s) related to the sequence listing

b. format of material

☒

on paper

☒

in electronic form

c. time of filing/furnishing

☐

contained in the international application as filed

☐

filed together with the international application in electronic form

☒

furnished subsequently to this Authority for the purpose of search

2. ☒ 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:

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/034647

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. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-36 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:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ 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.:

1-40, 119-124 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-40,119-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient in a dose of 400 mg to 1.3 grams at a frequency of one to three doses within a period of one month, article of manufacture comprising CD20 antibody, whereby CD20 antibody is Rituximab.

2. claims: 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos 2 and 8.

3. claims: 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 39 and 40.

4. claims: 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 32 and 33.

5. claims: 1-124 (all partially)

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO 8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO 2.

6. claims: 41-118 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure 42 The method of claim 41 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody whereby CD20 antibody is Rituximab.

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		International application No PCT/US2005/034647
A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/28		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, WPI Data, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SPECKS U ET AL: "RESPONSE OF WEGENER'S GRANULOMATOSIS TO ANTI-CD20 CHIMERIC MONOCLONAL ANTIBODY THERAPY" ARTHRITIS AND RHEUMATISM, LIPPINCOTT, PHILADELPHIA, US, vol. 44, no. 12, December 2001 (2001-12), pages 2836-2840, XP002406917 ISSN: 0004-3591	1,37,119
Y	page 2837, left-hand column, paragraph 1 page 2838, left-hand column, paragraph 2 figure 1 ----- -/--	2-36, 38-40, 120-124
☒ Further documents are listed in the continuation of Box C. ☒ 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 10 November 2006		Date of mailing of the international search report 23 -02- 2007
Name and mailing address of the ISA/ 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		Authorized officer Gruber, Andreas

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International application No
PCT/US2005/034647

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/035607 A2 (GENMAB AS [DK]; TEELING JESSICA [NL]; RUULS SIGRID [NL]; GLENNIE MARTI) 29 April 2004 (2004-04-29)	1,37,119
Y	page 14	2-36, 38-40, 120-124
	page 53, line 20 - line 24	
X	ERIKSSON P: "Short-term outcome and safety in 5 patients with ANCA-positive vasculitis treated with rituximab." KIDNEY & BLOOD PRESSURE RESEARCH, vol. 26, no. 4, 2003, page 294, XP002406918 & 11TH INTERNATIONAL VASCULITIS AND ANCA WORKSHOP; PRAGUE, CZECH REPUBLIC; OCTOBER 02-05, 2003 ISSN: 1420-4096 the whole document	1,37,119
A	WO 01/97858 A2 (IDEC PHARMA CORP [US]) 27 December 2001 (2001-12-27) page 1 page 55, paragraph 2; claims 1,6-8	1-40, 119-124
A	WO 00/67796 A (GENENTECH INC [US]; IDEC PHARMACEUTICALS INC [US]) 16 November 2000 (2000-11-16) page 3, paragraph 3 page 23, line 17 - line 22	1-40, 119-124
A	WO 00/74718 A (IMMUNOMEDICS INC [US]; GOLDENBERG DAVID M [US]; HANSEN HANS J [US]) 14 December 2000 (2000-12-14) page 18, paragraph 1; claims 2,7,12; examples 5,6	1-40, 119-124
A	LAMPRECHT P ET AL: "Rituximab induces remission in refractory HCV associated cryoglobulinaemic vasculitis." ANNALS OF THE RHEUMATIC DISEASES, vol. 62, no. 12, December 2003 (2003-12), pages 1230-1233, XP002406916 ISSN: 0003-4967 page 1232, right-hand column, paragraph 1 page 1233, left-hand column, last paragraph	1-40, 119-124

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Information on patent family members				International application No	
				PCT/US2005/034647	
Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 2004035607	A2	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
			JP 2006507844	T	09-03-2006
			KR 20050049552	A	25-05-2005
			MX PA05004022	A	05-10-2005
WO 0197858	A2	27-12-2001	AU 6836301	A	02-01-2002
			CA 2411102	A1	27-12-2001
			EP 1299128	A2	09-04-2003
			JP 2004512262	T	22-04-2004
WO 0067796	A	16-11-2000	AT 311199	T	15-12-2005
			AU 777970	B2	04-11-2004
			AU 4714300	A	21-11-2000
			AU 2005200462	A1	03-03-2005
			BR 0011197	A	19-02-2002
			CA 2372603	A1	16-11-2000
			CN 1378459	A	06-11-2002
			DE 60024436	D1	05-01-2006
			DE 60024436	T2	17-08-2006
			DK 1176981	T3	10-04-2006
			EP 1176981	A1	06-02-2002
			EP 1637160	A2	22-03-2006
			ES 2254174	T3	16-06-2006
			HK 1043312	A1	28-07-2006
			HU 0201009	A2	29-07-2002
			JP 2002544174	T	24-12-2002
			MX PA01011279	A	02-07-2002
			NO 20015417	A	07-01-2002
			NZ 514914	A	24-09-2004
			PL 351962	A1	14-07-2003
WO 0074718	A	14-12-2000	AU 782160	B2	07-07-2005
			AU 5600500	A	28-12-2000
			CA 2375912	A1	14-12-2000
			DE 05075555	T1	08-02-2007
			EP 1194167	A1	10-04-2002
			JP 2003501401	T	14-01-2003

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From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.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.
PCT/US2005/034647

International filing date (day/month/year)
28.09.2005

Priority date (day/month/year)
05.10.2004

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/28

Applicant
GENENTECH, INC.

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

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

2. FURTHER ACTION

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

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

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

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

Name and mailing address of the ISA:



European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

Gruber, Andreas

Telephone No. +31 70 340-8997



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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/034647

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

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

a. type of material:

- ☒ a sequence listing
- ☐ table(s) related to the sequence listing

b. format of material:

- ☒ on paper
- ☒ in electronic form

c. time of filing/furnishing:

- ☐ contained in the international application as filed.
- ☐ filed together with the international application in electronic form.
- ☒ furnished subsequently to this Authority for the purposes of search.

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

4. Additional comments:

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/034647

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. 1-36 (all partially)

because:

☒ the said international application, or the said claims Nos. 1-36 (all with regard to industrial applicability) relate to the following subject matter which does not require an international search (*specify*):

see separate sheet

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

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

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

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

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

☐ See Supplemental Box for further details

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/034847

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:
- ☐ paid additional fees
 - ☐ paid additional fees under protest and, where applicable, the protest fee
 - ☐ paid additional fees under protest but the applicable protest fee was not paid
 - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☐ all parts.
 - ☒ the parts relating to claims Nos. 1-40, 119-124 (all partially)

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

1. Statement

Novelty (N)	Yes: Claims	2-36, 38-40, 120-124
	No: Claims	1, 37, 119
Inventive step (IS)	Yes: Claims	
	No: Claims	1-40, 119-124
Industrial applicability (IA)	Yes: Claims	37-40, 119-124
	No: Claims	

2. Citations and explanations

see separate sheet

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/034647

Box No. VIII Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

The present application describes treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis), e.g. Wegener's granulomatosis or microscopic polyangiitis, by administering a CD20 antibody.

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: WO 01/97858 A2 (IDEC PHARMA CORP [US]) 27 December 2001
- D2: WO 00/67796 A (GENENTECH INC [US]; IDEC PHARMACEUTICALS INC [US]) 16 November 2000
- D3: WO 00/74718 A (IMMUNOMEDICS INC [US]; GOLDENBERG DAVID M [US]; HANSEN HANS J [US])
- D4: SPECKS U ET AL: "RESPONSE OF WEGENER'S GRANULOMATOSIS TO ANTI-CD20 CHIMERIC MONOCLONAL ANTIBODY THERAPY" ARTHRITIS AND RHEUMATISM, vol. 44, no. 12, December 2001, pages 2838-2840
- D5: WO 2004/035607 A2 (GENMAB AS [DK]; TEELING JESSICA [NL]; RUULS SIGRID [NL]; GLENNIE MARTI) 29 April 2004

Re Item III

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

Claims 1-36 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 IV

Lack of unity of invention

The application does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT, and multiple inventions were found, as follows:

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

Invention 1: claims 1-40, 119-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient in a dose of 400 mg to 1.3 grams at a frequency of one to three doses within a period of one month, article of manufacture comprising CD20 antibody, whereby CD20 antibody is Rituximab.

Inventions 2: claims 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos 2 and 8.

Inventions 3: claims 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 39 and 40.

Inventions 4: claims 1-124 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 32 and 33.

Inventions 5: claims 1-124 (all partially)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO 8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO 2.

Invention 6: claims 41-118 (all partially)

A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure 42 The method of claim 41 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody whereby CD20 antibody is Rituximab.

The concept or problem to be solved by the subject-matter of claims 1-124 may be regarded as the provision of methods for treating anti-neutrophil cytoplasmic antibodies (ANCA)- associated vasculitis in a subject (page 1, paragraph 2) using a CD20 antibody (e.g. claim 1).

However, this problem has been solved already:

WO0197858 (D1) discloses (the references in parentheses applying to this document): treatment of e.g. vasculitis (page 55, paragraph 2) comprising anti-CD20 antibody Rituximab (page 1) or an equivalent to Rituximab (claim 1) at a concentration of 0.1 mg to 500 mg/m² or more per week, or with a dosage of about 375 mg/m² weekly for four weeks (claims 6-8).

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

WO0067796 (D2) discloses (the references in parentheses applying to this document): treatment of vasculitis (page 3, paragraph 3) with Rituximab in the range from about 20mg/m² to about 1000mg/m² (page 23, lines 17-22).

WO0074718 (D3) treatment of Wegener's granulomatosis (claim 7) by administration as continuous infusion or by single or multiple boluses (page 18, first paragraph) of 20 to 2000 mg per dose (claim 2) anti-CD20 antibody (claim 12), further disclosed is Rituximab (examples 5,6).

Document D4 discloses treatment of Wegener's granulomatosis comprising administration of 375mg/m² of Rituximab 4 times per week (the whole document, e.g. abstract, page 2838, left-hand column, paragraph 2; Fig. 1).

Note: D4 discloses the treatment of a 66-year old man (page 2837, left-hand column, first paragraph) which is expected to corresponds to a body surface area of at least 1.5 m², and thus to an amount of Rituximab of at least 560 mg per dose.

WO2004035607 (D5) discloses the treatment of Wegener's granulomatosis with CD20 antibodies (page 14) administered by infusion in a weekly dosage of 10 to 500 mg/m², and may be repeated, e. g., 1 to 8 times (page 53, lines 20-24).

In the light of the prior art, it should be concluded that there is no common concept underlying the subject-matter of claims 1-124.

The technical problem to be solved by the present application is to provide a treatment that reduces the frequency of infusions of active drug within a month's time, that reduces the risk of toxic effects of currently used drugs such as steroids and chemotherapeutic agents, and to reduce the risk of disease flares, relapses, and recurrences in patients with ANCA-associated vasculitis, and to sustain remission and maintain sustained remission for a prolonged period of time (page 11, lines 17-21). The solutions to this problem and/or the special technical features, representing a contribution over the prior art, of the different inventions in the present application are as follows:

Solution 1: A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

administering a CD20 antibody to the patient in a dose of 400 mg to 1.3 grams at a frequency of one to three doses within a period of one month, article of manufacture comprising CD20 antibody, whereby CD20 antibody is Rituximab. This solution is known from the prior art and no special technical feature is present.

- Solution 2:** A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos 2 and 8.
- Solution 3:** A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 39 and 40.
- Solution 4:** A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS 32 and 33.
- Solution 5:** A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient, article of manufacture comprising CD20 antibody, whereby CD20 antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO 8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO 2.
- Solution 6:** A method of treating anti-neutrophil cytoplasmic antibodies-associated

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

vasculitis (ANCA-associated vasculitis) in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure 42 The method of claim 41 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody whereby CD20 antibody is Blituximab.

Thus, at least one solution, i.e. solution 1, to the problem is already disclosed and the remaining solutions may be seen as alternative solutions to the problem.

The inventions described in claims 1-124 are different from each other, and there is no special technical feature that links these inventions together. Thus, the technical relationship between the different inventions described in claims 1-124 required by Rule 13.2 PCT is lacking, and the requirement for unity of invention referred to in Rule 13.1 PCT is not fulfilled.

- 1 The following opinion is given for the subject-matter searched, i.e. invention 1 as described above:

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

- 2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claims 1,37,119 is not new in the sense of Article 33(2) PCT.

- 2.1 The document D5 makes the disclosure as stated above.

Thus, the subject-matter of independent claims 1,37,119 is not new in the sense of Article 33(2) PCT.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

- 2.2 The document D4 makes the disclosure as stated above and the unclear statement "about one month" does not allow to distinguish the subject-matter of claim 1 from D4.

Thus, the subject-matter of independent claims 1,37,119 is not new in the sense of Article 33(2) PCT.

- 3 Dependent claims 2-36,38-40,120-124 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows: In view of the prior art (e.g. documents D1-D5), the skilled person would regard the subject-matter of these claims as a normal option. The subject-matter of claims 2-36,38-40,120-124 does therefore not involve an inventive step in the sense of Article 33(3) PCT.
- 4 The subject-matter of claims 37-40,119-124 is susceptible of industrial application (Article 33(4) PCT).
- 5 For the assessment of the present claims 1-36 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.

Re Item VIII

Certain observations on the international application

- 6 The term "about" used throughout the claims is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT.
- 7 The expression "incorporated by reference" and similar expressions on:

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/00 951 271.6

- page 1 line 11,
- page 60, line 28

should have been deleted from the description. If matter in the documents referred to is essential to satisfy the requirements of Art. 5 PCT, then this matter should be expressly incorporated into the description.

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file JH/LS

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PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

From the INTERNATIONAL BUREAU

To:

HASAK, Janet E.
GENENTECH, INC.
1 DNA Way
South San Francisco, California 94080-4990
ETATS-UNIS D'AMERIQUE

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 10 January 2006 (10.01.2006)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference P2177R1	
International application No. PCT/US2005/034847	
International filing date (day/month/year) 28 September 2005 (28.09.2005)	
International publication date (day/month/year)	Priority date (day/month/year) 05 October 2004 (05.10.2004)
Applicant GENENTECH, INC. et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. (If applicable) The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
3. (If applicable) An asterisk (*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
05 October 2004 (05.10.2004)	60618,104	US	18 December 2005 (18.12.2005)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Sean M. Taylor Facsimile No. (41-22) 338.90.90 Telephone No. +41 22 338 9811

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From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 10 January 2006 (10.01.2006)	To: HASAK, Janet E. GENENTECH, INC. 1 DNA Way South San Francisco, California 94080-4990 ETATS-UNIS D'AMERIQUE	
Applicant's or agent's file reference P2177R1		IMPORTANT NOTIFICATION
International application No. PCT/US2005/034847		International filing date (day/month/year) 28 September 2005 (28.09.2005)
International publication date (day/month/year)		Priority date (day/month/year) 05 October 2004 (05.10.2004)
Applicant GENENTECH, INC. et al		

- 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).
- (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(e) 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.
- (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(e) 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
05 October 2004 (05.10.2004)	60/616,104	US	16 December 2005 (16.12.2005)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Sean M. Taylor Facsimile No. (41-22) 338.90.90 Telephone No. +41 22 338 9811

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UNITED STATES DEPARTMENT OF COMMERCE
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FEBRUARY 09, 2005

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GENETECH, INC.
JANET HASAK
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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: 02/04/2005

REEL/FRAME: 015662/0714
NUMBER OF PAGES: 3

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).
DOCKET NUMBER: PR2177

ASSIGNOR:

BRUNETTA, PAUL G.

DOC DATE: 02/02/2005

ASSIGNEE:

GENENTECH INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CALIFORNIA

94080-4990

SERIAL NUMBER: 60616104

FILING DATE: 10/05/2004

PATENT NUMBER:

ISSUE DATE:

TITLE: METHOD FOR TREATING VASCULITIS

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FROM 003/002

PER 001/001

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STEVEN POST, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

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Docket No. PR2177

ASSIGNMENT

WHEREAS, Paul G. Brunetta, a citizen of United States of America, residing at 1257 Hampshire Street, San Francisco, California 94110 (hereinafter "ASSIGNOR"), has invented a new and useful invention in

METHOD FOR TREATING VASCULITIS

for which a provisional application Serial No. 60/616,104, if received (Docket No. PR2177) has been filed by him on 05 October 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 expenses 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: 2/2/05


Paul G. Brunetta

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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 Prepared Using	PCT-SAFE [EASY mode] Version 3.50 (Build 0002.173)
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	P2177R1
I	Title of invention	METHOD FOR TREATING VASCULITIS
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)	BRUNETTA, Paul G.
III-1-5	Address	1257 Hampshire Street San Francisco, California 94110 United States of America
III-1-6	State of nationality	US
III-1-7	State of residence	US

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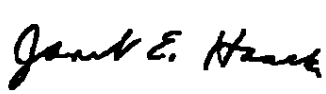
Original (for SUBMISSION)

IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent:
IV-1-1	Name (LAST, First)	HASAK, Janet E.
IV-1-2	Address	GENENTECH, INC. 1 DNA Way South San Francisco, California 94080-4990 United States of America
IV-1-3	Telephone No.	650-225-1896
IV-1-4	Facsimile No.	650-952-9881
IV-1-5	Agent's registration No.	28,616
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.; ELLIOTT, Jennifer L.; EVANS, David W.; JOHNSTON, Sean A.; KRESNAK, Mark T.; KUBINEC, Jeffrey S.; LEE, Wendy M.; 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	05 October 2004 (05.10.2004)
VI-1-2	Number	60/616,104
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

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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	69	-
IX-3	Claims	7	-
IX-4	Abstract	1	✓
IX-5	Drawings	8	-
IX-7	TOTAL	89	
IX-8	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	reference no. none	-
IX-17	PCT-SAFE physical media	-	✓
IX-19	Figure of the drawings which should accompany the abstract	8	
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative		
X-1-1	Name (LAST, First)	HASAK, Janet E.	
X-1-2	Name of signatory		
X-1-3	Capacity		

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Original (for SUBMISSION)

FOR RECEIVING OFFICE USE ONLY

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

FOR INTERNATIONAL BUREAU USE ONLY

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

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PATENT
Attorney Docket P2177R1
Express Mail No. EV 385655662 US
Mailed: September 28, 2005

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METHOD FOR TREATING VASCULITIS

Related Applications

10 This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application number 60/616,104 filed October 5, 2004, the contents of which are incorporated herein by reference.

Field of the Invention

15 The present invention concerns methods for treating anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis in a subject, and kits with instructions for such uses.

Background of the Invention

Vasculitis

20 Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, vasculitis, and lupus, among others, remain clinically important diseases in humans. As the name implies, autoimmune diseases wreak their havoc through the body's own immune system. While the pathological mechanisms differ among individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) present.

25 Vasculitis is defined by inflammation of the blood-vessel wall and forms the pathological foundation of a diverse group of individual disease entities. Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, which is a common primary systemic vasculitis, includes microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome, renal-limited vasculitis (idiopathic necrotizing crescentic glomerulonephritis) (Falk *et al.* *N. Engl. J. Med.*, 318: 1651-1657 (1988)), and certain types of drug-induced vasculitis. Jennette *et al.* *Arthritis Rheum* 37:187-92 (1994); Jennette and Falk, *N. Engl. J. Med.* 337:1512-1523 (1997). The diseases mentioned above affect people of all ages but are most common in older adults in their 50s and 60s, and they affect men and women equally. Pettersson *et al.* *Clin. Nephrol.*, 43: 141-149 (1995); Falk *et al.*, *Ann. Intern. Med.* 113: 656-663 (1990).

30 ANCA are specific antibodies for antigens in cytoplasmic granules of neutrophils and monocyte lysosomes, first reported in 1982. Niles *et al.*, *Arch. Intern. Med.*, 156:440-445 (1996). ANCA were originally detected by indirect immunofluorescence on ethanol-fixed neutrophils. Wiik, "Delineation of a standard procedure for indirect immunofluorescence detection of ANCA" *APMIS Suppl.* 6: 12-13 (1989). At least three different patterns of fluorescence have been distinguished: a cytoplasmic/classic pattern (cANCA) with accentuation of the fluorescence intensity in the area with the nuclear lobes, a perinuclear pattern (pANCA), and a more diffuse cytoplasmic staining pattern (atypical ANCA). Approximately 90% of the sera that produce a cANCA pattern react with proteinase 3 (PR3), a serine protease from the azurophilic granules of myeloid cells. Jennette and Falk, *N. Engl. J. Med.*, *supra*. In patients with primary systemic vasculitis predominantly affecting medium- and small-sized blood vessels, approximately 75% of the sera producing a perinuclear pattern (pANCA) react with myeloperoxidase (MPO), a myeloid lysosomal enzyme. Cohen

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Tervaert *et al.*, *Am. J. Med.* 91: 59-66 (1991). In ANCA-positive patients with other non-vasculitic diseases, often antigenic specificities are recognized. The diagnostic potential of PR3-ANCA and MPO-ANCA is now fairly well established. In a patient with signs and symptoms of vasculitis, ANCA with specificity for PR3 (PR3-ANCA) suggests a diagnosis of Wegener's granulomatosis, whereas ANCA with a specificity for MPO (MPO-ANCA) is highly sensitive for microscopic polyangiitis, idiopathic necrotizing crescentic glomerulonephritis, or active Churg-Strauss syndrome. Cohen Tervaert *et al.*, *Sarcoidosis Vasc. Diffuse Lung Dis.* 13: 241-245 (1996). See also Xiao *et al.*, *J. Clin. Invest.* 110: 955-963 (2002), which describe an animal model that offers strong support for a direct pathogenic role for ANCA IgG in human glomerulonephritis and vasculitis, and Pope *et al.*, *J. Allergy Clin. Immunol.*, 103: 885-894 (1999) showing that in Wegener's granulomatosis, B-cell activation is related to active disease, whereas T-cell activation persists during remission of the disease, which points to an intrinsic disordered immune system in this disease. See also Cupps *et al.*, *J. Immunol.*, 128: 2453-2457 (1982) regarding the role of cyclophosphamide in suppressing human B lymphocyte function.

Within the spectrum of primary vasculitic syndromes, the ANCA-related syndromes form a distinct group with overlapping features. Most patients have a prodromal flu-like onset consisting of malaise, myalgias, arthralgias, fever, and weight loss. This flu-like onset appears within days to weeks before the onset of overt vasculitic or nephritic disease. Wegener's granulomatosis is differentiated from the others by the presence of necrotizing granulomatous inflammation of the upper and lower respiratory tract, which is usually accompanied by systemic necrotizing small vessel vasculitis and glomerulonephritis. Churg-Strauss syndrome is differentiated by the presence of (a history of) asthma, allergic rhinitis, systemic eosinophilia, in addition to systemic vasculitis with or without glomerulonephritis. Microscopic polyangiitis is characterized by necrotizing and/or crescentic glomerulonephritis and a multi-system vasculitis involving small vessels. Microscopic polyangiitis shares many features with Wegener's granulomatosis and Churg-Strauss syndrome, but lacks necrotizing granulomatous inflammation of the respiratory tract and asthma. Jennette *et al.*, *Arthritis Rheum.*, *supra*. In idiopathic necrotizing and/or crescentic glomerulonephritis the vasculitic process is limited to the kidneys. Because the treatment of patients with microscopic polyangiitis or Wegener's granulomatosis is essentially the same when there is major organ injury, it is unnecessary to distinguish conclusively between these closely related variants of ANCA-associated vasculitis before initiating treatment. Jennette *et al.* *Arthritis Rheum.*, *supra*.

Before treatment became available, patients with generalized Wegener's granulomatosis had a median survival of five months. In the early 1970s, Fauci and Wolff introduced a regimen combining daily cyclophosphamide therapy given for one year after remission was achieved with prednisone therapy initiated at a dose of 1 mg per kilogram of body weight per day and tapered on an alternate-day schedule. This treatment has reproducibly been found to induce remission in 80 to 100 percent of patients and can result in long-term survival. In fact, prolonged immunosuppressive therapy (greater than 1 year) with cyclophosphamide and steroids is effective in inducing disease remission and preventing early relapses in most vasculitic disorders. Balow *et al.*, "Vasculitic diseases of the kidney, polyarteritis, Wegener's granulomatosis, necrotizing and crescentic glomerulonephritis, and other disorders." In: Schrier and Gottechalk (eds): *Diseases of the kidney*, 5th edition, (Little, Brown and Company, Boston, 1993), pp. 2095-2117; Jayne *et al.*, *N. Engl. J. Med.*, 349: 36-44 (2003); Gaskin *et al.*, "Systemic vasculitis" In: Cameon *et al.*

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(eds): *Oxford textbook of clinical nephrology*. (Oxford University Press, Oxford, 1992), pp. 612-636; Fauci *et al.*, *Ann. Intern. Med.* 89: 660-676 (1978); Fauci *et al.*, *Ann. Intern. Med.* 98: 76-85 (1983); Hoffman *et al.*, *Ann. Int. Med.*, 116: 488-498 (1992); and Andrassy *et al.*, *Clin. Nephrol.*, 35: 139-147 (1991).

5 However, when therapy is tapered and discontinued, relapses are common. In one study, in which patients with Wegener's granulomatosis were followed for a mean of eight years, relapse occurred in 50 percent of patients. Further, continuous use of cyclophosphamide to sustain remission is not recommended, since this treatment regimen is associated with severe and potentially lethal adverse effects such as the occurrence of opportunistic infections and the development of malignancies. For example, repeated courses of cyclophosphamide are associated with bone-marrow suppression, infection, cystitis, infertility,
10 myelodysplasia, and transitional-cell carcinoma of the bladder. In some instances, such toxic effects preclude further use of cyclophosphamide. Stillwell *et al.*, *Arthritis Rheum.*, 31: 465-470 (1988); Radies *et al.*, *Arthritis Rheum.* 38: 1120-1127 (1995). Therefore, cyclophosphamide is tapered or stopped and replaced by azathioprine once remission is achieved to prevent adverse effects, a policy tested in a rigorous multi-center trial and proven to be equally effective in the follow-up for 18 months. Gaskin *et al. supra*, 1992; Jayne, *Rheumatology* 39: 585-595 (2000). Azathioprine is considered less effective in inducing remission than
15 cyclophosphamide, but its long-term toxicity is much lower. Bouroncle *et al.*, *Am. J. Med.*, 42: 314-318 (1967); Norton *et al.*, *Arch. Intern. Med.*, 121: 554-560 (1968).

Other alternative maintenance therapy regimens include methotrexate ((de Groot *et al.*, *Arthritis Rheum.*, 39: 2052-2061 (1996)), cyclosporine A (Haubitz *et al.*, *Nephrol. Dial. Transplant.* 13: 2074-2076 (1998)), mycophenolate (Nowack *et al.*, *J. Am. Soc. Nephrol.* 10: 1965-1971 (1999)), or trimethoprim-sulfamethoxazole (Stegeman *et al.*, *N. Engl. J. Med.*, 335: 16-20 (1996)). See also Sanders, *et al. N. Engl. J. Med.* 349: 2072-2073 (2003). Since, however, relapses are frequently observed in ANCA-associated vasculitis, treatment in such cases has to be intensified or reinstituted. Hoffman *et al. supra*; Gordon *et al.*, *Q J. Med.*, 86: 779-789 (1993); Nachman *et al.*, *J. Am. Soc. Nephrol.*, 7: 33-39 (1996); Guillevin *et al.*, *Medicine* 78: 26-37 (1999); Reinhold-Keller *et al.*, *Arthritis. Rheum.* 43: 1021-1032 (2000); Langford, *New Eng. J. Med.*, 349: 3-4 (July 2003).
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Tumor necrosis factor-alpha (TNF-alpha) blockade with infliximab is a potential therapy for ANCA-associated vasculitis, both for initial therapy and in the management of refractory disease. Infliximab was effective at inducing remission in 88% of patients with ANCA-associated vasculitis and permitted reduction in
30 steroid doses. Booth *et al.*, *J. Am. Soc. Nephrol.* 15:717-721 (2004). In addition, Stone *et al.*, *Arthritis and Rheumatism*, 44: 1149-1154 (2001) disclosed that the TNF-alpha inhibitor etanercept (ENBREL®), given 25 mg subcutaneously twice weekly in combination with standard treatment for Wegener's granulomatosis, was well-tolerated in the patients with few adverse events, but intermittently active disease (occasionally severe) was common.

35 Patients with Churg-Strauss syndrome usually respond to high-dose corticosteroid therapy alone, although some cases may require the addition of cytotoxic drugs. Jayne and Rasmussen, *Mayo Clin. Proc.* 72:737-47 (1997). Co-morbid conditions that accelerate vascular damage, *e.g.*, hypertension, diabetes, hypercholesterolemia, and smoking, should be appropriately controlled.

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In drug-induced vasculitis, the offending agent should be stopped. Antihistamines and non-steroidal anti-inflammatory drugs help alleviate skin discomfort and reduce associated arthralgias and myalgias. Severe cutaneous disease may warrant oral corticosteroid therapy. Jennette *et al.*, *Arthritis Rheum*, *supra*.

5 The persistence or reappearance of ANCA is a risk factor for the development of a relapse of disease activity, suggesting a pathophysiological role *in vivo* for these autoantibodies. Stegeman *et al.*, *Ann. Intern. Med.*, 120: 12-17 (1994); De'Oliviera *et al.*, *Am. J. Kidney Dis.*, 25: 380-389 (1995); Jayne *et al.*, *Q. J. Med.*, 88: 127-133 (1995). Relapses of Wegener's granulomatosis are frequently preceded by rises in the titer of cANCA as detected by indirect immunofluorescence (Cohen Tervaert *et al.*, *Arch. Intern. Med.*, 149: 2461-2465 (1989)), and can be prevented by treatment with immunosuppressives based on rises in cANCA (Cohen
10 Tervaert *et al.*, *Lancet*, 336: 706-711 (1990)).

For a general discussion on ANCA-associated vasculitis, see Lhote and Guillevin, *Rheum. Dis. Clin. North Am.* 21:911-947 (1995); "ANCA-associated vasculitis: occurrence, prediction, prevention, and outcome of relapses" by Maarten Boomsma, PhD Thesis, Thesis University Groningen, ISBN 90-367-1451-6 (M.M. Boomsma, Groningen, 2001) (<http://www.ub.rug.nl/eldoc/dis/medicine/m.m.boomsma/thesis.pdf>):
15 Kamesh *et al.*, *J. Am. Soc. Nephrol.* 13:1953-1960 (2002); and Jayne, *Kidney & Blood Pressure Research* 26:231-239 (2003).

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
20 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 naïve 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
25 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 molecules 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
30 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
35 (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

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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.*). Rituximab 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 rituximab 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, rituximab has been shown to have anti-proliferative effects in tritiated thymidine-incorporation assays and to induce apoptosis directly, while other anti-CD19 and anti-CD20 antibodies do not. Maloney *et al.* *Blood* 88(10):637a (1996). Synergy between rituximab and chemotherapies and toxins has also been observed experimentally. In particular, rituximab 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 rituximab 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* 83:435-445 (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 NHL at a dose of 375 mg/m² weekly for four doses. In April 2001, the Food and Drug Administration (FDA) approved additional claims for the treatment of low-grade NHL: re-treatment (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.* 21:1746-1751 (2003); Hainsworth *et al.*, *J. Clin. Oncol.* 20:4261-4267 (2002). Also, rituximab has been used in the treatment of malignant and nonmalignant plasma cell disorders. Treon and Anderson, *Semin. Oncol.* 27: 79-85 (2000).

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.* 30:824-828 (2002). Rituximab has been reported to potentially relieve signs and symptoms of, for example, rheumatoid arthritis (RA) (Leandro *et al.*, *Ann. Rheum. Dis.* 61:883-888 (2002); Edwards *et al.*, *Arthritis Rheum.*, 46 (Suppl. 9): S46 (2002); Stahl *et al.*, *Ann. Rheum. Dis.*, 62 (Suppl. 1): OP004 (2003); Shaw *et al.* *Ann. Rheum. Dis.* 62 Suppl 2:ii55-ii59 (2003); Weyand and Goronzy, *Ann. N.Y. Acad. Sci.* 987: 140-149 (2003); Emery *et al.*, *Arthritis Rheum.* 48(9): S439 (2003)), lupus (Eisenberg, *Arthritis. Res. Ther.* 5:157-159 (2003); Anolik *et al.*, *Arthritis Rheum.* 48: 455-459 (2003); Leandro *et al.* *Arthritis Rheum.* 46: 2673-2677 (2002); Gorman *et al.*, *Lupus.* 13: 312-316 (2004); Tomietto *et al.*, *Thromb. Haemast.* 92: 1150-

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1153 (2004)), immune thrombocytopenic purpura (D'Arena *et al.*, *Leuk. Lymphoma* 44:561-562 (2003); Stasi *et al.*, *Blood*, 98: 952-957 (2001); Saleh *et al.*, *Semin. Oncol.*, 27 (Supp 12):99-103 (2000); Zaja *et al.*, *Haematologica*, 87: 189-195 (2002); Zaja *et al.*, *Haematologica* 88: 538-546 (2003); Cooper *et al.*, *Br. J. Haematol.* 125: 232-239 (2004); Ratanatharathorn *et al.*, *Ann. Int. Med.*, 133: 275-279 (2000)), pure red cell aplasia (Auner *et al.*, *Br. J. Haematol.*, 116: 725-728 (2002)); autoimmune anemia (Zaja *et al.*, *Haematologica* 87:189-195 (2002) (erratum appears in *Haematologica* 87:336 (2002); Raj *et al.*, *J. Pediatr. Hematol. Oncol.* 26: 312-314 (2004); Zecca *et al.*, *Blood* 101: 3857-3861 (2003); Quartier *et al.*, *Lancet* 358: 1511-1513 (2001)), autoimmune cytopenias (Robak, *Eur. J. Haematol.* 72: 79-88 (2004)); cold agglutinin disease (Layios *et al.*, *Leukemia*, 15: 187-8 (2001); Berentsen *et al.*, *Blood*, 103: 2925-2928 (2004); Berentsen *et al.*, *Br. J. Haematol.*, 115: 79-83 (2001); Bauduer, *Br. J. Haematol.*, 112: 1083-1090 (2001); Damiani *et al.*, *Br. J. Haematol.*, 114: 229-234 (2001); Lee and Kueck, *Blood* 92: 3490-3491 (1998)), type B syndrome of severe insulin resistance (Coll *et al.*, *N. Engl. J. Med.*, 350: 310-311 (2004), mixed cryoglobulinemia (DeVita *et al.*, *Arthritis Rheum.* 46 Suppl. 9:S206/S469 (2002); Zaja *et al.*, *Haematologica* 84: 1157-1158 (1999)), myasthenia gravis (Zaja *et al.*, *Neurology*, 55: 1062-63 (2000); Wylam *et al.*, *J. Pediatr.*, 143: 674-677 (2003)), Wegener's granulomatosis (Specks *et al.*, *Arthritis & Rheumatism* 44: 2836-2840 (2001)), refractory pemphigus vulgaris (Dupuy *et al.*, *Arch Dermatol.*, 140:91-96 (2004)), dermatomyositis (Levine, *Arthritis Rheum.*, 46 (Suppl. 9):S1299 (2002)), Sjogren's syndrome (Somer *et al.*, *Arthritis & Rheumatism*, 49: 394-398 (2003)), active type-II mixed cryoglobulinemia (Zaja *et al.*, *Blood*, 101: 3827-3834 (2003)), pemphigus vulgaris (Dupay *et al.*, *Arch. Dermatol.*, 140: 91-95 (2004)), autoimmune neuropathy (Pestronk *et al.*, *J. Neurol. Neurosurg. Psychiatry* 74:485-489 (2003); Nobile-Orazio, *Curr. Opin. Neurol.* 17: 599-605 (2004); Rojas-Garcia *et al.*, *Neurology* 61: 1814-1816 (2003); Renaud *et al.*, *Muscle Nerve* 27: 611-615 (2003)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli *et al.*, *Neurology* 60(Suppl. 1) PO5.128:A395 (2003)), acquired factor VIII inhibitors (Wiestner *et al.*, *Blood* 100: 3426-3428 (2002); and relapsing-remitting multiple sclerosis (RRMS). Cross *et al.* (abstract) "Preliminary Results from a Phase II Trial of Rituximab in MS" Eighth Annual Meeting of the Americas Committees for Research and Treatment in Multiple Sclerosis, 20-21 (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, and rituximab plus cyclophosphamide (CTX). The treatment regimen of rituximab was one gram 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 was considered to be mild to moderate in severity and was 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 4.66 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.* 46:2287-2293 (2002).

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The reported safety profile of rituximab in a small number of patients with neurologic disorders, including autoimmune neuropathy (Pestronk *et al.*, *supra*), opsoclonus-myoclonus syndrome (Panzatelli *et al.*, *supra*), and RRMS (Cross *et al.*, *supra*), was similar to that reported in oncology or RA. In an ongoing investigator-sponsored trial (IST) of rituximab in combination with interferon-beta (IFN- β) or glatiramer acetate in patients with RRMS (Cross *et al.*, *supra*), 1 of 10 treated patients was admitted to the hospital for overnight observation after experiencing moderate fever and rigors following the first infusion of rituximab, while the other 9 patients completed the four-infusion regimen without any reported adverse events.

Patents and patent publications concerning CD20 antibodies and CD20-binding molecules 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 and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); WO 2000/27433 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); WO 2001/10461 (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, WO 2001/74388 and 6,896,885B5 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2005/0123540 (Hanna *et al.*); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858; US 2005/0112060, and US Patent No. 6,846,476 (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 Devica); 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.); US Patent Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721. (Kaminaki *et al.*); US Patent Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, 6,652,852, 6,893,625 (Robinson *et al.*); US Patent No. 6,410,391 (Raubitschek *et al.*); US Patent No. 6,224,866 and WO00/20864 (Barbora-Guillem, E.); WO 2001/13945 (Barbora-Guillem, E.); WO 2000/67795 (Goldenberg); US 2003/0133930 and WO 2000/74718 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO 2004/058298 (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, US 2002/0009427, and US 2003/0185796 (Wolin *et al.*); WO 2003/061694 (Sing and Siegal); US 2003/0219818 (Bohen *et al.*); US 2003/0219433 and WO 2003/068821 (Hansen *et al.*); US 2003/0219818 (Bohen *et al.*); US 2002/0136719 (Shenoy *et al.*); WO 2004/032828 and US 2005/0180972 (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.*); US 2001/0056066 (Bugelski *et al.*); WO 1995/03770 (Bhat *et al.*); US 2003/0219433 A1 (Hansen *et al.*); WO 2004/035607 (Teeling *et al.*); WO 2004/056312 (Lowman *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.*); WO 2005/016969 (Carr *et al.*); US 2005/0069545 (Carr *et al.*); WO

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2005/014618 (Chang *et al.*); US 2005/0079174 (Barbera-Guillem and Nelson); US 2005/0106108 (Leung and Hansen); WO2005/044859 and US 2005/0123546 (Umana *et al.*); WO 2005/070963 (Allan *et al.*); US 2005/0186216 (Ledbetter and Hayden-Ledbetter); and US Patent No. 6,897,044 (Braslawski *et al.*).

Publications concerning treatment 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); Perotta *et al.*. "Rituxan in the treatment of chronic idiopathic thrombocytopenic purpura (ITP)". *Blood*, 94: 49 (abstract) (1999); 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*, *supra*; 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 and Rheumatism*, 46:2673-2677 (2002), wherein during a 2-week period, each patient received two 500-mg infusions of rituximab, two 750-mg infusions of cyclophosphamide, and high-dose oral corticosteroids, and wherein two of the patients treated relapsed at 7 and 8 months, respectively, and have been retreated, although with different protocols; "Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy" Weide *et al.*. *Lupus*, 12: 779-782 (2003), wherein a patient was treated with rituximab (375 mg/m² x 4, repeated at weekly intervals) and further rituximab applications were delivered every 5-6 months and then maintenance therapy was received with rituximab 375 mg/m² every three months, and a second patient with refractory SLE was treated successfully with rituximab and is receiving maintenance therapy every three months, with both patients responding well to rituximab therapy; Edwards and Cambridge, "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" *Rheumatology* 40:205-211 (2001); Cambridge *et al.*. "B lymphocyte depletion in patients with rheumatoid arthritis: serial studies of immunological parameters" *Arthritis Rheum.*, 46 (Suppl. 9): S1350 (2002); Cambridge *et al.*, "Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis" *Arthritis Rheum.*, 48: 2146-2154 (2003); Edwards *et al.*. "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" *Biochem Soc. Trans.*, *supra*; 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); Edwards *et al.*. "Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis" *N Engl. J. Med.* 350:2572-2582 (2004); Pavelka *et al.*, *Ann. Rheum. Dis.* 63: (S1):289-290 (2004); Emery *et al.*, *Arthritis Rheum.* 50 (S9):S659 (2004); Levine and Pestronk. "IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using rituximab" *Neurology* 52: 1701-1704 (1999); Uchida *et al.*. "The innate mononuclear phagocyte network depletes B lymphocytes through Fc receptor-dependent mechanisms during anti-CD20 antibody immunotherapy" *J. Exp. Med.* 199: 1659-1669 (2004); Gong *et al.*, "Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy" *J. Immunol.* 174: 817-826 (2005); Hamaguchi *et al.*, "The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice" *J. Immunol.* 174: 4389-4399 (2005); Cragg *et al.*. "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.* 8:140-174 (2005); Eisenberg, "Mechanisms of autoimmunity" *Immunol. Res.* 27: 203-218 (2003); De Vita *et al.*, "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hideshida *et al.* "Treatment of DMARD-

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refractory rheumatoid arthritis with rituximab." Presented at the *Annual Scientific Meeting of the American College of Rheumatology*, Oct 24-29; New Orleans, LA 2002; Tusciano, 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 and published Tusciano, *Arthritis Rheum.* 46: 3420 (2002); "Pathogenic roles of B cells in human autoimmunity; insights from the clinic" Martin and Chan, *Immunity* 20:517-527 (2004); Silverman and Weisman, "Rituximab therapy and autoimmune disorders, prospects for anti-B cell therapy". *Arthritis and Rheumatism*, 48: 1484-1492 (2003); Kazkaz and Isenberg, "Anti B cell therapy (rituximab) in the treatment of autoimmune diseases", *Current opinion in pharmacology*, 4: 398-402 (2004); Virgolini and Vanda, "Rituximab in autoimmune diseases", *Biomedicine & pharmacotherapy*, 58: 299-309(2004); Klemmer *et al.*, "Treatment of antibody mediated autoimmune disorders with a AntiCD20 monoclonal antibody Rituximab". *Arthritis And Rheumatism* . 48: (9) 9.S (SEP). page: S624-S624 (2003); Kneitz *et al.*, "Effective B cell depletion with rituximab in the treatment of autoimmune diseases". *Immunobiology*, 206: 519-527 (2002); Arzoo *et al.*, "Treatment of refractory antibody mediated autoimmune disorders with an anti-CD20 monoclonal antibody (rituximab)" *Annals of the Rheumatic Diseases*, 61 (10), p 922-924 (2002) *Comment in Ann Rheum Dis.* 61: 863-866 (2002): "Future strategies in immunotherapy" by Lake and Dionne, in *Burger's Medicinal Chemistry and Drug Discovery* (2003 by John Wiley & Sons, Inc.) Article Online Posting Date: January 15, 2003 (Chapter 2 " Antibody-Directed Immunotherapy"); Liang and Tedder, *Wiley Encyclopedia of Molecular Medicine*, Section: CD20 as an Immunotherapy Target, article online posting date: 15 January, 2002 entitled "CD20"; Appendix 4A entitled "Monoclonal Antibodies to Human Cell Surface Antigens" by Stockinger *et al.*, eds: Coligan *et al.*, in *Current Protocols in Immunology* (2003 John Wiley & Sons, Inc) Online Posting Date: May, 2003; Print Publication Date: February, 2003; Penichet and Morrison, "CD Antibodies/molecules: Definition; Antibody Engineering" in *Wiley Encyclopedia of Molecular Medicine* Section: Chimeric, Humanized and Human Antibodies; posted online 15 January, 2002.

Further, see Looney "B cells as a therapeutic target in autoimmune diseases other than rheumatoid arthritis" *Rheumatology*, 44 Suppl 2: ii13-ii17 (2005); Chambers and Isenberg, "Anti-B cell therapy (rituximab) in the treatment of autoimmune diseases" *Lupus* 14(3): 210-214 (2005); Looney *et al.*, "B-cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalating trial of rituximab" *Arthritis Rheum.* 50: 2580-2589 (2004); Looney, "Treating human autoimmune disease by depleting B cells" *Ann Rheum. Dis.* 61: 863-866 (2002); Edelbauer *et al.*, "Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression Case report" *Pediatr. Nephrol.* 20(6): 811-813 (2005); D'Cruz and Hughes, "The treatment of lupus nephritis" *BMJ* 330(7488): 377-378 (2005); Looney, "B cell-targeted therapy in diseases other than rheumatoid arthritis" *J. Rheumatol. Suppl.* 73: 25-28; discussion 29-30 (2005); Sfikakis *et al.*, "Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial" *Arthritis Rheum.* 52(2): 501-513 (2005); Rastetter *et al.*, "Rituximab: expanding role in therapy for lymphomas and autoimmune diseases" *Annu. Rev. Med.* 55: 477-503 (2004); Silverman, "Anti-CD20 therapy in systemic lupus erythematosus: a step closer to the clinic" *Arthritis Rheum.* 52(2): 371-7 (2005), Erratum in: *Arthritis Rheum.* 52(4): 1342 (2005); Ahn *et al.*, "Long-term remission from life-threatening hypercoagulable state associated with lupus anticoagulant (LA) following rituximab therapy" *Am. J. Hematol.*

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78(2): 127-129 (2005); Tahir *et al.*, "Humanized anti-CD20 monoclonal antibody in the treatment of severe resistant systemic lupus erythematosus in a patient with antibodies against rituximab" *Rheumatology*, 44(4): 561-562 (2005). Epub 2005 Jan 11; Looney *et al.*, "Treatment of SLE with anti-CD20 monoclonal antibody" *Curr. Dir. Autoimmun.* 8: 193-205 (2005); Cragg *et al.*, "The biology of CD20 and its potential as a target for mAb therapy" *Curr. Dir. Autoimmun.* 8: 140-174 (2005); Gottenberg *et al.*, "Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases" *Ann. Rheum. Dis.* 64(6): 913-920 (2005) Epub 2004 Nov 18; Tokunaga *et al.*, "Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab" *Rheumatology* 44(2): 176-182 (2005), Epub 2004 Oct 19. See also Leandro *et al.*, "B cell repopulation occurs mainly from naive B cells in patient with rheumatoid arthritis and systemic lupus erythematosus" *Arthritis Rheum.*, 48 (Suppl 9): S1160 (2003).

Specks *et al.* "Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy" *Arthritis & Rheumatism* 44(12):2836-2840 (2001) discloses successful use of four infusions of 375mg/m² of rituximab and high-dose glucocorticoids to treat Wegener's granulomatosis. The therapy was repeated after 11 months when the cANCA recurred, but therapy was without glucocorticoids. At 8 months after the second course of rituximab, the patients' disease remained in complete remission. Further, in another study, rituximab was found to be a well-tolerated, effective remission induction agent for severe ANCA-associated vasculitis, when used in a dose of 375 mg/m² x 4 along with oral prednisone 1 mg/kg/day, which was reduced by week 4 to 40 mg/day, and to complete discontinuation over the following 16 weeks. Four patients were re-treated with rituximab alone for recurring/rising ANCA titers. Other than glucocorticoids, no additional immunosuppressive agents seem to be necessary for remission induction and maintenance of sustained remission (6 months or longer). See online abstract submission and invitation Keogh *et al.*, "Rituximab for Remission Induction in Severe ANCA-Associated Vasculitis: Report of a Prospective Open-Label Pilot Trial in 10 Patients", American College of Rheumatology, Session Number: 28-100, Session Title: Vasculitis, Session Type: ACR Concurrent Session, Primary Category: 28 Vasculitis, Session 10/18/2004 (www.abstractsonline.com/viewer/SearchResults.asp). See also Keogh *et al.*, *Kidney Blood Press. Res.* 26:293 (2003), wherein it is reported that eleven patients with refractory ANCA-associated vasculitis were treated with four weekly doses of 375 mg/m² of rituximab and high-dose glucocorticoids, resulting in remission.

Patients with refractory ANCA-associated vasculitis were administered rituximab along with immunosuppressive medicaments such as intravenous cyclophosphamide, mycophenolate mofetil, azathioprine, or leflunomide, with apparent efficacy. Eriksson, "Short-term outcome and safety in 5 patients with ANCA-positive vasculitis treated with rituximab", *Kidney and Blood Pressure Research*, 26: 294 (2003) (five patients with ANCA-associated vasculitis treated with rituximab 375 mg/m² once a week for 4 weeks responded to the treatment); Jayne *et al.*, "B-cell depletion with rituximab for refractory vasculitis" *Kidney and Blood Pressure Research*, 26: 294-295 (2003) (six patients with refractory vasculitis receiving four weekly infusions of rituximab at 375 mg/m² with cyclophosphamide along with background immunosuppression and prednisolone experienced major falls in vasculitic activity). A further report of using rituximab along with intravenous cyclophosphamide at 375 mg/m² per dose in 4 doses for administering to

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patients with refractory systemic vasculitis is provided in Jayne, poster 88 (11th International Vasculitis and ANCA workshop), 2003 American Society of Nephrology. See also Stone and Specks, "Rituximab Therapy for the Induction of Remission and Tolerance in ANCA-associated Vasculitis", in the Clinical Trial Research Summary of the 2002-2003 Immune Tolerance Network,

5 <http://www.immunetolerance.org/research/autoimmune/trials/stone.html>, in which a trial of rituximab in ANCA-associated vasculitis is proposed for a total length of 18 months. See also Eriksson, *J. Internal Med.*, 257: 540-548 (2005) regarding nine patients with ANCA-positive vasculitis who were successfully treated with two or four weekly doses of 500 mg of rituximab, as well as Keogh *et al.*, *Arthritis and Rheumatism*, 52: 262-268 (2005), who reported that in 11 patients with refractory ANCA-associated vasculitis, treatment or re-
10 treatment with four weekly doses of 375 mg/m² of rituximab induced remission by B lymphocyte depletion, the study being conducted between January 2000 and September 2002.

As to the activity of a humanized anti-CD20 antibody, see, for example, Vugmeyster *et al.*, "Depletion of B cells by a humanized anti-CD20 antibody PRO70769 in *Macaca fascicularis*" *J. Immunother.* 28: 212-219 (2005). For discussion of a human monoclonal antibody, see Baker *et al.*, "Generation and
15 characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator" *Arthritis Rheum.* 48: 3253-3265 (2003)

There remains a need for approaches to treatment that reduce the frequency of infusions of active drug within a month's time. Further, there is a need to reduce the risk of toxic effects of currently used drugs such as steroids and chemotherapeutic agents, and to reduce the risk of disease flares, relapses, and
20 recurrences in patients with ANCA-associated vasculitis, and to sustain remission and maintain sustained remission for a prolonged period of time.

Summary of the Invention

The present invention involves administration of a CD20 antibody that provides a safe and active treatment regimen in subjects with ANCA-associated vasculitis, including selection of an efficacious dosing
25 regimen and scheduled or unscheduled re-treatment. This antagonist is effective both in initial therapy and in the management of refractory disease.

Accordingly, the invention is as claimed. In a first aspect, the present invention concerns treating ANCA-associated vasculitis in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

30 In a further aspect, the invention provides an article of manufacture comprising: a container comprising a CD20 antibody and a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the CD20 antibody of about 400 mg to 1.3 grams, at a frequency of one to three doses, is administered to the patient within a period of about one month.

In preferred embodiments of the above inventive aspects, the vasculitis is Wegener's granulomatosis or microscopic polyangiitis, and/or a second medicament is administered in an effective amount to the patient,
35 wherein the CD20 antibody is a first medicament. Such medicament may be one or more medicaments. More preferably, such second medicament is a chemotherapeutic agent, an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, a hormone, or a combination thereof.

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In still further aspects, the present invention relates to a method of treating ANCA-associated vasculitis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

5 In one preferred embodiment of this lattermost method involving multiple antibody exposures, the present invention relates to a method of treating ANCA-associated vasculitis in a subject comprising administering to the subject an effective amount of a CD20 antibody 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as about 1 to 4 doses of antibody, more preferably as a single dose or as
10 two or three separate doses of antibody.

A specific preferred embodiment herein is a method of treating ANCA-associated vasculitis in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until
15 from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably in such a method, the antibody exposures are of about 0.5 to 4 grams each.

In another preferred embodiment of these lattermost methods, a second medicament is administered with the initial exposure and/or later exposures, wherein the antibody is a first medicament. In a preferred
20 embodiment, the second medicament is one or more of those set forth above as preferred. In a more preferred embodiment, the second medicament is a steroid and/or an immunosuppressive agent. In a still preferred embodiment, a steroid is administered with the first exposure, but not with the second exposure, or is administered in lower amounts than are used with the initial exposure.

In still another preferred embodiment of these lattermost aspects, the subject has never been
25 previously treated with a CD20 antibody, and/or no other medicament than the CD20 antibody is administered to the subject to treat the vasculitis. In another preferred embodiment, the initial and second antibody exposures are with the same antibody, and more preferably all antibody exposures are with the same antibody. In another preferred embodiment, the subject is in remission after the initial or later antibody exposures, preferably when provided the second antibody exposure. More preferably, the subject is in remission when
30 provided all antibody exposures. Most preferably, such subject is in remission at least about six months after the last antibody exposure provided.

In yet another preferred embodiment of these lattermost aspects, the subject has an elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination of two or more
35 thereof.

Additionally, in further aspects, the invention provides an article of manufacture comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating ANCA-associated vasculitis in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to

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provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

Preferably, such package insert is provided with instructions for treating ANCA-associated vasculitis 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as about one to four doses, preferably as a single dose or as two or three separate doses of antibody.

In a specific aspect, an article of manufacture is provided comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.

The treatments herein preferably reduce, minimize, or eliminate the need for co-, pre-, or post-administration of excessive amounts of second medicaments such as immunosuppressive agents and/or chemotherapeutic agents that are ordinarily standard treatment for such subjects, to avoid as much as possible the side effects of such standard treatment, as well as reduce costs and increase convenience to the subject, such as convenience of time and frequency of administration..

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 L chain (SEQ ID NO:13)

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

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

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FIG. 5 is a sequence alignment comparing the light-chain amino acid sequences of the humanized 2H7.v16 variant (SEQ ID NO:2) and humanized 2H7.v138 variant (SEQ ID NO:28).

FIG. 6 is a sequence alignment comparing the heavy-chain amino acid sequences of the humanized 2H7.v16 variant (SEQ ID NO:8) and humanized 2H7.v138 variant (SEQ ID NO:29).

5 FIG. 7 shows an alignment of the mature 2H7.v16 and 2H7.v511 light chains (SEQ ID NOS: 13 and 30, respectively), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

FIG. 8 shows an alignment of the mature 2H7.v16 and 2H7.v511 heavy chains (SEQ ID NOS:14 and 31, respectively), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

10 FIG. 9A shows the sequence of the humanized 2H7.v114 variable light-chain domain (SEQ ID NO:32); FIG 9B shows the sequence of the humanized 2H7.v114 variable heavy-chain domain (SEQ ID NO:33); and FIG 9C shows the sequence of the humanized 2H7.v114 full-length heavy chain (SEQ ID NO:34), with Kabat variable-domain residue numbering and Eu constant-domain residue numbering.

Detailed Description of the Preferred Embodiments

15 L 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.

20 A "B-cell surface marker" or "B-cell surface antigen" herein is an antigen expressed on the surface of a B cell that can be targeted with an antagonist that binds thereto. 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 RPI05, FcRH2, B-cell CR2, CCR6, P2X5, HLA-DOB, CXCR5, FCER2, BR3, Bt1g, NAG14, SLGC16270, 25 FcRH1, IRTA2, ATWD578, FcRH3, IRTA1, FcRH6, BCMA, and 239287. The B-cell surface marker of particular interest is preferentially expressed on B cells compared to other non-B-cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. The preferred B-cell surface markers herein are CD20 and CD22.

30 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.

35 The "CD22" antigen, or "CD22," also known as BL-CAM or Lyb8, is a type I integral membrane glycoprotein with molecular weight of about 130 (reduced) to 140kD (unreduced). It is expressed in both the cytoplasm and cell membrane of B-lymphocytes. CD22 antigen appears early in B-cell lymphocyte differentiation at approximately the same stage as the CD19 antigen. Unlike other B-cell markers, CD22 membrane expression is limited to the late differentiation stages comprised between mature B cells (CD22+) and plasma cells (CD22-). The CD22 antigen is described, for example, in Wilson *et al.*, *J. Exp. Med.* 40 173:137 (1991) and Wilson *et al.*, *J. Immunol.* 150:5013 (1993).

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5 An "antagonist" is a molecule that, upon binding to CD20 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 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 as 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). Antagonists included within the scope of the present invention include antibodies, synthetic or native-sequence peptides, immunoadhesins, and small-molecule antagonists that bind to CD20, optionally conjugated with or fused to a cytotoxic agent. The preferred antagonist comprises an antibody.

10 An "antibody antagonist" herein is an 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 as 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).

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.

20 "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.

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

30 An "antibody that binds to a B-cell surface marker" is a molecule that, upon binding to a B-cell surface marker, 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 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 as 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). In one preferred embodiment, the antibody induces a major clinical response. In another preferred embodiment, the B-cell surface marker is CD20, so that the antibody that binds to a B-cell surface marker is an antibody that binds to CD20, or a "CD20 antibody." A particularly preferred embodiment is a CD20 antibody that induces a major clinical response. For purposes herein, a "major clinical response" is defined as achieving an American College of Rheumatology 70 response (ACR 70) for six consecutive months. ACR response scores are categorized as ACR 20, ACR 50 and ACR 70 with ACR 70 being the highest level of sign and symptom control in this evaluation system. ACR response scores measure improvement in rheumatoid arthritis disease activity, including joint swelling and tenderness, pain, level of

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disability and overall patient and physician assessment. An example of a different type of antibody that induces a major clinical response as recognized by the FDA and as defined herein is etanercept (ENBREL®).

Examples of CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labelled 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 labelled with ¹²⁵I to generate the "131I-B1" or "Iodine 131 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 (WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (US Patent No. 5,677,180); a humanized 2H7 (WO 2004/056312 (Lowman *et al.*) and as set forth below); HUMAX-CD20™ fully human, high-affinity antibody targeted at the CD20 molecule in the cell membrane of B-cells (Genmab, Denmark; see, for example, Glennie and van de Winkel, *Drug Discovery Today* 8: 503-510 (2003) and Cragg *et al., Blood* 101: 1045-1052 (2003)); the human monoclonal antibodies set forth in WO04/035607 (Teeling *et al.*); AME-133™ antibodies (Applied Molecular Evolution); A20 antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, hA20, respectively) (US 2003/0219433, Immunomedics); and monoclonal antibodies L27, G28-2, 93-1B3, B-C1 or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.*, In: *Leukocyte Typing III* (McMichael, Ed., p. 440, Oxford University Press (1987)). The preferred CD20 antibodies herein are chimeric, humanized, or human CD20 antibodies, more preferably rituximab, a humanized 2H7, chimeric or humanized A20 antibody (Immunomedics), and HUMAX-CD20™ human CD20 antibody (Genmab).

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 which retain the ability to bind CD20.

Purely for the purposes herein and unless indicated otherwise, a "humanized 2H7" refers to a humanized CD20 antibody, 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 (V_LI), 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. See also WO 2004/056312 (Lowman *et al.*).

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

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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). Another preferred embodiment is where the antibody is 2H7.v138 comprising the light- and heavy-chain amino acid sequences of SEQ ID NOS:28 and 29, respectively, as shown in Figs. 5 and 6, which are alignments of such sequences with the corresponding light- and heavy-chain amino acid sequences of 2H7.v16. Alternatively, such preferred intact humanized 2H7 antibody is 2H7.v477, which has the light- and heavy-chain sequences of 2H7.v138 except for the amino acid substitution of N434W. 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.*).

The most preferred humanized 2H7 variants are those having the variable light-chain domain of SEQ ID NO:2 and the variable heavy-chain domain of SEQ ID NO:8, *i.e.*, those with or without substitutions in the Fc region, and those having a variable heavy-chain domain with alteration N100A or D56A and N100A in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2, *i.e.*, those with or without substitutions in the Fc region. If substitutions are made in the Fc region, they are preferably one of those set forth in the table below.

In a summary of various preferred embodiments of the invention, the V 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 L chain as that of v16.

2H7 version	Heavy chain (V _H) changes	Light chain (V _L) changes	Fc changes
16			-
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

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

DIQMTQSPSSLSASVGDRVITTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWSFNPTFGQGTKVEIKR (SEQ ID NO:2):

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and the variable heavy-chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYYSNSYWFYFDVWGQGLVTVSS (SEQ ID
NO:8).

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

DIQMTQSPSSLSASVGDRVTTCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWSFNPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADYEKHKVYACEVTHQGLSSP
10 VTKSFNRGEC (SEQ ID NO:13);

and the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYYSNSYWFYFDVWGQGLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
15 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYYSNSYWFYFDVWGQGLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
20 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
25 KALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

In another preferred embodiment, the intact humanized 2H7 antibody comprises the light-chain
amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSR
FSGSGSGTDFTLTISLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLT
30 SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:28)

35 and the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASG
YTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGRFTISVDKSKNTLYLQMNSL
RAEDTAVYYCARVYYYSASYWFYFDVWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAA
40 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC

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VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKC
KVSNAALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSYMHEALHNHYTQKSL
SLSPGK (SEQ ID NO:29).

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"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 is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (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).

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"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.

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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 FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Dabron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991); Capel *et al.*, *Immunomethods* 4:25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-341 (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)).

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

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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, IgA₁, 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, Rosenberg 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

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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.

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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.

"ANCA-associated vasculitis" or "anti-neutrophil cytoplasmic antibodies-associated vasculitis" or "AAV" as used herein is an autoimmune disease or disorder involving systemic vasculitis (or inflammation of blood-vessel walls) in which circulating anti-neutrophil cytoplasmic antibodies (ANCA) are normally present in the blood of the subject, or other clinical manifestations are present that define the vasculitis, as noted below. The term "ANCA-associated vasculitis" as used herein applies to ANCA-associated vasculitis no matter what the type and stage or severity, and no matter what symptoms are evident, provided the diagnosis is made. Examples of ANCA-associated vasculitis include microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome, renal-limited vasculitis (idiopathic necrotizing crescentic glomerulonephritis), and certain types of drug-induced vasculitis. Diagnoses for ANCA-associated vasculitis and its various manifestations include those set forth below.

Several diagnostic tests are commonly used in people suspected of having ANCA-associated vasculitis. Features that may aid in defining the specific type of vasculitic disorder include the type of organ involvement, presence and type of ANCA (myeloperoxidase-ANCA or proteinase 3-ANCA), presence of serum cryoglobulins, and the presence of evidence for granulomatous inflammation.

Exemplary auto-antibodies associated with ANCA-associated vasculitis include elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), such as autoantibodies directed against proteinase 3 (PR3) or against myeloperoxidase (MPO), or a combination thereof.

The ANCA antibodies can be detected using antigen-specific immunochemical assay to characterize PR3-ANCA and MPO-ANCA. Niles *et al.*, *supra*. Since an ELISA test for ANCA is associated with a substantially higher positive predictive value and likelihood ratios for ANCA-associated vasculitis, ELISA tests may be performed only on samples that are positive for ANCA by immunofluorescence. Stone *et al.*, *Arthritis Care and Research*, 13: 424-34 (2000); *Comment on Arthritis Care Res.* 13: 341-342 (2000); Russell *et al.*, *Clin. Immunol.*, 103: 196-203 (2002).

About 10 percent of patients with microscopic polyangiitis (the most common type of ANCA-associated vasculitis) and Wegener's granulomatosis have negative assays for ANCA; however, this finding does not completely rule out these diseases, and ANCA titers do not always correlate with disease activity. Jennette and Falk, *N. Engl J Med.*, *supra*. On the other hand, a positive ANCA assay result is not solely diagnostic of ANCA-associated vasculitis.

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Table 1 summarizes the potential clinical manifestations of ANCA-associated vasculitis, which should be suspected in any patient who presents with a multisystem disease not caused by an infectious or malignant process (e.g., renal dysfunction, skin rashes, pulmonary manifestations, or neurologic manifestation). Constitutional symptoms are common. The frequency and combination of various system involvements vary among individual disease entities. See also Guillevin *et al.* *Arthritis Rheum.* 42:421-430 (1999); Pettersson *et al.*, *Clin. Nephrol.* 43:141-149 (1995); Savage *et al.*, *Lancet* 349:553-558 (1997); Guillevin *et al.*, *Br. J. Rheumatol* 35:958-964 (1996).

Table 1
Clinical Manifestations of ANCA-Associated Vasculitis

System	Manifestations
Constitutional	Fever, weight loss, anorexia, general malaise
Musculoskeletal	Myalgia, arthralgia
Skin	Palpable purpura, urticaria
Kidneys	Proteinuria, hematuria, renal insufficiency, renal failure, necrotizing glomerulonephritis
Respiratory tract	Dyspnea, cough, hemoptysis; lung infiltrate, interstitial lung disease, pulmonary hemorrhage
Nervous system	Peripheral neuropathy, especially mononeuritis
Gastrointestinal tract	Fecal blood, elevated liver enzymes; diarrhea, nausea, vomiting, abdominal pain

The most common cutaneous lesion is palpable purpura—a slightly raised, non-blanching eruption that usually begins in the lower extremities. Occasionally, the rash is vesicular or slightly ulcerated. Urticaria can also be a manifestation of ANCA-associated vasculitis. Unlike non-vasculitic allergic urticaria, vasculitic urticaria lasts more than one day and may evolve into purpuric lesions. The presence of hypocomplementemia may indicate that the vasculitis is immune complex-mediated rather than ANCA-associated vasculitis.

Renal involvement in vasculitis may progress to renal failure. Results of biopsy of the kidney commonly reveal glomerulonephritis. Focal necrosis, crescentic formation and the absence or paucity of immunoglobulin deposits characterize glomerulonephritis in patients with ANCA-associated vasculitis. Pettersson *et al.*, *Clin. Nephrol.* 43:141-149 (1995). Lung involvement ranges from fleeting focal infiltrates or interstitial disease to massive pulmonary hemorrhagic alveolar capillaritis. The latter is the most life-threatening feature of small-vessel vasculitis.

It is important, however, to differentiate ANCA-associated vasculitis from other diseases that result in multi-system manifestations. Diseases with widespread embolization to different organs (e.g., atheroembolic disease, endocarditis, antiphospholipid syndrome, and atrial myxoma) can produce similar clinical presentations. Kelley, "Vasculitis and related disorders" In: *Textbook of rheumatology*, 5th ed. (Philadelphia: Saunders, 1997), pp.1079-1101. Persons with sepsis can also present with multisystem involvement. It is also important to realize that ANCA-associated vasculitis may be secondary to infections or

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malignancy. Some viral, bacterial, and fungal infections may be complicated by vasculitis, which is predominantly a dermal vasculitis. The diagnosis is suggested by the clinical history. Malignancy, such as lymphomas, leukemia, myeloproliferative, and myelodysplastic syndromes, may be associated with ANCA-associated vasculitis; however, solid tumors are less commonly associated with such vasculitis. Underlying infectious or malignant causes should be thoroughly evaluated before the diagnosis of ANCA-associated vasculitis is made—even if the ANCA assay result is positive.

Table 2 depicts some of the clinical features that may help in the diagnosis of the specific type of vasculitis. Laboratory assessment should include a complete blood cell count and routine chemistry profile, urinalysis, fecal occult blood test, and chest radiography. There may be normocytic anemia, thrombocytosis, elevated erythrocyte sedimentation rate, increased liver function, or evidence of renal involvement. ANCA serum levels should also be measured. Other laboratory tests that should be performed to exclude ANCA-associated vasculitis include anti-nuclear antibody, rheumatoid factor, cryoglobulins, complement, antibodies to hepatitis B and C, and human immunodeficiency virus (HIV) testing. Chest and sinus computed tomographic scans may also be performed, if appropriate. Pathologic examination of the involved tissue (e.g., skin, nerve, lung, or kidney) may aid in documenting the type of ANCA-associated vasculitis. Biopsy should be obtained from symptomatic and accessible sites. Biopsies from asymptomatic sites have a low yield of positive results.

Table 2
Clinical Features That Favor Diagnosis
of a Specific Type of Vasculitis

Clinical features	Probable type of vasculitis
Pulmonary and renal symptoms	Wegener's granulomatosis; Microscopic polyangiitis
Pulmonary-dermal symptoms	Cryoglobulinemia; Henoch-Schonlein purpura
Asthma and eosinophilia	Churg-Strauss syndrome
Upper respiratory tract involvement	Wegener's granulomatosis (e.g., sinusitis and otitis media)

Information in Table 2 is from Jennette and Falk, *N. Engl. J. Med.*, *supra*, and Kelley, *supra*.

Wegener's granulomatosis is characterized by necrotizing granulomas of the upper and lower respiratory tract together with glomerulonephritis and systemic vasculitis, which involves usually the medium-sized vessels, with formation of granulomas and necrosis of the parenchyma. Kelley, *supra*. Upper respiratory tract signs and symptoms include sinusitis, nasal ulcers, otitis media, or hearing loss. Upper respiratory tract signs and symptoms are seen in 70 percent of patients and pulmonary infiltrates or nodules that may cavitate develop in 85 percent of patients. Kelley, *supra*. Serum antiprotease 3-ANCA (c-ANCA) is positive in 75 to 90 percent, although 20 percent may have positive p-ANCA. Open lung biopsy is the most definitive diagnostic test. Sinus biopsy is diagnostic in only 30 percent of cases because inflammatory findings are often nonspecific and renal biopsy is also relatively nonspecific. Radiographic findings are of mid and lower zone opacities, which are diffuse, and both alveolar and interstitial. Nodules, which may cavitate, are rare in children. CT scanning may show diffuse, ill-defined perivascular opacities. Wegener's

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granulomatosis can affect patients at any age, with the peak incidence during the fourth decade of life and is slightly more common in men. Duna *et al.*, *Rheum. Dis. Clin. North Am.* 21:949-986 (1995). The most definite way to diagnose Wegener's granulomatosis is by performing a biopsy of an involved organ site (usually the sinuses, lung or kidney) to confirm the presence of vasculitis and granulomas, which together are diagnostic of the disease.

Microscopic polyangiitis is characterized by the presence of ANCA and few or no immune deposits in the involved vessels. Savage *et al.*, *Lancet* 349:553-558 (1997). The kidneys are the most commonly affected organs in 90 percent of patients who have this type of vasculitis. Kelley, *supra*. Patients present with variable combinations of renal manifestations, palpable purpura, abdominal pain, cough, and hemoptysis. Most patients have positive MPO-ANCA (p-ANCA), although PR3- ANCA (c-ANCA) may be also present in 40 percent of patients. The most common age of onset is 40 to 60 years and most common sex is men.

Churg-Strauss syndrome is a rare disease and has three phases: allergic rhinitis and asthma, eosinophilic infiltrative disease resembling pneumonia, and systemic small vessel vasculitis with granulomatous inflammation. Guillevin *et al.*, *Br. J. Rheumatol.*, 35:958-964 (1996). The vasculitic phase usually develops within three years of the onset of asthma. Almost all patients have more than 10 percent eosinophils in the blood. Coronary arteritis and myocarditis are the principal causes of morbidity and mortality. The age of onset varies from 15 to 70 years and is more common in men. Drug-induced vasculitis usually develops within seven to 21 days after a drug is started and may be confined to the skin. Jennette and Falk, *N. Engl. J. Med.*, *supra*. Skin lesions are identical to those seen in systemic small vessel vasculitis. Drugs cause approximately 10 percent of vasculitic skin lesions. Drugs that have been implicated include penicillin, aminopenicillins, sulfonamides, allopurinol, thiazides, quinolones, hydantoins, and propylthiouracil. Some drugs, such as propylthiouracil and hydralazine (APRESOLINETM), appear to cause vasculitis by inducing ANCA.

Another way to test for active disease and determine which patients/subjects are eligible for treatment is to determine the Birmingham Vasculitis Activity Score/Wegener's granulomatosis (BVAS/WG) value of the patient, whether major or minor. This score is an index of vasculitis activity and is designed to document clinical features that are directly due to active Wegener's granulomatosis. It has been found to be a valid and reliable disease-specific indicator for Wegener's granulomatosis. Stone *et al.*, *Arthritis & Rheumatism*, 44: 912-920 (2001). It can also be used for other ANCA-associated vasculitis diseases. The instrument separates the features that represent new or worse disease activity from those that represent persistent activity. Typically, the patient's BVAS/WG score is 3 or greater (or has been 3 or greater within 28 days of treatment). Each major item on the BVAS/WG evaluation form is scored 3 points. Each minor item is scored 1 point. However, another distinction used is that acute disease, either first presentation or relapse, shows a BVAS/WG of at least 10, whereas persistent disease shows a BVAS/WG of at least 4. Lymphopenia may also be a good marker for Wegener's granulomatosis. Izzedine *et al.*, *Nephron* 92:466-471 (2002).

A "subject" herein is a human subject, including a patient, eligible for treatment for ANCA-associated vasculitis who is experiencing or has experienced one or more signs, symptoms, or other indicators of ANCA-associated vasculitis, has been diagnosed with ANCA-associated vasculitis, whether, for example, newly diagnosed or previously diagnosed and now experiencing a recurrence or relapse, or is at risk for developing ANCA-associated vasculitis. The subject may have been previously treated with CD20 antibody

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or not so treated. A subject eligible for treatment of ANCA-associated vasculitis may optionally be identified as one who has been screened, as in the blood, for elevated levels of infiltrating CD20 cells or is screened using an assay to detect auto-antibodies, wherein autoantibody production is assessed qualitatively, and preferably quantitatively.

5 A "patient" herein is a human subject eligible for treatment for ANCA-associated vasculitis who is experiencing or has experienced one or more signs, symptoms, or other indicators of ANCA-associated vasculitis, whether, for example, newly diagnosed or previously diagnosed and now experiencing a recurrence or relapse. The patient may have been previously treated with CD20 antibody or not so treated. A patient eligible for treatment of ANCA-associated vasculitis may optionally be identified as one who is screened
10 using an assay to detect auto-antibodies, such as those noted above, wherein autoantibody production is assessed qualitatively, and preferably quantitatively.

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

"Treatment" of a patient herein refers to therapeutic treatment. Those patients in need of treatment are those diagnosed with ANCA-associated vasculitis.

For purposes herein, a patient or subject is in "remission" if he/she has no symptoms of active
20 ANCA-associated vasculitis disease, such as those detectable by the methods disclosed herein, and has had no recurrence of ANCA titers or rising ANCA titers coinciding with or following reconstitution of B cells, since sustained or recurring ANCA levels have been found to be predictive of relapses in patients in clinical remissions from Wegener's granulomatosis. Boomsma *et al.*, *Arthritis Rheum.*, 43: 2025-2033 (2000). Those who are not in remission include, for example, those experiencing a disease flare after reconstitution of B
25 cells, those suffering organ damage such as kidney damage, or those who are asymptomatic but have had a recurrence of ANCA or an ANCA titer rise coinciding with or following reconstitution of B cells. Such subjects and patients experiencing a return of symptoms, including active disease and/or damage to organs, or exhibiting recurring or rising ANCA titers, are those who have "relapsed" or had a "recurrence."

A "symptom" of ANCA-associated vasculitis is any morbid phenomenon or departure from the
30 normal in structure, function, or sensation, experienced by the subject or patient and indicative of disease, such as those noted above.

The expression "effective amount" refers to an amount of the antibody or antagonist that is effective for treating ANCA-associated vasculitis.

"Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses
35 administered over a period of time of about 1 day to about 5 weeks. The doses may be given at one time or at a fixed or at irregular time intervals over this period of exposure, such as, for example, one dose weekly for four weeks or two doses separated by a time interval of about 13-17 days. Initial and later antibody exposures are separated in time from each other as described in detail herein.

An exposure not being administered or provided until a certain time "from the initial exposure" or
40 from any prior exposure means that the time for the second or later exposure is measured from the time any of

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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 cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatins; calystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma11 and calicheamicin omega11 (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994))); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucarins A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol;

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the doses from the prior exposure were administered, if more than one dose was administered in that exposure. For example, when two doses are administered in an initial exposure, the second exposure is not given until at least about 16-54 weeks as measured from the time the first or the second dose was administered within that prior exposure. Similarly, when three doses are administered, the second exposure may be measured from the time of the first, second, or third dose within the prior exposure. Preferably, "from the initial exposure" or from any prior disclosure is measured from the time of the first dose.

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); non-steroidal 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, including SOLU-MEDROL[®] methylprednisolone sodium succinate, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); anti-malarial agents such as chloroquine and hydroxychloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antibodies including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor(TNF)-alpha antibodies (infliximab (REMICADE[®]) or adalimumab), anti-TNF-alpha immunoadhesin (etanercept), anti-TNF-beta antibodies, anti-interleukin-2 (IL-2) antibodies and anti-IL-2 receptor antibodies, and anti-Interleukin-6 (IL-6) receptor antibodies and antagonists; 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; transforming growth factor-beta (TGF-beta); streptodornase; RNA or DNA from the host; FK506; RS-61443; , chlorambucil; 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); BAFF antagonists such as BAFF antibodies and BR3 antibodies and zTNF4 antagonists (for review, see Mackay and Mackay, *Trends Immunol.*, 23:113-5 (2002) and see also definition below); biologic agents that interfere with T cell helper signals, such as anti-CD40 receptor or anti-CD40 ligand (CD154), including blocking antibodies to CD40-CD40 ligand (e.g., Duric *et al.*, *Science*, 261: 1328-30 (1993); Mohan *et al.*, *J. Immunol.*, 154: 1470-80 (1995)) and CTLA4-Ig (Finck *et al.*, *Science*, 265: 1225-7 (1994)); and T-cell receptor antibodies (EP 340,109) such as T10B9. Some preferred immunosuppressive agents herein include cyclophosphamide, chlorambucil, azathioprine, leflunomide, MMF, or methotrexate.

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. ²¹¹At, ¹³¹I, ¹²⁵I, ⁹⁰Y, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁵³Sm, ²¹²Bi, ³²P and radioactive isotopes of Lu), chemotherapeutic agents, and toxins

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plpobromar; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, *e.g.*, paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and doxorubicin (TAXOTERE®); chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

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

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-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15, including PROLEUKIN® rIL-2; 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,

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including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

5 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; estradiol; hormone-replacement therapy; androgens such as calusterone, dromostanolone propionate, epitioctanol, mepitioctane, or testolactone; prolactin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); prolactin, placental lactogen, mouse gonadotropin-associated peptide, gonadotropin-releasing hormone; 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.

15 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.

25 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 *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.

35 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™).

40 Examples of "disease-modifying anti-rheumatic drugs" or "DMARDs" include hydroxycloquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methotrexate).

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azathioprine, D-penicillamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine including cyclosporine A and topical cyclosporine, *staphylococcal* protein A (Goodyear and Silverman, *J. Exp. Med.*, 197, (9), p1125-39 (2003)), including salts and derivatives thereof, *etc.*

5 Examples of "non-steroidal anti-inflammatory drugs" or "NSAIDs" include aspirin, acetylsalicylic acid, ibuprofen, naproxen, indomethacin, sulindac, tolmetin, COX-2 inhibitors such as celecoxib (CELEBREX®; 4-(5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide and valdecoxib (BEXTRA®), and meloxicam (MOBIC®), including salts and derivatives thereof, *etc.* Preferably, they are aspirin, naproxen, ibuprofen, indomethacin, or tolmetin.

10 Examples of "integrin antagonists or antibodies" herein include an LFA-1 antibody, such as efalizumab (RAPTIVA®) commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab (ANTEGREN®) available from Biogen, or 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), alkanolic acid derivatives (WO 2000/32575), substituted phenyl
15 derivatives (US Pat. Nos. 6,677,339 and 6,348,463), aromatic amine derivatives (US Pat. No. 6,369,229), ADAM disintegrin domain polypeptides (US2002/0042368), antibodies to alphavbeta3 integrin (EP 633945), aza-bridged bicyclic amino acid derivatives (WO 2002/02556), *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
20 corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone, such as SOLU-MEDROL® methylprednisolone sodium succinate), dexamethasone or dexamethasone triamcinolone, hydrocortisone, and betamethasone. The preferred corticosteroids herein are prednisone, methylprednisolone, hydrocortisone, or dexamethasone.

The terms "BAFF," "BAFF polypeptide," "TALL-1" or "TALL-1 polypeptide," and "BLyS" when
25 used herein encompass "native-sequence BAFF polypeptides" and "BAFF variants". "BAFF" is a designation given to those polypeptides that have any one of the amino acid sequences shown below:

Human BAFF sequence (SEQ ID NO:16):

1 MDDSTEREQSRLTSLCKKREEMKLKECVSILPRKESPSVRSSKDGKLLAATLLLALLSCC
30 61 LTVVSFYQVAALQGDLSLRAELQGHHAELPAGAGAPKAGLEEAPAVTAGLKIFEPPAP
121 GEGNSSQNSRNKRAVQGPEETVTQDCLQLIADSETPTIQKGSYTFVPWLLSFKRGSALLEE
181 KENKILVKETGYFFIYGQVLYTDKTYAMGHLIQRKKVHVFGDELSLVTLFRCIQNMPETL
241 PNNSCYSAGIAKLEEGDELQLAIPRENAQISLDGQVTFFGALKLL

35 Mouse BAFF sequence (SEQ ID NO:17):

1 MDESAKTLPPPCLCFCEKGEDMKVGYPITPQKEEGAWFGICRDGRLLAATLLLALLSS
30 61 SFTAMSLYQLAALQADLMNLRMELQSYRGSATPAAAGAPELTAGVKLLTPAAPRPHNSSR
121 GHRNRRAFQGPETEVDVLSAPPAPCLPGCRHSQHDDNGMNLNRNIIQDCLQLIADSDTP
40 181 TIRKGTYTTFVPWLLSFKRGNALKEENKIVVRQTGYFFIYSQVLYTDPFAMGHVIRKK
241 VHVFGDELSLVTLFRCIQNMPKTL PNNSCYSAGIARLEEGDEIQLAIPRENAQISRNGDD
301 TFFGALKLL

45 and homologs and fragments and variants thereof, which have the biological activity of the native BAFF. A biological activity of BAFF can be selected from the group consisting of promoting B cell survival, promoting

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B cell maturation and binding to BR3. Variants of BAFF will preferably have at least 80% or any successive integer up to 100% including, more preferably, at least 90%, and even more preferably, at least 95% amino acid sequence identity with a native sequence of a BAFF polypeptide.

5 A "native-sequence" BAFF polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding BAFF polypeptide derived from nature. For example, BAFF exists in a soluble form following cleavage from the cell surface by furin-type proteases. Such native-sequence BAFF polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means.

10 The term "native-sequence BAFF polypeptide" or "native BAFF" specifically encompasses naturally occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally occurring variant forms (e.g., alternatively spliced forms), and naturally occurring allelic variants of the polypeptide. The term "BAFF" includes those polypeptides described in Shu *et al.*, *J. Leukocyte Biol.*, 65:680 (1999); GenBank Accession No. AF136293; WO 1998/18921 published May 7, 1998; EP 869,180 published October 7, 1998; WO 1998/27114 published June 25, 1998; WO 1999/12964 published March 18, 1999; WO 1999/33980 published July 8, 1999; Moore *et al.*, *Science*, 285:260-263 (1999); Schneider *et al.*, *J. Exp. Med.*, 189:1747-1756 (1999) and Mukhopadhyay *et al.*, *J. Biol. Chem.*, 274:15978-15981 (1999).

15 The term "BAFF antagonist" as used herein is used in the broadest sense, and includes any molecule that (1) binds a native-sequence BAFF polypeptide or binds a native-sequence of BR3 to partially or fully block BR3 interaction with BAFF polypeptide, and (2) partially or fully blocks, inhibits, or neutralizes native-sequence BAFF activity. In one preferred embodiment the BAFF receptor to be blocked is the BR3 receptor. Native BAFF activity promotes, among other things, B-cell survival and/or B-cell maturation. In one
20 embodiment, the inhibition, blockage or neutralization of BAFF activity results in a reduction in the number of B cells. A BAFF antagonist according to this invention will partially or fully block, inhibit, or neutralize one or more biological activities of a BAFF polypeptide, *in vitro* and/or *in vivo*. In one embodiment, a biologically active BAFF potentiates any one or a combination of the following events *in vitro* and/or *in vivo*:
25 an increased survival of B cells, an increased level of IgG and/or IgM, an increased numbers of plasma cells, and processing of NF- κ B2/100 to p52 NF- κ B in splenic B cells (e.g., Batten *et al.*, *J. Exp. Med.* 192:1453-1465 (2000); Moore *et al.*, *Science* 285:260-263 (1999); Kayagaki *et al.*, *Immunity* 17:515-524 (2002)).

30 As mentioned above, a BAFF antagonist can function in a direct or indirect manner to partially or fully block, inhibit or neutralize BAFF signaling, *in vitro* or *in vivo*. For instance, the BAFF antagonist can directly bind BAFF. For example, BAFF antibodies that bind within a region of human BAFF comprising residues 162-275 and/or a neighboring residue of a residue selected from the group consisting of 162, 163, 206, 211, 231, 233, 264 and 265 of human BAFF such that the antibody sterically hinders BAFF binding to BR3 are contemplated, where such residue numbers refer to SEQ ID NO:16. In another example, a direct
35 binder is a polypeptide comprising any portion of a BAFF receptor that binds BAFF such as an extracellular domain of a BAFF receptor, or fragments and variants thereof that bind native BAFF. In another example, BAFF antagonists include the polypeptides having a sequence of a polypeptide comprising the sequence of Formula I:

40 $X_1-C-X_3-D-X_5-L-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-C-X_{14}-X_{15}-X_{16}-X_{17}$ (Formula I) (SEQ ID NO:18)
wherein X_1 , X_3 , X_5 , X_7 , X_8 , X_9 , X_{10} , X_{11} , X_{12} , X_{14} , X_{15} and X_{17} are any amino acid except cysteine; and
wherein X_{16} is an amino acid selected from the group consisting of L, F, I and V; and

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wherein the polypeptide does not comprise a cysteine within seven amino acid residues N-terminal to the most N-terminal cysteine C and C-terminal to the most C-terminal cysteine C of Formula I.

In one embodiment, a polypeptide comprising the sequence of Formula I has the two Cs joined by disulfide bonding: $X_3I.X_7X_8$ forming the conformation of a type I beta turn structure with the center of the turn between L and X_7 ; and has a positive value for the dihedral angle phi of X_8 . In one embodiment, X_{10} is selected from the group consisting of W, F, V, L, I, Y, M and a non-polar amino acid. In another embodiment, X_{10} is W. In another embodiment, X_3 is an amino acid selected from the group consisting of M, V, I, L, I, Y, F, W and a non-polar amino acid. In another embodiment, X_5 is selected from the group consisting of V, L, P, S, I, A and R. In another embodiment, X_7 is selected from the group consisting of V, T, I and L. In another embodiment, X_8 is selected from the group consisting of R, K, G, N, H and a D-amino acid. In another embodiment, X_9 is selected from the group consisting of H, K, A, R and Q. In another embodiment, X_{11} is I or V. In another embodiment, X_{12} is selected from the group consisting of P, A, D, E and S. In another embodiment, X_{14} is L. In one specific embodiment, the sequence of Formula I is a sequence selected from the group consisting of ECFDLLVRAWVPCSVLK (SEQ ID NO:19), ECFDLLVRHWVPCGLLR (SEQ ID NO:20), ECFDLLVRRWVPCMLG (SEQ ID NO:21), ECFDLLVRSWVPCHMLR (SEQ ID NO:22), ECFDLLVRHWVACGLLR (SEQ ID NO:23), and QCFDLRNAWVPCSVLK (SEQ ID NO:24). In a preferred embodiment, the BAFF antagonist comprises any one of the amino acid sequences selected from the group consisting of SEQ ID NO: 19, 20, 21, 22, and 23.

In still another example, BAFF antagonists include the polypeptides having a sequence of a polypeptide comprising the sequence of Formula II:

$X_1-C-X_2-D-X_3-L-V-X_4-X_5-W-V-P-C-X_6-X_{13}-L-X_{17}$ (Formula II) (SEQ ID NO:25)

wherein X_1 , X_2 , X_3 , X_4 , X_5 , X_{13} and X_{17} are any amino acid, except cysteine; and

wherein the polypeptide does not comprise a cysteine within seven amino acid residues N-terminal to the most N-terminal cysteine C and C-terminal to the most C-terminal cysteine C of Formula II.

In one embodiment, a polypeptide comprising the sequence of Formula II has a disulfide bond between the two Cs and has the conformation of $X_3LX_7X_8$ forming a type I beta turn structure with the center of the turn between L and X_7 ; and has a positive value for the dihedral angle phi of X_8 . In another embodiment of Formula II, X_2 is an amino acid selected from the group consisting of M, A, V, L, I, Y, F, W and a non-polar amino acid. In another embodiment of Formula II, X_3 is selected from the group consisting of V, L, P, S, I, A and R. In another embodiment of Formula II, X_5 is selected from the group consisting of R, K, G, N, H and D-amino acid. In another embodiment of Formula II, X_6 is selected from the group consisting of H, K, A, R and Q.

In a further embodiment, the BAFF receptor from which the extracellular domain or BAFF-binding fragment or BAFF-binding variant thereof is derived is TACI, BR3 or BCMA. Alternatively, the BAFF antagonist can bind an extracellular domain of a native-sequence BR3 at its BAFF binding region to partially or fully block, inhibit or neutralize BAFF binding to BR3 *in vitro*, *in situ*, or *in vivo*. For example, such indirect antagonist is an anti-BR3 antibody that binds in a region of BR3 comprising residues 23-38 of human BR3 as defined below (SEQ ID NO:26) or a neighboring region of those residues such that binding of human BR3 to BAFF is sterically hindered.

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In some embodiments, a BAFF antagonist according to this invention includes BAFF antibodies and immunoadhesins comprising an extracellular domain of a BAFF receptor, or fragments and variants thereof that bind native BAFF. In a further embodiment, the BAFF receptor from which the extracellular domain or BAFF-binding fragment or BAFF-binding variant thereof is derived is TACI, BR3 or BCMA. In a still
5 another embodiment, the immunoadhesin comprises an amino acid sequence of that of Formula I or Formula II as set forth above, including an amino acid sequence selected from any one of the group consisting of SEQ ID NOS: 19, 20, 21, 22, 23, and 24.

According to one embodiment, the BAFF antagonist binds to a BAFF polypeptide or a BR3 polypeptide with a binding affinity of 100nM or less. According to another embodiment, the BAFF
10 antagonist binds to a BAFF polypeptide or a BR3 polypeptide with a binding affinity of 10nM or less. According to yet another embodiment, the BAFF antagonist binds to a BAFF polypeptide or a BR3 polypeptide with a binding affinity of 1nM or less.

The terms "BR3", "BR3 polypeptide" or "BR3 receptor" when used herein encompass "native-sequence BR3 polypeptides" and "BR3 variants" (which are further defined herein). "BR3" is a designation
15 given to those polypeptides comprising the following amino acid sequence and homologs thereof, and variants or fragments thereof that bind native BAFF:

Human BR3 sequence (SEQ ID NO:26):

1 MRRGPRSLRGRDAPAPTPCVPAECFDLLVRHCVACGLI.RTPRPKPAGASSPAPRTALQPO
20 61 ESVGAGAGEAALPLPGLLFGAPALLGLALVLALVLVGLVSWRRRQRRLRGASSAEAPDGD
121 KDAPEPLDKVIILSPGISDATAPAWPPPGEDPGTTTPGHSVPVPATELGSTELVTTKTAG
181 PEQQ.

The BR3 polypeptides of the invention can be isolated from a variety of sources, such as from human
25 tissue types or from another source, or prepared by recombinant and/or synthetic methods. The term BR3 includes the BR3 polypeptides described in WO 2002/24909 and WO 2003/14294.

A "native-sequence" BR3 polypeptide or "native BR3" comprises a polypeptide having the same amino acid sequence as the corresponding BR3 polypeptide derived from nature. Such native-sequence BR3 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The
30 term "native-sequence BR3 polypeptide" specifically encompasses naturally occurring truncated, soluble or secreted forms (e.g., an extracellular domain sequence), naturally occurring variant forms (e.g., alternatively spliced forms) and naturally occurring allelic variants of the polypeptide. The BR3 polypeptides of the invention include the BR3 polypeptide comprising or consisting of the contiguous sequence of amino acid residues 1 to 184 of a human BR3 (SEQ ID NO:26).

A BR3 "extracellular domain" or "ECD" refers to a form of the BR3 polypeptide that is essentially free of the transmembrane and cytoplasmic domains. ECD forms of BR3 include a polypeptide comprising
35 any one of the amino acid sequences selected from the group consisting of amino acids 1-77, 2-62, 2-71, 1-61, 7-71, 23-38 and 2-63 of human BR3. The invention contemplates BAFF antagonists that are polypeptides comprising any one of the above-mentioned ECD forms of human BR3 and variants and fragments thereof
40 that bind a native BAFF.

Mini-BR3 is a 26-residue core region of the BAFF-binding domain of BR3, i.e., the amino acid sequence: TPCVPAECFD LLVRHCVACG LLRTPR (SEQ ID NO:27)

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"BR3 variant" means a BR3 polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a native-sequence, full-length BR3 or BR3 ECD and binds a native-sequence BAFF polypeptide. Optionally, the BR3 variant includes a single cysteine-rich domain. Such BR3 variant polypeptides include, for instance, BR3 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the BR3 ECD that bind a native sequence BAFF polypeptide are also contemplated. According to one embodiment, a BR3 variant polypeptide will have at least about 80% amino acid sequence identity, at least about 81% amino acid sequence identity, at least about 82% amino acid sequence identity, at least about 83% amino acid sequence identity, at least about 84% amino acid sequence identity, at least about 85% amino acid sequence identity, at least about 86% amino acid sequence identity, at least about 87% amino acid sequence identity, at least about 88% amino acid sequence identity, at least about 89% amino acid sequence identity, at least about 90% amino acid sequence identity, at least about 91% amino acid sequence identity, at least about 92% amino acid sequence identity, at least about 93% amino acid sequence identity, at least about 94% amino acid sequence identity, at least about 95% amino acid sequence identity, at least about 96% amino acid sequence identity, at least about 97% amino acid sequence identity, at least about 98% amino acid sequence identity or at least about 99% amino acid sequence identity with a human BR3 polypeptide or a specified fragment thereof (e.g., ECD). BR3 variant polypeptides do not encompass the native BR3 polypeptide sequence. According to another embodiment, BR3 variant polypeptides are at least about 10 amino acids in length, at least about 20 amino acids in length, at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, or at least about 70 amino acids in length.

In one preferred embodiment, the BAFF antagonists herein are immunoadhesins comprising a portion of BR3, TACI or BCMA that binds BAFF, or variants thereof that bind BAFF. In other embodiments, the BAFF antagonist is a BAFF antibody. A "BAFF antibody" is an antibody that binds BAFF, and preferably binds BAFF within a region of human BAFF comprising residues 162-275 of the human BAFF sequence disclosed herein under the "BAFF" definition (SEQ ID NO:16). In another embodiment, the BAFF antagonist is BR3 antibody. A "BR3 antibody" is an antibody that binds BR3, and is preferably one that binds BR3 within a region of human BR3 comprising residues 23-38 of the human BR3 sequence disclosed herein under the "BR3" definition (SEQ ID NO:26). In general, the amino acid positions of human BAFF and human BR3 referred to herein are according to the sequence numbering under human BAFF and human BR3, SEQ ID NOS: 16 and 26, respectively, disclosed herein under the "BAFF" and "BR3" definitions.

Other examples of BAFF-binding polypeptides or BAFF antibodies can be found in, e.g., WO 2002/092620, WO 2003/014294, Gordon *et al.*, *Biochemistry* 42(20):5977-5983 (2003), Kelley *et al.*, *J. Biol. Chem.*, 279(16):16727-16735 (2004), WO 1998/18921, WO 2001/12812, WO 2000/68378 and WO 2000/40716.

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.*

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A "medicament" is an active drug to treat the ANCA-associated vasculitis or its symptoms or side effects.

II. Therapy

5 In one aspect, the present invention provides a method of treating ANCA-associated vasculitis in a patient comprising administering an antagonist, preferably an antibody, that binds to a B-cell surface marker (more preferably a CD20 antibody) to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

10 Thus, the invention contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering an antibody that binds to a B-cell surface marker to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

The invention also contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering an antagonist that binds to a B-cell surface marker to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

15 The invention also contemplates a method of treating ANCA-associated vasculitis in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.

20 In a preferred embodiment of each of these aspects, the dose is about 500 mg to 1.2 grams, more preferably about 750 mg to 1.1 grams. In another preferred embodiment, the antibody is administered in two to three doses, more preferably in two doses, but alternatively three doses. In a still preferred embodiment, the antibody is administered within a period of about 2 to 3 weeks, more preferably about two weeks, but alternatively three weeks.

25 In another embodiment, the present invention provides a method of treating ANCA-associated vasculitis in a subject eligible for treatment, comprising administering an effective amount of an antibody that binds to a B-cell surface marker (preferably a CD20 antibody) to the subject to provide an initial antibody exposure (of preferably about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, and still more preferably about 1.5 to 2.5 grams) followed by a second antibody exposure (of preferably about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, still more preferably about 1.5 to 2.5 grams), the second exposure not being provided until from about 16 to 54 weeks (preferably from about 20 to 30 weeks, more preferably from about 46 to 54 weeks) from the initial exposure.

30 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. Such re-treatment may be scheduled or unscheduled, but is preferably a scheduled redosing, particularly to protect organs such as kidneys from damage.

35 The method preferably comprises administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure (preferably of about 0.5 to 4 grams, more preferably about 1.5 to 3.5 grams, still more preferably about 1.5 to 2.5 grams), the third exposure not being provided until from about 46 to 60 weeks (preferably about 46 to 55, more preferably about 46 to 52 weeks) from the initial exposure. Preferably, no further antibody exposure is provided until at least about 70-75 weeks from the

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initial exposure, and still more preferably no further antibody exposure is provided until about 74 to 80 weeks from the initial exposure.

Any one or more of the antibody exposures herein may be provided to the subject as a single dose of antibody, or as separate doses, for example, about 1-4 separate doses of the antibody (*e.g.*, constituting a first and second dose, or a first, second, and third dose, or a first, second, third, and fourth dose, *etc.*). The particular number of doses (whether one, two or three or more) employed for each antibody exposure is dependent, for example, on the type of ANCA-associated vasculitis treated, the type of antibody employed, whether, what type, and how much and how many of a second medicament is employed as noted below, and the method and frequency of administration. Where separate doses are administered, the later dose (for example, second or third dose) is preferably administered from about 1 to 20 days, more preferably from about 6 to 16 days, and most preferably from about 14 to 16 days from the time the previous dose was administered. The separate doses are preferably administered within a total period of between about 1 day and 4 weeks, more preferably between about 1 and 20 days (*e.g.*, within a period of 6-18 days). In one such aspect, the separate doses are administered about weekly, with the second dose being administered about one week from the first dose and any third or subsequent dose being administered about one week from the second dose. Each such separate dose of the antibody is preferably about 0.5 to 1.5 grams, more preferably about 0.75 to 1.3 grams.

In a most preferred embodiment, a method of treating ANCA-associated vasculitis in a subject is provided comprising administering an effective amount of an antibody that binds to a B-cell surface marker (*e.g.*, a CD20 antibody) to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably in such a method, the antibody exposures are of about 0.5 to 4 grams each, and most preferably the amounts given above.

In one embodiment, the subject is provided at least about three 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 24 weeks. 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 separate doses of the antibody. However, not every antibody exposure need be provided as a single dose or as separate doses.

In one preferred embodiment, about 2-3 grams of the CD20 antibody is administered as the initial exposure. If about 3 grams are administered, then about 1 gram of the CD20 antibody is administered weekly for about three weeks as the initial exposure. If about 2 grams of the CD20 antibody is administered as the initial exposure, then about 1 gram of the CD20 antibody is administered followed in about two weeks by another about 1 gram of the antibody as the initial exposure. In a preferred aspect, the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams. In an alternative preferred aspect, the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.

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In all the inventive methods set forth herein, the CD20 or B-cell surface marker 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 CD20 antibody herein is a chimeric, humanized, or human CD20 antibody, more preferably rituximab, a humanized 2H7 (*e.g.* comprising the variable domain sequences in SEQ ID Nos. 2 and 8, or comprising a variable heavy-chain domain with alteration N100A or D56A and N100A in SEQ ID NO:8 and a variable light-chain domain with alteration M32L or S92A, or M32L and S92A in SEQ ID NO:2), chimeric or humanized A20 antibody (Immunomedics), or HUMAX-CD20™ human CD20 antibody (Genmab). Still more preferred is rituximab or a humanized 2H7. Also, while the ANCA-associated vasculitis in all methods herein can be any such disease, in one preferred embodiment, it is Wegener's granulomatosis or microscopic polyangiitis.

In a further embodiment of all the methods herein, the subject or patient has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the ANCA-associated vasculitis and/or has never been previously treated with an antagonist (for example, antibody) to a B-cell surface marker (*e.g.* never been previously treated with a CD20 antibody). In a still further aspect, the subject or patient may have had a relapse with the ANCA-associated vasculitis or suffered organ damage such as kidney damage before being treated in any of the methods above, including after the initial or a later antibody exposure. However, preferably, the patient or subject has not relapsed with the vasculitis and more preferably has not had such a relapse before at least the initial treatment.

In another embodiment, the subject or patient has been previously treated with drug(s) to treat the vasculitis and/or has been previously treated with such antibody or antagonist. In another embodiment, the antagonist (for example, CD20 antibody) is the only medicament administered to the subject or patient to treat the vasculitis. In another embodiment, the antagonist (*e.g.*, CD20 antibody) is one of the medicaments used to treat the vasculitis. In a further embodiment, the subject or patient does not have a malignancy. In a still further embodiment, the subject or patient does not have rheumatoid arthritis. In a still further embodiment, the subject or patient does not have multiple sclerosis. In a yet further embodiment, the subject or patient does not have lupus or Sjögren's syndrome. In yet another embodiment, the subject or patient does not have an autoimmune disease other than ANCA-associated vasculitis. In yet another aspect of the invention, the ANCA-associated vasculitis is not associated with a different autoimmune disease or with a risk of developing a different autoimmune disease. For purposes of these lattermost statements, an "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. Without being limited to any one theory, B cells demonstrate a pathogenic effect in human autoimmune diseases through a multitude of mechanistic pathways, including autoantibody production, immune complex formation, dendritic and T-cell activation, cytokine synthesis, direct chemokine release, and providing a nidus for ectopic neolymphogenesis. Each of these pathways participates to different degrees in the pathology of autoimmune diseases.

In still another embodiment, the subject or patient has a BVAS/WG score of less than 3, more preferably less than about 2, still more preferably less than about 1, and most preferably 0 (complete remission) at about three months, preferably about six months, and most preferably about one year or greater, after administration of the antagonist or antibody. Specific embodiments of this BVAS response are

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achieving a BVAS/WG score of less than 2 at three months after administration, or less than 1 (for example, 0.2 or 0.4) at 14 weeks or three months after administration, or less than 1 (for example, 0.6) at 6 months after administration, or most preferably, 0 at three or six months after administration. In another embodiment, the amount of steroid such as prednisone as compared to start of treatment is lessened without substantially affecting the lowered BVAS/WG score. Thus, for example, a subject or patient at a set interval after treatment (such as three months or six months after treatment) preferably has a lowered BVAS/WG score from baseline and is being administered less of a dose of a steroid from baseline (baseline being at the start of administration). In a still further embodiment, a step is included in the treatment method to test for the subject's or patient's response to treatment after the administration step to determine that the level of response is effective to treat the vasculitis. For example, a step is included to test the BVAS/WG score after administration and compare it to a baseline BVAS/WG score obtained before administration to determine if treatment is effective by measuring if, and by how much, it has been lowered. This test may be repeated at various scheduled or unscheduled time intervals after the administration to determine maintenance of any partial or complete remission. Alternatively, the methods herein comprise a step of testing the patient or subject, before administration, to see if one or more biomarkers are present for ANCA-associated vasculitis, such as one or more autoantibodies, a BVAS/WG score, or symptoms unique to ANCA-associated vasculitis, as set forth above. In another method, a step may be included to check the patient's or subject's clinical history, as detailed above, for example, to rule out infections or malignancy as causes, for example, primary causes, of the patient's or subject's condition, prior to administering the antibody or antagonist to the subject or patient. Preferably, the ANCA-associated vasculitis is primary (*i.e.*, the leading disease), and is not secondary, such as secondary to infection or malignancy, whether solid or liquid tumors.

In a preferred embodiment of the multi-exposure method herein, the subject is in remission after the initial or any later antibody exposures. More preferably, the multi-exposure method herein involves scheduled re-dosing or re-treating such that the patient is in remission when provided the second, and preferably all antibody exposures. Such re-dosing is scheduled to prevent any relapse, recurrence, or organ damage, rather than to treat it therapeutically. Most preferably, the subject is in remission for at least about six months, and still most preferably at least about nine months, and even still most preferably at least about a year since the last antibody exposure used in the re-treatment method.

In yet another embodiment, the subject is treated with the same CD20 antibody for at least two antibody exposures, and preferably for each antibody exposure. Thus, the initial and second antibody exposures are preferably with the same antibody, and more preferably all antibody exposures are with the same antibody, *i.e.*, treatment for the first two exposures, and preferably all exposures, is with one type of antibody that binds to a B-cell surface marker, such as CD20 antibody, *e.g.*, all with rituximab or all with the same humanized 2H7.

In any of the methods herein, one may administer to the subject or patient along with the antagonist or antibody that binds a B-cell surface marker an effective amount of a second medicament (where the antagonist or antibody that binds a B-cell surface marker (*e.g.*, the CD20 antibody) is a first medicament). The second medicament may be one or more medicaments, and include, for example, a cytotoxic agent, chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist or antibody, or any combination thereof. The type of such second

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medicament depends on various factors, including the type of vasculitis, the severity of the vasculitis, the condition and age of the patient, the type and dose of first medicament employed, *etc.*

5 Examples of such additional medicaments include a chemotherapeutic agent, an interferon class drug such as interferon-alpha (*e.g.*, from Amarillo Biosciences, Inc.), IFN-beta-1a (REBIF[®] and AVONEX[®]) or IFN-beta-1b (BETASERON[®]), an oligopeptide such as glatiramer acetate (COPAXONE[®]), an agent blocking
10 CD40-CD40 ligand, a cytotoxic or immunosuppressive agent (such as mitoxantrone (NOVANTRONE[®]), methotrexate, cyclophosphamide, chlorambucil, leflunomide, and azathioprine), intravenous immunoglobulin (gamma globulin), lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, CAMPATH[™] antibodies, anti-CD4, cladribine, a polypeptide construct with at least two domains comprising a de-immunized, autoreactive antigen or its fragment that is specifically recognized by the Ig receptors of
15 autoreactive B-cells (WO 2003/68822), total body irradiation, bone marrow transplantation), 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/ANTEGREN[®] available from Biogen, or others as noted above), drugs that treat symptoms secondary or related to ANCA-associated vasculitis
20 (*e.g.*, fungal and other infections) such as those noted herein, steroid such as corticosteroid (*e.g.*, prednisolone, methylprednisolone such as SOLU-MEDROL[™] methylprednisolone sodium succinate for injection, prednisone such as low-dose prednisone, dexamethasone, or glucocorticoid, *e.g.*, via joint injection, including systemic corticosteroid therapy), non-lymphocyte-depleting immunosuppressive therapy (*e.g.*, MMF or cyclosporine), cholesterol-lowering drug of the "statin" class (which includes cerivastatin (BAYCOL[™]),
25 fluvastatin (LESCOL[™]), atorvastatin (LIPITOR[™]), lovastatin (MEVACOR[™]), pravastatin (PRAVACHOL[™]), and simvastatin (ZOCOR[™])), estradiol, testosterone (optionally at elevated dosages; Stuve *et al. Neurology* 8:290-301 (2002)), androgen, hormone-replacement therapy, a TNF inhibitor such as an antibody to TNF-alpha, DMARD, NSAID, plasmapheresis or plasma exchange, trimethoprim-sulfamethoxazole (BACTRIM[™], SEPTRA[™]), mycophenolate mofetil, H2-blockers or proton-pump
30 inhibitors (during the use of potentially ulcerogenic immunosuppressive therapy), levothyroxine, cyclosporin A (*e.g.* SANDIMMUNE[®]), somatostatin analogue, cytokine, anti-cytokine antagonist or antibody, anti-metabolite, immunosuppressive agent, rehabilitative surgery, radioiodine, thyroidectomy, BAFF antagonist such as BAFF or BR3 antibodies or immunoadhesins, anti-CD40 receptor or anti-CD40 ligand (CD154), anti-IL-6 receptor antagonist/antibody, another B-cell surface antagonist or antibody such as a humanized 2H7 or
other humanized or human CD20 antibody with rituximab, *etc.*

Preferred such medicaments are a chemotherapeutic agent, a cytotoxic agent, anti-integrin, gamma globulin, anti-CD4, cladribine, trimethoprim-sulfamethoxazole, an H2-blocker, a proton-pump inhibitor, a corticosteroid, cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, androgen, hormone-replacement drug, a TNF inhibitor, DMARD, NSAID (to treat, for example, musculoskeletal
35 symptoms), levothyroxine, cyclosporin A, somatostatin analogue, cytokine antagonist or cytokine-receptor antagonist, anti-metabolite, BAFF antagonist such as BAFF antibody or BR3 antibody, especially a BAFF antibody, immunosuppressive agent, and another B-cell surface marker antibody, such as a combination of rituximab and a humanized 2H7 or other humanized CD20 antibody.

The more preferred such medicaments are a chemotherapeutic agent, an immunosuppressive agent,
40 including an antibody against TNF-alpha, an antibody against CD40-CD40 ligand, and a BAFF antagonist

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such as a BAFF or BR3 antibody, a DMARD, a cytotoxic agent, an integrin antagonist, a NSAID, a cytokine antagonist, or a hormone, or a combination thereof. Immunosuppressants may be required, for example, for very active disease with major organ involvement, and include such agents as cyclophosphamide (CYTOXAN®), chlorambucil, leflunomide, MMF, azathioprine (IMURAN®), and methotrexate. BAFF antagonists may be useful in combination with the first medicament for efficacy.

Still more preferred are a steroid, chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone, or a combination thereof, most preferably a steroid and/or an immunosuppressive agent, still most preferably, a corticosteroid and/or immunosuppressive agent.

In one particularly preferred embodiment, the second medicament is or comprises one or more steroids, for example, a corticosteroid, which is preferably prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone. Such steroid is preferably administered in lower amounts than are used if the first medicament, e.g., CD20 antibody, is not administered to a patient treated with steroid. In a preferred aspect, the steroid is not administered with any second antibody exposure or is administered with the second exposure but in lower amounts than are used with the initial antibody exposure. Also preferred is wherein the steroid is not administered with third or later antibody exposures.

In a still further particularly preferred aspect, the second medicament is an immunosuppressive agent, more preferably cyclophosphamide, MMF, chlorambucil, azathioprine, leflunomide, or methotrexate, and preferably administered at least with the initial antibody exposure. In one embodiment, azathioprine, methotrexate, or MMF are preferably used instead of cyclophosphamide for the maintenance of remission.

In a yet further preferred aspect, the second medicament is a combination of one or more steroids and immunosuppressive agent.

Prophylactic treatment of the ANCA-associated vasculitis with fluconazole (DIFLUCAN™) orally for fungal infection may also be used, as well as trimethoprim-sulfamethoxazole (480 mg) three times weekly for prophylactic treatment of patients with *pneumocystis carinii*. Jayne and Rasmussen, *supra*.

All these second medicaments may be used in combination with each other or by themselves with the first medicament, so that the expression "second medicament" as used herein does not mean it is the only medicament besides the first medicament, respectively. Thus, the second medicament need not be one medicament, but may constitute or comprise more than one such drug.

These second medicaments as set forth herein 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. If such second medicaments are used at all, preferably, they are used in lower amounts than if the first medicament were not present, especially in subsequent dosings beyond the initial dosing with the first medicament, so as to eliminate or reduce side effects caused thereby.

For the re-treatment method herein, where a second medicament is administered in an effective amount with an antibody exposure, it may be administered with any exposure, for example, only with one exposure, or with more than one exposure. In one embodiment, the second medicament is administered with the initial exposure. In another embodiment, the second medicament is administered with the initial and second exposures. In a still further embodiment, the second medicament is administered with all exposures. It is preferred that after the initial exposure, such as of steroid, the amount of such second medicament is

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reduced or eliminated so as to reduce the exposure of the subject to an agent with side effects such as prednisone, prednisolone, methylprednisolone, and cyclophosphamide.

As a specific example, treatment of patients with microscopic polyangiitis and Wegener's granulomatosis has three phases: (1) induction of remission, (2) maintenance of remission, and (3) treatment of relapse. Current induction therapy often consists of cyclophosphamide (CYTOXAN®) and corticosteroids. This includes a high dose of intravenous methylprednisolone for several days (e.g., one to five days), plus oral prednisone tapered over a period of time, such as 3-5 months. For aggressive disease, use of high-dose intravenous methylprednisolone for three days is recommended, combined with intravenous or oral cyclophosphamide. Tapering doses of prednisone preferably follows, along with cyclophosphamide maintenance for 12 to 18 months. When the first medicament is employed, such amount and frequency of dosing are preferably reduced further, since the lowest dosage of steroids that controls the disease should be used. Infection should be considered if the symptoms appear to exacerbate. For patients in sustained remission at 12 months, it is preferred that the use of all such second medicaments is discontinued at a faster rate with the first medicament herein administered than without it. Patients whose symptoms are under good control must, nevertheless, be closely followed at six-month intervals for signs and symptoms of relapse. During treatment with these agents, complete blood counts and liver function tests should be performed periodically.

The combined administration of a second medicament includes co-administration (concurrent administration), using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents (medicaments) simultaneously exert their biological activities.

The antibody or antagonist herein is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous (i.v.), intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, e.g., US 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody or antagonist may suitably be administered by pulse infusion, e.g., with declining doses of the antibody or antagonist. Preferably, the dosing is given intravenously or subcutaneously, and more preferably by intravenous infusion(s).

If multiple exposures of antibody are provided, each exposure may be provided using the same or a different administration means. In one embodiment, each exposure is by intravenous administration. In another embodiment, each exposure is given by subcutaneous administration. In yet another embodiment, the exposures are given by both intravenous and subcutaneous administration.

In one embodiment, the CD20 antibody is administered as a slow intravenous infusion rather than an intravenous push or bolus. For example, a steroid such as prednisolone or methylprednisolone (e.g., about 80-120 mg i.v., more specifically about 100 mg i.v.) is administered about 30 minutes prior to any infusion of the CD20 antibody. The CD20 antibody is, for example, infused through a dedicated line.

For the initial dose of a multi-dose exposure to CD20 antibody, or for the single dose if the exposure involves only one dose, such infusion is preferably commenced at a rate of about 50 mg/hour. This may be escalated, e.g., at a rate of about 50 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. However, if the subject is experiencing an infusion-related reaction, the infusion rate is preferably

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reduced, *e.g.*, to half the current rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such dose of CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed at about 255 minutes (4 hours 15 min.). Optionally, the subjects receive a prophylactic treatment of acetaminophen/paracetamol (*e.g.*, about 1 g) and diphenhydramine HCl (*e.g.*, about 50 mg or equivalent dose of similar agent) by mouth about 30 to 60 minutes prior to the start of an infusion.

If more than one infusion (dose) of CD20 antibody is given to achieve the total exposure, the second or subsequent CD20 antibody infusions in this infusion embodiment are preferably commenced at a higher rate than the initial infusion, *e.g.*, at about 100 mg/hour. This rate may be escalated, *e.g.*, at a rate of about 100 mg/hour increments every about 30 minutes to a maximum of about 400 mg/hour. Subjects who experience an infusion-related reaction preferably have the infusion rate reduced to half that rate, *e.g.*, from 100 mg/hour to 50 mg/hour. Preferably, the infusion of such second or subsequent dose of CD20 antibody (*e.g.*, an about 1000-mg total dose) is completed by about 195 minutes (3 hours 15 minutes).

A discussion of methods of producing, modifying, and formulating such antibodies follows.

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.

CD20 antigen to be used for production of, or screening for, antibody(ies) may be, *e.g.*, a soluble form of CD20 or a portion thereof, containing the desired epitope. Alternatively, or additionally, cells expressing CD20 at their cell surface can be used to generate, or screen for, antibody(ies). Other forms of CD20 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 (*s.c.*) or intraperitoneal (*i.p.*) 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 sulfo succinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, 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

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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*

5 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.

10 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).

15 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)).

20 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.

25 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.*,
30 *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

35 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).

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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

5 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.

10 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

15 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 Plöckthum, *Immunol. Rev.*, 130:151-188 (1992).

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage

20 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.*, *BioTechnology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a

25 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.

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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

35 and another antigen-combining site having specificity for a different antigen.

In addition, antibodies comprising a variant Fc region with high affinity for FcγR are useful for treating diseases where an enhanced efficacy of effector cell function is desired, such as autoimmune diseases.

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as set forth, for example, in US 2003/0037000 and WO 2004/63351 (MacroGenics, Inc. STAVENHAGEN *et al.*).

(III) *Humanized antibodies*

5 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); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for
10 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.

15 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.*,
20 *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)).

25 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
30 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
35 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

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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. 5,391,669, 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 *et al.*, *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.

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(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 CD20 antigen. Other such antibodies may bind CD20 and further bind a second B-cell surface marker. Alternatively, an anti-CD20 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 CD20-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 co-expression 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 Trautnecker *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).

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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 (scFv) 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).

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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 (CD20) antibody may be conjugated to a drug as described in WO2004/032828.

5 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.

10 Alternatively, the antibody is conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics is 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_1^1 , α_3^1 , α_9^1 , N-acetyl- γ_1^1 , PSAG and θ^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, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

25 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^{121} , 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 imidocsters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as 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,

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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.

5 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).

10 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 convert it into its more active, cytotoxic form.

15 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 serratio 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
20 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
25 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
30 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 non-proteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody
35 fragments, such as Fab', linked to one or more PEG molecules are an especially preferred embodiment of the invention.

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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 WO 97/38731 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 protein or peptide antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

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

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include 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

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substitutional mutagenesis of antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 3 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 3, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 3

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)

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(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)

5 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;

10 (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.

15 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).

20 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.

35 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.

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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.

5 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).

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

20 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 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; 25 US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 30 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)).

35 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

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substitutions in an Fc region of an 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, *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).

WO 00/42072 (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.* IgG₁, IgG₂, IgG₃, or IgG₄) 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,

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glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; 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™, PLURONIC™ or polyethylene glycol (PEG).

5 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 10 mg/mL rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection.
10 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.

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

The formulation herein may also contain more than one active compound (a second medicament as noted above) as necessary, preferably those with complementary activities that do not adversely affect each other. The type and effective amounts of such medicaments depend, for example, on the amount of antibody
20 present in the formulation, and clinical parameters of the subjects. The preferred such medicaments are noted above.

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-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems
25 (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are
30 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-
35 hydroxybutyric acid.

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

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VI. Articles of Manufacture

In another embodiment of the invention, articles of manufacture containing materials useful for the treatment of ANCA-associated vasculitis described above are provided. In one aspect, the article of manufacture comprises (a) a container comprising an antagonist that binds to a B-cell surface marker (e.g., an antibody that so binds, including a CD20 antibody) (preferably the container comprises the antagonist or antibody and a pharmaceutically acceptable carrier or diluent within the container); and (b) a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the antagonist or antibody of about 400 mg to 1.3 grams at a frequency of one to three doses is administered to the patient within a period of about one month.

Thus, the invention provides an article of manufacture comprising: a container comprising a CD20 antibody, or an antibody or antagonist that binds to a B-cell surface marker; and a package insert with instructions for treating ANCA-associated vasculitis in a patient, wherein the instructions indicate that a dose of the CD20 antibody, or the antibody or antagonist that binds to a B-cell surface marker, of about 400 mg to 1.3 grams, at a frequency of one to three doses, is administered to the patient within a period of about one month.

In a preferred embodiment, the article of manufacture herein further comprises a container comprising a second medicament, wherein the antagonist or antibody is a first medicament. This article further comprises instructions on the package insert for treating the patient with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone. The preferred second medicaments are those preferred as set forth above, and most preferred is a steroid or an immunosuppressive agent or both.

In another aspect, the invention provides an article of manufacture comprising: (a) a container comprising an antibody that binds to a B-cell surface marker (e.g., a CD20 antibody) (preferably the container comprises the antibody and a pharmaceutically acceptable carrier or diluent within the container); and (b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.

Preferably, such package insert is provided with instructions for treating ANCA-associated vasculitis 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, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure and each of the antibody exposures is provided to the subject as about one to four doses, preferably as a single dose or as two or three separate doses of antibody.

In a specific aspect, an article of manufacture is provided comprising:

(a) a container comprising an antibody that binds to a B-cell surface marker (e.g., a CD20 antibody) (preferably the container comprises the antibody and a pharmaceutically acceptable carrier or diluent within the container); and

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(b) a package insert with instructions for treating ANCA-associated vasculitis 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 followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure, and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. Preferably, the antibody exposures are of about 0.5 to 4 grams.

In a preferred embodiment of these inventive aspects, the article of manufacture herein further comprises a container comprising a second medicament, wherein the antibody is a first medicament, and which article further comprises instructions on the package insert for treating the subject with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone, most preferably a steroid or an immunosuppressive agent, or both.

In all of these aspects, the package insert is 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 ANCA-associated vasculitis 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 antagonist or antibody. The label or package insert indicates that the composition is used for treating ANCA-associated vasculitis in a patient or subject eligible for treatment with specific guidance regarding dosing amounts and intervals of antagonist or antibody and any other medicament being provided. The article of manufacture may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or 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.

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

Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study assesses the superiority of efficacy and safety of rituximab (MABTHERA[®]/RITUXAN[™]) in a certain dosing regimen compared to placebo for treatment of signs and symptoms in patients with Wegener's granulomatosis exhibiting one or more symptoms of systemic disease.

Rituximab (1000 mg i.v. x 2) is administered i.v. in two initial doses at days 1 and 15 with 1 mg/kg of oral prednisone daily (which is reduced to 40 mg/day at week 4, and tapered using a standardized tapering regimen resulting in complete discontinuation of prednisone over the following 3-5 months). This experimental regimen is compared to the same regimen except using rituximab placebo instead of rituximab, with 1:1 randomization between the two arms of the study, with about 48 patients per arm (total 96 patients).

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Active disease is defined as a Birmingham Vasculitis Activity Score/Wegener's granulomatosis (BVAS/WG) score of greater than 0. For trial inclusion, the patient's BVAS/WG score must be 3 or greater (or have been 3 or greater within 28 days of randomization). Each major item on the BVAS/WG evaluation form is scored 3 points. Each minor item is scored 1 point. In determining the degree of disease activity, the investigator will distinguish between active vasculitis (new/worse BVAS/WG items as opposed to persistent items) and permanent organ damage (caused by previously active vasculitis).

A severe flare is a new occurrence of one or more major BVAS/WG items. (Major items have a * on the BVAS/WG scoring sheet.) Generally, such flares are treated by increases in prednisone dose or cyclophosphamide dose.

Severe Wegener's granulomatosis occurs in a patient whose disease is not classifiable as limited, by definition below.

A limited flare is a new occurrence of one or more minor BVAS/WG items. Generally, such flares are treated with increases in prednisone dose or an increase in methotrexate dose.

Limited Wegener's granulomatosis occurs in a patient who meets the modified American College of Rheumatology (ACR) criteria for a diagnosis of Wegener's granulomatosis but who does not have disease that poses an immediate threat to either a critical individual organ or to the patient's life. Specifically, this means that:

- The patient has no red blood cell casts in the urine.
- If hematuria (but no +RBC casts) is present, the serum creatinine must be less than or equal to 1.4 and there must be evidence of no rise of creatinine more than 25% above the patient's baseline.
- Pulmonary involvement must be circumscribed, such that the room air pO_2 is > 70 mmHg or the room air O_2 saturation by pulse oximetry is $> 92\%$. Pulmonary hemorrhage may be treated as limited disease provided there is no evidence of progression of the process. In the absence of data on progression, pulmonary hemorrhage may be treated as severe disease at the discretion of the physician.
- No disease may exist within any other critical organ (e.g., the gastrointestinal tract, eyes, central nervous system) that, without the immediate institution of maximal therapy (i.e., pulse methylprednisolone and daily oral cyclophosphamide), threatens the function of that organ and/or the patient's life.

A newly-diagnosed patient is a patient on his/her first treatment course of corticosteroids and/or a chemotherapeutic or immunosuppressive agent for Wegener's granulomatosis, with no history of increase in immunosuppressive therapy prior to entry into the study.

For the purpose of scoring BVAS/WG, persistent disease is defined as the presence of ongoing disease activity that was present at the previous trial evaluation (i.e., not new or worse activity).

A purified protein derivative skin test may be used to detect latent tuberculosis infection.

A refractory patient is a patient with a history of immunosuppressive therapy (corticosteroids and/or an immunosuppressive agent or chemotherapeutic agent) prior to the initiation of treatment for the Wegener's granulomatosis activity that makes the patient eligible for this study.

Patients will be considered to be in remission when their BVAS/WG score is 0.

This rituximab-based regimen challenges the current standard of care, and is intended to limit patient exposure to steroids and its known toxicities, and to demonstrate improved net clinical benefit. Patients are monitored for disease activity, use of additional immunosuppressants, steroid usage, and safety events over

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the trial length of one year, with the primary efficacy endpoint of the trial measured at 3 months, with follow-up to 16.8 months. Safety follow-up is required until 12 months following the last dose of rituximab or return of ANCA into the normal range, whichever occurs later.

5 The primary objective is to determine the proportion of patients achieving a BVAS/WG score of 0 and successful prednisone taper at 6 months, and no pre-specified adverse events.

10 It is predicted and expected that rituximab (or a humanized 2H7 substituted for rituximab) is effective in inducing remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and to permit reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean prednisolone dose (mg/day) is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. If the mean BVAS/WG at entry is 3.6, at 6 months of treatment, it is expected to decrease to a statistically significant value of 0.6. Intermittently active disease is expected to be observed in fewer than 70% of the patients. In contrast, the control arm is expected to show much less decrease in BVAS/WG and C-reactive protein, and in steroid use, and fewer patients in remission.

Example 2

20 Study of Efficacy of Rituximab in Patients with Microscopic Polyangiitis

The protocol in Example 1 is followed except that the patients are treated for microscopic polyangiitis. It is expected that similar results will be observed as for Wegener's granulomatosis, i.e., that remission, as measured by the BVAS/WG score of 0, is expected to occur in at least 80% of the patients treated in the study arm and that steroid use is expected to decrease over the course of the study, which results are expected to be much better, in a statistically significant sense, than the control results.

Example 3

30 Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study assesses the superiority of efficacy and safety of re-treatment with rituximab (MABTHERA[®]/RITUXAN[®]) compared to placebo in adult subjects with Wegener's granulomatosis. Study I examines acute disease, either first presentation or relapse (BVAS ≥ 10 ; $n = 16$); study II examines persistent disease (BVAS ≥ 4 ; $n = 16$). Patients receive rituximab (1 g i.v.) in three initial doses at days 1, 8, and 15 for Studies I and II. Concomitant therapy in Study I includes 1 mg/kg/day oral prednisone tapered according to the regimen in Example 1 and cyclophosphamide (according to standard treatment). Study II patients receive rituximab and 1 mg/kg/day oral prednisone tapered according to the regimen in Example 1. All subjects receive a second rituximab/placebo infusion course of 1000 mg i.v. separated by 14 days at weeks 24 and 26, respectively, without steroids or cyclophosphamide, whether the patients exhibited symptoms or were in complete remission. Courses of rituximab treatment must be separated by a minimum interval of 16 weeks.

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The experimental regimens are compared to rituximab placebo + the same doses of tapered oral prednisone and cyclophosphamide (Study I), or tapered oral prednisone (Study II).

5 Changes in immunosuppressive drugs are not permitted during the studies, unless mandated by toxicity, and requests to taper a drug other than the oral prednisone must be discussed in advance with the Medical Monitor. Study personnel will be trained on how to properly administer rituximab. Subjects may be hospitalized for observation, particularly for their first infusion, at the discretion of the investigator. Rituximab must be administered under close supervision, and full resuscitation facilities must be immediately available.

10 Patients are monitored each month for 12 months for disease activity, use of additional immunosuppressants, flares of disease, prednisone usage, and safety events over the 52 weeks of the study. The primary efficacy endpoint of the trial is at 52 weeks, and efficacy measures are assessed by a unique Examining Assessor who is not involved with patient treatment or other study procedures. The patients are assessed for their BVAS/WG scores and successful prednisone taper. At the end of 52 weeks, subjects who received rituximab placebo or rituximab but demonstrate a BVAS/WG score of 0 and successful prednisone taper at 6 months will complete study participation. Subjects who received rituximab but have not demonstrated such score at 52 weeks are observed for 6 months following the last course of rituximab or until a BVAS/WG score of 0, whichever occurs first. Sites will be informed as to whether a subject must continue in follow-up, but not whether the subject received placebo or rituximab. Safety follow-up is required until 12 months following the last dose of rituximab or a BVAS/WG score of 0, whichever occurs later.

20 These rituximab-based regimens challenge the current standard of care, and are expected to demonstrate improved net clinical benefit, with the primary objective to determine the proportion of patients achieving the primary endpoint of a BVAS/WG score of 0, and successful prednisone taper.

25 It is predicted and expected that administration of rituximab or a humanized 2H7 to the subject in the protocols of Studies I and II set forth above will induce remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and permit reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean steroid use for both Studies I and II is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. These results are expected to be significantly better than those of the control arms for Studies I and II.

30 It is also expected that at about week 48-54, another 2-g dose of the rituximab given all at once or spread out over about 14-16 days in 1-gram amounts would be effective to treat Wegener's granulomatosis for the entire second year (causing a BVAS/WG score of 0 in at least 80% of the enrolled patients), with or without the steroid(s) and/or other immunosuppressive agents, with a marked improvement over control patients receiving rituximab placebo rather than rituximab. Thus, the rituximab (or a humanized 2H7) would be administered initially within about the 2-week time period, followed by another treatment at about 4-8 months, followed by another treatment at about one year from initial treatment (measured from the time any one of the doses was given), followed by treatment at about two years from initial treatment, with expected success, in about one-gram x 2-4 dosing for each treatment, administered together, about weekly, or about

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every other week over about two to four weeks. This re-treatment protocol is expected to be successfully used for several years with little or no adverse effects.

Example 4

Second Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

This study is the same as in Example 3 except that the initial dose of rituximab or rituximab placebo is given as 1000 mg i.v. x 2 (on day 0, with the second infusion occurring on Day 15 +/- 1 day), and the subsequent course of rituximab or placebo infusions administered at weeks 24 and 26 and consisting of 2 biweekly doses is administered only to those subjects in remission, e.g., those not exhibiting increasing disease activity, as by rising ANCA titers, sustained elevated ANCA titers, and other symptoms. All other criteria are the same.

It is predicted and expected that administration of rituximab or a humanized 2H7 to the subject in the scheduled redosing protocol set forth above will be effective in inducing remission (achieving BVAS/WG scores of 0) in at least 80% of the enrolled patients with Wegener's granulomatosis and in permitting reduction in steroid doses over the control arm. BVAS/WG is expected to decrease from the score at entry to about 0.2 to 0.4 at week 14. C-reactive protein (mg/L) is expected to decrease from the level at entry to a range of about 3 to 11 by week 14. Mean prednisolone dose (mg/day) is expected to decrease from the value at entry to a statistically significant lower value at week 14. Relapse is expected to occur in fewer than five patients after a mean of 27 weeks. These results are expected to be significantly better than those of the control.

It is also expected that at about week 48-54, another 2-g dose of the CD20 antibody (e.g., rituximab or a humanized 2H7) given all at once or spread out over about 14-16 days in 1-gram amounts would be effective to treat Wegener's granulomatosis for the entire second year (with at least 80% of the enrolled patients having a BVAS/WG score of 0), with or without the prednisone taper and i.v. methylprednisolone and/or other immunosuppressive agents. Thus, the CD20 antibody would be administered initially within about the 2-week time period, followed by another treatment at about 4-8 months, followed by another treatment at about one year from initial treatment (measured from the time any one of the doses was given), followed by treatment at about two years from initial treatment, with expected success, in about one-gram x 2-4 dosing for each treatment, administered together, about weekly, or about every other week over about two to four weeks. The results of this treatment would be expected to be much better than those of the control with placebo. This re-treatment protocol is expected to be successfully used for several years with little or no adverse effects.

Example 5

Third Re-treatment Study of Efficacy of Rituximab in Patients with Wegener's Granulomatosis

It is expected that Example 4 results would be successful if the patients were initially treated with rituximab and then re-treated with rituximab one year after first being treated, using the same dosing

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and other protocol of Example 4 except that rituximab is given at one-year intervals rather than six-month intervals.

Example 6

Study of Efficacy of Rituximab in Subjects with Generalized ANCA-Associated Vasculitis

5 A randomized, multi-center, double-masked, placebo-controlled trial is performed in patients with generalized ANCA-associated vasculitis using rituximab. Two-hundred patients are randomized to either (1) conventional treatment (cyclophosphamide and corticosteroids, followed by azathioprine); or (2) rituximab (plus corticosteroids, initially) for remission induction, using 1 gram of rituximab on day 1 and again on day 15.

10 The primary clinical comparison is the ability of rituximab in this dosing regimen and corticosteroids to induce disease remissions, as measured by the cumulative disease activity at six months. Consistent with the standard duration of treatment for ANCA-associated vasculitis, patients in the conventional therapy arm will receive cyclophosphamide for up to 6 months followed by azathioprine, to complete a total length of treatment of 18 months. To assess the ability of rituximab to restore B-cell tolerance, patients in both arms of the trial will be followed for a total of 18 months.

15 It is expected that rituximab (or a humanized 2H7 substituted for rituximab) will induce stable remissions in the patients with ANCA-associated vasculitis and will re-establish B-cell tolerance to the ANCA target antigens in at least two thirds of the patients. It is also expected that rituximab or other CD20 antibody will be at least as effective as the conventional treatment regimen for induction and maintenance of disease remission, offering substantial advantages over standard therapy by virtue of its superior side-effect profile, e.g., much less toxic than chemotherapeutics and steroids, and better at restoring tolerance.

Example 7

Re-treatment Study of Efficacy of Rituximab in Subjects with Severe Wegener's Granulomatosis or Severe Microscopic Polyangiitis

25 Twenty patients with active severe Wegener's granulomatosis or severe microscopic polyangiitis, positive ANCA test, and BVAS/WO score of at least 3, who are unresponsive to cyclophosphamide or have contraindications for cyclophosphamide use, are enrolled. See the definition of severe Wegener's granulomatosis in Example 1. The remission induction regimen consists of oral prednisone (1 mg/kg/day) and rituximab (1 gram at day 1 and 1 gram at day 15). By week 4, the prednisone is reduced to 40 mg/day. A standardized tapering regimen follows, resulting in complete discontinuation of prednisone over the following 16 weeks. This is compared with the same regimen, but with rituximab placebo rather than rituximab (control study). The protocol stipulates re-treatment with the same remission induction regimen at 6 months for all patients, whether they are experiencing a disease flare after reconstitution of B cells, whether they are asymptomatic with a recurrence of ANCA or ANCA titer rise coinciding or following reconstitution of B cells, or whether in complete remission. This re-treatment regimen includes the 1 g x 2 two weeks apart for

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rituximab and rituximab placebo. A clinical flare in the absence of B cells is considered treatment failure. The patients are assessed monthly for one year.

It is expected that the patients in the treatment arm will tolerate rituximab infusions well and that their B cells will be depleted swiftly and all will achieve complete remission (BVAS/WG of 0) by three months. All patients in the treatment arm are expected to complete the glucocorticoid taper by 6 months. It is expected that after 12 months, no patient in the treatment arm will experience a clinical flare, and that B cells will return in most, if not all, such patients in the 12 months. Other than glucocorticoids, no additional immunosuppressive agents are expected to be necessary for induction of remission and maintenance of sustained remission (6 months or longer) in the rituximab-treated patients.

Example 8

Humanized 2H7 Variants Useful Herein

Useful for purposes herein are humanized 2H7 antibodies comprising 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:35), 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:36), for example, SEQ ID NO:6 (Fig. 1A),

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

CDR H2 sequence of AIYPCNGXTSYNQKFKG wherein X is D or A (SEQ ID NO:37), for example, SEQ ID NO:11 (Fig. 1B), and

CDR H3 sequence of VVYYSSXXYWYFDV wherein the X at position 6 is N, A, Y, W, or D, and the X at position 7 is S or R (SEQ ID NO:38), for example, SEQ ID NO:12 (Fig. 1B).

The humanized 2H7 antibodies herein include those with heavy-chain amino acid sequences containing a C-terminal lysine and those without. 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 non-native-sequence 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 sequence of SEQ ID NO:13 or 30, and heavy-chain amino acid sequence of SEQ ID NO:14, 15, 29, 31, 34, or 39, the sequence of SEQ ID NO:39 being given below.

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

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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. 6,737,056, L. 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. 6,737,056, L. 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. 6,528,624, 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 in SEQ ID NO:8 of N100A; or D56A and N100A; or D56A, N100Y, and S100aR; and a variable light domain with alteration in SEQ ID NO:2 of M32L; or S92A; or M32L and S92A.

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 Table 4 below. Unless otherwise indicated, the 2H7 variants will have the same light chain as that of v16.

Table 4
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, K322A, K334A,
138	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A,
477	D56A, N100A	M32L, S92A	S298A, K326A, E333A, K334A, N434W
375	-	-	K334L
588	-	-	S298A, K326A, E333A, K334A
511	D56A, N100Y, S100aR	M32L, S92A	S298A, K326A, E333A, K334A

One preferred humanized 2H7 comprises 2H7.v16 variable light-domain sequence:

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DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWSFNPPFTFGQGTKVEIKR (SEQ ID NO:2);

and 2H7.v16 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
5 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYFDVWGQGLTVTVSS (SEQ ID
NO:8).

Where the humanized 2H7.v16 antibody is an intact antibody, it may comprise the light-chain amino
acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
10 DFTLTISLQPEDFATYYCQQWSFNPPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO:13);

and the heavy-chain amino acid sequence of SEQ ID NO:14 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
15 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSNSYWFYFDVWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
20 TPPVLDSDGSFPLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKSLSLSPG (SEQ ID NO:15).

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWAFNPPFTFGQGTKVEIKR (SEQ ID NO:39)

and 2H7.v511 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFK
25 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWFYFDVWGQGLTVTVSS (SEQ ID
NO:40).

Where the humanized 2H7.v511 antibody is an intact antibody, it may comprise the light-chain
amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGT
30 DFTLTISLQPEDFATYYCQQWAFNPPFTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC (SEQ ID NO:30)

and the heavy-chain amino acid sequence of SEQ ID NO: 31 or:

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EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWYFDVWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
5 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
AALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG (SEQ ID NO:41).

See Figures 7 and 8, which align the mature light and heavy chains, respectively, of humanized
2H7.v511 with humanized 2H7.v16 using the C-terminal lysine sequence for the heavy chain..

10 Where the humanized 2H7.v31 antibody is an intact antibody, it may comprise the light-chain amino
acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPI.IYAPSNLASGVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
15 VTKSFNRGEC (SEQ ID NO:13)

and the heavy-chain amino acid sequence of SEQ ID NO:15 or:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFK
GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWYFDVWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
20 GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG (SEQ ID NO:42) or:
EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFK
25 GRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVYYSYRYWYFDVWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSN
AALPAPIAATISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
30 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG (SEQ ID NO:43).

A preferred embodiment herein is where the antibody is humanized 2H7 comprising the variable
domain sequences in SEQ ID NOS:2 and 8 (version 16). Another preferred embodiment herein is where the
antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40 (version
511). Further preferred is where the antibody is humanized 2H7 comprising the variable domain sequences in
35 SEQ ID NOS:32 and 33 (see Figure 9 re version 114), such as one comprising the variable light-chain domain
in SEQ ID NO:32 and the heavy-chain amino acid sequence of SEQ ID NO:34. Further preferred is wherein
the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A
and N100A, or D56A, N100Y, and S100A in SEQ ID NO:8 and a variable light-chain domain with alteration
M32L, or S92A, or M32L and S92A in SEQ ID NO:2.

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WHAT IS CLAIMED IS:

1. A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient comprising administering a CD20 antibody to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month.
- 5 2. The method of claim 1 wherein the dose is about 500 mg to 1.2 grams.
3. The method of claim 1 or 2 wherein the dose is about 750 mg to 1.1 grams.
4. The method of any one of claims 1-3 wherein the antibody is administered in two to three doses.
5. The method of any one of claims 1-4 wherein the antibody is administered in three doses.
6. The method of any one of claims 1-5 wherein the antibody is administered within a period of about 2
- 10 to 3 weeks.
7. The method of claim 6 wherein the period is about three weeks.
8. The method of any one of claims 1-7 wherein the ANCA-associated vasculitis is Wegener's granulomatosis.
9. The method of any one of claims 1-7 wherein the ANCA-associated vasculitis is microscopic polyangiitis.
- 15 10. The method of any one of claims 1-9 wherein a second medicament is administered in an effective amount, wherein the CD20 antibody is a first medicament.
11. The method of claim 10 wherein the second medicament is more than one medicament.
12. The method of claim 10 or 11 wherein the second medicament is a chemotherapeutic agent, an
- 20 immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a hormone, or a combination thereof.
13. The method of claim 12 wherein the second medicament is a steroid or an immunosuppressive agent or both.
- 25 14. The method of claim 13 wherein the second medicament is a steroid.
15. The method of claim 14 wherein the steroid is a corticosteroid.
16. The method of claim 15 wherein the steroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.
17. The method of any one of claims 14-16 wherein the steroid is administered in lower amounts than
- 30 are used if the CD20 antibody is not administered to a patient treated with steroid.
18. The method of claim 13 wherein the second medicament is an immunosuppressive agent.
19. The method of claim 18 wherein the immunosuppressive agent is cyclophosphamide, chlorambucil, mycophenolate mofetil, leflunomide, azathioprine, or methotrexate.
20. The method of claim 19 wherein the immunosuppressive agent is cyclophosphamide.
- 35 21. The method of claim 13 wherein the second medicament is a steroid and an immunosuppressive agent.
22. The method of any one of claims 1-21 wherein the patient has never been previously treated with a CD20 antibody.
23. The method of any one of claims 1-22 wherein the patient has not relapsed with the vasculitis.
- 40 24. The method of any one of claims 1-22 wherein the antibody is a naked antibody.

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25. The method of any one of claims 1-22 wherein the antibody is conjugated with another molecule.
26. The method of claim 25 wherein the other molecule is a cytotoxic agent.
27. The method of any one of claims 1-26 wherein the antibody is administered intravenously.
28. The method of any one of claims 1-26 wherein the antibody is administered subcutaneously.
- 5 29. The method of any one of claims 1-28 wherein no other medicament than the CD20 antibody is administered to the subject to treat the ANCA-associated vasculitis.
30. The method of any one of claims 1-29 wherein the antibody is rituximab.
31. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos. 2 and 8.
- 10 32. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40.
33. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:32 and 33.
- 15 34. The method of any one of claims 1-29 wherein the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2.
35. The method of any one of claims 1-34 wherein the patient has a BVAS/WG score of 0 at six months after administration of the antibody.
- 20 36. The method of any one of claims 1-35 wherein the patient has an elevated level of a anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination thereof.
37. An article of manufacture comprising:
- a. a container comprising a CD20 antibody; and
- 25 b. a package insert with instructions for treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient, wherein the instructions indicate that a dose of the CD20 antibody of about 400 mg to 1.3 grams at a frequency of one to three doses is administered to the patient within a period of about one month.
- 30 38. The article of claim 37 further comprising a container comprising a second medicament, wherein the CD20 antibody is a first medicament, further comprising instructions on the package insert for treating the patient with the second medicament.
39. The article of claim 38 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.
- 35 40. The article of claim 38 or 39 wherein the second medicament is a steroid or an immunosuppressive agent or both.

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41. A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure followed by a second antibody exposure, wherein the second exposure is not provided until from about 16 to 54 weeks from the initial exposure.
- 5 42. The method of claim 41 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.
43. The method of claim 41 or 42 wherein the second exposure is not provided until from about 20 to 30 weeks from the initial exposure.
- 10 44. The method of any one of claims 41-43 wherein the second exposure is not provided until from about 46 to 54 weeks from the initial exposure.
45. The method of any one of claims 41-44 wherein each of the initial and second antibody exposures is provided in amounts of about 0.5 to 4 grams.
46. The method of any one of claims 41-45 wherein each of the initial and second antibody exposures is provided in amounts of about 1.5 to 3.5 grams.
- 15 47. The method of any one of claims 41-46 wherein each of the initial and second antibody exposures is provided in amounts of about 1.5 to 2.5 grams.
48. The method of any one of claims 41-47 additionally comprising administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure, wherein the third exposure is not provided until from about 46 to 60 weeks from the initial exposure.
- 20 49. The method of claim 48 wherein the third antibody exposure is provided in an amount of about 0.5 to 4 grams.
50. The method of claim 48 or 49 wherein the third antibody exposure is provided in an amount of about 1.5 to 3.5 grams.
- 25 51. The method of any one of claims 48-50 wherein the third antibody exposure is provided in an amount of about 1.5 to 2.5 grams.
52. The method of any one of claims 48-51 wherein the third exposure is not provided until from about 46 to 55 weeks from the initial exposure.
53. The method of any one of claims 48-52 wherein no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.
- 30 54. The method of claim 53 wherein no further antibody exposure is provided until about 74 to 80 weeks from the initial exposure.
55. The method of any one of claims 41 or 43-54 wherein one or more of the antibody exposures is provided to the subject as a single dose of antibody.
- 35 56. The method of any one of claims 41-55 wherein each antibody exposure is provided to the subject as a single dose of antibody.

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57. The method of any one of claims 41 or 43-54 wherein one or more of the antibody exposures is provided to the subject as separate doses of the antibody.
58. The method of any one of claims 41-54 or 57 wherein each antibody exposure is provided as separate doses of the antibody.
- 5 59. The method of claim 57 wherein the separate doses are from about 2 to 3 doses.
60. The method of any one of claims 57-59 wherein the separate doses constitute a first and second dose.
61. The method of any one of claims 57-59 wherein the separate doses constitute a first, second, and third dose.
- 10 62. The method of any one of claims 57-61 wherein a later dose is administered from about 1 to 20 days from the time the previous dose was administered.
63. The method of any one of claims 57-62 wherein a later dose is administered from about 6 to 16 days from the time the previous dose was administered.
64. The method of any one of claims 57-63 wherein a later dose is administered from about 14 to 16 days from the time the previous dose was administered.
- 15 65. The method of any one of claims 57-64 wherein the separate doses are administered within a total period of between about 1 day and 4 weeks.
66. The method of any one of claims 57-65 wherein the separate doses are administered within a total period of between about 1 and 25 days.
- 20 67. The method of any one of claims 57-66 wherein the separate doses are administered about weekly, with the second dose being administered about one week from the first dose and any later dose being administered about one week from the previous dose.
68. The method of any one of claims 57-67 wherein each separate dose of antibody is about 0.5 to 1.5 grams.
- 25 69. The method of any one of claims 57-68 wherein each separate dose of antibody is about 0.75 to 1.3 grams.
70. The method of any one of claims 41-69 wherein 4 to 20 antibody exposures are administered to the subject.
71. The method of any one of claims 41-70 wherein a second medicament is administered in an effective amount with an antibody exposure, wherein the CD20 antibody is a first medicament.
- 30 72. The method of claim 71 wherein the second medicament is administered with the initial exposure.
73. The method of claim 71 or 72 wherein the second medicament is administered with the initial and second exposures.
74. The method of any one of claims 71-73 wherein the second medicament is administered with all exposures.

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75. The method of any one of claims 71-74 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a disease-modifying anti-rheumatic drug (DMARD), a cytotoxic agent, an integrin antagonist, a non-steroidal anti-inflammatory drug (NSAID), a cytokine antagonist, or a hormone, or a combination thereof.
- 5 76. The method of any one of claims 71-75 wherein the second medicament comprises a steroid or an immunosuppressive agent or both.
77. The method of any one of claims 71-76 wherein the second medicament is a steroid.
78. The method of claim 77 wherein the steroid is a corticosteroid.
79. The method of claim 78 wherein the steroid is prednisone, prednisolone, methylprednisolone, hydrocortisone, or dexamethasone.
- 10 80. The method of any one of claims 77-79 wherein the steroid is administered in lower amounts than are used if the CD20 antibody is not administered to a subject treated with steroid.
81. The method of any one of claims 71-76 wherein the second medicament is an immunosuppressive agent.
- 15 82. The method of claim 81 wherein the immunosuppressive agent is cyclophosphamide, chlorambucil, leflunomide, mycophenolate mofetil, azathioprine, or methotrexate.
83. The method of claim 82 wherein the immunosuppressive agent is cyclophosphamide.
84. The method of any one of claims 71-76 wherein the second medicament comprises a steroid and an immunosuppressive agent.
- 20 85. The method of claim 72 wherein the second medicament is not administered with the second exposure, or is administered in lower amounts than are used with the initial exposure.
86. The method of any one of claims 41-85 wherein about 2-3 grams of the CD20 antibody is administered as the initial exposure.
87. The method of claim 86 wherein about 1 gram of the CD20 antibody is administered weekly for about three weeks as the initial exposure.
- 25 88. The method of claim 86 or 87 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.
89. The method of any one of claims 86-88 wherein the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.
- 30 90. The method of claim 86 wherein about 1 gram of the CD20 antibody is administered followed in about two weeks by another about 1 gram of the antibody as the initial exposure.
91. The method of claim 90 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.
- 35 92. The method of claim 90 or 91 wherein the second exposure is at about six months from the initial exposure and is administered as about 1 gram of the antibody followed in about two weeks by another about 1 gram of the antibody.

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93. The method of any one of claims 86-92 wherein a steroid is administered to the subject before or with the initial exposure.
94. The method of claim 93 wherein the steroid is not administered with the second exposure or is administered with the second exposure but in lower amounts than are used with the initial exposure.
- 5 95. The method of claim 93 or 94 wherein the steroid is not administered with third or later exposures.
96. The method of any one of claims 41-95 wherein the subject has never been previously treated with a CD20 antibody.
97. The method of any one of claims 41-96 wherein the subject is in remission after the initial or a later antibody exposure.
- 10 98. The method of any one of claims 41-96 wherein the subject is in remission when provided the second antibody exposure.
99. The method of claim 98 wherein the subject is in remission when provided all antibody exposures.
100. The method of any one of claims 41-99 wherein the initial and second antibody exposures are with the same CD20 antibody.
- 15 101. The method of any one of claims 41-100 wherein all antibody exposures are with the same CD20 antibody.
102. The method of any one of claims 41-101 wherein the antibody is a naked antibody.
103. The method of any one of claims 41-101 wherein the antibody is conjugated with another molecule.
104. The method of claim 103 wherein the other molecule is a cytotoxic agent.
- 20 105. The method of any one of claims 41-104 wherein the antibody is administered intravenously.
106. The method of claim 105 wherein the antibody is administered intravenously for each antibody exposure.
107. The method of any one of claims 41-104 wherein the antibody is administered subcutaneously.
108. The method of claim 107 wherein the antibody is administered subcutaneously for each antibody exposure.
- 25 109. The method of any one of claims 41-108 wherein no other medicament than the CD20 antibody is administered to the subject to treat the ANCA-associated vasculitis.
110. The method of any one of claims 41-109 wherein the ANCA-associated vasculitis is Wegener's granulomatosis.
- 30 111. The method of any one of claims 41-109 wherein the ANCA-associated vasculitis is microscopic polyangiitis.
112. The method of any one of claims 41-111 wherein the antibody is rituximab.
113. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID Nos. 2 and 8.

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114. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:39 and 40.
115. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:32 and 33.
- 5 116. The method of any one of claims 41-111 wherein the antibody is humanized 2H7 comprising a variable heavy-chain domain with alteration N100A, or D56A and N100A, or D56A, N100Y, and S100aR in SEQ ID NO:8 and a variable light-chain domain with alteration M32L, or S92A, or M32L and S92A in SEQ ID NO:2.
- 10 117. The method of any one of claims 41-116 wherein the subject has a BVAS/WG score of 0 at six months after administration of the antibody.
118. The method of any one of claims 41-117 wherein the subject has an elevated level of anti-nuclear antibodies (ANA), anti-rheumatoid factor (RF) antibodies, creatinine, blood urea nitrogen, anti-endothelial antibodies, anti-neutrophil cytoplasmic antibodies (ANCA), or a combination thereof.
119. An article of manufacture comprising:
- 15 a. a container comprising a CD20 antibody; and
- b. a package insert with instructions for treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) 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 followed by a second antibody exposure, wherein the
- 20 second exposure is not provided until from about 16 to 54 weeks from the initial exposure.
120. The article of claim 119 wherein each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody.
121. The article of claim 119 or 120 wherein each of the initial and second antibody exposures is provided in an amount of 0.5 to 4 grams.
- 25 122. The article of any one of claims 119-121 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.
123. The article of claim 122 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a
- 30 hormone.
124. The article of claim 123 wherein the second medicament is a steroid or an immunosuppressive agent or both.

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METHOD FOR TREATING VASCULITIS

ABSTRACT OF THE DISCLOSURE

5 A method of treating anti-neutrophil cytoplasmic antibodies-associated vasculitis (ANCA-associated vasculitis) in a patient eligible for treatment is provided involving administering an antagonist that binds to a B-cell surface marker, such as CD20 antibody, to the patient in a dose of about 400 mg to 1.3 grams at a frequency of one to three doses within a period of about one month. Another method of treating ANCA-associated vasculitis in a subject eligible for treatment is provided involving administering an effective amount of an antibody that binds to a B-cell surface marker to the subject to provide an initial exposure and a subsequent exposure to the antibody within certain dosing regimens. Further provided are articles of
10 manufacture useful for such methods.

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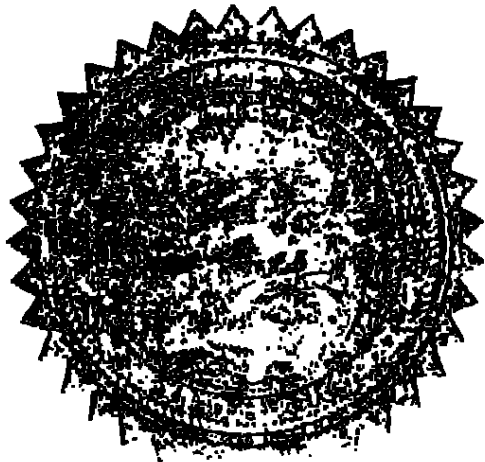
(Convention de La Haye du 5 octobre 1961)

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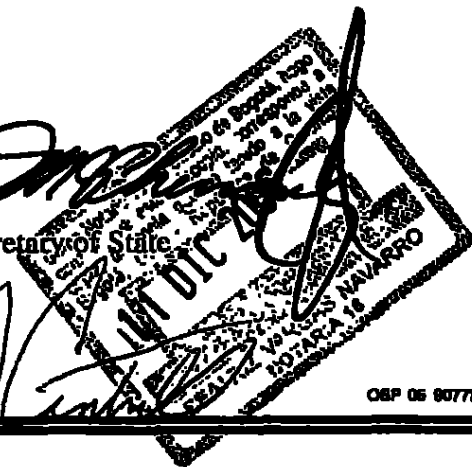
CERTIFIED

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



[Signature]
Secretary of State



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CALIF RNIA ALL-PURP SE ACKN WLEDGMENT

State of California

County of

San Mateo

} ss.

On December 6, 2005 before me, Aida Miclat Guian

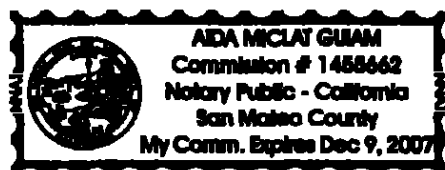
personally appeared Timothy R. Schwartz

Name and Title of Officer (e.g., "Jane Doe, Notary Public")

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 Guian
Signature of Notary Public

OPTIONAL

Though the information below is not required by law, it may prove valuable to persons relying on this document and could prevent fraudulent removal and reattachment of this form to another document.

Description of Attached Document

Title or Type of Document: _____

Document Date: _____

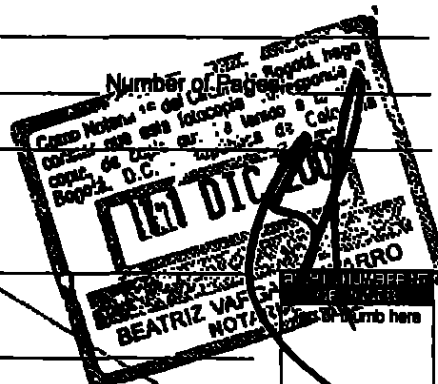
Signer(s) Other Than Named Above: _____

Capacity(ies) Claimed by Signer

Signer's Name: _____

- ☐ Individual
☐ Corporate Officer — Title(s): _____
☐ Partner — ☐ Limited ☐ General
☐ Attorney-in-Fact
☐ Trustee
☐ Guardian or Conservator
☐ Other: _____

Signer is Representing: _____



Brigard & Castro
BOGOTA - COLOMBIA

PODER

Yo (nosotros)

domiciliado(s) en

Nombro(amos) a: JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO y/o CARLOS H. PALACIO EASTMAN y/o HECTOR GOMEZ MEJIA, de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñas, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(s) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeran conducentes al resguardo de mis (nuestros intereses), con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer, y la de sustituirlo en todo o en parte y revocar sustituciones. Asimismo nombro(amos) a: JUAN PABLO CADENA SARMIENTO y/o BEATRIZ JARAMILLO y/o CARLOS H. PALACIO EASTMAN y/o HECTOR GOMEZ MEJIA, domiciliado(s) en la Carrera 7 No. 18-58 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

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. 18-58, Piso 9, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

South San Francisco, California
USA

Granted and signed in

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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TRADUCCION AL ESPAÑOL del Documento de Poder otorgado por Genentech, Inc. domiciliada en 1 DNA Way, South San Francisco, California 94080-4990, Estados Unidos de América.

**ESTADO DE CALIFORNIA
SECRETARIA DE ESTADO**

APOSTILLA

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

1. País: Estados Unidos de América
Esta documento público
2. ha sido firmado por AIDA MICLAT GUIAM
3. actuando en capacidad de Notario Público, Estado de California
4. lleva el sello/timbre de AIDA MICLAT GUIAM, Notario Público
Estado de California

CERTIFICADO

5. en Sacramento, California
6. el 15 de diciembre de 2005
7. por el Secretario de Estado Delegado, Estado de California
8. No. 267083
9. Sello/Timbre

10. Firma

(Firma)

Secretario de Estado



RECONOCIMIENTO TODO-PROPOSITO DE CALIFORNIA

Estado de California)
) ss.
Condado de San Mateo)

El 6 de diciembre de 2005, ante mi, AIDA MICLAT GUIAM, apareció personalmente
Thimoty R. Schwartz

☐ a quien conozco personalmente

☒ quien demostró ante mi en base a
evidencia satisfactoria ser la persona cuyo
nombre esta suscrito en el instrumento y
quien reconoció ante mi que ejecutó el
mismo en su capacidad autorizada, y que
por su firma en el instrumento la persona o
la entidad a nombre de la cual la persona
actuó, ejecutó el instrumento.

Hay un sello que dice:
AIDA MICLAT GUIAM
COMISIÓN # 14556612
Notario Público-California
Condado de San Mateo
Mi comisión expira Dic. 9 de 2007

Atestigua mi firma y sello oficial,

AIDA MICLAT GUIAM

OPCIONAL

Aunque la información en esta sección no es requerida por la ley, puede probar ser valiosa para las personas que se basan en el documento y puede
prevenir la remoción y readhesión fraudulenta de esta forma a otro documento

Descripción adicional de cualquier documento anexo

Título o tipo de documento: _____

Fecha del Documento: _____ Número de páginas: _____

Firmante(s) diferente(s) a (los) nombrado(s) arriba: _____

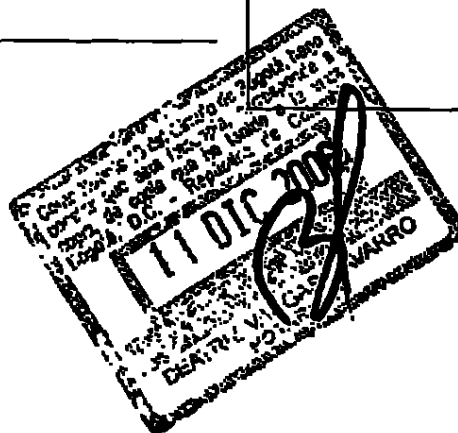
Capacidad(es) Revindicada(s) por el (los) firmante(s)

Nombre del firmante: _____

- ☐ Individuo
☐ Funcionario Corporativo – Título(s): _____
☐ Socio - ☐ Limitado ☐ General
☐ Abogado de hecho
☐ Fideicomisario
☐ Guardián o conservador
☐ Otro: _____

El firmante representa: _____

Huella Índice dedo del firmante
Parte alta del dedo aquí



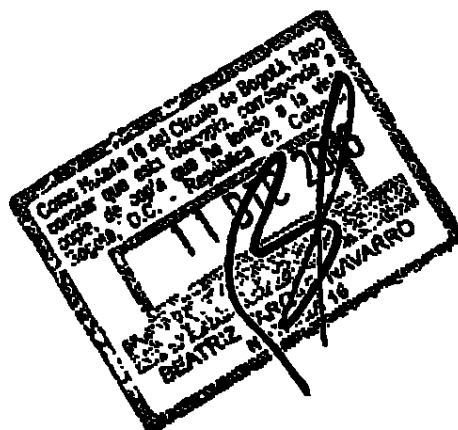
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27 de Agosto de 2002

(Firma)

Vicepresidente, Propiedad Intelectual
Genentech, Inc.



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Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America

This public document:

2. has been signed by Harriet Smith Windsor

3. acting in the capacity of Secretary of State of Delaware

4. bears the seal/stamp of Office of Secretary of State

Certified

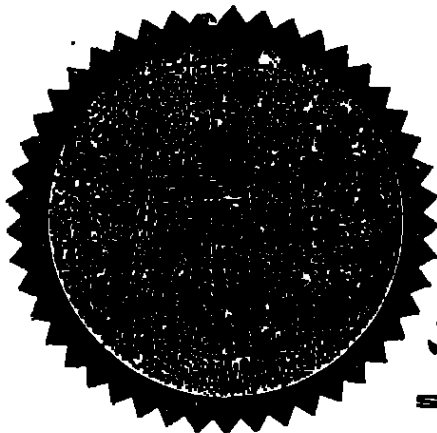
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254030

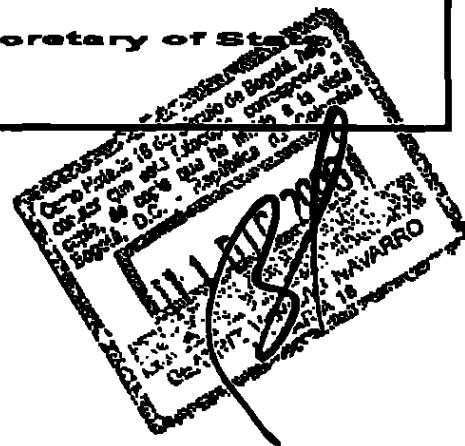
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



Delaware

PAGE 1

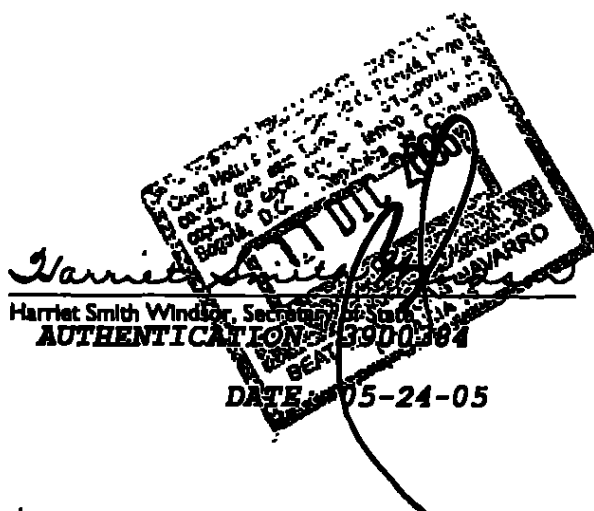
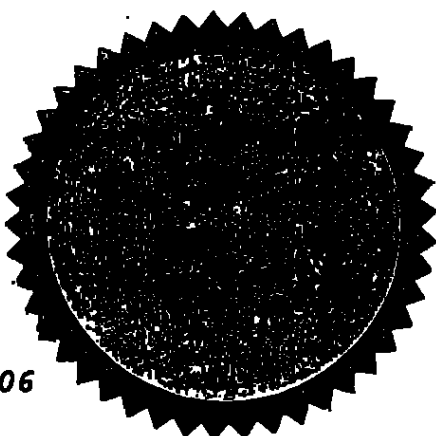
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The First State

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.

2112549

050426506



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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

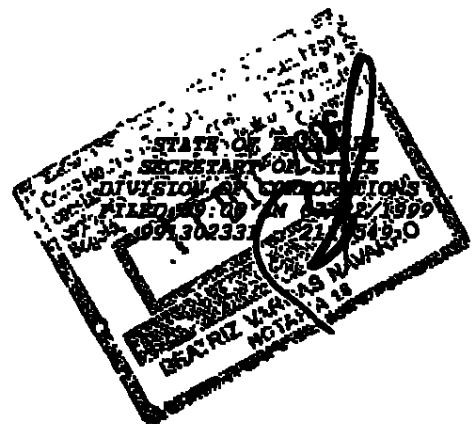
The name of the Corporation is: Genentech, Inc.

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").



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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.

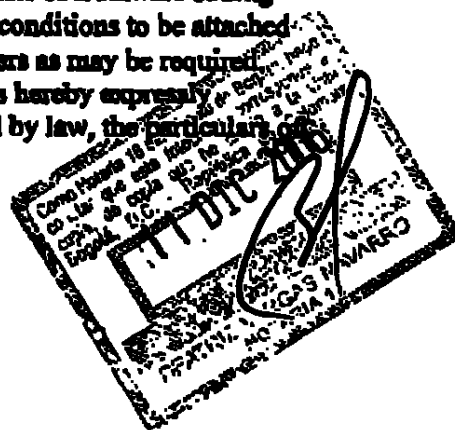
(b) The number of authorized shares of any class or classes of stock may be increased or decreased (but not below the number of shares thereof then outstanding) by the affirmative vote of a majority in voting power of the outstanding stock of the Corporation entitled to vote thereon irrespective of the 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.

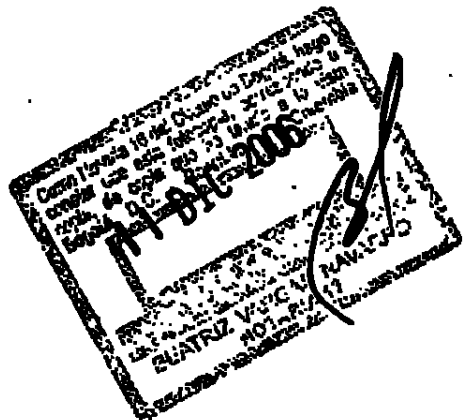
(a) *Series and Limits of Variations between Series.* Any unissued or treasury shares of the Preferred Stock may be issued from time to time in one or more series for such consideration as may be fixed from time to time by the Board of Directors. Before any shares of Preferred Stock of any particular 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



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the shares of such series (so far as not inconsistent with the provisions of this Article 4 applicable to all series of Preferred Stock), including, but not limited to, the following:

- (i) the distinctive designation of such series and the number of shares which shall constitute such series, which number may be increased (except where otherwise provided by the Board of Directors in creating such series) or decreased (but not below the number of shares thereof then outstanding) from time to time by like action of the Board of Directors;
- (ii) the annual rate of dividends payable on shares of such series, the conditions upon which such dividends shall be payable and the date from which dividends shall be cumulative in the event the Board of Directors determines that dividends shall be cumulative;
- (iii) whether such series shall have voting rights, in addition to the voting rights provided by law and, if so, the terms of such voting rights;
- (iv) whether such series shall have conversion privileges and, if so, the terms and conditions of such conversion, including, but not limited to, provision for adjustment of the conversion rate upon such events and in such manner as the Board of Directors shall determine;
- (v) whether or not the shares of such series shall be redeemable and, if so, the terms and conditions of such redemption, including the date or dates upon or after which they shall be redeemable, and the amount per share payable in case of redemption, which amount may vary under different conditions and at different redemption dates;
- (vi) whether such series shall have a sinking fund for the redemption or purchase of shares of that series and, if so, the terms and amount of such sinking fund;
- (vii) the rights of the shares of such series in the event of voluntary or involuntary liquidation, dissolution or winding up of the Corporation, and the relative rights of priority, if any, of payment of shares of that series; and
- (viii) any other relative rights, preferences and limitations of such series.



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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.

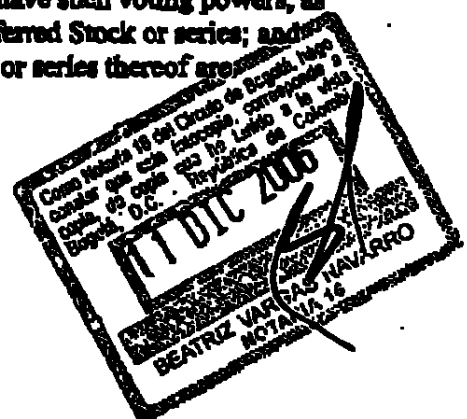
(b) Sections 2.02, 3.02, 3.03(a), 3.03(b), 3.04, 3.05, 3.14, and 5.06 of the bylaws may only be altered, amended, repealed, or rescinded by the affirmative vote of holders of capital stock entitled to vote thereon representing more than 60% of the shares entitled to be voted thereon.

(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



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divested of such rights to elect a specified number of directors, voting as a class, pursuant to the provisions of such Preferred Stock or series, the terms of office of all directors elected by the holders of such Preferred Stock or series, voting as a class pursuant to such provisions or elected to fill any vacancies resulting from the death, resignation or removal of directors so elected by the holders of such Preferred Stock or series, shall forthwith terminate and the authorized number of directors shall be reduced accordingly.

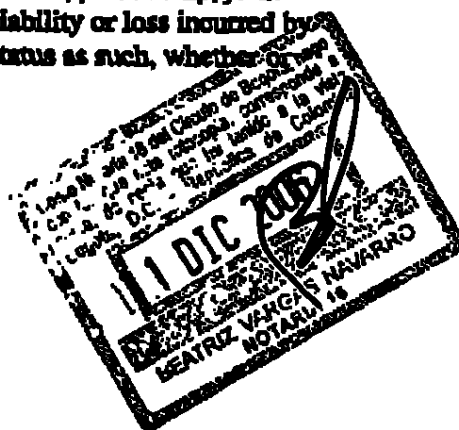
SECTION 6.02. Ballots. Elections of directors at an annual or special meeting of stockholders need not be by written ballot unless the bylaws of the Corporation shall provide otherwise.

SECTION 6.03. Elimination of Certain Personal Liability of Directors. (a) A director of the Corporation shall not be liable to the Corporation or its stockholders for monetary damages for breach of fiduciary duty as a director to the fullest extent permitted by Delaware Law.

(b) Each person (and the heirs, executors or administrators of such person) who was or is a party or is threatened to be made a party to, or is involved in any threatened, pending or completed action, suit or proceeding, whether civil, criminal, administrative or investigative, by reason of the fact that such person is or was a director of the Corporation or is or was serving at the request of the Corporation as a director of another corporation, partnership, joint venture, trust or other enterprise, shall be indemnified and held harmless by the Corporation to the fullest extent permitted by Delaware Law. The right to indemnification conferred in this Section 6.03 shall also include the right to be paid by the Corporation the expenses incurred in connection with any such proceeding in advance of its final disposition to the fullest extent authorized by Delaware Law. The right to indemnification conferred in this Section 6.03 shall be a contract right.

(c) The Corporation may, by action of its Board of Directors, provide indemnification to such of the officers, employees and agents of the Corporation to such extent and to such effect as the Board of Directors shall determine to be appropriate and authorized by Delaware Law.

(d) The Corporation shall have power to purchase and maintain insurance on behalf of any person who is or was a director, officer, employee or agent of the Corporation, or is or was serving at the request of the Corporation as a director, officer, employee or agent of another corporation, partnership, joint venture, trust or other enterprise against any expense, liability or loss incurred by such person in any such capacity or arising out of his status as such, whether or not



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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.

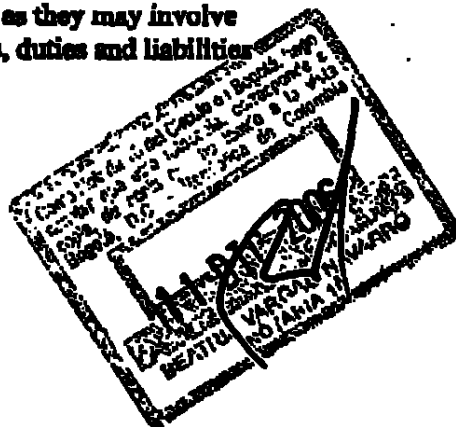
(f) Neither the alteration, amendment, repeal or rescission of this Section 6.03, nor the adoption of any provision of this Certificate of Incorporation or the bylaws of the Corporation, nor, to the fullest extent permitted by Delaware Law, any modification of law, shall eliminate or reduce the effect of this Section 6.03 in respect of any acts or omissions occurring prior to such alteration, amendment, repeal, rescission, adoption or modification.

ARTICLE 7

SECTION 7.01. *Section 203 of Delaware Law.* The Corporation hereby elects not to be governed by Section 203 of Delaware Law.

ARTICLE 8

SECTION 8.01. *Relationship With Roche.* In anticipation that the Corporation will cease to be a wholly owned subsidiary of Roche Holdings, Inc., but that Roche (as defined in Section 8.05) will remain a stockholder of the Corporation, and in anticipation that the Corporation (as defined in Section 8.05) and Roche may engage in the same or similar activities or lines of business and have an interest in the same areas of corporate opportunities, and in recognition of (i) the benefits to be derived by the Corporation through its continued contractual, corporate and business relations with Roche (including service of officers and directors of Roche as directors of the Corporation) and (ii) the difficulties attendant to any director, who desires and endeavors fully to satisfy such director's fiduciary duties, in determining the full scope of such duties in any particular situation, the provisions of this Article 8 are set forth to regulate, define and guide the conduct of certain affairs of the Corporation as they may involve Roche and its officers and directors, and the powers, rights, duties and liabilities



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of the Corporation and its officers, directors and stockholders in connection therewith.

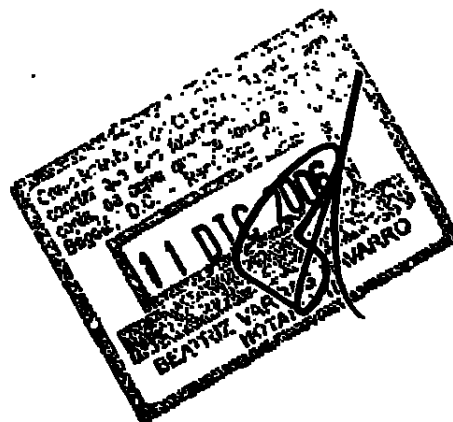
SECTION 8.02. *Similar Business Activities.* Except as Roche Holdings, Inc. may otherwise agree in writing.

(a) Roche shall not have a duty to refrain from engaging directly or indirectly in the same or similar business activities or lines of business as the Corporation, and

(b) neither Roche nor any officer or director thereof shall be liable to the Corporation or its stockholders for breach of any fiduciary duty by reason of any such activities of Roche or of such person's participation therein.

In the event that Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for both Roche and the Corporation, Roche shall have no duty to communicate or offer such corporate opportunity to the Corporation and shall not be liable to the Corporation or its stockholders for breach of any fiduciary duty as a stockholder of the Corporation or controlling person of a stockholder by reason of the fact that Roche pursues or acquires such corporate opportunity for itself, directs such corporate opportunity to another person or entity, or does not communicate information regarding, or offer, such corporate opportunity to the Corporation.

SECTION 8.03. *Corporate Opportunities.* In the event that a director, officer or employee of the Corporation who is also a director, officer or employee of Roche acquires knowledge of a potential transaction or matter that may be a corporate opportunity for the Corporation and Roche (whether such potential transaction or matter is proposed by a third-party or is conceived of by such director, officer or employee of the Corporation), such director, officer or employee shall be entitled to offer such corporate opportunity to the Corporation or Roche as such director, officer or employee deems appropriate under the circumstances in his sole discretion, and no such director, officer or employee shall be liable to the Corporation or its stockholders for breach of any fiduciary duty or duty of loyalty or failure to act in (or not opposed to) the best interests of the Corporation or the derivation of any improper personal benefit by reason of the fact that (i) such director, officer or employee offered such corporate opportunity to Roche (rather than the Corporation) or did not communicate information regarding such corporate opportunity to the Corporation or (ii) Roche pursues or acquires such corporate opportunity for itself or directs such corporate opportunity to another person or does not communicate information regarding such corporate opportunity to the Corporation.



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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.

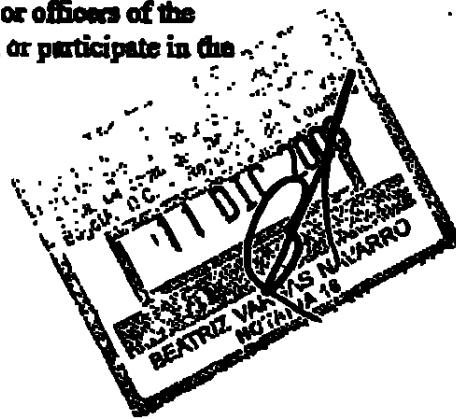
SECTION 8.05. *Definitions.* For purposes of this Article 8 and Article 9 only, (i) the term "Corporation" shall mean the Corporation and all corporations, partnerships, joint ventures, associations and other entities in which the Corporation beneficially owns (directly or indirectly) fifty percent or 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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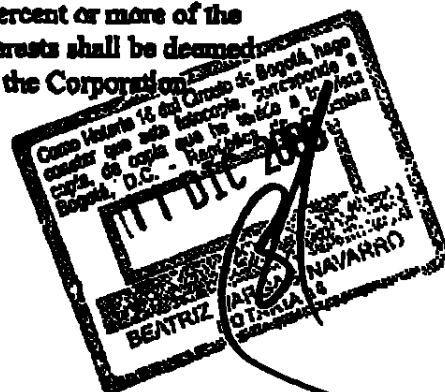
meeting of the Board of Directors or committee thereof which authorizes the contract, agreement, arrangement, transaction, amendment, modification or termination or solely because his or their votes are counted for such purpose, but any such contract, agreement, arrangement or transaction (or any amendment, 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 or director thereof in good faith takes any action or exercises any rights or gives or withholds any consent in connection with any agreement or contract between Roche and the Corporation. No vote cast or other action taken by any person who is an officer, director or other representative of Roche, which vote is cast or action is taken by such person in his capacity as a director of this Corporation, shall constitute an action of or the exercise of a right by or a consent of Roche for the purpose of any such agreement or contract.

SECTION 9.04. Deemed Consent by Stockholders. Any person or entity purchasing or otherwise acquiring any interest in any shares of capital stock of the Corporation shall be deemed to have notice of and to have consented to the provisions of this Article 9.

SECTION 9.05. Contracts Covered. For purposes of this Article 9, any contract, agreement, arrangement or transaction with the Corporation or any corporation, partnership, joint venture, association or other entity in which the Corporation beneficially owns (directly or indirectly) fifty percent or more of the outstanding voting stock, voting power or similar voting interests shall be deemed to be a contract, agreement, arrangement or transaction with the Corporation.



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SECTION 9.06. *Binding Effect.* Neither the alteration, amendment, change or repeal of any provision of this Article 9 nor the adoption of any provision inconsistent with any provision of this Article 9 shall eliminate or reduce the effect of this Article 9 in respect of any matter occurring, or any cause of action, suit or claim that, but for this Article 9, would accrue or arise, prior to such alteration, amendment, change, repeal or adoption.

SECTION 9.07. *Article 8.* The provisions of this Article 9 are in addition to the provisions of Article 8.



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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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preferenciales, por un valor de \$0.02 por acción ("Acción Preferencial"). Trecientos millones (300,000,000) de acciones serán designadas acciones comunes, por un valor de \$0.02 por acción ("Acción Común"). La acción común de la Corporación será toda de una misma clase.

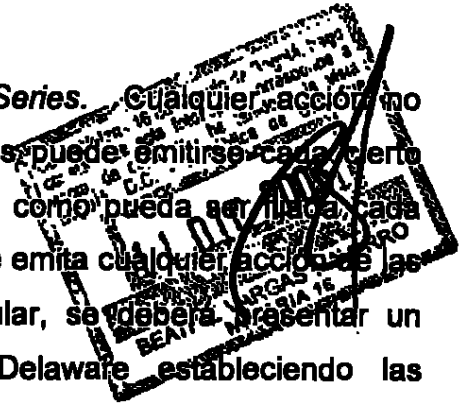
(b) El número de acciones autorizadas de cualquier clase o clases de acciones puede incrementarse o disminuirse (pero no por debajo del número del número de acciones de las mismas que en su momento están vigentes) por el voto afirmativo de una mayoría con poder de votación de las acciones vigentes de la Corporación con derecho a votar por la misma independiente de las consideraciones de la Sección 242(b)(2) de la Ley de Delaware.

SECCION 4.02. Acción Común. (a) Emisión y Consideración. Cualquier acción sin emitir o del tesoro del las Acciones Comunes puede emitirse para tal consideración como pueda fijarse de acuerdo con la ley aplicable cada cierto tiempo por la Junta Directiva.

(b) *Dividendos.* Sujeto a los derechos de los accionistas de las Acciones Preferenciales, los accionistas de Acciones Comunes deberán tener el derecho a recibir, cuando y como se declare por la Junta Directiva, de los activos de la Corporación los cuales están disponibles para ello por ley, dividendos pagables ya sea en dinero, propiedad, o acciones y los accionistas de la Acción Preferencial no tendrán derecho a participar en tales dividendos (a menos que la Junta Directiva determine lo contrario en cualquier resolución que condiciona la emisión de una serie de Acciones Preferenciales).

SECCIÓN 4.03. Acción Preferencial

(a) *Series y Limites de Variaciones entre Series.* Cualquier acción no emitida o del tesoro de las Acciones Preferenciales puede emitirse cada cierto tiempo en una o más series para tal consideración como pueda ser fijada cada cierto tiempo por la Junta Directiva. Antes de que se emita cualquier acción de las Acciones Preferenciales de cualquier serie particular, se deberá presentar un certificado ante el Secretario de Estado de Delaware estableciendo las designaciones, derechos, privilegios, restricciones, y condiciones que irán anexas a las Acciones Preferenciales de tal serie y tales otras cuestiones como puedan requerirse, y la Junta Directiva deberá fijar y determinar, y por la presente está expresamente autorizada para fijar y determinar, en la forma provista por la ley, los



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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

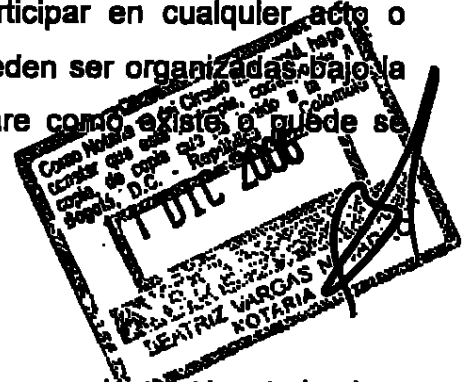
La dirección de la oficina registrada de la Corporación en el Estado de Delaware es Corporation Service Company, 1013 Centre Road, Ciudad de Wilmington, Condado de New castle, Delaware 19805. El nombre de su agente registrado en tal dirección es Corporation Service Company.

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 y 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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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;

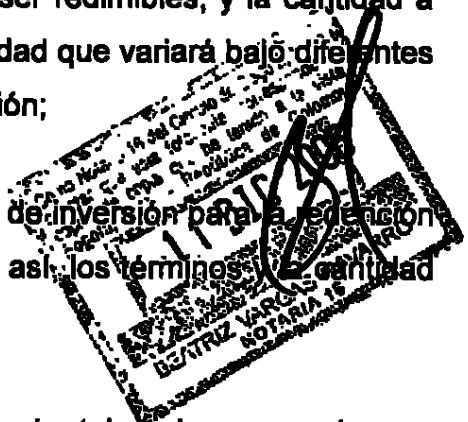
(iii) si la serie tendrá o no derechos de voto, además de los derechos de voto provistos por la ley, y de ser así, los términos de tales derechos de voto;

(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 serie en caso de una liquidación, disolución o cierre voluntaria o involuntaria de la Corporación, y



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los derechos relativos de prioridad, de haber, de pago de acciones de esa serie; y

(viii) cualquier otro derecho relativo, preferencia y limitación de tal serie.

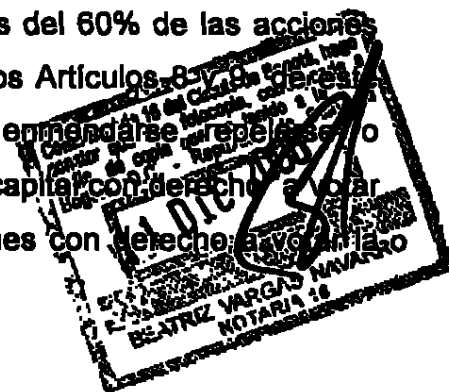
ARTICULO 5

SECCION 5.01. Enmiendas a los estatutos por los Directores. (a) En adición y no limitando los poderes conferidos por estatutos, la Junta Directiva esta expresamente autorizada para hacer, alterar, enmendar, rechazar y rescindir los estatutos de la Corporación, sujetos a las secciones 5.01(b) y 5.01(c) a continuación.

(b) Las secciones 2.02, 3.02, 3.03(a), 3.04, 3.05, 3.14 y 5.06 de los estatutos solo pueden alterarse, enmendarse, repelerse, o rescindirse por el voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 60% de las acciones con derecho a votar las mismas.

(c) Las secciones 3.03(c) y 3.15 de los estatutos solo pueden ser alteradas, enmendadas, repelidas, o rescindidas por le voto afirmativo de los accionistas de capital con derecho a votar las mismas que representen más del 90% de las acciones con derecho a votar las mismas.

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 del 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

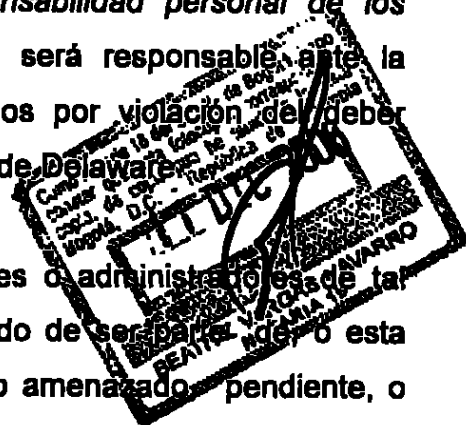
SECCION 6.01. Elección del Directorio por los Accionistas de Acción Preferencial. Durante cualquier periodo cuando los accionistas de cualquier

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 de deber fiduciario como director hasta donde lo permita la Ley de Delaware.

(b) Cada persona (y sus herederos, ejecutores o administradores de tal persona) quien fue o es una parte o esta amenazado de ser parte 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



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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 con 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. En este presente la Corporación elige no ser gobernada por la Sección 203 de la Ley de Delaware.



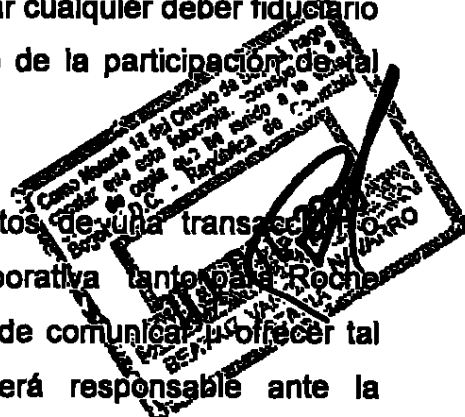
SECCION 8.01. *Relación con Roche.* Anticipándose a que la Corporación cese de ser un subsidiaria completamente de propiedad de Roche Holdings, Inc., pero que Roche (como se define en la sección 8.05) permanezca siendo un accionista de la Corporación, y anticipándose a que la Corporación (como se define 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 artículo 8 se establecen para regular, definir y guiar la conducta de ciertas cuestiones de la Corporación ya que pueden involucrar a Roche y sus funcionarios 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 de 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 hecho

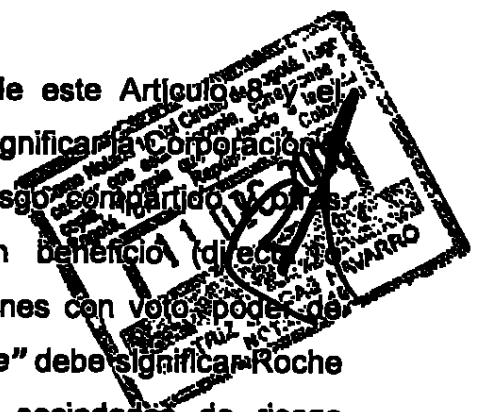


que Roche prosigue o adquiere tal oportunidad corporativa para si misma, dirige tal oportunidad corporativa a otra persona o entidad, o no comunica información respecto, u ofrece, tal oportunidad corporativa a la Corporación.

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, todas las corporaciones, sociedades, sociedades de riesgo compartido y otras entidades en las cuales la Corporación posee con beneficio (directo 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



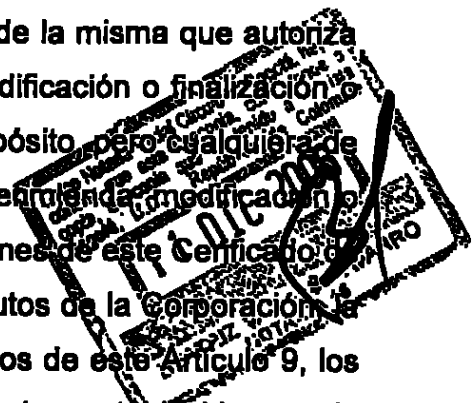
define en la Regla 12b-2 bajo el Acta de Intercambio de Valores de 1934, como se enmendó) de Roche Holdings, Inc.

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 Certificado de Incorporación Enmendado y vuelto a Redactar, los Estatutos de la Corporación, la Ley de Delaware y otras leyes aplicables. Para propósitos de este Artículo 9, los términos "*Corporación*" y "*Roche*" tienen los significados establecidos en la Sección 8.05.

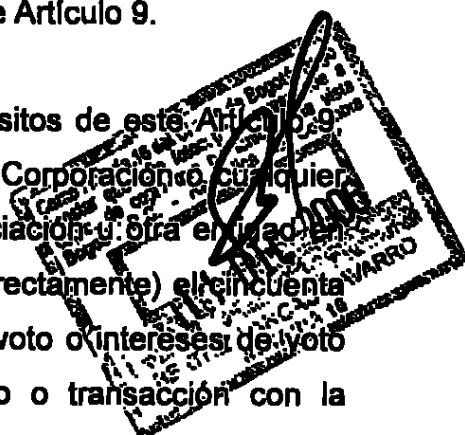


SECCION 9.02. Quórum. Los Directores de la Corporación que son también directores o funcionarios de Roche pueden contarse al determinar la presencia de un quórum en una reunión de la Junta Directiva o de un comité que autoriza o aprueba cualquiera de tal contrato, acuerdo, arreglo o transacción (o cualquier 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.

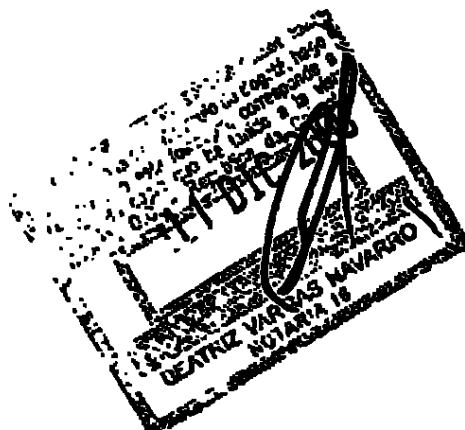
GENENTECH, INC.

Por: /s/ Stephen G. Juelsgaard

Nombre: Stephen G. Juelsgaard

Título: Vicepresidente

Consejo General y Secretaría



436
440

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

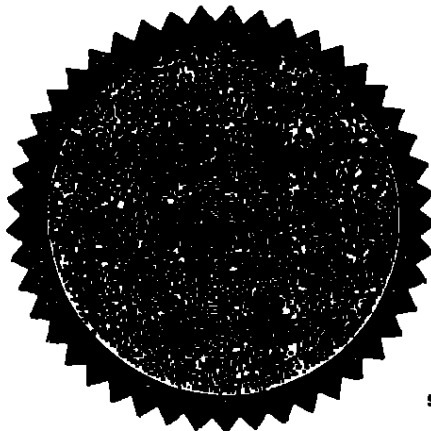
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

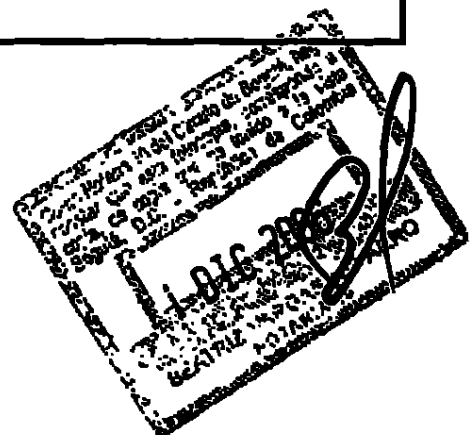
8. No. 0254031

9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor
Secretary of State



Delaware

PAGE 1

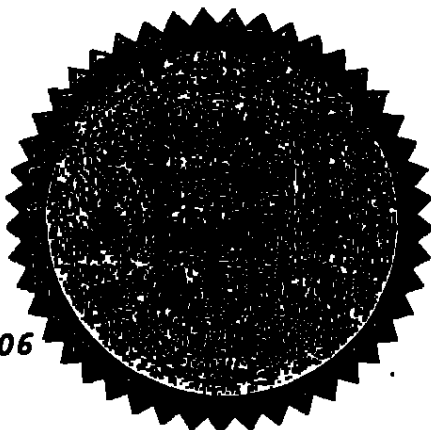
437
441

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE FIFTEENTH DAY OF MAY, A.D. 2000, AT 5 O'CLOCK P.M.

2112549

050426506



Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION

DATE

05-15-2000



498
442

CERTIFICATE OF AMENDMENT
OF AMENDED AND RESTATED
CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

"Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is seven hundred million (700,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value \$0.02 per share ("Preferred Stock"). Six hundred million (600,000,000) shares shall be designated common stock, par value \$0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

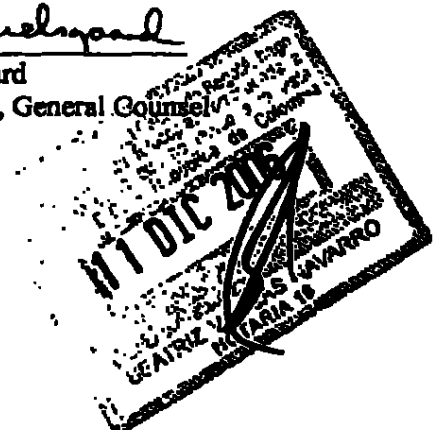
FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 15th day of May, 2000.

GENENTECH, INC.

By: Stephen G. Juelsgaard
Name: Stephen G. Juelsgaard
Title: Senior Vice President, General Counsel
and Secretary

75841



APOSTILLA

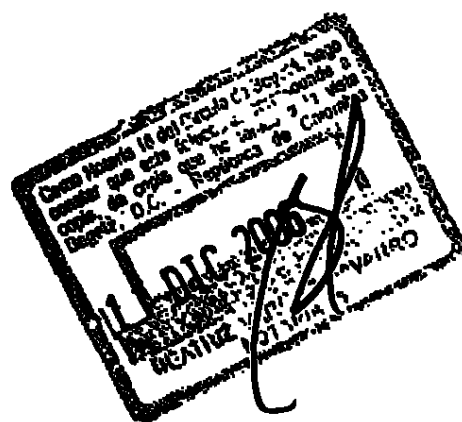
(Convención de La Haya del 5 de Octubre de 1961)

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



440
444

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
AUTENTICACION: 3900385

FECHA: 05 - 24 - 05

441
115

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

gmr
d46

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.

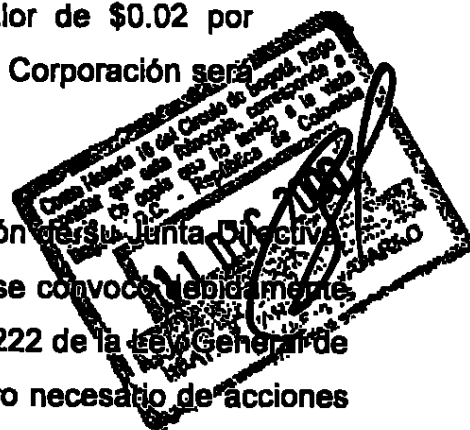
CERTIFICA POR LA PRESENTE:

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 enmienda 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 la 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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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

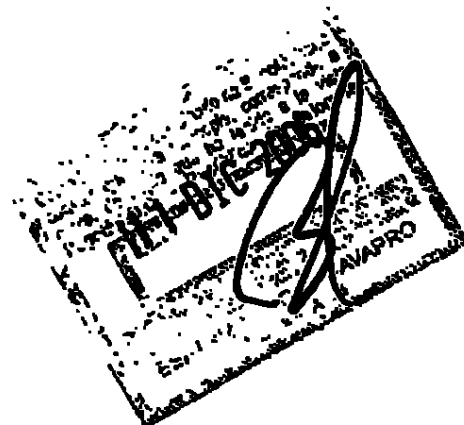
EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 15 de Mayo de 2000.

GENENTECH, INC.

Por: (Firma)

Nombre: Stephen G. Juelsgaard

Título: Vicepresidente, Consejo
y Secretaría General.



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

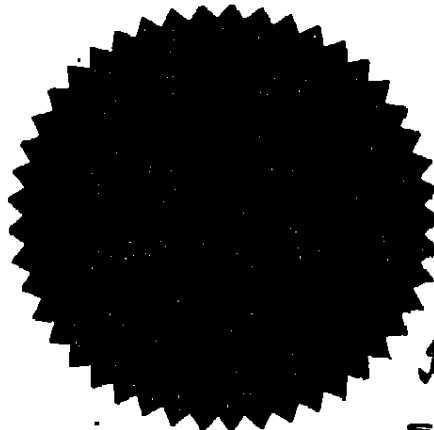
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

8. No. 0254033

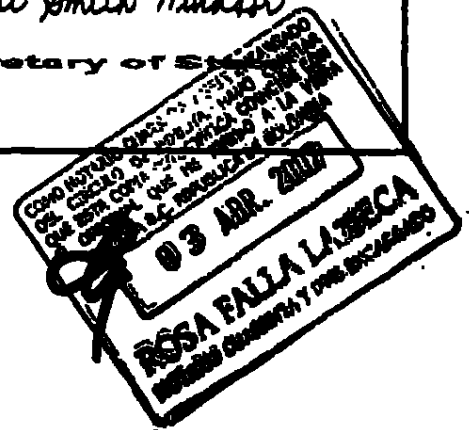
9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor

Secretary of State



Delaware

PAGE 1

The First State

I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE ATTACHED IS A TRUE AND CORRECT COPY OF THE CERTIFICATE OF AMENDMENT OF "GENENTECH, INC.", FILED IN THIS OFFICE ON THE SIXTEENTH DAY OF APRIL, A.D. 2004, AT 7:39 O'CLOCK P.M.

2112549

050426506

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State
AUTHENTICATION: 3900387

DATE: 05-24-04



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.

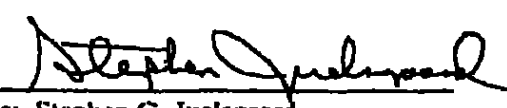
SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares 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.

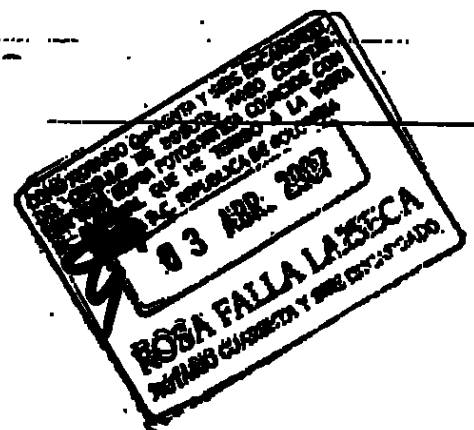
GENENTECH, INC.

By: 

Name: Stephen G. Juelsgaard

Title: Executive Vice President, General Counsel
and Secretary

#153087



547
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TRADUCCION AL ESPAÑOL del Certificado de Enmienda de Genentech, Inc.

APOSTILLA

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

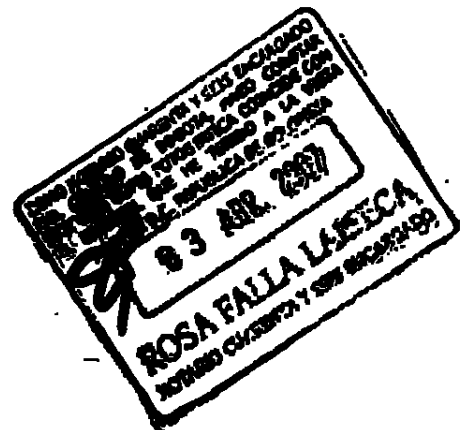
1. País: Estados Unidos de América
Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

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

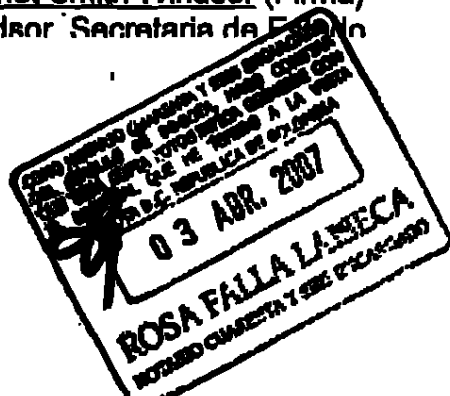


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DELAWARE
EL PRIMER ESTADO

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 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



453

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.

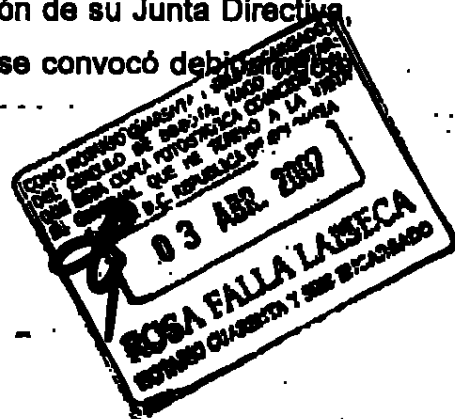
CERTIFICA POR LA PRESENTE:

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 enmienda cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

SECCION 4.01. *Acciones de Capital.* (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de tres mil cien millones (3,100,000,000) de acciones. Cien millones (100,000,000) de acciones serán designadas acciones preferenciales, por un valor de \$0.02 por acción ("Acción Preferencial"). Tres mil millones (3,000,000,000) de acciones serán designadas acciones comunes, por un valor de \$0.02 por acción ("Acción Común"). La acción común de la Corporación será toda de una misma clase.

SEGUNDO: Que por lo tanto, de acuerdo con la resolución de su Junta Directiva, la reunión ordinaria de accionistas de dicha corporación se convocó debidamente.



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TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 18 de Abril de 2004.

GENENTECH, INC.

Por: (Firma)

Nombre: Stephen G. Juelsgaard

Título: Vicepresidente, Consejo
y Secretaría General.



77 200
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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

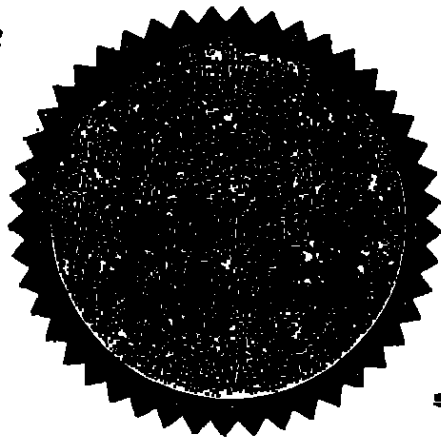
5. at Dover, Delaware

6. the twenty-fourth day of May, A.D. 2005

7. by Secretary of State, Delaware Department of State

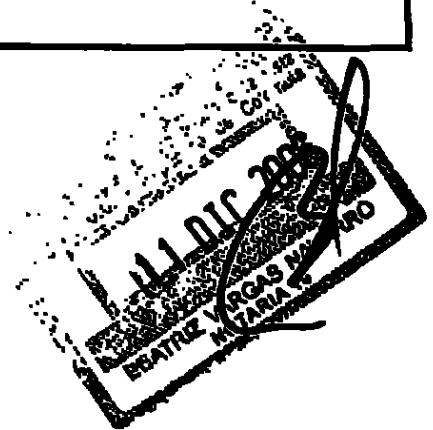
8. No. 0254032

9. Seal/Stamp:



10. Signature:

Harriet Smith Windsor
Secretary of State



Delaware

PAGE 1

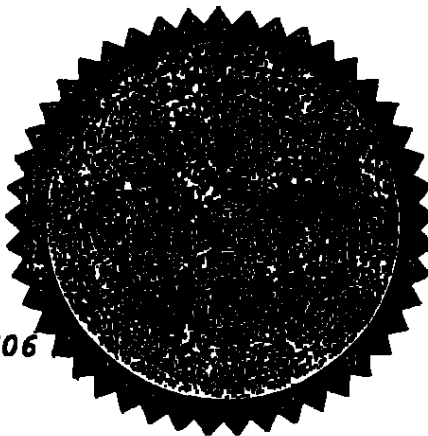
482
486

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.

2112549

050426506

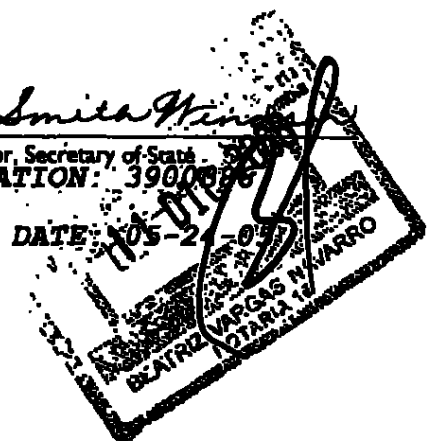


Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 3900886

DATE: 05-21-01



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457

CERTIFICATE OF AMENDMENT
OF AMENDED AND RESTATED
CERTIFICATE OF INCORPORATION

Genentech, Inc. a corporation organized and existing under and by virtue of the General Corporation Law of the State of Delaware.

DOES HEREBY CERTIFY:

FIRST: That at a meeting of the Board of Directors of Genentech, Inc. resolutions were duly adopted setting forth a proposed amendment of the Amended and Restated Certificate of Incorporation of said corporation, declaring said amendment to be advisable and calling a meeting of the stockholders of said corporation for consideration thereof. The resolution setting forth the proposed amendment is as follows:

RESOLVED, that the Amended and Restated Certificate of Incorporation of this corporation be amended by changing "Section 4.01(a)" of the Article thereof numbered "4" so that, as amended, said Section of said Article shall be and read as follows:

"Section 4.01. *Capital Stock.* (a) The Corporation is authorized to issue two classes of stock to be designated, respectively, preferred stock and common stock. The total number of shares which the Corporation is authorized to issue is one billion three hundred million (1,300,000,000) shares. One hundred million (100,000,000) shares shall be designated preferred stock, par value \$0.02 per share ("Preferred Stock"). One billion two hundred million (1,200,000,000) shares shall be designated common stock, par value \$0.02 per share ("Common Stock"). The Common Stock of the Corporation shall be all of one class."

SECOND: That thereafter, pursuant to resolution of its Board of Directors, the regular meeting of the stockholders of said corporation was duly called and held upon notice in accordance with Section 222 of the General Corporation Law of the State of Delaware at which meeting the necessary number of shares as required by statute were voted in favor of the amendment.

THIRD: That said amendment was duly adopted in accordance with the provisions of Section 242 of the General Corporation Law of the State of Delaware.

FOURTH: That the capital of said corporation shall not be reduced under or by reason of said amendment.

IN WITNESS WHEREOF, said Genentech, Inc. has caused this Certificate of Amendment to be signed by Stephen G. Juelsgaard, an Authorized Officer, this 10th day of May, 2001.

GENENTECH, INC.

By:

Name: Stephen G. Juelsgaard

Title: Senior Vice President, General Counsel
and Secretary

26797



APOSTILLA

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

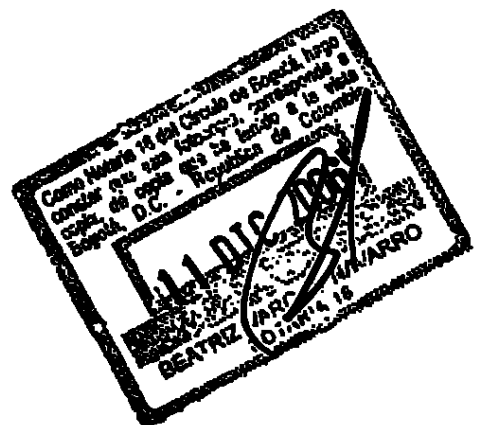
1. País: Estados Unidos de América
Este documento público
2. ha sido firmado por Harriet Smith Windsor
3. actuando en capacidad de Secretaria de Estado de Delaware
4. lleva el sello/timbre de la Oficina del Secretario de Estado

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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DELAWARE
EL PRIMER ESTADO

YO, HARRIET SMITH WINDSOR, SECRETARIA DE ESTADO DEL ESTADO DE DELAWARE, CERTIFICO POR LA PRESENTE QUE EL ANEXO ES UNA COPIA AUTÉNTICA Y CORRECTA DEL CERTIFICADO DE ENMIENDA DE "GENENTECH, INC", PRESENTADO EN ESTA OFICINA EL DECIMO DIA DE MAYO, A. D. 2001, A LAS 2:05 EN PUNTO DE LA TARDE.



Harriet Smith Windsor (Firma)
Harriet Smith Windsor, Secretaria de Estado
AUTENTICACIÓN: 3900386

FECHA: 05 - 24 - 05

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**CERTIFICADO DE TERCERA ENMIENDA DEL
CERTIFICADO DE INCORPORACIÓN
CORREGIDO Y VUELTO A REDACTAR**

Genentech, Inc. una corporación organizada y existente bajo y por virtud de la Ley General de Corporación del Estado de Delaware.

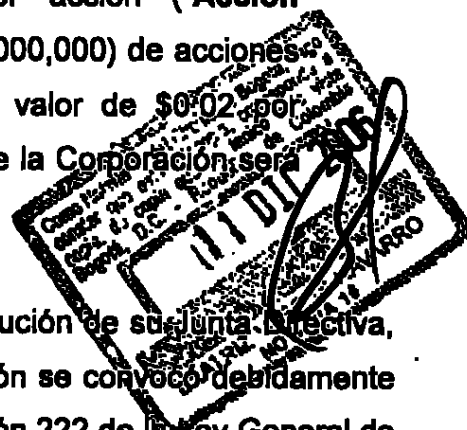
CERTIFICA POR LA PRESENTE:

PRIMERO: Que en una reunión de la Junta Directiva de Genentech, Inc. se adoptaron debidamente resoluciones que establecen una enmienda propuesta del certificado de Incorporación Corregido y vuelto a Redactar de dicha corporación, declarando dicha enmienda como aconsejable y llamando a una reunión de accionistas de dicha corporación para consideración de la misma. La resolución establece la enmienda propuesta de la siguiente manera:

RESUELVE, que el Certificado de Incorporación Corregido y vuelto a Redactar de esta corporación se enmiende cambiando "la Sección 4.01(a)" del Artículo allí numerado "4" tal que, como se enmienda, dicha Sección de dicho Artículo se lea de la siguiente manera:

SECCION 4.01. Acciones de Capital. (a) la Corporación está autorizada a emitir dos clases de acciones que deben designarse, respectivamente, acciones preferenciales y acciones comunes. El número total de acciones que la Corporación esta autorizada a emitir es de 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.



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TERCERO: Que dicha enmienda se adoptó debidamente de acuerdo con lo previsto en la Sección 242 de la Ley General de Corporación del Estado de Delaware.

CUARTO: Que el capital de dicha corporación no será reducido bajo o por razón de dicha enmienda

EN TESTIMONIO DE LO CUAL, dicha Genentech, Inc. ha causado este Certificado de Enmienda para que sea firmado por Stephen G. Juelsgaard, un Funcionario Autorizado, este 10 de Mayo de 2001.

GENENTECH, INC.

Por: (Firma)

Nombre: Stephen G. Juelsgaard

Título: Vicepresidente, Consejo
y Secretaría General.



REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [X] MODELO DE UTILIDAD []

- ◆ Indicación de que se solicita la concesión de una patente [X]
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- ◆ Descripción de la invención [X]
- ◆ Dibujos de ser estos pertinentes [X]
- ◆ Comprobante de pago de las tasas establecidas [X]

COMPLETA [] INCOMPLETA []

DISEÑO INDUSTRIAL []

- ◆ Indicación de que se solicita el registro de Diseño Industrial []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA [] INCOMPLETA []

ESQUEMA DE TRAZADO []

- ◆ Indicación de que se solicita el registro de un Esquema de Trazado []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica del esquema trazado []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA [] INCOMPLETA []

Eduar Naranjo

Firma Responsable

Fecha 04-04-07

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

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REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
CADENA SARMIENTO JUAN PABLO
Apoderado(a) y/o Representante de
GENENTECH, INC.

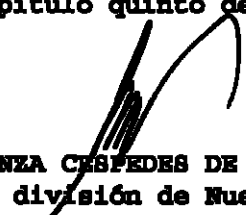
REFERENCIA	Radicación No.	7 34204
	Solicitud internacional	PCT/US2005/034647
	Fecha inicio fase nacional	05/05/2007
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	8890

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. Cuando se trate de documentos otorgados en el extranjero, deben legalizarse de conformidad con lo dispuesto por los artículos 259 y 260 del Código de Procedimiento Civil.

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 27 JUL 2007
adpall