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(54) Title: METHOD FOR TREATING LUPUS

Humanized 2H7.v16 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

(57) Abstract: A method of treating lupus 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 and an article of manufacture therefor.

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METHOD FOR TREATING LUPUS

Field of the Invention

The present invention concerns methods for treating lupus in a subject using special dosing regimens and protocols, and a kit with instructions for such use.

Background of the Invention

Lupus

Autoimmune diseases, such as systemic lupus erythematosus (SLE), myasthenia gravis (MG) and idiopathic thrombocytopenic purpura (ITP), 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 between individual types of autoimmune diseases, one general mechanism involves the binding of certain antibodies (referred to herein as self-reactive antibodies or autoantibodies) present in the sera of patients to self-nuclear or cellular antigens.

Lupus is an autoimmune disease involving antibodies that attack connective tissue. The disease is estimated to affect nearly 1 million Americans, primarily women between the ages of 20-40. The principal form of lupus is a systemic one (systemic lupus erythematosus; SLE). SLE is associated with the production of antinuclear antibodies, circulating immune complexes, and activation of the complement system. SLE has an incidence of about 1 in 700 women between the ages of 20 and 60. SLE can affect any organ system and can cause severe tissue damage. Numerous autoantibodies of differing specificity are present in SLE. SLE patients often produce autoantibodies having anti-DNA, anti-Ro, and anti-platelet specificity and that are capable of initiating clinical features of the disease, such as glomerulonephritis, arthritis, serositis, complete heart block in newborns, and hematologic abnormalities. These autoantibodies are also possibly related to central nervous system disturbances. Arbuckle *et al.* describes the development of autoantibodies before the clinical onset of SLE (Arbuckle *et al. N. Engl. J. Med.* 349(16): 1526-1533 (2003)).

Untreated lupus can be fatal as it progresses from attack of skin and joints to internal organs, including lung, heart, and kidneys (with renal disease being the primary concern). Lupus mainly appears as a series of flare-ups, with intervening periods of little or no disease manifestation.

Kidney damage, measured by the amount of proteinuria in the urine, is one of the most acute areas of damage associated with pathogenicity in SLE, and accounts for at least 50% of the mortality and morbidity of the disease.

The presence of antibodies immunoreactive with double-stranded native DNA is used as a diagnostic marker for SLE.

Currently, there are no really curative treatments for patients who have been diagnosed with SLE. From a practical standpoint, physicians generally employ a number of powerful immunosuppressive drugs such as high-dose corticosteroids, *e.g.*, prednisone, or azathioprine or cyclophosphamide, which are given during periods of flare-ups, but may also be given persistently for those who have experienced frequent flare-ups. Even with effective treatment, which reduces symptoms and prolongs life, many of these drugs have potentially harmful

side effects to the patients being treated. In addition, these immunosuppressive drugs interfere with the person's ability to produce all antibodies, not just the self-reactive anti-DNA antibodies. Immunosuppressants also weaken the body's defense against other potential pathogens, thereby making the patient extremely susceptible to infection and other potentially fatal diseases, such as cancer. In some of these instances, the side effects of current treatment modalities, combined with continued low-level manifestation of the disease, can cause serious impairment and premature death. Recent therapeutic regimens include cyclophosphamide, methotrexate, antimalarials, hormonal treatment (*e.g.*, DHEA), and anti-hormonal therapy (*e.g.*, the anti-prolactin agent bromocriptine).

Methods for treatment of SLE involving antibodies are also described. The method in Diamond *et al.* (US Patent No. 4,690,905) consists of generating monoclonal antibodies against anti-DNA antibodies (the monoclonal antibodies being referred to therein as anti-idiotypic antibodies) and then using these anti-idiotypic antibodies to remove the pathogenic anti-DNA antibodies from the patient's system. However, the removal of large quantities of blood for treatment can be a dangerous, complicated process. US Patent No. 6,726,909 discloses treating SLE wherein the antibody composition administered to the patient comprises purified anti-DNA anti-idiotypic antibodies and the administration requires an injection, or other equivalent mode of administration.

High-dose intravenous immune globulin (IVIG) infusions have also been used in treating certain autoimmune diseases. Up until the present time, treatment of SLE with IVIG has provided mixed results, including both resolution of lupus nephritis (Akashi *et al., J. Rheumatology* 17:375-379 (1990)), and in a few instances, exacerbation of proteinuria and kidney damage (Jordan *et al., Clin. Immunol. Immunopathol.* 53: S164-169 (1989)).

CD20 Antibodies and Treatment 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 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(2):435-445 (1994)).

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

Rituximab has also been studied in a variety of non-malignant autoimmune disorders, in which B cells and autoantibodies appear to play a role in disease pathophysiology. Edwards *et al., Biochem Soc. Trans.* 30:824–828 (2002). Rituximab has been reported potentially to 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); Emery *et al., Arthritis Rheum.* 48(9): S439 (2003)), lupus (Eisenberg, *Arthritis. Res. Ther.* 5/4:157–159 (2003); Leandro *et al. Arthritis Rheum.* 46: 2673–2677 (2002); Gorman *et al., Lupus,* 13: 312-316 (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); Zaia *et al., Haematolgica*, 87: 189-195 (2002); 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)), 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)), 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)), 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)), Sjögren'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)), paraneoplastic opsoclonus-myoclonus syndrome (Pranzatelli *et al. Neurology* 60(Suppl. 1) PO5.128:A395 (2003)), 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).

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-β (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, US 2003/0026804, and WO 2000/09160 (Grillo-Lopez, A.); WO 2000/27428 (Grillo-Lopez and White); WO 2000/27433 and US 2004/0213784 (Grillo-Lopez and Leonard); WO 2000/44788 (Braslawsky *et al.*); WO 2001/10462 (Rastetter, W.); 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 and WO 2001/74388 (Hanna, N.); US 2002/0058029 (Hanna, N.); US 2003/0103971 (Hariharan and Hanna); US 2002/0009444 and WO 2001/80884 (Grillo-Lopez, A.); WO 2001/97858 (White, C.); US 2002/0128488 and WO 2002/34790 (Reff, M.); WO 2002/060955 (Braslawsky *et al.*);WO 2002/096948 (Braslawsky *et al.*);WO 2002/079255 (Reff and Davies); US Patent No. 6,171,586 and WO 1998/56418 (Lam *et al.*); WO 1998/58964 (Raju, S.); WO 1999/22764 (Raju, S.); WO 1999/51642 and US Patent Nos. 6,194,551, 6,242,195, 6,528,624, and 6,538,124 (Idusogie *et al.*); WO 2000/42072 (Presta, L.); WO 2000/67796 (Curd *et al.*); WO 2001/03734 (Grillo-Lopez *et al.*); US 2002/0004587 and WO 2001/77342 (Miller and Presta); US 2002/0197256 (Grewal, I.); US 2003/0157108 (Presta, L.); WO 04/056312 (Lowman *et al.*); US 2004/0202658 and WO 2004/091657 (Benyunes, K.); WO 2005/000351 (Chan, A.); US 2005/0032130A1 (Beresini *et al.*); US 2005/0053602A1 (Brunetta, P.); US Patent Nos. 6,565,827, 6,090,365, 6,287,537, 6,015,542, 5,843,398, and 5,595,721, (Kaminski *et al.*); US Patent Nos. 5,500,362, 5,677,180, 5,721,108, 6,120,767, and 6,652,852 (Robinson *et al.*); US Pat No. 6,410,391 (Raubitschek *et al.)*; US Patent No. 6,224,866 and WO00/20864 (Barbera-Guillem, E.); WO 2001/13945 (Barbera-Guillem, E.); WO 2000/67795 (Goldenberg); US 2003/0133930 and WO 2000/74718 (Goldenberg and Hansen); US 2003/0219433 and WO 2003/68821 (Hansen *et al.*); WO2004/058298 (Goldenberg and Hansen); WO 2000/76542 (Golay *et al.*);WO 2001/72333 (Wolin and Rosenblatt); US Patent No. 6,368,596 (Ghetie *et al.*); US Patent No. 6,306,393 and US 2002/0041847 (Goldenberg, D.); US 2003/0026801 (Weiner and Hartmann); WO 2002/102312 (Engleman, E.); US 2003/0068664 (Albitar *et al.*); WO 2003/002607 (Leung, S.); WO 2003/049694, US2002/0009427, and US 2003/0185796 (Wolin *et al.*); WO 2003/061694 (Sing and Siegall); US 2003/0219818 (Bohen *et al.*); US 2003/0219433 and WO 2003/068821 (Hansen *et al.*); US 2003/0219818 (Bohen *et al.*); US 2002/0136719 (Shenoy *et al.*); WO 2004/032828 (Wahl *et al.*); and WO 2002/56910 (Hayden-Ledbetter). See also US Patent No. 5,849,898 and EP 330,191 (Seed *et al.*); EP332,865A2 (Meyer and Weiss); US Patent No. 4,861,579 (Meyer *et al.*); 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.*); 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 and US 2005/0069545 A1 (Carr *et al.*); WO 2005/014618 (Chang *et al.*); US 2005/0079174 (Barbera-Guillem and Nelson); and US 2005/0106108 (Leung and Hansen). Certain of these include, *inter alia*, treatment of lupus.

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/m^2 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/m^2 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); 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); DeVita *et al.*, "Efficacy of selective B cell blockade in the treatment of rheumatoid arthritis" *Arthritis & Rheum* 46:2029-2033 (2002); Hidashida *et al.* "Treatment of DMARD-refractory rheumatoid arthritis with rituximab." Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; Tuscano, J. "Successful treatment of infliximab-refractory rheumatoid arthritis with rituximab" Presented at the *Annual Scientific Meeting of the American College of Rheumatology*; Oct 24-29; New Orleans, LA 2002; "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), p922-4* (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; Specks *et al.* "Response

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Blood 94:646, abstract 2869 (1999); "Hypocomplementemic urticarial vasculitis with angioedema, a rare presentation of systemic lupus erythematosus: rapid response to rituximab" Saigal *et al. Journal of the American Academy of Dermatology* 49, (5 Suppl), pS283-5 (Nov 2003); "Anti-CD20 monoclonal antibody (rituximab, RTX) therapy in a hemodialysis (HD) patient (Pt) with severe systemic lupus erythematosis (SLE)" Valentine *et al. Journal of the American Society of Nephrology*, Volume: 13 , Number: Program and Abstracts Issue, Page: 683A , September 2002 , "Anti-CD20 monoclonal antibody (rituximab) for refractory autoimmune thrombocytopenia in a girl with systemic lupus erythematosus" ten Cate *et al. Rheumatology*, 43 (2): 244 (Feb 2004); "Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy" Weide *et al. Lupus*, 12 (10): 779-782 (2003); "Cytokine-receptor pairing: Accelerating discovery of cytokine function" Foster *et al. Nature Reviews Drug Discovery* 3/2: 160-170 (2004); "Rituximab: expanding role in therapy for lymphomas and autoimmune diseases" Rastetter *et al. Annual review of medicine* 55: 477-503 (2004); "B cell therapy for rheumatoid arthritis: The rituximab (anti-CD20) experience" Shaw *et al. Annals of the Rheumatic Diseases*, 62/SUPPL. 2, pp. 55-59 (2003); "Severe hypercoagulable state and concomitant life threatening bleeding successfully treated with rituximab" Ahn *et al., Blood*, 102/11: 108b (November 16 2003); and "Graft-versus-Kaposi's Sarcoma effect and reversal of Lupus Anticoagulant Syndrome and Thalassemia Intermedia after non-myeloablative allogeneic stem cell transplant" Patel *et al. Blood*, 98/11 Part 2: 370b (November 16 2001). See also "Remission of Proliferative Lupus Nephritis Following B Cell Depletion Therapy Is Preceded by Down-Regulation of the T Cell Costimulatory Molecule CD40 Ligand" Sfikakis *et al. Arthrit. Rheumat.*, 52 (2): 501-513 (2005) and "B Cell Deletion in SLE" presented by D. Isenberg at the 6th European Lupus Meeting on March 4, 2005, including slide 38 on retreatment of seven patients.

Persons afflicted with lupus such as those with SLE who show clinical evidence for lupus nephritis and those with lupus nephritis need a cost-efficient and safe treatment that will help ameliorate the tissue damage that leads ultimately to kidney failure and the need for chronic hemodialysis and/or renal transplantation caused by their condition.

Summary of the Invention

The present invention involves, at least in part, the selection of a dose for a CD20 antibody that provides a safe and active treatment regimen in subjects with lupus, such as SLE or lupus nephritis. Accordingly, the invention is as claimed. In a first aspect, the present invention concerns a method of treating lupus in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, 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.

In one embodiment of this first aspect, a second medicament is administered with the initial exposure and/or later exposures, wherein the CD20 antibody is a first medicament. In a preferred embodiment, the second medicament is a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial drug, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone. In a more preferred embodiment, the second medicament is an immunosuppressive agent, an anti-malarial agent, or a chemotherapeutic agent. In another preferred embodiment, the immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent is administered with the initial exposure. In other embodiments, it is administered with the second exposure and/or later exposures and/or with the initial exposure, and preferably with all exposures. In a still preferred

embodiment, a corticosteroid, hydroxychloroquine, chloroquine, quinacrine, methotrexate, cyclophosphamide, azathioprine, mycophenolate mofetil, or 6-mercaptopurine is administered. In another aspect, the immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent is not administered with the second exposure, or is administered in lower amounts than are used with the initial exposure. In this method, preferably the subject has never been previously treated with a CD20 antibody.

In another embodiment, no other medicament than the CD20 antibody is administered to the subject to treat the lupus.

In another preferred embodiment, the subject has an elevated level of infiltrating CD20 cells, anti-nuclear antibodies (ANA), anti-double-stranded DNA (dsDNA) antibodies, anti-Sm antibodies, anti-nuclear ribonucleoprotein antibodies, anti-phospholipid antibodies, anti-ribosomal P antibodies, anti-Ro/SS-A antibodies, anti-Ro antibodies, or anti-La antibodies, or a combination of two or more of such cells or antibodies.

Additionally, the invention provides an article of manufacture comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating lupus 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 a single dose or as two or three separate doses of antibody.

The invention herein involves a dosing amount and regimen that reduces or minimizes the need for treating a lupus subject more often than necessary with CD20 antibody. The invention herein also preferably reduces, minimizes, or eliminates the need for co-, pre-, or post-administration of an immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent that is 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 patient, such as time convenience. However, the invention also contemplates the use of such concomitant treatment.

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

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

Detailed Description of the Preferred Embodiments

I. Definitions

"Lupus" as used herein is an autoimmune disease or disorder involving antibodies that attack connective tissue. The principal form of lupus is a systemic one, systemic lupus erythematosus (SLE), including cutaneous SLE and subacute cutaneous SLE, as well as other types of lupus (including nephritis, extrarenal, cerebritis, pediatric, non-renal, discoid, and alopecia).

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.

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, 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.

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.

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.* 173:137 (1991) and Wilson *et al. J. Immunol.*150:5013 (1993).

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

Examples of CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labeled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from IDEC Pharmaceuticals, Inc. (US Patent No. 5,736,137; 2B8 deposited with ATCC under accession no. HB11388 on June 22, 1993); murine IgG2a "B1," also called "Tositumomab," optionally labeled with ^{131}I to generate the "131I-B1" or "iodine I131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, US Patent No. 5,595,721); murine monoclonal antibody "1F5" (Press *et al. Blood* 69(2):584-591 (1987) and variants thereof including "framework-patched" or humanized 1F5 (WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (US Patent No. 5,677,180); humanized 2H7; HUMAX-CD20™ antibodies (Genmab, Denmark); the human monoclonal antibodies set forth in WO 2004/035607 (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 terms "rituximab" or "RITUXAN®" herein refer to the genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen and designated "C2B8" in US Patent No. 5,736,137, including fragments thereof that retain the ability to bind CD20.

Purely for the purposes herein and unless indicated otherwise, "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 (VI), wherein the V_H region may be joined to a human IgG chain constant region, wherein the region may be, for example, IgG1 or IgG3. In a preferred embodiment, such antibody comprises the V_H sequence of SEQ ID NO:8 (v16, as shown in Fig. 1B), optionally also comprising the V_L sequence of SEQ ID NO:2 (v16, as shown in Fig. 1A), which may have the amino acid substitutions of D56A and N100A in the H chain and S92A in the L chain (v96). Preferably, the antibody is an intact antibody comprising the light- and heavy-chain amino acid sequences of SEQ ID NOS:13 and 14, respectively, as shown in Figs. 2 and 3. Another preferred embodiment is where the antibody is 2H7.v31 comprising the light- and heavy-chain amino acid sequences of SEQ ID NOS:13 and 15, respectively, as shown in Figs. 2 and 4. The antibody herein may further comprise at least one amino acid substitution in the Fc region that improves ADCC and/or CDC activity, such as one wherein the amino acid substitutions are S298A/E333A/K334A, more preferably 2H7.v31 having the heavy-chain amino acid sequence of SEQ ID NO:15 (as shown in Fig. 4). Any of these antibodies may further comprise at least one amino acid substitution in the Fc region that decreases CDC activity, for example, comprising at least the substitution K322A. See US Patent No. 6,528,624B1 (Idusogie *et al.)*.

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and the variable heavy-chain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID NO:8).

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:13);

and the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI

SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:14)

or the heavy-chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR
FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIAATI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:15).

In the preferred embodiment 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
31	-	-	S298A, E333A, K334A
96	D56A, N100A	S92A	
114	D56A, N10	M32L, S92A	S298A, E333A, K334A
115	D56A, N100A	M32L, S92A	S298A, E333A, K334A, E356D, M358L

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

"Human effector cells" are leukocytes that express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes that mediate ADCC include peripheral blood mononuclear cells (PBMC), natural-killer (NK) cells, monocytes, cytotoxic T cells, and neutrophils, with PBMCs and NK cells being preferred.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native-sequence human FcR. Moreover, a preferred FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (see Daëron *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet *Annu. Rev. Immunol* 9:457-92 (1991);

Capel *et al. Immunomethods* 4:25-34 (1994); and de Haas *et al. J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al, J. Immunol.* 117:587 (1976) and Kim *et al. J. Immunol.* 24:249 (1994)).

"Complement-dependent cytotoxicity" or "CDC" 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.

"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 ADCC.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability

to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

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

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy-chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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

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

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 1993/11161; 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.*, US 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 (US 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 Patent 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 sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells, since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

A "subject" herein is a human subject. Generally, such subject is eligible for treatment for lupus. For the purposes herein, such eligible subject is one that is experiencing or has experienced one or more signs, symptoms, or other indicators of lupus or has been diagnosed with lupus, whether, for example, newly diagnosed, previously diagnosed with a new flare, or chronically steroid dependent with a new flare, or is at risk for developing lupus. One eligible for treatment of lupus may optionally be identified as one who has been screened for elevated levels of infiltrating CD20 cells or is screened using an assay to detect auto-antibodies, such as those noted below, wherein autoantibody production is assessed qualitatively, and preferably quantitatively. Exemplary such auto-antibodies associated with SLE are anti-nuclear Ab (ANA), anti-double-stranded DNA (dsDNA) Ab, anti-Sm Ab, anti-nuclear ribonucleoprotein Ab, anti-phospholipid Ab, anti-ribosomal P Ab, anti-Ro/SS-A Ab, anti-Ro Ab, and anti-La Ab.

A nephritic lupus flare is defined as 1) an increase of > 30% in Scr within a 1-month period, or 2) a recurrence or appearance of nephrotic syndrome, or 3) a 3-fold increase in urinary protein with baseline proteinuria > 1 gm/24 hrs or as noted in Example 1. For lupus nephritis, the treatment eligibility may be evidenced by a nephritic flare as defined by renal criteria as noted below in Example 1.

Diagnosis of SLE may be according to current American College of Rheumatology (ACR) criteria. Active disease may be defined by one British Isles Lupus Activity Group's (BILAG) "A" criteria or two BILAG "B" criteria, as noted in Example 2. Some signs, symptoms, or other indicators used to diagnose SLE adapted from: Tan *et al.* "The Revised Criteria for the Classification of SLE" *Arth Rheum* 25 (1982) may be malar rash such as rash over the cheeks, discoid rash, or red raised patches, photosensitivity such as reaction to sunlight, resulting in the development of or increase in skin rash, oral ulcers such as ulcers in the nose or mouth, usually painless, arthritis, such as non-erosive arthritis involving two or more peripheral joints (arthritis in which the bones around the joints do not become destroyed), serositis, pleuritis or pericarditis, renal disorder such as excessive protein in the urine (greater than 0.5 gm/day or 3+ on test sticks) and/or cellular casts (abnormal elements derived from the urine and/or white cells and/or kidney tubule cells), neurologic signs, symptoms, or other indicators, seizures (convulsions), and/or psychosis in the absence of drugs or metabolic disturbances that are known to cause such effects, and hematologic signs, symptoms, or other indicators such as hemolytic anemia or leukopenia (white bloodcount below 4,000 cells per cubic millimeter) or lymphopenia (less than 1,500 lymphocytes per cubic millimeter) or thrombocytopenia (less than 100,000 platelets per cubic millimeter). The leukopenia and lymphopenia must

be detected on two or more occasions. The thrombocytopenia must be detected in the absence of drugs known to induce it. The invention is not limited to these signs, symptoms, or other indicators of lupus.

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

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

The expression "effective amount" refers to an amount of the antibody that is effective for preventing, ameliorating, or treating the lupus.

"Antibody exposure" refers to contact with or exposure to the antibody herein in one or more doses administered over a period of time of about 1 day to about 5 weeks. The doses may be given at one time or at a fixed or 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.

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 US Patent No. 4,665,077); nonsteroidal antiinflammatory 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 US Patent No. 4,120,649); anti-idiotypic antibodies for MHC antigens and MHC fragments; cyclosporin A; steroids such as corticosteroids or glucocorticosteroids or glucocorticoid analogs, *e.g.*, prednisone, methylprednisolone, and dexamethasone; dihydrofolate reductase inhibitors such as methotrexate (oral or subcutaneous); hydroxycloroquine; sulfasalazine; leflunomide; cytokine or cytokine receptor antibodies including anti-interferon-alpha, -beta, or -gamma antibodies, anti-tumor necrosis factor-alpha antibodies (infliximab or adalimumab), anti-TNF-alpha immunoahesin (etanercept), anti-tumor necrosis factor-beta antibodies, anti-interleukin-2 antibodies and anti-IL-2 receptor antibodies; anti-LFA-1 antibodies, including anti-CD11a and anti-CD18 antibodies; anti-L3T4 antibodies; heterologous anti-lymphocyte globulin; pan-T antibodies, preferably anti-CD3 or anti-CD4/CD4a antibodies; soluble peptide containing a LFA-3 binding domain (WO 1990/08187 published 7/26/90); streptokinase; TGF-beta; streptodornase; RNA or DNA from the host; FK506; RS-61443; deoxyspergualin; rapamycin; T-cell receptor (Cohen *et al.*, US Patent No. 5,114,721); T-cell-receptor fragments (Offner *et al.*, *Science*, 251: 430-432 (1991); WO 1990/11294; Ianeway, *Nature*, 341: 482 (1989); and WO 1991/01133); and T-cell-receptor antibodies (EP 340,109) such as T10B9.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as

small-molecule toxins or enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin, and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e. g.*, calicheamicin, especially calicheamicin gamma1I and calicheamicin omega1I (see, *e.g.*, Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE® doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin;

vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

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

The term "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence cytokines, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "hormone" refers to polypeptide hormones, which are generally secreted by glandular organs with ducts. Included among the hormones are, for example, growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH); prolactin, placental lactogen, mouse gonadotropin-associated peptide, inhibin; activin; mullerian-inhibiting substance; and thrombopoietin. As used herein, the term hormone includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence hormone, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "growth factor" refers to proteins that promote growth, and include, for example, hepatic growth factor; fibroblast growth factor; vascular endothelial growth factor; nerve growth factors such as NGF-β; platelet-derived growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; and colony-stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF). As used herein, the term growth factor includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native-sequence growth

factor, including synthetically produced small-molecule entities and pharmaceutically acceptable derivatives and salts thereof.

The term "integrin" refers to a receptor protein that allows cells both to bind to and to respond to the extracellular matrix and is involved in a variety of cellular functions such as wound healing, cell differentiation, homing of tumor cells, and apoptosis. They are part of a large family of cell adhesion receptors that are involved in cell-extracellular matrix and cell-cell interactions. Functional integrins consist of two transmembrane glycoprotein subunits, called alpha and beta, that are non-covalently bound. The alpha subunits all share some homology to each other, as do the beta subunits. The receptors always contain one alpha chain and one beta chain. Examples include Alpha6beta1, Alpha3beta1, Alpha7beta1, LFA-1 *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 hydroxycloroquine, sulfasalazine, methotrexate, leflunomide, etanercept, infliximab (plus oral and subcutaneous methrotrexate), azathioprine, D-penicillamine, gold salts (oral), gold salts (intramuscular), minocycline, cyclosporine, *staphylococcal* protein A immunoadsorption, including salts and derivatives thereof, *etc.*

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

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 Patent Nos. 6,677,339 and 6,348,463), aromatic amine derivatives (US Patent No. 6,369,229), ADAM disintegrin domain polypeptides (US 2002/0042368), 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 corticosteroids. Examples of synthetic corticosteroids include prednisone, prednisolone (including methylprednisolone), dexamethasone triamcinolone, and betamethasone.

A "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications, other therapeutic products to be combined with the packaged product, and/or warnings concerning the use of such therapeutic products, *etc.*

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

A "medicament" is an active drug to treat the lupus or its symptoms or side effects.

II. Treatment

The present invention provides a method of treating lupus 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 about 0.5 to 4 grams (preferably about 1.5 to 3.5 grams, more preferably about 1.5 to 2.5 grams) followed by a second antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 3.5 grams, more preferably about 1.5 to 2.5 grams), wherein the second exposure is not 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, and each of the antibody exposures is provided to the subject as a single dose or as two or three separate doses of antibody. For purposes of this invention, the second antibody exposure is the next time the subject is treated with the CD20 antibody after the initial antibody exposure, there being no intervening CD20 antibody treatment or exposure between the initial and second exposures.

The method preferably comprises administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure of about 0.5 to 4 grams (preferably about 1.5 to 3.5 grams, 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 two or three separate doses of the antibody (*i.e.*, constituting a first and second dose or a first, second, and third dose). The particular number of doses (whether one, two, or three) employed for each antibody exposure is dependent, for example, on the type of lupus treated, the type of antibody employed, whether and what type of second medicament is employed as noted below, and the method and frequency of administration. Where separate doses are administered, the second dose 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 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 one embodiment, the subject is provided at least about three exposures of the antibody, for example, from about 3 to 60 exposures, and more preferably about 3 to 40 exposures, most preferably, about 3 to 20 exposures. Preferably, such exposures are administered at intervals each of about 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.

The antibody may be a naked antibody or may be conjugated with another molecule such as a cytotoxic agent such as a radioactive compound. The preferred antibody herein is rituximab, humanized 2H7 (*e.g.* comprising the variable domain sequences in SEQ ID NOS:2 and 8), or HUMAX-CD20^{TM} antibody (Genmab), more preferably, rituximab or humanized 2H7.

In one embodiment, the subject has never been previously treated with drug(s), such as immunosuppressive agent(s), to treat the lupus and/or has never been previously treated with an antibody to a B-cell surface marker (*e.g.* never been previously treated with a CD20 antibody). In another embodiment, the subject has been previously treated with drug(s) to treat the lupus and/or has been previously treated with such antibody. In another embodiment, the CD20 antibody is the only medicament administered to the subject to treat the lupus. In another embodiment, the CD20 antibody is one of the medicaments used to treat the lupus. In a further embodiment, the subject does not have rheumatoid arthritis. In a still further embodiment, the subject does not have multiple sclerosis. In yet another embodiment, the subject does not have an autoimmune disease other than lupus. For purposes of this lattermost statement, an "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or organs or a co-segregate or manifestation thereof or resulting condition therefrom. In one embodiment, it refers to a condition that results from, or is aggravated by, the production by B cells of antibodies that are reactive with normal body tissues and antigens. In other embodiments, the autoimmune disease is one that involves secretion of an autoantibody that is specific for an epitope from a self antigen (*e.g.* a nuclear antigen).

The antibody is administered by any suitable means, including parenteral, topical, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and/or intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Intrathecal administration is also contemplated (see, *e.g.*, US 2002/0009444, Grillo-Lopez, A concerning intrathecal delivery of a CD20 antibody). In addition, the antibody may suitably be administered by pulse infusion, *e.g.*, with declining doses of the antibody. Preferably, the dosing is given intravenously or subcutaneously, and more preferably by intravenous infusion(s). 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, methylprednisolone (*e.g.*, about 80-120 mg i.v., more preferably 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 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.). Preferably, 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).

One may administer a second medicament with the antibody that binds a B-cell surface marker (*e.g.* with the CD20 antibody), such as a cytotoxic agent, chemotherapeutic agent, anti-malarial agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone, integrin, integrin antagonist, or antibody.

For instance, the antibody may be combined with a chemotherapeutic agent, an interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®), an oligopeptide such as glatiramer acetate (COPAXONE®), a cytotoxic agent (such as mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, and azathioprine), intravenous immunoglobulin (gamma globulin), lymphocyte-depleting therapy (*e.g.*, mitoxantrone, cyclophosphamide, CAMPATH™ antibodies, anti-CD4, cladribine, total body irradiation, bone marrow transplantation), corticosteroid (*e.g.*, methylprednisolone, prednisone such as low-dose prednisone, dexamethasone, or glucorticoid, *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™), 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)), hormone-replacement therapy, an anti-malarial drug such as, *e.g.*, hydroxychloroquine, chloroquine, or quinacrine, treatment for symptoms secondary or related to lupus *(e.g.,* spasticity, incontinence, pain, fatigue), a TNF inhibitor, DMARD, NSAID, anti-integrin antibody or antagonist, plasmapheresis, levothyroxine, cyclosporin A, somatastatin analogue, cytokine, anti-cytokine antagonist or antibody, anti-metabolite, immunosuppressive agent, rehabilitative surgery, radioiodine, thyroidectomy, another B-cell surface antagonist/antibody, *etc.*

More specific examples of such second medicaments, if the CD20 antibody is called the first medicament, include a chemotherapeutic agent, cytotoxic agent, anti-integrin, anti-malarial drug such as, *e.g.*, hydroxychloroquine, chloroquine, or quinacrine, gamma globulin, anti-CD4, cladribine, corticosteroid, MMF, cyclosporine, cholesterol-lowering drug of the statin class, estradiol, testosterone, hormone-replacement drug, TNF inhibitor, DMARD, NSAID, levothyroxine, cyclosporin A, somatastatin analogue, cytokine antagonist or cytokine-receptor antagonist, anti-metabolite, immunosuppressive agent, and/or another B-cell surface marker antibody, such as a combination of rituximab and humanized 2H7. Still more preferred is a chemotherapeutic agent, an immunosuppressive agent, a cytotoxic agent, an integrin antagonist, an anti-malarial drug, a cytokine antagonist, or a hormone, or a combination of one or more of these medicaments.

These second medicaments 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 CD20 antibody were not present, especially in subsequent dosings beyond the initial dosing with antibody, so as to eliminate or reduce side effects caused thereby.

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.

The combined administration 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 simultaneously exert their biological activities. In a preferred embodiment, after the initial exposure, the amount of such agent is reduced or eliminated so as to reduce the exposure of the subject to an agent with side effects such as prednisone and cyclophosphamide, especially when the agent is a corticosteroid. In another embodiment, the amount of the second medicament is not reduced or eliminated.

In a preferred embodiment, an immunosuppressive agent, an anti-malarial agent, or a chemotherapeutic agent is administered with the initial exposure, more preferably a corticosteroid, methotrexate, cyclophosphamide, hydroxychloroquine, chloroquine, quinacrine, azathioprine, mycophenolate mofetil, or 6-mercaptopurine. In another aspect, the immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent is not administered with the subsequent exposure, or is administered in lower amounts than with the initial exposure. However, such agent is optionally administered with more than one exposure, including all exposures, in the same or similar amounts as with the initial exposure.

If the lupus is lupus nephritis, preferably about 2-3 grams of the CD20 antibody is administered as the initial exposure, more preferably about 2 grams. In another preferred embodiment, if 3 grams are administered, about 1 gram of the CD20 antibody is administered weekly for about three weeks as the initial exposure. In another preferred embodiment, if 2 grams are administered, 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 another aspect, the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams. In still another 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.

Preferably, for lupus nephritis, a corticosteroid such as methylprednisolone and/or prednisone is administered to the subject before and/or with the CD20 antibody. Preferably, the subject receives IV methylprednisolone at about 1000 mg each day for two days upon the first antibody exposure. For the first antibody exposure, this treatment is preferably followed by oral prednisone at an initial dose of about 0.75 mg/kg/day for about 4 weeks and tapered to about 10-15 mg/day by about week 16. Preferably, about 100 mg IV methylprednisolone is given about 30-60 minutes prior to infusions of subsequent doses of CD20 antibody from the initial dose. Also preferred is administering the prednisone in lower amounts with the second exposure than are used with the initial exposure or wherein prednisone is not administered with the second exposure, or wherein the prednisone is administered in lower amounts with the second exposure than are used with the initial exposure, but not administered in third or later exposures. Additionally or alternatively, MMF is preferably administered with the initial antibody exposure, with concomitant administration of MMF and the corticosteroid being particularly preferred. Preferably, the MMF is given initially with the CD20 antibody at about 1500 mg/day in divided doses (3x/day) and the subject is titrated up to a target dose of about 3g/day in divided doses (3x/day) by about week 4, as tolerated. If reductions in dose are necessary, decreases will be allowed in about 250-500 mg decrements. In another aspect, cyclophosphamide may be administered to the subject with or without the corticosteroid at the initial antibody exposure. If cyclophosphamide is administered, it is preferably not administered with the second exposure or is administered with the second exposure but in lower amounts than are used with the initial exposure. Also preferred is wherein cyclophosphamide is not administered with third or later exposures.

If the lupus is systemic lupus erythematosus, preferably about 2 grams of the CD20 antibody is administered as the initial exposure. Also preferred is 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. Preferably, the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams. In another preferred embodiment, 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.

For SLE, preferably prednisone is administered before and/or with the initial exposure, such as a week before the initial exposure in an amount of about 0.4-1 mg/kg/day. More preferably, the subjects receive an initial oral prednisone regimen of 0.5 mg/kg/day, 0.75 mg/kg/day, or 1.0 mg/kg/day, based on their BILAG score and prestudy prednisone dose, over a 7-day period. At about day 16 after the initial CD20 antibody administration, subjects are preferably given a prednisone taper over about 10 weeks to achieve a prednisone dose of less than about 10 mg/day. Subjects will continue to taper their corticosteroid dose as tolerated to a target dose of less than or equal to about 5 mg/day. Still more preferred is administering the prednisone in lower amounts with the second exposure than are used with the initial exposure or wherein prednisone is not administered with the second exposure, or wherein the prednisone is administered in lower amounts with the second exposure than are used with the initial exposure, but not administered in third or later exposures. In another preferred aspect, in addition to prednisone, an anti-malarial drug such as, *e.g.*, hydroxychloroquine, chloroquine, or quinacrine, or methotrexate, mycophenolate mofetil, azathioprine, or 6-mercaptopurine is

administered. It may be administered during one or more exposures, such as the initial or second exposure or a later exposure or during all exposures. In such embodiment, the anti-malarial drug, methotrexate, mycophenolate mofetil, azathioprine, or 6-mercaptopurine is optionally only administered during the initial exposure or is optionally also administered with the second exposure but in lower amounts than are used with the initial exposure.

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

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope except for possible variants that arise during production of the monoclonal antibody, such variants generally being present in minor

amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete or polyclonal antibodies.

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

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

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

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

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

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

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-

SEPHAROSE™ crosslinked agarose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

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

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

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (US Patent No. 4,816,567; Morrison, *et al., Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin-coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

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

(iii) Humanized antibodies

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

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

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

(iv) Human antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain-joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al., Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al., Nature*, 362:255-258 (1993); Bruggermann *et al., Year in Immuno.*, 7:33 (1993); and US Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage-display technology (McCafferty *et al., Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V)-domain gene repertoires from unimmunized donors. According to this technique, antibody V-domain genes are cloned in-frame into either a major or minor coat-protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, *e.g.*, Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571

(1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, US Patent Nos. 5,565,332 and 5,573,905.

Human antibodies may also be generated by *in vitro*-activated B cells (see US Patent Nos. 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 1993/16185 and US Patent Nos. 5,571,894 and 5,587,458. The antibody fragment may also be a "linear antibody", *e.g.*, as described in US Patent No. 5,641,870. 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-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 coexpression of two immunoglobulin heavy-chain-light-chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the

product yields are low. Similar procedures are disclosed in WO 1993/08829, and in Traunecker *et al., EMBO J.*, 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1), containing the site necessary for light-chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy-chain-light-chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 1994/04690. For further details of generating bispecific antibodies, see, for example, Suresh *et al., Methods in Enzymology*, 121:210 (1986).

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

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 1991/00360, WO 1992/200373, 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, for example, 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 WO 2004/032828.

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^I, α_2^I, α_3^I, N-acetyl-γ_1^I, PSAG and θ^I_1 (Hinman *et al. Cancer Research* 53: 3336-3342 (1993) and Lode *et al. Cancer Research* 58: 2925-2928 (1998)).

Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. See, for example, WO 1993/21232 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 is available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO 1994/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker, or disulfide-containing linker (Chari *et al. Cancer Research* 52: 127-131 (1992)) may be used.

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

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

The antibodies of the present invention may also be conjugated with a prodrug-activating enzyme that converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO 1981/01145) to an active anti-cancer drug. See, for example, WO 1988/07378 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.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases, and cathepsins (such as cathepsins B and L), that are useful for converting peptide-

containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

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

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Antibody fragments, such as Fab', linked to one or more PEG molecules are an especially preferred embodiment of the invention.

The antibodies disclosed herein may also be formulated as liposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); US Patent Nos. 4,485,045 and 4,544,545; and WO 1997/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in US 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 a different residue. The sites of greatest interest for substitutional mutagenesis of antibodies include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile

Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their side chains (in A. L. Lehninger, in *Biochemistry*, second ed., pp. 73-75, Worth Publishers, New York (1975)):

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

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

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

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

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

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

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

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

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

(6) aromatic: Trp, Tyr, Phe.

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

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

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several

hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.* binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine-scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

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

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

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

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US 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.

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 that further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO

2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); and 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 2003/0157108, Presta, L; and WO 2004/056312, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,*-knockout CHO cells (Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004)).

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

It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.* so as to enhance ADCC and/or 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 ADCC. See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.*148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

WO 2000/42072 (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 CDC are described in WO 1999/51642 and US Patent Nos. 6,194,551, 6,242,195, 6,528,624, and 6,538,124 (Idusogie *et al.*). The antibodies comprise an amino acid substitution at one or more of amino acid positions 270, 322, 326, 327, 329, 313, 333, and/or 334 of the Fc region thereof.

To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in US Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Antibodies with substitutions in an Fc region thereof and increased serum half-lives are also described in WO 2000/42072 (Presta, L.).

Engineered antibodies with three or more (preferably four) functional antigen-binding sites are also contemplated (US 2002/0004587 A1, Miller *et al.*).

V. Pharmaceutical Formulations

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

Exemplary anti-CD20 antibody formulations are described in WO 1998/56418. 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 emulsifying agent 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 emulsifying agent, and Sterile Water for Injection, pH 6.5.

Lyophilized formulations adapted for subcutaneous administration are described, for example, 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.

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 necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent (*e.g.* mitoxantrone (NOVANTRONE®), methotrexate, cyclophosphamide, chlorambucil, or azathioprine), chemotherapeutic agent, immunosuppressive agent, cytokine, cytokine antagonist or antibody, growth factor, hormone (*e.g.*, testosterone or hormone replacement therapy), integrin, integrin antagonist or antibody (*e.g.*, an LFA-1 antibody such as efalizumab/RAPTIVA® commercially available from Genentech, or an alpha 4 integrin antibody such as natalizumab/ANTEGREN® available from Biogen, or others as noted above), interferon class drug such as IFN-beta-1a (REBIF® and AVONEX®) or IFN-beta-1b (BETASERON®), an oligopeptide such as

glatiramer acetate (COPAXONE™), intravenous immunoglobulin (gamma globulin), lymphocyte-depleting drug (*e.g.*, mitoxantrone, cyclophosphamide, CAMPATH™ antibodies, anti-CD4, or cladribine), non–lymphocyte-depleting immunosuppressive drug (*e.g.*, MMF or cyclosporine), cholesterol-lowering drug of the "statin" class, estradiol, drug that treats symptoms secondary or related to lupus (*e.g.*, spasticity, incontinence, pain, fatigue), a TNF inhibitor, DMARD, NSAID, corticosteroid (*e.g.*, methylprednisolone, prednisone, dexamethasone, or glucorticoid), levothyroxine, cyclosporin A, somatastatin analogue, anti-metabolite, another B-cell surface antagonist/antibody, *etc.*, in the formulation. The type and effective amounts of such other agents (called herein second medicaments, wherein the first medicament is the CD20 antibody) depend, for example, on the amount of antibody present in the formulation, the type of lupus being treated, and clinical parameters of the subjects.

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

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

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

VI. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of lupus described above is provided. Preferably, the article of manufacture comprises (a) a container comprising a composition comprising an antibody that binds to a B-cell surface marker (*e.g.*, a CD20 antibody) and a pharmaceutically acceptable carrier or diluent within the container; and (b) a package insert with instructions for treating lupus 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 a single dose or as two or three separate doses of antibody.

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 lupus and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable

by a hypodermic injection needle). At least one active agent in the composition is the antibody. The label or package insert indicates that the composition is used for treating lupus in a subject eligible for treatment with specific guidance regarding dosing amounts and intervals of antibody and any other drug being provided. The article of manufacture may further comprise a second container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and dextrose solution. The article of manufacture may still further comprise a second or third container comprising a second medicament, wherein the CD20 antibody is a first medicament, where the article further comprises instructions on the package insert for treating the subject with the second medicament. Exemplary second medicaments include a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone. The preferred second medicament is a chemotherapeutic agent, an anti-malarial agent, or an immunosuppressive agent, most preferably hydroxychloroquine, chloroquine, quinacrine, cyclophosphamide, prednisone, mycophenolate mofetil, methotrexate, azathiprine, or 6-mercaptopurine. More specifically, if the lupus is SLE, such second medicament is preferably a corticosteroid such as prednisone (along with optionally methotrexate, hydroxychloroquine, chloroquine, quinacrine, MMF, or azathioprine with or without 6-mercaptopurine), and if the lupus is lupus nephritis, the second medicament is preferably a corticosteroid such as prednisone as well as MMF or cyclophosphamide. 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 and Safety of Rituximab in Subjects with ISN/RPS 2003 Class III or IV Lupus Nephritis

This study assesses the superiority of efficacy and safety of rituximab (MABTHERA®/RITUXAN®) added to mycophenolate mofetil (MMF) and corticosteroids compared to MMF plus corticosteroids alone in subjects with active ISN/RPS 2003 class III or IV lupus nephritis. Rituximab (1000 mg x 2) is administered i.v. in two initial doses at days 1 and 15 with IV and oral corticosteroids, followed by 1 g x 2 at six months. This experimental regimen (rituximab added to MMF + corticosteroids) is compared to placebo (placebo added to MMF + corticosteroids). This rituximab-based regimen challenges the current standard of care, eliminates patient exposure to CYTOXAN® cyclophosphamide (CYC) and its known toxicities, and demonstrates improved net clinical benefit. Patients are monitored for disease activity, both renal and extrarenal, flares of disease, and safety events over the 52 weeks of the study. The primary efficacy endpoint of the trial is at 52 weeks. Safety follow-up is required until 12 months following the last dose of rituximab or B-cell recovery, whichever occurs later.

The primary objective is to determine the proportion of patients achieving either a complete or partial renal response.

A complete renal response is defined as:

1. normal creatinine or normalization of creatinine to baseline ($\pm$ 0.2 mg/dL) if baseline creatinine lower than normal range.
2. inactive urinary sediment (as evidenced by < 10 red blood cells (RBCs)/high power field (HPF) and absence of red cell casts).

3. urinary protein to creatinine ratio < 0.5.

A partial renal response is defined as:

1. stable (± 10% from screening values) or improved estimated glomerular filtration rate (GFR) (as calculated by the Modification in Diet in Renal Disease (MDRD) equation).
2. no worsening of urinary sediment from baseline.
3. if the baseline urine protein to creatinine ratio is ≤ 3.5, then observe a reduction in proteinuria to a urine protein to creatinine ratio of < 1.0, or if the baseline urine protein to creatinine ratio is > 3.5, then observe a reduction in proteinuria by ≥ 50% to a level lower than a urine protein to creatinine ratio of 3.5.

Consented subjects participate in a screening period of up to 14 days to determine eligibility. After screening, subjects not already on MMF will be initiated on MMF at 1500 mg/day in divided doses (3x/day). All subjects will be titrated up to a target dose of 3g/day in divided doses (3x/day) by week 4, as tolerated. If reductions in dose are necessary, decreases will be allowed in 250-500 mg decrements. At randomization, subjects, after either continuing or initiating MMF, will be started on methylprednisolone 1000 mg IV once a day for two days and then, starting on day 3, patients will be started on oral prednisone at 0.75 mg/kg/day tapering to 10 - 15 mg per day by week 16. Subjects will receive either rituximab or placebo at days 1 and 15 and days 168 and 182, with 100 mg IV methylprednisolone 30-60 minutes prior to infusions given on days 15, 168, and 182. Subjects who experience a worsening of renal function may be withdrawn and treated at the Investigator's discretion. These subjects will be counted as treatment failures but followed closely in safety follow-up.

Subjects are eligible for the study if all three of the criteria below have been met. They:

- have been diagnosed with ISN/RPS class III or IV lupus nephritis as evidenced by a renal biopsy done within 12 months of screening showing < 50% of glomeruli with sclerosis.
- have active disease as evidenced by proteinuria, with urine protein to creatinine ratio > 1.0 and *either* a renal biopsy within 3 months of screening showing ISN/RPS 2003 class III or IV lupus nephritis *or* an active urinary sediment with > 10 RBCs/HPF or presence of red cells casts.
- have an estimated GFR (as calculated by the MDRD equation) ≥ 30 ml/min for the 12 weeks prior to screening.

B-cell counts (CD19) are assessed at baseline, at the end of each course of rituximab/placebo, and every 4 weeks thereafter throughout the study. All B-cell counts will be conducted at the sponsor-assigned central laboratory. At the end of 78 weeks, subjects who received placebo rituximab or active rituximab but demonstrate B-cell recovery will complete study participation. Subjects who received rituximab but have not demonstrated B-cell recovery will be followed until B-cell recovery, defined by baseline or lower limit of normal, whichever is lower.

After week 52, subjects may be eligible for rituximab infusions. All subjects who receive a dose of rituximab after week 52 will be observed for 12 months after their last dose of rituximab or until B-cell recovery, whichever is later.

It is predicted and expected that administration of rituximab or humanized 2H7 to the subject in the protocol set forth above will ameliorate one or more signs, symptoms, or other indicators of lupus nephritis over the control. It is also expected that another 2-g dose of the CD20 antibody, given again between 12 and 18 months after initial therapy with the CD20 antibody either all at once or spread out over about 14-16 days in 1-gram amounts, would be effective in continuing the response of initial therapy or inducing another complete/partial response if the subject experiences a flare, with or without the prednisone 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 around 6 months, followed by another potential treatment at about one to one and a half years from initial treatment (measured from the time any one of the doses was given) with expected success. This re-treatment protocol is expected to be successfully used in the treatment of proliferative lupus nephritis.

Example 2

A Study to Evaluate the Efficacy and Safety of Rituximab in Subjects with Moderate-to- Severe Systemic Lupus Erythematosus

This study assesses the efficacy and safety of rituximab (MABTHERA®/RITUXAN®) added to prednisone and one additional immunosuppressant (MMF, methotrexate (MTX), azathioprine (AZA), or 6-mercaptopurine (6-MP)) compared with placebo in subjects with active SLE without active glomerulonephritis at enrollment for a Phase II/III trial. Subjects may qualify by exhibiting a severe Lupus Flare as defined by one new BILAG A criterion or two new BILAG B criteria and will receive an initial oral prednisone regimen of 0.5 mg/kg/day, 0.75 mg/kg/day, or 1.0 mg/kg/day, based on their BILAG score and prestudy prednisone dose, over a 7-day period. Subjects are then randomized to receive rituximab or placebo and at day 16 will initiate a prednisone taper over 10 weeks to achieve a prednisone dose of <10 mg/day. Subjects will continue to taper their corticosteroid dose as tolerated to a target dose of $\leq$ 5 mg/day. Subjects are monitored for disease activity, use of additional immunosuppressants, flares of disease, prednisone use, and safety events over the 52 weeks of the study. The primary efficacy endpoint of the trial will be at 52 weeks. Safety follow-up is required until 12 months following the last dose of rituximab or B-cell recovery, whichever occurs later.

The primary objective of this study is to assess the efficacy of rituximab compared with placebo in achieving and maintaining a major clinical response (MCR) or partial clinical response (PCR) in subjects with moderate-to-severe systemic lupus erythematosus (SLE), as assessed by the BILAG assessment. Clinical responses will be grouped by the following three mutually exclusive categories:

- Subjects who achieve an MCR.
- Subjects who do not achieve an MCR but achieve a PCR.
- Subjects who do not achieve either an MCR or PCR (i.e., non-clinical response (NCR)).

The MCR, PCR, and NCR are defined as follows:

- MCR: Subjects who achieve BILAG C scores or better at 24 weeks and maintain this response without developing a flare (one or more new domains with a BILAG A or B score) to 52 weeks.

- PCR: Subjects who achieve BILAG C scores or better at 24 weeks and maintain this response without developing a flare (one or more new domains with a BILAG B score) for 16 consecutive weeks or achieve a maximum of one domain with a BILAG B score at 24 weeks and maintain this response without developing a flare (one or more new domains with a BILAG B or new BILAG A score) to 52 weeks.
- NCR: All subjects who experience a severe flare (one new domain with a BILAG A score or two new domains with a BILAG B score) from Day 1 to Week 24 or any subject who fails to meet the definition of an MCR or PCR as defined above.

The secondary objectives or efficacy outcome measures of this study (comparing rituximab with placebo) will be to evaluate the following:

- Ability of rituximab to decrease overall SLE disease activity as measured by time-adjusted area under the curve minus baseline (AUCMB) scoring with the BILAG assessment over 52 weeks.
- Ability of rituximab to induce MCRs (excluding PCRs) or PCRs (including MCRs), as measured, *e.g.*, by the proportion of subjects who achieve an MCR (excluding PCR) and the proportion of subjects who achieve a PCR (including MCR) at Week 52.
- Safety and tolerability of rituximab.
- Ability of rituximab-treated subjects to achieve a BILAG C score or better at Week 24, as measured by, *e.g.*, the proportion of subjects who achieve a BILAG C score or better in all domains at Week 24.
- Ability of rituximab to prolong the time to a moderate or severe flare over 52 weeks.
- Ability of rituximab to improve quality of life as measured by SLE Expanded Health Survey physical function score from baseline at Week 52 (SF-36 index with additional elements specific to lupus).
- Corticosteroid-sparing in subjects receiving rituximab, as measured, *e.g.*, by the proportion of subjects who achieve an MCR with < 10 mg prednisone per day from Weeks 24 to 52.
- Pharmacokinetics of rituximab in subjects with SLE.

Consented subjects participate in a screening period lasting up to 7 days to determine eligibility. Subjects must present with active lupus determined by ACR criteria and one new BILAG category "A" or two new BILAG category "B" criteria without evidence of active glomerulonephritis while on a background immunosuppressant. At screening, subjects are initially treated with oral prednisone 0.5 mg/kg/day, 0.75 mg/kg/day, or 1.0 mg/kg/day for 7 days, based on initial BILAG score and pre-screening corticosteroid dose. Eligible subjects are randomized in a 2:1 ratio to receive rituximab 1000mg i.v. x 2 (days 1, 15) plus prednisone taper or rituximab placebo i.v. equivalent plus prednisone taper during the 52-week treatment and observation period. The first rituximab/placebo infusion occurs on Day 1 with the second infusion occurring on Day 15. A scheduled prednisone taper commences on study Day 16 and patients fractionally reduce prednisone to 10 mg/day p.o. over 10 weeks, followed by a continued taper to < 5 mg/day by week 52 as tolerated. 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. All subjects will be re-dosed with either rituximab or placebo at weeks 24 and 26. In addition, subjects will receive 100 mg IV solumedrol 30–60 minutes prior to each study drug (rituximab or placebo) infusion.

All subjects are instructed to continue baseline immunosuppressive medications (*e.g.*, MMF, AZA/6-MP, MTX) that were present at screening and to continue their anti-malarial medication (if indicated), as well as their baseline non-corticosteroid SLE medication(s), throughout the study, without alteration unless instructed by the treating Investigator. NSAIDs will be allowed to treat mild symptomatic disease. Requests to taper an immunosuppressive drug must be discussed in advance with the Medical Monitor. The following table lists the anti-malarial agents and dose ranges expected to be used during the course of the trial, if they are indicated.

Antimalarial Medication	Dose Range (oral)
Hydroxychloroquine	100 – 250 mg/day
Chloroquine	500 mg every day or every other day
Quinacrine	100 mg every day

Subjects who experience a protocol-defined moderate-to-severe SLE flare (treatment failure) may receive treatment with additional oral corticosteroids, if judged clinically appropriate by the Investigator. These subjects may be retreated with prednisone (0.5–1.0 mg/kg) based on the severity of disease. IV corticosteroids in equivalent doses may be allowed if gastrointestinal involvement temporarily precludes oral corticosteroids. Subjects who experience flares that are non-responsive to corticosteroids are those without improvement in their BILAG A or B symptoms after 2 weeks of increased corticosteroid treatment. They will be eligible to enroll for rescue treatment in an open-label extension trial, if desired by the subject and Investigator. Subjects who initiate a new immunosuppressive agent or any other new SLE medication will enter the safety follow-up period of the trial and will not receive further study drug if initiation of the concomitant medication occurs prior to the second study drug regimen (at 6 months).

Patients are assessed monthly for 12 months. B-cell counts are assessed at baseline, at the end of each course of rituximab/placebo infusion, and subsequently every 4 weeks throughout the treatment/observation period. All B-cell counts are performed by a central laboratory, and physicians will be blinded to B-cell counts. At the end of 78 weeks, subjects who received rituximab placebo or rituximab and demonstrate B-cell recovery, as defined by B-cell recovery to baseline or the lower limit of normal, whichever is lower, will complete study participation. Subjects who received rituximab but have not demonstrated B-cell recovery at 78 weeks will be observed until B-cell recovery.

It is predicted and expected that administration of rituximab or humanized 2H7 to the subject in the protocol set forth above will ameliorate one or more signs, symptoms, or other indicators of SLE over the control. It is also expected that another 2-g dose of the CD20 antibody, given again between 12 and 18 months after initial therapy with the CD20 antibody, either all at once or spread out over about 14-16 days in 1-gram amounts, would be effective in continuing the response of initial therapy or inducing another complete/partial response if the subject experiences a flare, with or without the prednisone 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 around 6 months, followed by another potential treatment at about one to one and a half years from initial treatment (measured from the time that any of the doses are given). This re-treatment protocol is expected to be successfully used in the treatment of SLE.

Example 3

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:18), for example, SEQ ID NO:4 (Fig. 1A),
CDR L2 sequence of SEQ ID NO:5 (Fig. 1A),
CDR L3 sequence QQWXFNPPT wherein X is S or A (SEQ ID NO:19), for example, SEQ ID NO:6 (Fig. 1A),
CDR H1 sequence of SEQ ID NO:10 (Fig. 1B),
CDR H2 sequence of AIYPGNGXTSYNQKFKG wherein X is D or A (SEQ ID NO:20), for example, SEQ ID NO:11 (Fig. 1B), and
CDR H3 sequence of VVYYSXXYWYFDV wherein the X at position 6 is N, A, Y, W, or D, and the X at position 7 is S or R (SEQ ID NO:21), for example, SEQ ID NO:12 (Fig. 1B).

The CDR sequences above are generally present within human variable light- and variable heavy-framework sequences, such as substantially the human consensus FR residues of human light-chain kappa subgroup I (V_LKI), 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 16, and heavy-chain amino acid sequence of SEQ ID NO:14, 15, 17, or 22, where SEQ ID NO:22 is indicated 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.

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

Table 2

Exemplary Humanized 2H7 Antibody Variants

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

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKR (SEQ ID NO:2);

and 2H7.v16 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSS (SEQ ID NO:8).

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR

EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:13);

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGDTSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSNSYWYFDVWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:15).

Another preferred humanized 2H7 antibody comprises 2H7.v511 variable light-domain sequence: DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKR (SEQ ID NO:23)

and 2H7.v511 variable heavy-domain sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYN QKFKGRFTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSS (SEQ ID NO:24).

See Figures 5 and 6, which align the mature light and heavy chains, respectively, of humanized 2H7.v511 with humanized 2H7.v16.

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

DIQMTQSPSSLSASVGDRVTITCRASSSVSYLHWYQQKPGKAPKPLIYAPSNLASGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQWAFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC (SEQ ID NO:13)

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

EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYNMHWVRQAPGKGLEWVGAIYPGNGATSYNQKFKGR FTISVDKSKNTLYLQMNSLRAEDTAVYYCARVVYYSYRYWYFDVWGQGTLVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNATYRVVSVLTVLHQDWLNGKEYKCKVSNAALPAPIAA TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:22).

A preferred embodiment herein is where the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:2 and 8. Another preferred embodiment herein is where the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:23 and 24.

WHAT IS CLAIMED IS:

1. A method of treating lupus in a subject comprising administering an effective amount of a CD20 antibody to the subject to provide an initial antibody exposure of about 0.5 to 4 grams followed by a second antibody exposure of about 0.5 to 4 grams, 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.

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

3. The method of claim 1 or 2 wherein the second exposure is not provided until from about 46 to 54 weeks from the initial exposure.

4. The method of any one of claims 1-3 wherein the initial and second antibody exposures are each provided in amounts of about 1.5 to 3.5 grams.

5. The method of any one of claims 1-4 wherein the initial and second antibody exposures are each provided in amounts of about 1.5 to 2.5 grams.

6. The method of any one of claims 1-5 additionally comprising administering to the subject an effective amount of the CD20 antibody to provide a third antibody exposure of about 0.5 to 4 grams, wherein the third exposure is not provided until from about 46 to 60 weeks from the initial exposure and the third antibody exposure is provided to the subject as a single dose or as two or three separate doses of antibody.

7. The method of claim 6 wherein the third antibody exposure is provided in an amount of about 1.5 to 3.5 grams.

8. The method of claim 6 or 7 wherein the third antibody exposure is provided in an amount of about 1.5 to 2.5 grams.

9. The method of any one of claims 6-8 wherein the third exposure is not provided until from about 46 to 55 weeks from the initial exposure.

10. The method of any one of claims 6-9 wherein no further antibody exposure is provided until at least about 70-75 weeks from the initial exposure.

11. The method of claim 10 wherein no further antibody exposure is provided until about 74 to 80 weeks from the initial exposure.

12. The method of any one of claims 1-11 wherein one or more of the antibody exposures is provided to the subject as a single dose of antibody.

13. The method of claim 12 wherein each antibody exposure is provided to the subject as a single dose of antibody.

14. The method of any one of claims 1-11 wherein one or more of the antibody exposures is provided to the subject as separate doses of the antibody.

15. The method of claim 14 wherein each antibody exposure is provided as separate doses of the antibody.

16. The method of claim 14 or 15 wherein the separate doses constitute a first and second dose.

17. The method of claim 14 or 15 wherein the separate doses constitute a first, second, and third dose.

18. The method of any one of claims 15-17 wherein the second or third dose is administered from about 1 to 20 days from the time the previous dose was administered.

19. The method of any one of claims 15-18 wherein the second or third dose is administered from about 6 to 16 days from the time the previous dose was administered.

20. The method of any one of claims 15-19 wherein the second or third dose is administered from about 14 to 16 days from the time the previous dose was administered.

21. The method of any one of claims 15-20 wherein the separate doses are administered within a total period of between about 1 day and 4 weeks.

22. The method of claim 21 wherein the separate doses are administered within a total period of between about 1 and 25 days.

23. The method of any one of claims 15-22 wherein the separate doses are administered about weekly, with the second dose being administered about one week from the first dose and any third dose being administered about one week from the second dose.

24. The method of any one of claims 15-23 wherein each separate dose of antibody is about 0.5 to 1.5 grams.

25. The method of any one of claims 15-24 wherein each separate dose of antibody is about 0.75 to 1.3 grams.

26. The method of any one of claims 1-25 wherein 4 to 20 antibody exposures are administered to the subject.

27. The method of any one of claims 1-26 wherein a second medicament is administered in an effective amount with an antibody exposure, wherein the CD20 antibody is a first medicament.

28. The method of claim 27 wherein the second medicament is administered with the initial exposure.

29. The method of claim 27 or 28 wherein the second medicament is administered with the initial and second exposures.

30. The method of any one of claims 27-29 wherein the second medicament is administered with all exposures.

31. The method of any one of claims 27-30 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

32. The method of any one of claims 27-31 wherein the second medicament is an immunosuppressive agent, an anti-malarial agent, or a chemotherapeutic agent.

33. The method of claim 32 wherein the immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent is administered with the initial exposure.

34. The method of claim 33 wherein a corticosteroid, methotrexate, cyclophosphamide, hydroxychloroquine, chloroquine, quinacrine, azathioprine, mycophenolate mofetil, or 6-mercaptopurine is administered.

35. The method of any one of claims 32-34 wherein the immunosuppressive agent, anti-malarial agent, or chemotherapeutic agent is not administered with the second exposure, or is administered in lower amounts than are used with the initial exposure.

36. The method of any one of claims 1-35 wherein the lupus is lupus nephritis.

37. The method of claim 36 wherein about 2 grams of the CD20 antibody is administered as the initial exposure.

38. The method of claim 36 or 37 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.

39. The method of any one of claims 36-38 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.

40. The method of any one of claims 36-39 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.

41. The method of any one of claims 36-40 wherein a corticosteroid is administered.

42. The method of claim 41 wherein the corticosteroid is methylprednisolone or prednisone or both.

43. The method of any one of claims 36-42 wherein mycophenolate mofetil is administered.

44. The method of any one of claims 36-43 wherein a third exposure to the CD20 antibody is made at about 1 year to 18 months from the initial exposure.

45. The method of any one of claims 1-35 wherein the lupus is systemic lupus erythematosus.

46. The method of claim 45 wherein about 2 grams of the CD20 antibody is administered as the initial exposure.

47. The method of claim 45 or 46 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.

48. The method of any one of claims 45-47 wherein the second exposure is at about six months from the initial exposure and is administered in an amount of about 2 grams.

49. The method of any one of claims 45-48 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.

50. The method of any one of claims 45-49 wherein prednisone is administered before or with the initial exposure.

51. The method of claim 50 wherein prednisone is administered in lower amounts with the second exposure than are used with the initial exposure or wherein prednisone is not administered with the second exposure or wherein the prednisone is administered in lower amounts with the second exposure than are used with the initial exposure but not administered in third or later exposures.

52. The method of claim 50 or 51 wherein additionally hydroxychloroquine, chloroquine, quinacrine, methotrexate, mycophenolate mofetil, azathioprine, or 6-mercaptopurine is administered.

53. The method of any one of claims 45-52 wherein a third exposure to the CD20 antibody is made at about 1 year to 18 months from the initial exposure.

54. The method of any one of claims 1-53 wherein the subject has never been previously treated with a CD20 antibody.

55. The method of any one of claims 1-54 wherein the antibody is a naked antibody.

56. The method of any one of claims 1-54 wherein the antibody is conjugated with another molecule.

57. The method of claim 56 wherein the other molecule is a cytotoxic agent.

58. The method of any one of claims 1-57 wherein the antibody is administered intravenously.

59. The method of claim 58 wherein the antibody is administered intravenously for each antibody exposure.

60. The method of any one of claims 1-57 wherein the antibody is administered subcutaneously.

61. The method of claim 60 wherein the antibody is administered subcutaneously for each antibody exposure.

62. The method of any one of claims 1-26, 36-40, 44-49, and 53-61 wherein no other medicament than the CD20 antibody is administered to the subject to treat the lupus.

63. The method of any one of claims 1-62 wherein the antibody is rituximab.

64. The method of any one of claims 1-62 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:2 and 8.

65. The method of any one of claims 1-62 wherein the antibody is humanized 2H7 comprising the variable domain sequences in SEQ ID NOS:23 and 24.

66. The method of any one of claims 1-65 wherein the subject has an elevated level of infiltrating CD20 cells, anti-nuclear antibodies (ANA), anti-double stranded DNA (dsDNA) antibodies, anti-Sm antibodies, anti-nuclear ribonucleoprotein antibodies, anti-phospholipid antibodies, anti-ribosomal P antibodies, anti-Ro/SS-A antibodies, anti-Ro antibodies, or anti-La antibodies, or a combination of two or more of such cells or antibodies.

67. An article of manufacture comprising:

(a) a container comprising a CD20 antibody; and

(b) a package insert with instructions for treating lupus 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 a single dose or as two or three separate doses of antibody.

68. The article of claim 67 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.

69. The article of claim 68 wherein the second medicament is a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial agent, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

70. The article of claim 68 or 69 wherein the second medicament is a chemotherapeutic agent, anti-malarial agent, or immunosuppressive agent.

71. The article of any one of claims 68-70 wherein the second medicament is methylprednisolone, prednisone, mycophenolate mofetil, methotrexate, hydroxychloroquine, chloroquine, quinacrine, azathiprine, or 6-mercaptopurine.

Sequence Alignment of Variable Light-Chain Domain

```
               |-------- FR1 ---------|     CDR1        |-----
                        10        20        30          40
2H7        QIVLSQSPAILSASPGEKVTMTC [RASSSVS-YMH] WYQQKP
           * ***    **     * **   *
hu2H7.v16  DIQMTQSPSSLSASVGDRVTITC [RASSSVS-YMH] WYQQKP
                                      * * * **
hum κI     DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

           FR2 ----|   CDR2    |---------- FR3 ----------
                   50         60        70        80
2H7        GSSPKPWIY [APSNLAS] GVPARFSGSGSGTSYSLTISRVEA
            **   *                *         ***    ****
hu2H7.v16  GKAPKPLIY [APSNLAS] GVPSRFSGSGSGTDFTLTISSLQP
               *       * * *
hum κI     GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

           --------|    CDR3      |-- FR4 --|
                   90          100
2H7        EDAATYYC [QQWSFNPPT] FGAGTKLELKR
             *                   *   * *
hu2H7.v16  EDFATYYC [QQWSFNPPT] FGQGTKVEIKR
                       **** *
hum κI     EDFATYYC [QQYNSLPWT] FGQGTKVEIKR
```

FIG._1A

Sequence Alignment of Variable Heavy-Chain Domain

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

             ---FR2---|       CDR2          |----- FR3 ------
                      50  a       60          70        80
2H7          PRQGLEWIG [AIYPGNGDTSYNQKFKG] KATLTVDKSSSTAYM
              **    *                      ** **    ** * *
hu2H7.v16    PGKGLEWVG [AIYPGNGDTSYNQKFKG] RFTISVDKSKNTLYL
                     *  * **** * * ****         * *
hum III      PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLTL

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

FIG._1B

Humanized 2H7.v16 Light Chain

DIQMTQSPSSLSASVGDRVTITCRASSSVSYMHWYQQKPGKAPKPLIYAPSNLASGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQWSFNPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:13)

FIG._2

Humanized 2H7.v16 Heavy Chain

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

FIG._3

Humanized 2H7.v31 Heavy Chain

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

FIG._4

Light Chain Alignment

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

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

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

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

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

FIG._5

Heavy Chain Alignment

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

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

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

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

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

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

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

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

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

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

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

FIG._6