B-CELL REDUCTION USING CD37-SPECIFIC AND CD20-SPECIFIC BINDING MOLECULES

[0001] The present application claims benefit under 35 U.S.C. § 119 of United States Patent Application No. 60/702,499, which was filed July 25, 2005, Unites States Patent Application No. 60/798,344, which was filed May 16, 2006, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention generally provides methods for B-cell reduction in an individual using CD37-specific binding molecules. In particular, the invention provides methods for B-cell reduction using CD37-specific binding molecules alone, or a combination of CD37-specific binding molecules and CD20-specific binding molecules, in some instances a synergistic combination. The invention further provides materials and methods for treatment of diseases involving aberrant B-cell activity.

BACKGROUND OF THE INVENTION

[0003] In its usual role, the human immune system protects the body from damage from foreign substances and pathogens. One way in which the immune system protects the body is by production of specialized cells called B lymphocytes or B-cells. B-cells produce antibodies that bind to, and in some cases mediate destruction of, a foreign substance or pathogen.

[0004] In some instances though, the human immune system and specifically the B lymphocytes of the human immune system go awry and disease results. There are numerous cancers that involve uncontrolled proliferation of B-cells. There are also numerous autoimmune diseases that involve B-cell production of antibodies that, instead of binding to foreign substances and pathogens, bind to parts of the body. Such antibodies are sometimes called autoantibodies. In addition, there are numerous autoimmune and inflammatory diseases that involve B-cells in their pathology,

for example, through inappropriate B-cell antigen presentation to T-cells, or through other pathways involving B-cells. For example, autoimmune-prone mice deficient in B-cells do not develop autoimmune kidney disease, vasculitis or autoantibodies. See Shlomchik et al., *J Exp. Med.*, 180:1295-306 (1994). Interestingly, these same autoimmune-prone mice which possess B-cells but are deficient in immunoglobulin production, do develop autoimmune diseases when induced experimentally as described by Chan et al., *J Exp. Med.*, 189:1639-48 (1999), indicating that B-cells play an integral role in development of autoimmune disease.

[0005] B-cells can be identified by molecules on their cell surface. CD20 was the first human B-cell lineage-specific surface molecule identified by a monoclonal antibody. It is a non-glycosylated, hydrophobic 35 kDa B-cell transmembrane phosphoprotein that has both its amino and carboxy ends situated inside the cell. See, Einfeld et al., EMBO J., 7:711-17 (1998). CD20 is expressed by all normal mature B-cells, but is not expressed by precursor B-cells or plasma cells. Natural ligands for CD20 have not been identified, and the function of CD20 in B-cell biology is still incompletely understood.

[0006] Another B-cell lineage-specific cell surface molecule is CD37. CD37 is a heavily glycosylated 40-52 kDa protein that belongs to the tetraspanin transmembrane family of cell surface antigens. It traverses the cell membrane four times forming two extracellular loops and exposing its amino and carboxy ends to the cytoplasm. CD37 is highly expressed on normal antibody-producing (sIg+)B-cells, but is not expressed on pre-B-cells or plasma cells. The expression of CD37 on resting and activated T cells, monocytes and granulocytes is low and there is no detectable CD37 expression on NK cells, platelets or erythrocytes. See, Belov et al., Cancer Res., 61(11):4483-4489 (2001); Schwartz-Albiez et al., J. Immunol., 140(3): 905-914 (1988); and Link et al., J. Immunol., 137(9): 3013-3018 (1988). Besides normal B-cells, almost all malignancies of B-cell origin are positive for CD37 expression, including CLL, NHL, and hairy cell leukemia [Moore et al., Journal of Pathology, 152: 13-21 (1987); Merson and Brochier, Immunology Letters, 19: 269-272 (1988); and Faure et al., American Journal of

Dermatopathology, 12 (3): 122-133 (1990)]. CD37 participates in regulation of B-cell function, since mice lacking CD37 were found to have low levels of serum IgG1 and to be impaired in their humoral response to viral antigens and model antigens. It appears to act as a nonclassical costimulatory molecule or by directly influencing antigen presentation via complex formation with MHC class II molecules. See Knobeloch et al., Mol. Cell. Biol., 20(15):5363-5369 (2000). CD37 also seems to play a role in TCR signaling. See Van Spriel et al., J. Immunol., 172: 2953-2961 (2004).

[0007] Research and drug development has occurred based on the concept that B-cell lineage-specific cell surface molecules such as CD37 or CD20 can themselves be targets for antibodies that would bind to, and mediate destruction of, cancerous and autoimmune disease-causing B-cells that have CD37 or CD20 on their surfaces. Termed "immunotherapy," antibodies made (or based on antibodies made) in a non-human animal that bind to CD37 or CD20 were given to a patient to deplete cancerous or autoimmune disease-causing B-cells.

[0008] One antibody to CD37 has been labeled with ^{131}I and tested in clinical trials for therapy of NHL. See Press et al., J. Clin. Oncol., 7(3): 1027-1038 (1989); Bernstein et al., Cancer Res. (Suppl.), 50: 1017-1021 (1990); Press et al., Front. Radiat. Ther. Oncol., 24: 204-213 (1990); Press et al., Adv. Exp. Med. Biol., 303: 91-96 (1991) and Brown et al., Nucl. Med. Biol., 24: 657-663 (1997). The antibody, MB-1, is a murine IgG1 monoclonal antibody that lacks Fc effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and MB-1 did not inhibit tumor growth in an *in vivo* xenograft model unless it had been labeled with an isotope (Buchsbaum et al., Cancer Res., 52(83): 6476-6481 (1992). Favorable biodistribution of ^{131}I-MB-1 was seen in lymphoma patients who had lower tumor burdens (<1 kg) and therapy of these patients resulted in complete tumor remissions lasting from 4 to 11 months (Press et al., 1989 and Bernstein et al. 1990).

[0009] In addition, an immunoconjugate composed of the drug adriamycin linked to G28-1, another anti-CD37 antibody, has been evaluated in mice and

showed effects through internalization and intracellular release of the drug. See Braslawsky et al., Cancer Immunol. Immunother., 33(6): 367-374 (1991).

[0010] Various groups have investigated the use of anti-CD20 antibodies to treat B-cell related diseases. One treatment consists of anti-CD20 antibodies prepared in the form of radionuclides for treating B-cell lymphoma (e.g., ^{131}I-labeled anti-CD20 antibody), as well as a ^{89}Sr-labeled form for the palliation of bone pain caused by prostate and breast cancer metastases [Endo, *Gan To Kagaku Ryoho*, 26: 744-748 (1999)].

[0011] Another group developed a chimeric monoclonal antibody specific for CD20, consisting of heavy and light chain variable regions of mouse origin fused to human IgG1 heavy chain and human kappa light chain constant regions. The chimeric antibody reportedly retained the ability to bind to CD20 and the ability to mediate ADCC and to fix complement. See, Liu et al., J. Immunol. 139:3521-26 (1987). Yet another chimeric anti-CD20 antibody was made from IDEC hybridoma C2B8 and was named rituximab. The mechanism of anti-tumor activity of rituximab is thought to be a combination of several activities, including ADCC, complement fixation, and triggering of signals that promote apoptosis in malignant B-cells, although the large size of the chimeric antibody prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B-cells, thereby limiting its anti-tumor activities. ADCC is 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. Complement fixation, or complement-dependent cytotoxicity (CDC) is 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. The large size of rituximab prevents optimal diffusion of the molecule into lymphoid tissues that contain malignant B-cells, thereby limiting these anti-tumor activities.

[0012] Rituximab, typically administered in 4 weekly infusions, is currently used to treat low-grade or follicular B-cell non-Hodgkin's lymphoma

[McLaughlin et al., Oncology, 12: 1763-1777 (1998); Leget et al., Curr. Opin. Oncol.,10: 548-551 (1998)] and in relapsed stage III/IV follicular lymphoma [White et al., Pharm. Sci. Technol. Today, 2: 95-101 (1999)]. Other disorders treatable with rituximab include follicular centre cell lymphoma (FCC), mantle cell lymphoma (MCL), diffuse large cell lymphoma (DLCL), and small lymphocytic lymphoma (SLL) [Nguyen et al., Eur J Haematol., 62:76-82 (1999)]. Rituximab administered in weekly infusions is also used to treat CLL [Lin et al., Sem Oncol., 30:483-92 (2003)].

[0013] Anti-CD20 antibodies have also been used to treat patients suffering from autoimmune diseases associated with B-cell production of autoantibodies. For example, rituximab has demonstrated significant clinical benefit in depleting CD20+ B-cells in patients with multiple autoimmune/inflammatory diseases including RA [Edwards, N Engl J Med., 350:2546-2548 (2004); Cambridge et al., Arthritis Rheum., 48:2146-54 (2003)]. RA patients received continued doses of methotrexate (MTX) and a 4 dose course of rituximab infusion (Edwards, supra). These patients showed improved American College of Rheumatology (ACR) responses compared to control groups.

[0014] In a trial for the treatment of systemic lupus erythematosus (SLE) [Leandro et al., Arthritis Rheum., 46:2673-2677 (2002)], patients were administered two infusions of high dose rituximab, and demonstrated B-cell reduction and improved disease state. In a second study of B-cell reduction in SLE [Looney et al., Arthritis Rheum., 50:2580-2589 (2004)], patients were given a single infusion of 100 mg/m2 (low dose), a single infusion of 375 mg/m2 (intermediate dose), or as 4 infusions (1 week apart) of 375 mg/m2 (high dose) rituximab. These patients demonstrated B-cell reduction and improved disease scores, but the treatment did not alter the level of autoantibody. Trials of rituximab have also been carried out in Waldenstrom's macroglobulinemia [Treon et al., Immunother., 24:272-279 (2000)], where patients showed increased hematocrit (HCT) and platelet (PLT) counts after 4 infusions of rituximab.

[0015] Recent reports of rituximab treatment in patients suffering from multiple sclerosis, an autoimmune disease affecting the central nervous system, indicate that a course of treatment depletes peripheral B-cells but has little effect on B-cells in cerebrospinal fluid. See Monson et al., Arch. Neurol., 62: 258-264 (2005).

[0016] Additional publications concerning the use of rituximab include: Stashi et al. "Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura" Blood 98:952-957 (2001); Matthews, R. "Medical Heretics" New Scientist (7 Apr., 2001); Leandro et al. "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" Ann Rheum Dis 61:833-888 (2002); Leandro et al. "Lymphocyte depletion in rheumatoid arthritis: early evidence for safety, efficacy and dose response. Arthritis and Rheumatism 44(9): S370 (2001); Leandro et al. "An open study of B lymphocyte depletion in systemic lupus erythematosus", Arthritis Rheum. 46:2673-2677 (2002); Edwards et al., "Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes" Rheumatology 40:205-211 (2001); Edwards et al. "B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders" Biochem. Soc. Trans. 30(4):824-828 (2002); Edwards et al. "Efficacy and safety of rituximab, a B-cell targeted chimeric monoclonal antibody: A randomized, placebo controlled trial in patients with rheumatoid arthritis. Arthritis Rheum. 46: S197 (2002); Levine et al., "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; October 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; October 24-29; New Orleans, La. 2002.

[0017] Problems associated with rituximab therapy remain. For example, the majority of cancer patients treated with rituximab relapse, generally within about 6-12 months, and fatal infusion reactions within 24 hours of rituximab infusion have been reported. These fatal reactions followed an infusion reaction complex that included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation or cardiogenic shock. Acute renal failure requiring dialysis with instances of fatal outcome has also been reported in the setting of tumor lysis syndrome following treatment with rituximab, as have severe mucocutaneous reactions, some with fatal outcome. Additionally, high doses of rituximab are required for intravenous injection because the molecule is large, approximately 150 kDa, and, as noted above, diffusion into the lymphoid tissues where many tumor cells reside is limited.

[0018] Because normal mature B-cells also express CD37 and CD20, normal B-cells are depleted by anti-CD37 (Press et al., 1989) or anti-CD20 antibody therapy [Reff *et al.*, *Blood,* 83:435-445 (1994)]. After treatment is completed, however, normal B-cells can be regenerated from CD37- and CD20-negative B-cell precursors; therefore, patients treated with anti-CD37 or anti-CD20 therapy do not experience significant immunosuppression.

[0019] Monoclonal antibody technology and genetic engineering methods have led to development of immunoglobulin molecules for diagnosis and treatment of human diseases. Protein engineering has been applied to improve the affinity of an antibody for its cognate antigen, to diminish problems related to immunogenicity, and to alter an antibody's effector functions. The domain structure of immunoglobulins is amenable to engineering, in that the antigen binding domains and the domains conferring effector functions may be exchanged between immunoglobulin classes and subclasses. Immunoglobulin structure and function are reviewed, for example, in Harlow et al., Eds., Antibodies: A Laboratory Manual, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring Harbor (1988). An extensive introduction as well as detailed information about all aspects of recombinant antibody technology can be found in the textbook "Recombinant Antibodies"

(John Wiley & Sons, NY, 1999). A comprehensive collection of detailed antibody engineering lab Protocols can be found in R. Kontermann and S. Dübel (eds.), "The Antibody Engineering Lab Manual" (Springer Verlag, Heidelberg/New York, 2000).

[0020] Recently, smaller immunoglobulin molecules have been constructed to overcome problems associated with whole immunoglobulin therapy. Single chain Fv (scFv) comprise an antibody heavy chain variable domain joined via a short linker peptide to an antibody light chain variable domain [Huston et al., *Proc. Natl. Acad. Sci. USA*, 85: 5879-5883 (1988)]. In addition to variable regions, each of the antibody chains has one or more constant regions. Light chains have a single constant region domain. Thus, light chains have one variable region and one constant region. Heavy chains have several constant region domains. The heavy chains in IgG, IgA, and IgD antibodies have three constant region domains, which are designated CH1, CH2, and CH3, and the heavy chains in IgM and IgE antibodies have four constant region domains, CH1, CH2, CH3 and CH4. Thus, heavy chains have one variable region and three or four constant regions.

[0021] The heavy chains of immunoglobulins can also be divided into three functional regions: the Fd region (a fragment comprising V.sub.H and CH1, i.e., the two N-terminal domains of the heavy chain), the hinge region, and the Fc region (the "fragment crystallizable" region, derived from constant regions and formed after pepsin digestion). The Fd region in combination with the light chain forms an Fab (the "fragment antigen-binding"). Because an antigen will react stereochemically with the antigen-binding region at the amino terminus of each Fab the IgG molecule is divalent, i.e., it can bind to two antigen molecules. The Fc contains the domains that interact with immunoglobulin receptors on cells and with the initial elements of the complement cascade. Thus, the Fc fragment is generally considered responsible for the effector functions of an immunoglobulin, such as complement fixation and binding to Fc receptors.

[0022] Because of the small size of scFv molecules, they exhibit very rapid clearance from plasma and tissues and more effective penetration into tissues

than whole immunoglobulin. An anti-tumor scFv showed more rapid tumor penetration and more even distribution through the tumor mass than the corresponding chimeric antibody [Yokota et al., *Cancer Res.*, 52, 3402-3408 (1992)]. Fusion of an scFv to another molecule, such as a toxin, takes advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. [Chaudary et al., *Nature*, 339:394 (1989); Batra et al., *Mol. Cell. Biol.*, 11:2200 (1991)].

[0023] Despite the advantages of scFv molecules, several drawbacks to their use exist. While rapid clearance of scFv may reduce toxic effects in normal cells, such rapid clearance may prevent delivery of a minimum effective dose to the target tissue. Manufacturing adequate amounts of scFv for administration to patients has been challenging due to difficulties in expression and isolation of scFv that adversely affect the yield. During expression, scFv molecules lack stability and often aggregate due to pairing of variable regions from different molecules. Furthermore, production levels of scFv molecules in mammalian expression systems are low, limiting the potential for efficient manufacturing of scFv molecules for therapy [Davis et al, *J Biol. Chem.*, 265:10410-10418 (1990); Traunecker et al., *EMBO J*, 10: 3655-3659 (1991). Strategies for improving production have been explored, including addition of glycosylation sites to the variable regions [Jost, C. R. U.S. Pat. No. 5,888,773, Jost et al, *J. Biol. Chem.*, 69: 26267-26273 (1994)].

[0024] Another disadvantage to using scFv for therapy is the lack of effector function. An scFv without the cytolytic functions, ADCC and complement dependent-cytotoxicity (CDC), associated with the constant region of an immunoglobulin may be ineffective for treating disease. Even though development of scFv technology began over 12 years ago, currently no scFv products are approved for therapy.

[0025] Alternatively, it has been proposed that fusion of an scFv to another molecule, such as a toxin, could take advantage of the specific antigen-binding activity and the small size of an scFv to deliver the toxin to a target tissue. Chaudary *et al.*, *Nature* 339:394 (1989); Batra *et al.*, *Mol. Cell. Biol.* 11:2200 (1991). Conjugation or fusion of toxins to scFvs has thus been

offered as an alternative strategy to provide potent, antigen-specific molecules, but dosing with such conjugates or chimeras can be limited by excessive and/or non-specific toxicity due to the toxin moiety of such preparations. Toxic effects may include supraphysiological elevation of liver enzymes and vascular leak syndrome, and other undesired effects. In addition, immunotoxins are themselves highly immunogenic upon administration to a host, and host antibodies generated against the immunotoxin limit potential usefulness for repeated therapeutic treatments of an individual.

[0026] Other engineered fusion proteins, termed small, modular immunopharmaceutical (SMIP™) products, are described in co-owned US Patent Publications 2003/133939., 2003/0118592, and 2005/0136049, and co-owned International Patent Publications WO02/056910, WO2005/037989., and WO2005/017148, which are all incorporated by reference herein. SMIP products are novel binding domain-immunoglobulin fusion proteins that feature a binding domain for a cognate structure such as an antigen, a counterreceptor or the like; an IgG1, IGA or IgE hinge region polypeptide or a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues; and immunoglobulin CH2 and CH3 domains. SMIP products are capable of ADCC and/or CDC.

[0027] Although there has been extensive research carried out on antibody-based therapies, there remains a need in the art for improved methods to treat diseases associated with aberrant B-cell activity. The methods of the present invention described and claimed herein provide such improved methods as well as other advantages.

SUMMARY OF THE INVENTION

[0028] The present invention provides methods for reducing B-cells using CD37-specific binding molecules. In some methods of the invention, use of combinations of CD37-specific binding molecules (one or more CD37-specific binding molecules) and CD20-specific binding molecules (one or more CD20-

specific binding molecules) results in increased B-cell reduction. In some of these methods, the combinations are synergistic. In a related aspect, the invention provides a method of treating an individual having, or suspected of having, a disease associated with aberrant B-cell activity.

[0029] The present invention also provides humanized CD37-specific binding molecules (e.g., humanized TRU-016 constructs) and methods for reducing B-cells using these molecules. In some embodiments of the methods of the invention, uses of combinations of humanized TRU-016 constructs with one or more CD20-specific binding molecules is contemplated. In another aspect, the invention provides methods of treating individuals having, or suspected of having, a disease associated with aberrant B-cell activity. Related aspects of the invention are drawn to methods of preventing any such disease and methods of ameliorating a symptom associated with such a disease comprising administering a dose of a humanized CD37-specific binding molecule effective to treat or prevent such disease, or to ameliorate a symptom of such disease.

[0030] "Aberrant B-cell activity" refers to B-cell activity that deviates from the normal, proper, or expected course. For example, aberrant B-cell activity may include inappropriate proliferation of cells whose DNA or other cellular components have become damaged or defective. Aberrant B-cell activity may include cell proliferation whose characteristics are associated with a disease caused by, mediated by, or resulting in inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. Such diseases may be characterized, for example, by single or multiple local abnormal proliferations of cells, groups of cells or tissue(s), whether cancerous or non-cancerous, benign or malignant. Aberrant B-cell activity may also include aberrant antibody production, such as production of autoantibodies, or overproduction of antibodies typically desirable when produced at normal levels. It is contemplated that aberrant B-cell activity may occur in certain subpopulations of B-cells and not in other subpopulations. Aberrant B-cell activity may also include inappropriate stimulation of T-cells, such as by

inappropriate B-cell antigen presentation to T-cells or by other pathways involving B-cells.

[0031] "Treatment" or "treating" refers to either a therapeutic treatment or prophylactic/preventative treatment. A therapeutic treatment may improve at least one symptom of disease in an individual receiving treatment or may delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases.

[0032] A "therapeutically effective dose" or "effective dose" of a CD20-specific binding molecule refers to that amount of the compound sufficient to result in amelioration of one or more symptoms of the disease being treated. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously. The invention specifically contemplates that one or more specific binding molecules may be administered according to methods of the invention, each in an effective dose.

[0033] "An individual having, or suspected of having, a disease associated with aberrant B-cell activity" is an individual in whom a disease or a symptom of a disorder may be caused by aberrant B-cell activity, may be exacerbated by aberrant B-cell activity, or may be relieved by regulation of B-cell activity. Examples of such diseases are a B-cell cancer (for example, B-cell lymphoma, a B-cell leukemia or a B-cell myeloma), a disease characterized by autoantibody production or a disease characterized by inappropriate T-cell stimulation caused by inappropriate B-cell antigen presentation to T-cells or caused by other pathways involving B-cells.

[0034] In one exemplary aspect, an individual treated by methods of the invention demonstrates a response to treatment that is better than, or improved relative to, the response to treatment with rituximab. A response which is improved over treatment with rituximab refers to a clinical response wherein treatment by a method of the invention results in a clinical response in a patient that is better than a clinical response in a patient receiving

rituximab therapy, such as rituximab. An improved response is assessed by comparison of clinical criteria well-known in the art and described herein. Exemplary criteria include, but are not limited to, duration of B cell depletion, reduction in B cell numbers overall, reduction in B cell numbers in a biological sample, reduction in tumor size, reduction in the number of tumors existing and/or appearing after treatment, and improved overall response as assessed by patients themselves and physicians, e.g., using an International Prognostic Index. The improvement may be in one or more than one of the clinical criteria. An improved response with the method of the invention may be due to an inadequate response to previous or current treatment with rituximab, for example, because of toxicity and/or inadequate efficacy of the rituximab treatment.

[0035] B-cell cancers include B-cell lymphomas [such as various forms of Hodgkin's disease, non-Hodgkins lymphoma (NHL) or central nervous system lymphomas], leukemias [such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia and chronic myoblastic leukemia] and myelomas (such as multiple myeloma). Additional B cell cancers include small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

[0036] Disorders characterized by autoantibody production are often considered autoimmune diseases. Autoimmune diseases include, but are not limited to: arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, polychondritis, psoriatic arthritis, psoriasis, dermatitis, polymyositis/dermatomyositis, inclusion body myositis, inflammatory myositis,

toxic epidermal necrolysis, systemic scleroderma and sclerosis, CREST syndrome, responses associated with inflammatory bowel disease, Crohn's disease, ulcerative colitis, respiratory distress syndrome, adult respiratory distress syndrome (ARDS), meningitis, encephalitis, uveitis, colitis, glomerulonephritis, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE), subacute cutaneous lupus erythematosus, discoid lupus, lupus myelitis, lupus cerebritis, juvenile onset diabetes, multiple sclerosis, allergic encephalomyelitis, neuromyelitis optica, rheumatic fever, Sydenham's chorea, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis and Churg-Strauss disease, agranulocytosis, vasculitis (including hypersensitivity vasculitis/angiitis, ANCA and rheumatoid vasculitis), aplastic anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, central nervous system (CNS) inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Behcet disease, Castleman's syndrome, Goodpasture's syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson syndrome, solid organ transplant rejection, graft versus host disease (GVHD), pemphigoid bullous, pemphigus, autoimmune polyendocrinopathies, seronegative spondyloarthropathies, Reiter's disease, stiff-man syndrome, giant cell arteritis, immune complex nephritis, IgA nephropathy, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), Henoch-Schonlein purpura, autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including

autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, autoimmune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM) and Sheehan's syndrome; autoimmune hepatitis, lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barre' Syndrome, large vessel vasculitis (including polymyalgia rheumatica and giant cell (Takayasu's) arteritis), medium vessel vasculitis (including Kawasaki's disease and polyarteritis nodosa), polyarteritis nodosa (PAN) ankylosing spondylitis, Berger's disease (IgA nephropathy), rapidly progressive glomerulonephritis, primary biliary cirrhosis, Celiac sprue (gluten enteropathy), cryoglobulinemia, cryoglobulinemia associated with hepatitis, amyotrophic lateral sclerosis (ALS), coronary artery disease, familial Mediterranean fever, microscopic polyangiitis, Cogan's syndrome, Whiskott-Aldrich syndrome and thromboangiitis obliterans.

[0037] Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the joints, leading to swelling, pain, and loss of function. Patients having RA for an extended period usually exhibit progressive joint destruction, deformity, disability and even premature death.

[0038] Crohn's disease and a related disease, ulcerative colitis, are the two main disease categories that belong to a group of illnesses called inflammatory bowel disease (IBD). Crohn's disease is a chronic disorder that causes inflammation of the digestive or gastrointestinal (GI) tract. Although it can involve any area of the GI tract from the mouth to the anus, it most commonly affects the small intestine and/or colon. In ulcerative colitis, the GI involvement is limited to the colon.

[0039] Crohn's disease may be characterized by antibodies against neutrophil antigens, i.e., the "perinuclear anti-neutrophil antibody" (pANCA), and Saccharomyces cervisiae, i.e. the "anti-Saccharomyces cervisiae antibody" (ASCA). Many patients with ulcerative colitis have the pANCA antibody in their blood, but not the ASCA antibody, while many Crohn's patients exhibit ASCA antibodies, and not pANCA antibodies. One method of

evaluating Crohn's disease is using the Crohn's disease Activity Index (CDAI), based on 18 predictor variables scores collected by physicians. CDAI values of 150 and below are associated with quiescent disease; values above that indicate active disease, and values above 450 are seen with extremely severe disease [Best et al., "Development of a Crohn's disease activity index." Gastroenterology 70:439-444 (1976)]. However, since the original study, some researchers use a 'subjective value' of 200 to 250 as an healthy score.

[0040] Systemic Lupus Erythematosus (SLE) is an autoimmune disease caused by recurrent injuries to blood vessels in multiple organs, including the kidney, skin, and joints. In patients with SLE, a faulty interaction between T cells and B-cells results in the production of autoantibodies that attack the cell nucleus. There is general agreement that autoantibodies are responsible for SLE, so new therapies that deplete the B-cell lineage, allowing the immune system to reset as new B-cells are generated from precursors, would offer hope for long lasting benefit in SLE patients.

[0041] Multiple sclerosis (MS) is also an autoimmune disease. It is characterized by inflammation of the central nervous system and destruction of myelin, which insulates nerve cell fibers in the brain, spinal cord, and body. Although the cause of MS is unknown, it is widely believed that autoimmune T cells are primary contributors to the pathogenesis of the disease. However, high levels of antibodies are present in the cerebral spinal fluid of patients with MS, and some theories predict that the B-cell response leading to antibody production is important for mediating the disease.

[0042] Autoimmune thyroid disease results from the production of autoantibodies that either stimulate the thyroid to cause hyperthyroidism (Graves' disease) or destroy the thyroid to cause hypothyroidism (Hashimoto's thyroiditis). Stimulation of the thyroid is caused by autoantibodies that bind and activate the thyroid stimulating hormone (TSH) receptor. Destruction of the thyroid is caused by autoantibodies that react with other thyroid antigens.

[0043] Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands.

[0044] Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction.

[0045] Myasthenia Gravis (MG) is a chronic autoimmune neuromuscular disorder characterized by autoantibodies that bind to acetylcholine receptors expressed at neuromuscular junctions leading to weakness of the voluntary muscle groups.

[0046] Psoriasis, is characterized by autoimmune inflammation in the skin and also associated with arthritis in 30% of cases, scleroderma, inflammatory bowel disease, including Crohn's disease and ulcerative colitis,

[0047] Also contemplated is the treatment of idiopathic inflammatory myopathy (IIM), including dermatomyositis (DM) and polymyositis (PM). Inflammatory myopathies have been categorized using a number of classification schemes. Miller's classification schema (Miller, *Rheum Dis Clin North Am.* 20:811-826, 1994) identifies 2 idiopathic inflammatory myopathies (IIM), polymyositis (PM) and dermatomyositis (DM).

[0048] Polymyositis and dermatomyositis are chronic, debilitating inflammatory diseases that involve muscle and, in the case of DM, skin. These disorders are rare, with a reported annual incidence of approximately 5 to 10 cases per million adults and 0.6 to 3.2 cases per million children per year in the United States (Targoff, *Curr Probl Dermatol.* 1991, 3:131-180). Idiopathic inflammatory myopathy is associated with significant morbidity and mortality, with up to half of affected adults noted to have suffered significant impairment (Gottdiener et al., *Am J Cardiol.* 1978, 41:1141-49). Miller (*Rheum Dis Clin North Am.* 1994, 20:811-826 and *Arthritis and Allied Conditions*, Ch. 75, Eds. Koopman and Moreland, Lippincott Williams and Wilkins, 2005) sets out five groups of criteria used to diagnose IIM, i.e., Idiopathic Inflammatory Myopathy Criteria (IIMC) assessment, including muscle weakness, muscle biopsy evidence of degeneration, elevation of serum levels of muscle-associated enzymes, electromagnetic triad of myopathy, evidence of rashes in dermatomyositis, and also includes evidence of autoantibodies as a secondary criteria.

[0049] IIM associated factors, including muscle-associated enzymes and autoantibodies include, but are not limited to, creatine kinase (CK), lactate dehydrogenase, aldolase, C-reactive protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and antinuclear autoantibody (ANA), myositis-specific antibodies (MSA), and antibody to extractable nuclear antigens.

[0050] A "binding molecule" according to the invention can be, for example, a protein (a "protein" may be polypeptide or peptide), nucleic acid, carbohydrate, lipid, or small molecule compound that binds to a target. A type of proteinaceous binding molecule contemplated by the invention is an antibody or an antibody fragment that retains binding activity. A binding molecule may be modified according to methods standard in the art to improve its binding affinity, diminish its immunogenicity, alter its effector functions and/or improve its availability in the body of an individual. Such modifications may include, for example, amino acid sequence modifications or expression as a fusion protein. Such fusion proteins are also binding molecules according to the invention. An exemplary binding molecule of the invention is a small modular immunopharmaceutical (SMIP™).

[0051] A binding molecule that is "specific" for a target binds to that target with a greater affinity than any other target. For example, a CD37-specific binding molecule binds to CD37 with a greater affinity than to any other target and a CD20-specific binding molecule binds to CD20 with a greater affinity than to any other target. Binding molecules of the invention may have affinities for their targets of a Ka of greater than or equal to about 10^4 M^{-1}, preferably of greater than or equal to about 10^5 M^{-1}, more preferably of greater than or equal to about 10^6 M^{-1} and still more preferably of greater than or equal to about 10^7 M^{-1}. Affinities of even greater than about 10^7 M^{-1} are still more preferred, such as affinities equal to or greater than about 10^7 M^{-1}, about 10^8 M^{-1}, and about 10^9 M^{-1}, and about 10^{10} M^{-1}. Affinities of binding molecules according to the present invention can be readily determined using conventional techniques, for example those described by Scatchard et al., Ann. N.Y. Acad. Sci. 51:660 (1949).

[0052] Certain CD37-specific binding molecules contemplated by the invention have affinities for CD37 of about 0.5 to about 10nM. Certain CD20-specific binding molecules contemplated by the invention have affinities for CD20 of about 1 to about 30 nM.

[0053] Another characteristic of certain CD37-binding molecules and CD20-binding molecules contemplated by the invention is they exhibit a half life in circulation of about 7 to about 30 days.

[0054] CD37-specific antibodies that characterized the CD37 antigen in the Thrid HLDA Workshop were HD28, G28-1, HH1, BI14, WR17 and F93G6. See, Ling and MacLennan, pp. 302-335 in Leucocyte Typing III. White Cell Differentiation Antigens, Oxford University Press (1987). Other CD37-specific antibodies that have been described include RFB-7, Y29/55, MB-1, M-B371, M-B372 and IPO-24. See, Moldenhaurer, J. Biol., Regul. Homeost. Agents, 14: 281-283 (2000) which states that all these antibodies recognize only one CD37 epitope. Schwartz-Albiez et al., 14: 905-914 (1988) indicates that the epitope is situated in the carbohydrate moiety of CD37. Another CD37-specific antibody is S-B3 (Biosys).

[0055] Patents and patent publications describing CD20 antibodies include U.S. Pat. Nos. 5,776,456, 5,736,137, 6,399,061, and 5,843,439, as well as US patent application Nos. US 2002/0197255A1 and US 2003/0021781A1 (Anderson et al.); U.S. Pat. No. 6,455,043B1 and WO00/09160 (Grillo-Lopez, A.); WO00/27428 (Grillo-Lopez and White); WO00/27433 (Grillo-Lopez and Leonard); WO00/44788 (Braslawsky et al.); WO01/10462 (Rastetter, W.); WO01/10461 (Rastetter and White); WO01/10460 (White and Grillo-Lopez); US appln No. US2002/0006404 and WO02/04021 (Hanna and Hariharan); US appln No. US2002/0012665 A1 and WO01/74388 (Hanna, N.); US appln No. US2002/0009444A1, and WO01/80884 (Grillo-Lopez, A.); WO01/97858 (White, C.); US appln No. US2002/0128488A1 and WO02/34790 (Reff, M.);WO02/060955 (Braslawsky et al.);WO02/096948 (Braslawsky et al.);WO02/079255 (Reff and Davies); U.S. Pat. No. 6,171,586B1, and WO98/56418 (Lam et al.); WO98/58964 (Raju, S.); WO99/22764 (Raju, S.);WO99/51642, U.S. Pat. No. 6,194,551B1, U.S. Pat. No. 6,242,195B1,

U.S. Pat. No. 6,528,624B1 and U.S. Pat. No. 6,538,124 (Idusogie et al.); WO00/42072 (Presta, L.); WO00/67796 (Curd et al.); WO01/03734 (Grillo-Lopez et al.); US appln No. US 2002/0004587A1 and WO01/77342 (Miller and Presta); US appln No. US2002/0197256 (Grewal, I.); U.S. Pat. Nos. 6,090,365B1, 6,287,537B1, 6,015,542, 5,843,398, and 5,595,721, (Kaminski et al.); U.S. Pat. Nos. 5,500,362, 5,677,180, 5,721,108, and 6,120,767 (Robinson et al.); U.S. Pat No. 6,410,391B1 (Raubitschek et al.); U.S. Pat. No. 6,224,866B1 and WO00/20864 (Barbera-Guillem, E.); WO01/13945 (Barbera-Guillem, E.); WO00/67795 (Goldenberg); WO00/74718 (Goldenberg and Hansen); WO00/76542 (Golay et al.);WO01/72333 (Wolin and. Rosenblatt); U.S. Pat. No. 6,368,596B1 (Ghetie et al.); US Appln No. US2002/0041847A1, (Goldenberg, D.); US Appln no. US2003/0026801A1 (Weiner and Hartmann); WO02/102312 (Engleman, E.), each of which is expressly incorporated herein by reference. See, also, U.S. Pat. No. 5,849,898 and EP appln No. 330,191 (Seed et al.); U.S. Pat. No. 4,861,579 and EP332,865A2 (Meyer and Weiss); and WO95/03770 (Bhat et al.).

[0056] Rituximab has been approved for human clinical use as Rituxan®. Rituxan® is considered to be a CD20-specific binding molecule of the invention.

[0057] Small, modular immunopharmaceuticals (SMIPs) are considered to be one type of binding molecules of the invention. Methods for making SMIPs have been described previously in co-owned U.S. application no. 10/627,556 and US Patent Publ. 20030133939, 20030118592, and 20050136049, which are incorporated herein by reference in their entirety. SMIPs are novel binding domain-immunoglobulin fusion proteins that generally feature a binding domain for a cognate structure such as an antigen, a counterreceptor or the like, an IgG1, IGA or IgE hinge region polypeptide or a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues, and immunoglobulin CH2 and CH3 domains. In one embodiment, the binding domain molecule has one or two cysteine (Cys) residues in the hinge region. In a related embodiment, when the binding domain molecule comprises two

Cys residues, the first Cys, which is involved in binding between the heavy chain and light chain, is not deleted or substituted with an amino acid.

[0058] The binding domain of molecules useful in methods of the invention are contemplated as having one or more binding regions, such as variable light chain and variable heavy chain binding regions derived from one or more immunoglobulin superfamily members, such as an immunoglobulin. These regions, moreover, are typically separated by linker peptides, which may be any linker peptide known in the art to be compatible with domain or region joinder in a binding molecule. Exemplary linkers are linkers based on the Gly_4Ser linker motif, such as $(Gly_4Ser)_n$, where n=1-5. The molecules for use in the methods of the invention also contain sufficient amino acid sequence derived from a constant region of an immunoglobulin to provide an effector function, preferably ADCC and/or CDC. Thus, the molecules will have a sequence derived from a CH2 domain of an immunoglobulin or CH2 and CH3 domains derived from one or more immunoglobulins. SMIPs are capable of ADCC and/or CDC but are compromised in their ability to form disulfide-linked multimers.

[0059] The invention includes humanized CD37-specific SMIP polypeptides that exhibit at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37. In one aspect, the humanized CD37-specific SMIP polypeptides comprise any amino acid sequence selected from the group consisting of SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 80, 82, 84, 86, and 88. In another aspect, the humanized CD37-specific SMIP polypeptides comprise at least one amino acid modification in a complementarity-determining region (CDR) selected from the group consisting of: light chain CDR1, heavy chain CDR1, light chain CDR2, heavy chain CDR2, light chain CDR3, and heavy chain CDR3.

[0060] In one embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR1 of the light chain comprises the amino acid sequence of SEQ ID NO: 61 (RASENVYSYLA). The invention also includes a humanized CD37-specific SMIP polypeptide, wherein CDR1 of

the light chain comprises the amino acid sequence of SEQ ID NO: 62 (RTSENVYSYLA). The invention further includes a humanized CD37-specific SMIP polypeptide, wherein CDR1 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 63 (GYMNM).

[0061] In another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR2 of the light chain comprises the amino acid sequence of SEQ ID NO: 64 (FAKTLAE). The invention also includes a humanized CD37-specific SMIP polypeptide, wherein CDR2 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 65 (NIDPYYGGTTTYNRKFKG).

[0062] In a further embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 66 (QHHSDNPWT). The invention further includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 67 (SVGPFDY). The invention further includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 68 (SVGPFDS). The invention also includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 69 (SVGPMDY).

[0063] In another aspect, the invention includes a humanized CD37-specific SMIP polypeptide comprising at least one, at least two, or at least three sequence(s) of the light chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 61, 62, 64, and 66. In yet another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide comprising a light chain CDR1 amino acid sequence of SEQ ID NOS: 61 or 62, or a variant thereof in which one or two amino acids of SEQ ID NOS: 61 or 62 has been changed; a light chain CDR2 amino acid sequence of SEQ ID NO: 64, or a variant thereof in which one or two amino acids of SEQ ID NO: 64 has been changed; and a light chain CDR3 amino acid sequence of SEQ ID NO: 66, or a variant thereof in which one or two amino acids of SEQ ID NO: 66 has been changed.

[0064] In still another aspect, the invention includes a humanized CD37-specific SMIP polypeptide comprising at least one, at least two, or at least three of the heavy chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 63, 65, and 67-69. In a further embodiment, the invention includes a humanized CD37-specific SMIP polypeptide comprising a heavy chain CDR1 amino acid sequence of SEQ ID NO: 63, or a variant thereof in which one or two amino acids of SEQ ID NO: 63 has been changed; a heavy chain CDR2 amino acid sequence of SEQ ID NO: 65, or a variant thereof in which one or two amino acids of SEQ ID NO: 65 has been changed; and a heavy chain CDR3 amino acid sequence selected from the group consisting of SEQ ID NOS: 67-69, or a variant thereof in which one or two amino acids of any one of SEQ ID NOS: 67-69 has been changed.

[0065] The invention also includes humanized CD37-specific SMIP polypeptides comprising at least one amino acid modification in a framework region (FR) selected from the group consisting of: light chain FR1, heavy chain FR1, light chain FR2, heavy chain FR2, light chain FR3, heavy chain FR3, light chain FR4, and heavy chain FR4. In one embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein the first framework region (FR1) of the light chain comprises the amino acid sequence of SEQ ID NO: 70 (EIVLTQSPATLSLSPGERATLSC). In another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR1 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 71 (EVQLVQSGAEVKKPGESLKISCKGSGYSFT). In still another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR2 of the light chain comprises the amino acid sequence of SEQ ID NO: 72 (WYQQKPGQAPRLLIY). In a further embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR2 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 73 (WVRQMPGKGLEWMG). In yet another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 74 (GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC). In yet another embodiment, the invention includes a humanized CD37-specific

SMIP polypeptide, wherein FR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 75 (QVTISADKSISTAYLQWSSLKASDTAMYYCAR). In yet another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the light chain comprises the amino acid sequence of SEQ ID NO: 76 (FGQGTKVEIK). In yet another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 77 (WGQGTLVTVSS). In yet another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 78 (WGRGTLVTVSS).

[0066] The invention further includes humanized CD37-specific SMIP polypeptides comprising at least one, at least two, or at least three sequence(s) of the light chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 70, 72, 74, and 76. In one embodiment, the invention includes a humanized CD37-specific SMIP polypeptide comprising a light chain FR1 amino acid sequence of SEQ ID NO: 70, or a variant thereof in which one or two amino acids of SEQ ID NO: 70 has been changed; a light chain FR2 amino acid sequence of SEQ ID NO: 72, or a variant thereof in which one or two amino acids of SEQ ID NO: 72 has been changed; a light chain FR3 amino acid sequence of SEQ ID NO: 74, or a variant thereof in which one or two amino acids of SEQ ID NO: 74 has been changed; and a light chain FR4 amino acid sequence of SEQ ID NO: 76, or a variant thereof in which one or two amino acids of SEQ ID NO: 76 has been changed.

[0067] In addition, the invention includes humanized CD37-specific SMIP polypeptides comprising at least one, at least two, or at least three sequence(s) of the heavy chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 71, 73, 75, 77, and 78. In one embodiment, the invention includes a humanized CD37-specific SMIP polypeptide comprising a heavy chain FR1 amino acid sequence of SEQ ID NO: 71, or a variant thereof in which one or two amino acids of SEQ ID NO: 71 has been changed; a heavy chain FR2 amino acid sequence of SEQ ID NO: 73, or a

variant thereof in which one or two amino acids of SEQ ID NO: 73 has been changed; a heavy chain FR3 amino acid sequence of SEQ ID NO: 75, or a variant thereof in which one or two amino acids of SEQ ID NO: 75 has been changed; and a heavy chain FR4 amino acid sequence of SEQ ID NOS: 77 or 78, or a variant thereof in which one or two amino acids of SEQ ID NOS: 77 or 78 has been changed.

[0068] The invention also includes an isolated nucleic acid molecule comprising a nucleotide sequence encoding a humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37. Such an isolated nucleic acid molecule may comprise a nucleotide sequence selected from the group consisting of: SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 79, 81, 83, 85, and 87. In one embodiment, the invention includes vectors that comprise these nucleic acid molecules and host cells that comprise the vectors.

[0069] The invention also includes processes of producing the polypeptides described herein, comprising culturing the host cells under suitable conditions to express the polypeptides, and optionally isolating the polypeptides from the culture.

[0070] In yet another aspect, the invention includes compositions comprising the humanized CD37-specific SMIP polypeptides of the invention and a pharmaceutically acceptable carrier.

[0071] The invention further includes using the CD37-specific SMIP or CD37-specific binding molecules described herein in any of the methods of the invention. Such methods include the use of any of the CD37-specific SMIP or CD37-specific binding molecule comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 80, 82, 84, 86, and 88.

[0072] In yet another aspect, the invention includes kits for reducing B-cells comprising the compositions of the invention; and protocols for using the kits to reduce B cells. Such kits may further comprise one or more CD20-specific binding molecule(s). The invention contemplates that such a CD20-specific binding molecule is TRU-015.

[0073] The invention also includes humanized CD37-specific SMIP polypeptides comprising a CDR1, a CDR2, and a CDR3, that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2. Such CD37-specific SMIP polypeptides may further comprise a human framework domain separating each of CDR1, CDR2, and CDR3.

[0074] In another aspect, the invention includes a humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37 and comprises a hinge region polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 90, 92, 94, 96, 98, 100, 102, 104, 106, 108 110, 112, 114, 115, 116, 118, 120, 122, 124, 126 and 127.

[0075] The invention also contemplates a humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37 and comprises a linker comprising (Gly4Ser)n, wherein n is 1, 2, 3, 4, 5, or 6.

[0076] In still a further aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR1 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 128 (RTSQNVYSYLA), 129 (RTSESVYSYLA), 130 (RASQSVYSYLA), 131 (RASQSVSSYLA) and 132 (RASQSVSYYLA). In another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR1 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 133 (SYMNM) and 134 (SYWIG). In a further embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR2 of the light chain comprises the amino acid

sequence selected from the group consisting of SEQ ID NOS: 135 (AASSLQS), 136 (GASTRAT) and 137 (DASNRAT). In still another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR2 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 138 (IIYPGDSDTRYSPSFQG) and 139 (RIDPSDSYTNYSPSFQG).

[0077] The invention also includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 220 (QHHSDNPWT). In another embodiment, the invention includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 211 (SVGPMDY), 212 (SVGPFDY), 213 (SVGPMDV), 214 (SVGPFDS), 215 (SVGPFDP), 216 (SVGPFQH), 217 (SVGPFDV), 218 (SVGPFDI) and 219 (SVGPFDL).

[0078] In still a further aspect, the invention includes CD37-specific SMIP polypeptides with alternative framework regions. In one aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR1 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 170-181. In another aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR1 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 140-146. In a still further aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR2 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 182-193. In yet another aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR2 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 147-153. In an additional aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR3 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 194-205. In yet another aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR3 of the

heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 154-160. In a further aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 206-210. In yet another aspect, the invention includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 161-169.

[0079] Exemplary CD37-specific SMIPs useful in the invention include, but are not limited to: G28-1 scFv (SSS-S) H WCH2 WCH3, consists of a G28-1 single chain Fv in which all three cysteine residues in the connection or hinge regions are mutated to serine residues, and wild type CH2 and CH3 domains; G28-1 scFv IgAH WCH2 WCH3, comprising an IgA hinge and WT IgG1 domains; G28-1 scFv VHL11S (SSS-S) H WCH2 CH3 in which all three cysteine residues in the connection or hinge regions are mutated to serine residues and the leucine at position 11 of the heavy chain variable region is substituted with a serine; G28-1 scFv VH L11S (CSS-S) H WCH2 CH3, in which cysteine residues were substituted at the second and third positions with serine; G28-1 scFv VHL11S (CSC-S) H WCH2 CH3, in which cysteine residues were substituted at the second position with serine; G28-1 scFv VH11S (SSC-P) H WCH2 WCH3 (referred to as TRU-016 herein), in which the first and second cysteine residues in the connection or hinge regions are mutated to serine residues and the leucine at position 11 of the heavy chain variable region is substituted with a serine; G28-1 scFv VH11S (SCS-S) H WCH2 WCH3, in which the first and third cysteine residues in the hinge regions are mutated to serine residues; G28-1 scFv VHL11S (CCS-P) H WCH2 WCH3, in which the third cysteine residue in the hinge region is substituted with a serine; G28-1scFv VHL11S (SCC-P) H WCH2 WCH3, in which the first cysteine is substituted with a serine; G28-1 scFv VH L11S mIgE CH2 CH3 CH4, comprising mouse IgE CH 2-4 regions in which the leucine at position 11 of the heavy chain variable region is substituted with a serine; G28-1 scFv VH L11S mIgA WIgACH2 T4CH3, comprising a mouse IgA hinge with a wild type IgA CH2 and a truncated IgA CH3 domain lacking

the 4 carboxy amino acids GTCY; G28-1 scFv VHL11S hIgE CH2 CH3 CH4, comprising IgE CH regions in which the leucine at position 11 of the heavy chain variable region is substituted with a serine; and G28-1 scFv VHL11S hIgAH WIgACH2 TCH3, comprising an IgA hinge, a wild type IgA CH2 and a truncated IgA CH2 and a truncated IgA CH3 domain lacking the 4 carboxy amino acids GTCY.

[0080] Exemplary CD20-specific SMIPs useful in the invention include SMIPs derived from the anti-CD20 monoclonal antibody 2H7 described in US Patent Publ. 2003133939. and 20030118592. The SMIPs include 2H7scFv-Ig or a derivative thereof. Derivatives includes CytoxB-MHWTG1C, which has a human IgG1 Fc domain and a mutant IgG1 hinge domain; CytoxB-MHMG1C, which comprises a mutated Fc domain; MG1H/MG1C, which comprises an Fc receptor with a mutated leucine residue 234; CytoxB-IgAHWTHG1C, comprising a portion of the human IgA hinge fused to wild-type human Fc domain; 2H7 scFv-llama IgG1, comprising the llama IgG1 hinge and CH2CH3 regions, 2H7 scFv-llama IgG2, comprising the llama IgG2 hinge and CH2CH3 regions; 2H7 scFv-llama IgG3, comprising the llama IgG3 hinge and CH2CH3 regions.

[0081] 2H7 scFv MTH (SSS) WTCH2CH3, in which all three cysteine residues in the connection or hinge regions are mutated to serine residues, and wild type CH2 and CH3 domains; 2H7 scFv MTH (SSC), in which the first two cysteine residues were substituted with serine residues; 2H7 scFv MTH (SCS), in which the first and third cysteines were substituted with serine residues; 2H7 scFv MTH (CSS) WTCH2CH3, in which cysteine residues were substituted at the second and third positions with serine; 2H7 scFv VH11SER IgG MTH (SSS) WTCH2CH3, in which the leucine at position 11 in the heavy chain variable region is substituted with serine; 2H7 scFv IgA hinge-IgG1 CH2-CH3, comprising an IgA hinge region and WT IgG1 domains; 2H7 scFv IgA hinge-CH2-CH3, comprising IgA hinge, CH2-3 regions; 2H7 IgAWH IgACH2-T4CH3, comprising an IgA hinge, a wild type IgA CH2 and a truncated IgA CH3 domain lacking the 4 carboxy amino acids GTCY.

[0082] Derivatives with mutations in the IgG CH3 region include 2H7 scFv MTH WTCH2 MTCH3 Y405, in which phenylalanine residue at position 405 (numbering according to Kabat *et al. supra*) was substituted with tyrosine; 2H7 scFv MTH WTCH2 MTCH3 A405, in which phenylalanine position at 405 was substituted with an alanine; scFv MTH WTCH2 MTCH3 A407, in which tyrosine residue at position 407 was substituted with an alanine; scFv MTH WTCH2 MTCH3 Y405A407, comprising the two mutations; and scFv MTH WTCH2 MTCH3 A405A407 comprising two mutations.

[0083] 2H7 scFv MTH (CCS) WTCH2CH3 is a construct with the third cysteine residue in the IgG1 hinge region substituted with a serine residue. The 2H7 scFv IgG MTH (SSS) MTCH2WTCH3 SMIP comprises mutant hinge (MT (SSS)) and a mutant CH2 domain in which the proline at residue 238 (according to Ward *et al.*,) was substituted with a serine.

[0084] 2H7scFv-Ig derivatives also include 2H7 scFv mutants with point mutations in the variable heavy chain region. The following constructs all comprise mutations in which the leucine at position 11 in the heavy chain variable region is substituted with serine: 2H7 scFv VH11SER IgG MTH (SSS-S) WTCH2CH3, 2H7scFv VHL11S (CSS-S) H WCH2 WCH3, comprising a mutated hinge region as set out above; 2H7scFv VHL11S (CSC-S) H WCH2 WCH3 comprising a mutated hinge region as set out above; 2H7 scFv VHL11S IgAH IgACH2 T4CH3, comprises the IgA hinge, WT IgA CH2 and truncated IgA CH3; 2H7 scFv VHL11S IgECH2 CH3 CH4, comprising the IgE CH 2-4 regions; 2H7 VHL11S scFv (SSS-S) IgECH3CH4, comprising a mutated hinge region and IgE CH3 and CH4 regions; 2H7 scFv VH L11S mIgE CH2 CH3 CH4, comprises mouse IgE regions; 2H7 scFv VH L11S mIgAH WIGACH2 T4CH3 comprises the mutations described above and a mouse IgA constant region consisting of a wild type CH2 region and a mutated CH3 region; 2H7 scFv VH L11S (SSS-S) H K322S CH2 WCH3 comprises a mutation in the human IgG1 CH2 region at residue 322, where lysine was changed to serine; 2H7 scFv VH L11S (CSS-S) H K322S CH2 WCH3 comprises a mutated hinge region as described above, and a mutated CH2 region as previously described; 2H7 scFv VH L11S (SSS-S) H P331S

CH2 WCH3, comprises a mutated hinge region as described above, and a mutated CH2 region in which proline at residue 331 was changed to a serine; 2H7 scFv VH L11S (CSS-S) H P331S CH2 WCH3 comprises a mutated hinge region and a proline to serine mutation at residue 331 in the CH2 region; 2H7 scFv VH L11S (SSS-S) H T256N CH2 WCH3, comprises a mutated hinge region and a threonine to asparagine mutation at residue 256 in the CH2 region; 2H7 scFv VH L11S (SSS-S) H RTPE/QNAK (255-258) CH2 WCH3, comprises a mutated hinge region and a series of mutations in which residues 255-258 have been mutated from arginine, threonine, proline, glutamic acid to glutamine, asparagines, alanine and lysine, respectively; 2H7 scFv VH L11S (SSS-S) H K290Q CH2 WCH3, comprises a mutated hinge regions and a lysine to glutamine change at position 290; 2H7 scFv VH L11S (SSS-S) H A339P CH2 WCH3, comprises a mutated hinge region and an alanine to proline change at position 339; SMIP 2H7 scFv (SSS-S) H P238SCH2 WCH3, comprises a mutated hinge region and an proline to serine change at position 238 in CH2, which is the same as 2H7 scFv IgG MTH (SSS) MTCH2WTCH3. 2H7 scFv IgAH IGAHCH2 T18CH3 comprises a wild type IgA hinge and CH2 region and a CH3 region with an18 amino acid truncation at the carboxy end.

[0085] A binding molecule of the invention may comprise a native or engineered extracellular domain from another protein which improves the binding molecule activity. In one embodiment, the extracellular domain is selected from the group consisting of CD154 and CTLA4.

[0086] A "synergistic combination" of CD37-specific binding molecules and CD20-specific binding molecules is a combination that has an effect that is greater than the sum of the effects of the binding molecules when administered alone.

[0087] In one aspect of the invention, the binding molecules are administered in one or more pharmaceutical compositions. To administer the binding molecules to human or test animals, it is preferable to formulate the binding molecules in a composition comprising one or more pharmaceutically acceptable carriers. The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce

allergic, or other adverse reactions when administered using routes well-known in the art, as described below. "Pharmaceutically acceptable carriers" include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like.

[0088] In addition, compounds may form solvates with water or common organic solvents. Such solvates are contemplated as well.

[0089] The binding molecule compositions may be administered orally, topically, transdermally, parenterally, by inhalation spray, vaginally, rectally, or by intracranial injection. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramusclar, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and or surgical implantation at a particular site is contemplated as well. Generally, compositions are essentially free of pyrogens, as well as other impurities that could be harmful to the recipient. Injection, especially intravenous, is preferred.

[0090] Pharmaceutical compositions of the present invention containing binding molecules used in a method of the invention may contain pharmaceutically acceptable carriers or additives depending on the route of administration. Examples of such carriers or additives include water, a pharmaceutical acceptable organic solvent, collagen, polyvinyl alcohol, polyvinylpyrrolidone, a carboxyvinyl polymer, carboxymethylcellulose sodium, polyacrylic sodium, sodium alginate, water-soluble dextran, carboxymethyl starch sodium, pectin, methyl cellulose, ethyl cellulose, xanthan gum, gum Arabic, casein, gelatin, agar, diglycerin, glycerin, propylene glycol, polyethylene glycol, Vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose, a pharmaceutically acceptable surfactant and the like. Additives used are chosen from, but not limited to, the above or combinations thereof, as appropriate, depending on the dosage form of the present invention.

[0091] Formulation of the pharmaceutical composition will vary according to the route of administration selected (e.g., solution, emulsion). An appropriate composition comprising the antibody to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers

[0092] A variety of aqueous carriers, e.g., water, buffered water, 0.4% saline, 0.3% glycine, or aqueous suspensions may contain the active compound in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyl-eneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate.

[0093] The binding molecule compositions can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins. Any suitable lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can

lead to varying degrees of antibody activity loss and that use levels may have to be adjusted to compensate.

[0094] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active compound in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above.

[0095] The concentration of binding molecule in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. Thus, a typical pharmaceutical composition for parenteral injection could be made up to contain 1 mL sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 mL of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980). An effective dosage of antibody is within the range of 0.01 mg to 1000 mg per kg of body weight per administration.

[0096] The pharmaceutical compositions may be in the form of a sterile injectable aqueous, oleaginous suspension, dispersions or sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, vegetable oils, Ringer's solution and

isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0097] In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0098] Compositions useful for administration may be formulated with uptake or absorption enhancers to increase their efficacy. Such enhancers include for example, salicylate, glycocholate/linoleate, glycholate, aprotinin, bacitracin, SDS, caprate and the like. See, *e.g.*, Fix (*J. Pharm. Sci.*, 85:1282-1285, 1996) and Oliyai and Stella (*Ann. Rev. Pharmacol. Toxicol.*, 32:521-544, 1993).

[0099] In addition, the properties of hydrophilicity and hydrophobicity of the compositions contemplated for use in the invention are well balanced, thereby enhancing their utility for both *in vitro* and especially *in vivo* uses, while other compositions lacking such balance are of substantially less utility. Specifically, compositions contemplated for use in the invention have an appropriate degree of solubility in aqueous media which permits absorption and bioavailability in the body, while also having a degree of solubility in lipids which permits the compounds to traverse the cell membrane to a putative site

of action. Thus, antibody compositions contemplated are maximally effective when they can be delivered to the site of target antigen activity.

[00100] In one aspect, methods of the invention include a step of administration of a binding molecule composition.

[00101] Methods of the invention are performed using any medically-accepted means for introducing a therapeutic directly or indirectly into a mammalian individual, including but not limited to injections, oral ingestion, intranasal, topical, transdermal, parenteral, inhalation spray, vaginal, or rectal administration. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, and intracisternal injections, as well as catheter or infusion techniques. Administration by, intradermal, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and or surgical implantation at a particular site is contemplated as well.

[00102] In one embodiment, administration is performed at the site of a cancer or affected tissue needing treatment by direct injection into the site or via a sustained delivery or sustained release mechanism, which can deliver the formulation internally. For example, biodegradable microspheres or capsules or other biodegradable polymer configurations capable of sustained delivery of a composition (*e.g.*, a soluble polypeptide, antibody, or small molecule) can be included in the formulations of the invention implanted near the cancer.

[00103] Therapeutic compositions may also be delivered to the patient at multiple sites. The multiple administrations may be rendered simultaneously or may be administered over a period of time. In certain cases it is beneficial to provide a continuous flow of the therapeutic composition. Additional therapy may be administered on a period basis, for example, hourly, daily, weekly or monthly.

[00104] Binding molecule compositions of the invention may comprise one, or may comprise more than one, binding molecules. Also contemplated by the present invention is the administration of binding molecule compositions in

conjunction with a second agent. Second agents contemplated by the invention are listed in paragraphs below.

[00105] A second agent may be a B-cell-associated molecule. Other B-cell-associated molecules contemplated by the invention include binding molecules which bind to B-cell surface molecules that are not CD37 or CD20. B-cell-associated molecules, include but are not limited to, CD19 (B-lymphocyte antigen CD19, also referred to as B-lymphocyte surface antigen B4, or Leu-12), CD21, CD22 (B-cell receptor CD22, also referred to as Leu-14, B-lymphocyte cell adhesion molecule, or BL-CAM), CD23, CD40 (B-cell surface antigen CD40, also referred to as Tumor Necrosis Factor receptor superfamily member 5, CD40L receptor, or Bp50), CD80 (T lymphocyte activation antigen CD80, also referred to as Activation B7-1 antigen, B7, B7-1, or BB1), CD86 (T lymphocyte activation antigen CD86, also referred to as Activation B7-2 antigen, B70, FUN-1, or BU63), CD137 (also referred to as Tumor Necrosis Factor receptor superfamily member 9), CD152 (also referred to as cytotoxic T-lymphocyte protein 4 or CTLA-4), L6 (Tumor-associated antigen L6, also referred to as Transmembrane 4 superfamily member 1, Membrane component surface marker 1, or M3S1), CD30 (lymphocyte activation antigen CD30, also referred to as Tumor Necrosis Factor receptor superfamily member 8, CD30L receptor, or Ki-1), CD50 (also referred to as Intercellular adhesion molecule-3 (ICAM3), or ICAM-R), CD54 (also referred to as Intercellular adhesion molecule-1 (ICAM1), or Major group rhinovirus receptor), B7-H1 (ligand for an immunoinhibitory receptor expressed by activated T cells, B-cells, and myeloid cells, also referred to as PD-L1; see Dong, *et al.*, "B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion," *Nat. Med.*, 5:1365-1369 (1999), CD134 (also referred to as Tumor Necrosis Factor receptor superfamily member 4, OX40, OX40L receptor, ACT35 antigen, or TAX-transcriptionally activated glycoprotein 1 receptor), 41BB (4-1BB ligand receptor, T-cell antigen 4-1BB, or T-cell antigen ILA), CD153 (also referred to as Tumor Necrosis Factor ligand superfamily member 8, CD30 ligand, or CD30-L), CD154 (also referred to as Tumor Necrosis Factor ligand superfamily member 5, TNF-related activation protein, TRAP, or T cell antigen Gp39) and Toll

receptors. The above list of construct targets and/or target antigens is exemplary only and is not exhaustive.

[00106] Cytokines and growth factors are second agents contemplated by the invention and include, without limitation, one or more of TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin. Pharmaceutical compositions in accordance with the invention may also include other known angiopoietins, for example Ang-1, Ang-2, Ang-4, Ang-Y, and/or the human angiopoietin-like polypeptide, and/or vascular endothelial growth factor (VEGF). Growth factors for use in pharmaceutical compositions of the invention include angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor α, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil chemotactic factor 2α, cytokine-induced neutrophil chemotactic factor 2β, β endothelial cell growth factor, endothelin 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6, fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor 8b, fibroblast growth factor 8c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophic factor receptor α1, glial cell line-derived neutrophic factor receptor α2, growth related protein, growth related protein α, growth related protein β, growth related protein γ, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor,

leukemia inhibitory factor receptor α, nerve growth factor, nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor α, platelet derived growth factor receptor β, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor α, transforming growth factor β, transforming growth factor β1, transforming growth factor β1.2, transforming growth factor β2, transforming growth factor β3, transforming growth factor β5, latent transforming growth factor β1, transforming growth factor β binding protein I, transforming growth factor β binding protein II, transforming growth factor β binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

[00107] Examples of chemotherapeutic agents contemplated as second agents include, but are not limited to, alkylating agents, such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide, ifosfamide, melphalan, and chlorambucil); nitrosoureas (e.g., carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU)); ethyleneimines and methyl-melamines (e.g., triethylenemelamine (TEM), triethylene thiophosphoramide (thiotepa), and hexamethylmelamine (HMM, altretamine)); alkyl sulfonates (e.g., buslfan); and triazines (e.g., dacabazine (DTIC)); antimetabolites, such as folic acid analogs (e.g., methotrexate, trimetrexate, and pemetrexed (multi-targeted antifolate)); pyrimidine analogs (such as 5-fluorouracil (5-FU), fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, and 2,2'-difluorodeoxycytidine); and purine analogs (e.g, 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, 2-chlorodeoxyadenosine (cladribine, 2-CdA)); Type I topoisomerase inhibitors such as camptothecin (CPT), topotecan, and irinotecan; natural products,

such as epipodophylotoxins (e.g., etoposide and teniposide); and vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine); anti-tumor antibiotics such as actinomycin D, doxorubicin, and bleomycin; radiosensitizers such as 5-bromodeozyuridine, 5-iododeoxyuridine, and bromodeoxycytidine; platinum coordination complexes such as cisplatin, carboplatin, and oxaliplatin; substituted ureas, such as hydroxyurea; and methylhydrazine derivatives such as N-methylhydrazine (MIH) and procarbazine.

[00108] Non-limiting examples of chemotherapeutic agents, radiotherapeutic agents and other active and ancillary agents are also shown in Table 1.

TABLE 1

Alkylating agents
Nitrogen mustards
mechlorethamine
cyclophosphamide
ifosfamide
melphalan
chlorambucil

Nitrosoureas
carmustine (BCNU)
lomustine (CCNU)
semustine (methyl-CCNU)

Ethylenemine/Methyl-melamine
thriethylenemelamine (TEM)
triethylene thiophosphoramide (thiotepa)
hexamethylmelamine (HMM, altretamine)

Alkyl sulfonates
busulfan

Triazines
dacarbazine (DTIC)

Antimetabolites
Folic Acid analogs
methotrexate

Natural products
Antimitotic drugs

Taxanes
paclitaxel
Vinca alkaloids
vinblastine (VLB)
vincristine
vinorelbine
Taxotere® (docetaxel)
estramustine
estramustine phosphate

Epipodophylotoxins
etoposide
teniposide

Antibiotics
actimomycin D
daunomycin (rubido-mycin)
doxorubicin (adria-mycin)
mitoxantroneidarubicin
bleomycin
splicamycin (mithramycin)
mitomycinC
dactinomycin
aphidicolin

Trimetrexate
Pemetrexed
(Multi-targeted antifolate)

Pyrimidine analogs
5-fluorouracil
fluorodeoxyuridine
gemcitabine
cytosine arabinoside
(AraC, cytarabine)
5-azacytidine
2,2´- difluorodeoxy-cytidine

Purine analogs
6-mercaptopurine
6-thioguanine
azathioprine
2'-deoxycoformycin
(pentostatin)
erythrohydroxynonyl-adenine (EHNA)
fludarabine phosphate
2-chlorodeoxyadenosine
(cladribine, 2-CdA)

Type I Topoisomerase Inhibitors
camptothecin
topotecan
irinotecan

Biological response modifiers
G-CSF
GM-CSF

Differentiation Agents
retinoic acid derivatives

Hormones and antagonists
Adrenocorticosteroids/ antagonists
prednisone and equivalents
dexamethasone
ainoglutethimide

Progestins
hydroxyprogesterone caproate
medroxyprogesterone acetate
megestrol acetate

Estrogens
diethylstilbestrol
ethynyl estradiol/ equivalents

Antiestrogen
tamoxifen

Enzymes
L-asparaginase
L-arginase

Radiosensitizers
metronidazole
misonidazole
desmethylmisonidazole
pimonidazole
etanidazole
nimorazole
RSU 1069
EO9
RB 6145
SR4233
nicotinamide
5-bromodeozyuridine
5-iododeoxyuridine
bromodeoxycytidine

Miscellaneous agents
Platinium coordination complexes
cisplatin
Carboplatin
oxaliplatin
Anthracenedione
mitoxantrone

Substituted urea
hydroxyurea

Methylhydrazine derivatives
N-methylhydrazine (MIH)
procarbazine

Adrenocortical suppressant
mitotane (*o,p´*- DDD)
ainoglutethimide

Cytokines
interferon (α, β, γ)
interleukin-2

Photosensitizers
hematoporphyrin derivatives
Photofrin®
benzoporphyrin derivatives
Npe6
tin etioporphyrin (SnET2)
pheoboride-a
bacteriochlorophyll-a
naphthalocyanines
phthalocyanines

zinc phthalocyanines

Androgens
testosterone propionate
fluoxymesterone/equivalents

Antiandrogens
flutamide
gonadotropin-releasing
hormone analogs
leuprolide

Nonsteroidal antiandrogens
flutamide

Radiation
X-ray
ultraviolet light
gamma radiation
visible light
infrared radiation
microwave radiation

[00109] Second agents contemplated by the invention for treatment of autoimmune diseases are referred to as immunosuppressive agents, which act to suppress or mask the immune system of the individual being treated. Immunosuppressive agents include, for example, non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, glucocorticoids, disease-modifying antirheumatic drugs (DMARDs) for the treatment of arthritis, or biologic response modifiers. Compositions in the DMARD description are also useful in the treatment of many other autoimmune diseases aside from RA.

[00110] Exemplary NSAIDs are chosen from the group consisting of ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors such as Vioxx and Celebrex, and sialylates. Exemplary analgesics are chosen from the group consisting of acetaminophen, oxycodone, tramadol of proporxyphene hydrochloride. Exemplary glucocorticoids are chosen from the group consisting of cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include, but are not limited to, molecules directed against cell surface markers (e.g., CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists (e.g. etanercept (Enbrel), adalimumab (Humira) and infliximab (Remicade)), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include, but are not limited to, azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine,

leflunomide, sulfasalazine, hydroxychloroquine, Gold [oral (auranofin) and intramuscular] and minocycline.

[00111] It is contemplated the binding molecule composition and the second agent may be given simultaneously in the same formulation. Alternatively, the agents are administered in a separate formulation but concurrently, with concurrently referring to agents given within 30 minutes of each other.

[00112] In another aspect, the second agent is administered prior to administration of the binding molecule composition. Prior administration refers to administration of the second agent within the range of one week prior to treatment with the antibody, up to 30 minutes before administration of the antibody. It is further contemplated that the second agent is administered subsequent to administration of the binding molecule composition. Subsequent administration is meant to describe administration from 30 minutes after antibody treatment up to one week after antibody administration.

[00113] It is further contemplated that when the binding molecule is administered in combination with a second agent, wherein the second agent is a cytokine or growth factor, or a chemotherapeutic agent, the administration may also include use of a radiotherapeutic agent or radiation therapy. The radiation therapy administered in combination with an antibody composition is administered as determined by the treating physician, and at doses typically given to patients being treated for cancer.

[00114] The amounts of binding molecule in a given dose will vary according to the size of the individual to whom the therapy is being administered as well as the characteristics of the disorder being treated. In exemplary treatments, it may be necessary to administer about 1 mg/day, about 5 mg/day, about 10 mg/day, about 20 mg/day, about 50 mg/day, about 75 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 250 mg/day, about 500 mg/day or about 1000 mg/day. The doses may also be administered based on weight of the patient, at a dose of about 0.01 to about 50 mg/kg. In a related embodiment, the binding molecule may be administered in a dose range of about 0.015 to about 30 mg/kg. In an

additional embodiment, the binding molecule is administered in a dose of about 0.015, about 0.05, about 0.15, about 0.5, about 1.5, about 5, about 15 or about 30 mg/kg.

[00115] These compositions may be administered in a single dose or in multiple doses. Standard dose-response studies, first in animal models and then in clinical testing, reveal optimal dosages for particular disease states and patient populations.

[00116] The administration of the binding molecule composition decreases the B-cell population by at least 20% after a single dose of treatment. In one embodiment, the B-cell population is decreased by at least about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90 or about 100%. B-cell reduction is defined as a decrease in absolute B-cell count below the lower limit of the normal range. B-cell recovery is defined as a return of absolute B-cell count to either of the following: 70% of subject's baseline value or normal range.

[00117] The administration of CD20-specific binding molecules also results in enhanced apoptosis in particular B-cell subsets. Apoptosis refers to the induction of programmed cell death of a cell, manifested and assessed by DNA fragmentation, cell shrinkage, cell fragmentation, formation of membrane vesicles, or alteration of membrane lipid composition as assessed by annexin V staining.

[00118] Further, the administration of binding molecule compositions of the invention results in desired clinical effects in the disease or disorder being treated. For example, in patients affected by rheumatoid arthritis, in one aspect the administration improves the patient's condition by a clinically significant amount [e.g., achieves the American College of Rheumatology Preliminary Detection of Improvement (ACR20)], and/or an improvement of 20% in tender and swollen joint and 20% improvement in 3/5 remaining ACR measures (Felson et al., *Arthritis Rheum.* 1995, 38:727-35). Biological measures for improvement in an RA patient after administration of CD37-specific and CD20-specific binding molecules include measurement of changes in cytokine levels, measured via protein or RNA levels. Cytokines of

interest include, but are not limited to, TNF-α, IL-1, interferons, Blys, and APRIL. Cytokine changes may be due to reduced B cell numbers or decreased activated T cells. In RA patients, markers relevant to bone turnover (bone resorption or erosion) are measured before and after administration of CD20-specific binding molecules. Relevant markers include, but are not limited to, alkaline phosphatase, osteocalcin, collagen breakdown fragments, hydroxyproline, tartrate-resistant acid phosphatase, and RANK ligand (RANKL). Other readouts relevant to the improvement of RA include measurement of C reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), rheumatoid factor, CCP (cyclic citrullinated peptide) antibodies and assessment of systemic B cell levels and lymphocyte count via flow cytometry. Specific factors can also be measured from the synovium of RA patients, including assessment of B cell levels in synovium from synovium biopsy, levels of RANKL and other bone factors and cytokines set out above.

[00119] In a related aspect, the effects of combination administration on other diseases is measured according to standards known in the art. For example, it is contemplated that Crohn's disease patients treated according to the invention achieve an improvement in Crohn's Disease Activity Index (CDAI) in the range of about 50 to about 70 units, wherein remission is at 150 units (Simonis et al, *Scand. J Gastroent.* 1998, 33:283-8). A score of 150 or 200 is considered normal, while a score of 450 is considered a severe disease score. It is further desired that administration of the CD37-specific and CD20-specific binding molecules results in a reduction in perinuclear anti-neutrophil antibody (pANCA) and anti-Saccharomyces cervisiae antibody (ASCA) in individuals affected by inflammatory bowel disease.

[00120] It is further contemplated that adult and juvenile myositis patients treated according to the invention achieve an improvement in core set of evaluations, such as 3 out of 6 of the core set measured improved by approximately 20%, with not more than 2 of the core measurements worse by approximately 25% (see Rider et al., *Arthritis Rheum.* 2004, 50:2281-90).

[00121] It is further contemplated that SLE patients treated according to the invention achieve an improvement in Systemic Lupus Activity Measure

(SLAM) or SLE Disease Activity Index (SLEDAI) score of at least 1 point (Gladman et al, *J Rheumatol* 1994, 21:1468-71) (Tan et al., Arthritis Rheum. 1982, 25:1271-7). A SLAM score of >5, or SLEDAI score >2, is considered clinically active disease. A response to treatment may be defined as improvement or stabilization over the in 2 disease activity measures (the SLE Disease Activity Index [SLEDAI] and the Systemic Lupus Activity Measure) and 2 quality of life measures (patient's global assessment and the Krupp Fatigue Severity Scale) (Petri et al., *Arthritis Rheum.* 2004, 50:2858-68.) It is further contemplated that administration of the binding molecule to SLE patients results in a reduction in anti-double-stranded DNA antibodies. Alternatively, improvement may be gauged using the British Isles Lupus Assessment Group Criteria (BILAG).

[00122] It is further contemplated that multiple sclerosis patients treated according to the invention achieve an improvement in clinical score on the Kurtzke Expanded Disability status scale (EDSS) (Kurtzke, F., *Neurology* 1983, 33:1444-52) of at least 0.5, or a delay in worsening of clinical disease of at least 1.0 on the Kurtzke scale (Rudick et al., *Neurology* 1997, 49:358-63).

[00123] It is further contemplated that patients suffering from IIM receiving CD37-specific and CD20-specific binding molecules achieve a reduction in at least one of five criteria set out in the Idiopathic Inflammatory Myopathy Criteria (IIMC) assessment (Miller, F., *supra*). It is further contemplated that administration to IIM patients results in a reduction in IIM associated factors selected from the group consisting of creatine kinase (CK), lactate dehydrogenase, aldolase, C-reactive protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and antinuclear autoantibody (ANA), myositis-specific antibodies (MSA), and antibody to extractable nuclear antigens. Alternatively, patients meet 3 out of 6 of the criteria set out in Rider et al., Arthritis Rheum., 50(7):2281-2290 (2004), with worsening in no more than 2 criteria.

[00124] In some embodiments, patients suffering from a B cell cancer receive treatment according to the invention and demonstrate an overall beneficial response to the treatment, based on clinical criteria well-known and

commonly used in the art, and as described below, such as a decrease in tumor size, decrease in tumor number and/or an improvement in disease symptoms.

[00125] Exemplary clinical criteria are provided by the U.S. National Cancer Institute (NCI), which has divided some of the classes of cancers into the clinical categories of "indolent" and "aggressive" lymphomas. Indolent lymphomas include follicular cell lymphomas, separated into cytology "grades," diffuse small lymphocytic lymphoma/chronic lymphocytic leukemia (CLL), lymphoplasmacytoid/Waldenstrom's Macroglobulinemia, Marginal zone lymphoma and Hairy cell leukemia. Aggressive lymphomas include diffuse mixed and large cell lymphoma, Burkitt's lymphoma/diffuse small non-cleaved cell lymphoma, Lymphoblastic lymphoma, Mantle cell lymphoma and AIDS-related lymphoma. In some cases, the International Prognostic Index (IPI) is used in cases of aggressive and follicular lymphoma. Factors to consider in the IPI include Age (<60 years of age versus >60 years of age), serum lactate dehydrogenase (levels normal versus elevated), performance status (0 or 1 versus 2-4) (see definition below), disease stage (I or II versus III or IV), and extranodal site involvement (0 or 1 versus 2-4). Patients with 2 or more risk factors have less than a 50% chance of relapse-free and overall survival at 5 years.

[00126] Performance status in the aggressive IPI is defined as follows: Grade Description: 0 Fully active, able to carry on all pre-disease performance without restriction; 1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work; 2 Ambulatory and capable of all selfcare but unable to carry out any work activities, up to and about more than 50% of waking hours; 3 Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours; 4 Completely disabled, unable to carry on any selfcare, totally confined to bed or chair; and, 5 Dead. (See., The International Non-Hodgkin's Lymphoma Prognostic Factors Project. A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med. 329:987–94, 1993)

[00127] Typically, the grade of lymphoma is clinically assessed using the criterion that low-grade lymphoma usually presents as a nodal disease and is often indolent or slow-growing. Intermediate- and high-grade disease usually presents as a much more aggressive disease with large extranodal bulky tumors.

[00128] The Ann Arbor classification system is also used to measure progression of tumors, especially non-Hodgkins lymphomas. In this system, stages I, II, III, and IV of adult NHL can be classified into A and B categories depending on whether the patient has well-defined generalized symptoms (B) or not (A). The B designation is given to patients with the following symptoms: unexplained loss of more than 10% body weight in the 6 months prior to diagnosis, unexplained fever with temperatures above 38° C. and drenching night sweats. Definitions of the stages are as follows: Stage I-involvement of a single lymph node region or localized involvement of a single extralymphatic organ or site. Stage II-involvement of two or more lymph node regions on the same side of the diaphragm or localized involvement of a single associated extralymphatic organ or site and its regional lymph nodes with or without other lymph node regions on the same side of the diaphragm. Stage III-involvement of lymph node regions on both sides of the diaphragm, possibly accompanying localized involvement of an extralymphatic organ or site, involvement of the spleen, or both. Stage IV-disseminated (multifocal) involvement of one or more extralymphatic sites with or without associated lymph node involvement or isolated extralymphatic organ involvement with distant (non-regional) nodal involvement. For further details, see The International Non-Hodgkin's Lymphoma Prognostic Factors Project: A predictive model for aggressive non-Hodgkin's lymphoma, New England J. Med. (1993) 329:987-994.

[00129] In one aspect, a therapeutic effect of the methods according to the invention is determined by the level of response, for example a partial response is defined as tumor reduction to less than one-half of its original size. A complete response is defined as total elimination of disease confirmed by clinical or radiological evaluation. In one embodiment, the

individual receiving treatment according to the invention demonstrates at least a partial response to treatment.

[00130] According to the Cheson criteria for assessing NHL developed in collaboration with the National Cancer Institute (Cheson et al., *J Clin Oncol.* 1999, 17:1244; Grillo-Lopez et al., *Ann Oncol.* 2000, 11:399-408), a complete response is obtained when there is a complete disappearance of all detectable clinical and radiographic evidence of disease and disease-related symptoms, all lymph nodes have returned to normal size, the spleen has regressed in size, and the bone marrow is cleared of lymphoma.

[00131] An unconfirmed complete response is obtained when a patient shows complete disappearance of the disease and the spleen regresses in size, but lymph nodes have regressed by more than 75% and the bone marrow is indeterminate. An unconfirmed complete response meets and exceeds the criteria for partial response. An overall response is defined as a reduction of at least 50 percent in overall tumor burden.

[00132] Similar criteria have been developed for various other forms of cancers or hyperproliferative diseases and are readily available to a person of skill in the art. See, e.g., Cheson et al., *Clin Adv Hematol Oncol.* 2006, 4:4-5, which describes criteria for assessing CLL; Cheson et al., *J Clin Oncol.* 2003, 21:4642-9, which describes criteria for AML; Cheson et al., *Blood* 2000, 96:3671-4, which describes criteria for myelodysplastic syndromes.

[00133] In another aspect, a therapeutic response in patients having a B cell cancer is manifest as a slowing of disease progression compared to patients not receiving therapy. Measurement of slowed disease progression or any of the above factors may be carried out using techniques well-known in the art, including bone scan, CT scan, gallium scan, lymphangiogram, MRI, PET scans, ultrasound, and the like.

[00134] It will also be apparent that dosing may be modified if traditional therapeutics are administered in combination with therapeutics of the invention.

[00135] As an additional aspect, the invention includes kits which comprise one or more compounds or compositions useful in the methods of the invention packaged in a manner which facilitates their use to practice methods of the invention. In a simplest embodiment, such a kit includes a compound or composition described herein as useful for practice of a method of the invention packaged in a container such as a sealed bottle or vessel, with a label affixed to the container or included in the package that describes use of the compound or composition to practice the method of the invention. Preferably, the compound or composition is packaged in a unit dosage form. The kit may further include a device suitable for administering the composition according to a preferred route of administration or for practicing a screening assay. The kit may include a label that describes use of the binding molecule composition(s) in a method of the invention.

[00136] The present invention also comprises articles of manufacture. Such articles comprise CD37-specific binding molecules or CD37-specific and CD20-specific binding molecules, optionally together with a pharmaceutical carrier or diluent, and at least one label describing a method of use of the binding molecules according to the invention. Such articles of manufacture may also optionally comprise at least one second agent for administration in connection with the binding molecules.

[00137] The present invention also calls for use of a composition comprising a CD37-specific binding molecule or CD37-specific and CD20-specific binding molecules in the manufacture of a medicament for the treatment or prophylaxis of a disease involving aberrant B-cell activity.

BRIEF DESCRIPTION OF THE DRAWING

[00138] Figure 1A diagrams the structure of the TRU-016 molecule; Figure 1B shows the results of SDS-PAGE analysis, demonstrating that the expressed protein migrates at a Mr of approximately 110 kDa under nonreducing conditions, and approximately 52 kDa when subjected to reducing conditions; and Figure 1C shows that the TRU-016 molecule

demonstrates high level, specific binding to human peripheral blood B lymphocytes, and a much lower level of binding to other subpopulations of cells in the non-B cell lymphocyte gate (CD19 negative population) when analyzed by flow cytometry.

[00139] Figure 2A-E shows binding inhibition by different CD37 targeted reagents.

[00140] Figure 3A demonstrates FITC C1q binding to TRU-016 molecular forms incubated with Ramos B Cells in normal human serum with and without cobra venom factor (CVF); Figure 3B shows CDC activity of TRU-016 molecular forms incubated with Ramos B Cells in normal human serum with and without CVF; and Figure 3C shows CDC activity of TRU-016 molecular forms incubated with Ramos B cells and human or rabbit complement.

[00141] Figure 4 shows the HPLC size exclusion chromatography (SEC) traces obtained from GPC purification of the TRU-016, plotting absorbance versus retention time for the different fractions collected.

[00142] Figure 5A shows the binding properties of SEC fractions; Figure 5B shows the CDC activity of SEC fractions; and Figure 5C shows the ADCC activity of SEC fractions.

[00143] Figure 6 shows the CDC activity of TRU-015, rituxan, TRU-016, or a combination thereof on Ramos B cells.

[00144] Figure 7 shows that the effect of TRU-016 on CDC activity of TRU-015 on DHL-4 B cells.

[00145] Figure 8 shows the effect of TRU-016 on the CDC activity of TRU-015 and rituxan.

[00146] Figure 9 shows the effect of TRU-016 on TRU-015 in a CDC assay.

[00147] Figure 10 shows the effect of TRU-016 on rituxan in a CDC assay.

[00148] Figure 11 shows the interaction of TRU-015 and TRU-016 in an ADCC assay using BJAB cells.

[00149] Figure 12 shows the interaction of TRU-015 and TRU-016 in an ADCC assay using Daudi cells.

[00150] Figure 13 shows the interaction of TRU-015 and TRU-016 in an ADCC assay using Ramos cells.

[00151] Figure 14 shows the effect of rituxan, TRU-016, and a combination thereof on the specific killing of BJAB cells.

[00152] Figure 15 shows the effect of rituxan, TRU-016, and a combination thereof on the specific killing of BJAB cells.

[00153] Figure 16 shows the effect of TRU-015, TRU-016, and a combination thereof on the specific killing of BJAB cells.

[00154] Figure 17 shows the effect of TRU-015, TRU-016, and a combination thereof on the specific killing of BJAB cells.

[00155] Figure 18A-D shows that TRU-016 dimer forms do not mediate CDC alone, but potentiate the CDC activity of Rituximab *in vitro*.

[00156] Figure 19A-B demonstrates that protein A purified TRU-016 induces apoptosis of Ramos and Daudi cells, while dimer forms require crosslinking.

[00157] Figure 20 shows that TRU-016 preferentially depletes normal B cells from PBMC cultures.

[00158] Figure 21 demonstrates the efficacy of TRU-016 compared to huIgG, rituxan, and the combination treatment of TRU-016 and rituxan on tumor volume in animals.

[00159] Figure 22A and B shows that TRU-016 dimer forms exhibit significant anti-tumor activity, as measured by effect on tumor volume and percent survival in a mouse xenograft tumor model.

[00160] Figure 23 demonstrates that TRU-016 dimers do not augment CDC activity resulting from treatment with MHCII, CD19, CD80/86, or CD45 specific reagents.

[00161] Figure 24 shows the percent survival of mice with Ramos tumors (up to 90 days) after treatment with TRU-016, rituximab, or a combination thereof.

[00162] Figures 25 and 26 show the percent survival of mice with Daudi tumors (up to 90 days) after treatment with TRU-016 or rituximab.

[00163] Figure 27 shows that TRU-016 effectively reduced relative cell viability in cells treated with fludarabine, thereby potentiating the cytotoxic effect of fludarabine alone.

[00164] Figure 28 shows that TRU-016 induced greater cell toxicity than herceptin or rituximab in rituximab-resistant cell lines.

[00165] Figure 29 shows that TRU-016 induced tyrosine phosphorylation in CD19+ primary CLL B cells.

[00166] Figure 30A shows the consensus amino acid sequence of humanized TRU-016 construct no. 019001 (SEQ ID NO: 6) and TRU-016 (SEQ ID NO: 2) with Kabat numbering; Figure 30B shows amino acid sequence alignments of three humanized TRU-16 constructs (019001, 019008, and 109009).

[00167] Figure 31 shows the DNA and amino acid sequence alignments of three humanized constructs of TRU-016 (019001, 019041, and 019044).

[00168] Figure 32 shows the FASTA formatted sequence alignments of the same three humanized constructs of TRU-016 (019001, 019041, and 019044).

EXAMPLES

[00169] Additional aspects and details of the invention will be apparent from the following examples, which are intended to be illustrative rather than limiting. Example 1 describes the production of a CD37-specific binding molecule; Example 2 demonstrates that TRU-016 and various CD37-specific antibodies recognize the same or overlapping epitopes; Example 3 shows that TRU-016 is deficient in binding C1q and activating the classical complement

activation pathway; Example 4 demonstrates activity and binding of TRU-016 multimers; Example 5 describes the production of a CD20-specific binding molecule; Example 6 shows that combinations of TRU-016 with TRU-015 or rituxan synergistically increase apoptosis in B cells; Example 7 shows that combinations of TRU-016 with CD20-specific antibodies or SMIPs synergistically increase CDC; Example 8 demonstrates that TRU-016 augments the ADCC and the CDC activity of CD20-specific antibodies and SMIPS; Example 9 demonstrates that TRU-016 induces apoptosis in B cells; Example 10 shows that combinations of a CD37-specific SMIP with a CD20-specific antibody synergistically reduce tumor volume in a murine tumor xenograft model; Example 11 shows that a CD37-specific SMIP alone also reduces tumor volume in a murine tumor xenograft model; Example 12 demonstrates that TRU-016 does not affect the CDC activity of other B cell surface receptors; Example 13 demonstrates that TRU-016 does not augment the CDC activity of various targeted receptors, including MHCII, CD19, CD80/86, and CD40; Example 14 provides additional data showing that TRU-016 increases survival *in vivo* in mice with tumors; Example 15 demonstrates that TRU-016 potentiates fludarabine-induced cell death in CLL cells *in vitro*; Example 16 shows that TRU-016 induces direct cytotoxicity in rituximab-resistant cells; Example 17 shows that TRU-016 induces tyrosine phosphorylation in CD19+ primary CLL B cells; and Example 18 provides humanized TRU-016 molecules.

Example 1

Production of a CD37-Specific Binding Molecule

[00170] CD37-specific SMIPs are described in co-owned U.S. Application No. 10/627,556 and U.S. Patent Publication Nos. 2003/133939, 2003/0118592 and 2005/0136049. An exemplary SMIP, TRU-016, is produced as described below.

[00171] TRU-016 [G28-1 scFv VH11S (SSC-P) H WCH2 WCH3] is a recombinant single chain protein that binds to the CD37 antigen. The binding

domain was based on the G28-1 antibody sequence previously disclosed in the patent publications listed in the preceding paragraph, which disclosure is incorporated herein by reference. The binding domain is connected to the effector domain, the CH2 and CH3 domains of human IgG1, through a modified hinge region. TRU-016 exists as a dimer in solution and the dimer has a theoretical molecular weight of approximately 106,000 daltons.

[00172] Total RNA from the G28-1 hybridoma was isolated using Trizol RNA (Gibco) reagent according to the manufacturer's instructions. cDNA was prepared using 5 μg RNA, random primers and Superscript II Reverse Transcriptase (GIBCO BRL). The variable domains were cloned using pools of degenerate primers for the different murine VK or VH gene families. The variable domains from the G28-1 hybridoma were cloned into PCR 2.1 TOPO cloning vectors (Invitrogen) and DNA from transformants with correct size inserts was sequenced. Heavy and light chain variable regions from correct clones were then used as templates for sewing PCR amplification of a G28-1 scFv joined together in the VL-VH orientation with a 15 aa (gly4ser)3 linker. The anti-CD37 scFv was attached to a modified human IgG1 hinge, CH2, and CH3 domains (see Figure 1A). In order to ensure adequate expression by mammalian cells, modifications of the variable regions were selected that allowed significant increases in expression by mammalian cells. Specifically, a leucine was changed to a serine at position 11 of the scFV. The predicted mature peptide is 473 amino acids long.

[00173] The polynucleotide sequence encoding TRU-016 and the amino acid sequence of TRU-016 are respectively set out in SEQ ID NOs: 1 and 2.

[00174] TRU-016 was produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian cell expression system. Transfected CHO cells that produce the SMIP were cultured in a bioreactor using proprietary media.

[00175] TRU-016 SMIPs were purified from CHO culture supernatants by Protein A affinity chromatography. Using dPBS, a 50 mL rProtein A FF sepharose column (GE Healthcare rProtein A Sepharose FF, Catalog # 17-0974-04) was equilibrated at 5.0 mls/min (150 cm/hr) for 1.5 column volumes

(CV). The culture supernatant was loaded to the rProtein A Sepharose FF column at a flow rate of 1.7mls/min using the AKTA Explorer 100 Air (GE healthcare AKTA Explorer 100 Air, Catalog # 18-1403-00), capturing the recombinant TRU-016. The column was washed with dPBS for 5 Column Volumes (CV), then 1.0 M NaCl, 20mM Sodium Phosphate, pH 6.0, and then with 25 mM NaCl, 25mM NaOAc, pH 5.0. These washing steps removed nonspecifically bound CHO host cell proteins from the rProtein A column that contribute to product precipitation after elution.

[00176] The recombinant TRU-016 was eluted from the column with 100mM Glycine, pH 3.5. 10mL fractions of the eluted product were recovered and the eluted product was then brought to pH 5.0 with 20% of the eluted volume of 0.5 M 2-(N-Morpholino)ethanesulfonic acid (MES) pH6.0. This eluted product was prepared for GPC purification by concentration of the sample to approximately 25 mg/mL TRU-016 and then filter sterilized in preparation for GPC purification.

[00177] Purified protein was then subjected to GPC size exclusion chromatography (SEC) to achieve further purification of the TRU-016 (dimer) molecule from higher molecular weight aggregates. Using dPBS, an XK 50/100 column (GE healthcare XK 50/100 empty chromatography column, Catalog # 18-8753-01) containing 1 L of Superdex 200 FF sepharose was equilibrated at 12.6 mls/min (38cm/hr) for 1.5 column volumes (CV). A maximum volume of 54 mls (3% CV) of sample was applied to the column. The column continued to run at 12.6 ml/min and the eluted protein was fractionated in 40 mL fractions. Each fraction was analyzed for product quality using an analytic HPLC, and the eluted fractions were pooled for >95% POI (non-aggregated) TRU-016. This resultant pool was filter sterilized at 0.22μm. The material was then concentrated and formulated with 20 mM sodium phosphate and 240 mM sucrose, with a resulting pH of 6.0. The composition is filtered before filling into glass vials at a concentration of 10 mg/mL. Each glass vial contains 5 mL of TRU-016 (50 mg/vial).

[00178] TRU-016 protein was also subject to SDS-PAGE analysis on 4-20% Novex Tris-glycine gels (Invitrogen, San Diego, CA). Samples were

loaded using Novex Tris-glycine SDS sample buffer (2X) under reducing (addition of 1/10 volume NuPAGE sample reducing agent) or non-reducing conditions after heating at 95°C for 3 minutes, followed by electrophoresis at 150V for 90 minutes. Electrophoresis was performed using 1X Novex Tris-Glycine SDS Running Buffer (Invitrogen). Gels were stained after electrophoresis in Coomassie SDS PAGE R-250 stain for 30 minutes with agitation, and destained for at least one hour. The predicted molecular weight of the mature peptide is 51.5 kDa. Under reducing conditions, fusion protein migrates at the expected molecular weight. Under non-reducing conditions, the molecule migrates at approximately 150 kDa (Figure 1B).

[00179] Experiments were also performed to determine that the binding specificity of the parent antibody to the CD37 cell surface receptor is preserved in TRU-016. Human PBMCs were isolated over LSM density gradients and incubated with unconjugated TRU-016 and PE-conjugated anti-human CD19. Cells were washed and incubated with 1:100 FITC GAH IgG (Fc specific) for 45 minutes on ice. Cells were washed and analyzed by two-color flow cytometry on a FACsCalibur instrument using Cell Quest software. Cells were gated for B lymphocytes or non-B lymphocytes by CD19 staining.

[00180] With increasing concentrations of TRU-016, the FITC signal on the B lymphocyte (CD19 positive gate) increased rapidly from 0.01-1.0 μg/ml, until it reached saturation at approximately 1 μg/mL or a mean fluorescence intensity (MFI) of 1000. In contrast, the staining of the non-B lymphocyte population is detectable, but very low, and increases slowly with increasing concentration of scFvIg. Thus, the staining pattern of the G28-1 murine monoclonal antibody is preserved with TRU-016 (Figure 1C).

[00181] The CD37-binding molecules according to the invention describe structures (binding domains derived from antibodies, hinge variants, CH2CH3 regions being the same or different, and various isotypes).

Example 2

TRU-016 and Various CD37-Specific Antibodies Bind

the Same or Overlapping Epitopes on CD37

[00182] Experiments were performed to identify the CD37 epitope bound by TRU-016 and other previously described CD37-specific antibodies.

[00183] Unconjugated MB371 (#555457) and FITC-conjugated MB371 (#555456) were obtained from BD Pharmingen (San Jose, CA), FITC-conjugated BL14 (#0457) from Immunotech/Beckman Coulter (Fullerton, CA), FITC-conjugated NMN46 (#RDI-CBL 136FT) and unconjugated NMN46 (#RDI-CBL 136) from RDI (Flanders, NJ), FITC-conjugated IPO24 (#186-040) and unconjugated IPO-24 (#186-020) from Ancell Corporation (Bayport, MN), FITC-conjugated HHI (#3081) and unconjugated HH1 (#3080) from DiaTec.Com (Oslo, Norway) and FITC-conjugated WR17 (YSRTMCA483F) and unconjugated WR17 (YSRTMCA483S) from Accurate Chemical & Scientific (Westbury, NY). TRU-016 protein was produced as described in Example 1.

[00184] TRU-016 was conjugated to FITC at Trubion using a Molecular Probes Fluororeporter FITC Labeling Kit (F6434) according to manufacturer's instructions as follows: TRU-016 protein peak of interest (POI) at 13.5 mg/mL was adjusted to 5 mg/mL with PBS. 1 mg (200 ul) was added to kit tubes with a stirbar, and 1M NaHCO3 (adjusted to pH 8.5 with 6N NaOH), was added to a final concentration of 0.1M. 50 ul DMSO was added to 370 ug of FITC and was added to the tubes at molar ratios of 15, 20, 30 and 40 FITC : protein using the following formula to determine the ul of FITC to add: [ul of FITC solution to add = 5 mg/mL protein x 0.2 mL x 389 x 100 x desired molar ratio/Molecular weight of TRU-016 (110,000)].

[00185] Reactions were shielded from light and stirred continuously for 75 minutes at room temperature. Reactions were added to spin columns prepared as described in the kit and spun at 1100 g for 5 minutes to buffer exchange into PBS with azide and remove unconjugated FITC. The OD at 280nM and 494 nM was determined with 2 ul drops on the Nanodrop; the

extinction coefficient for TRU-016 was experimentally determined for this instrument by reading dilutions of the starting unconjugated SMIP, the concentration of each of the conjugates was 4.25 mg/ml and the following FITC:protein rations were determined: 2.7 FITC/TRU-016 at a ratio of 15; 3.7 FITC/TRU-016 at a ratio of 20; 4.4 FITC/TRU-016 at a ratio of 30; and 5.1 FITC/TRU-016 at a ratio of 40.

[00186] BSA was added to 3 mg/mL to help stabilize the protein. Binding of each fraction was assessed at dilutions ranging from 100-24,300x on Ramos and 3200-25,600 on human PBMC. All bound, but the MR30 ratio was chosen for further use since it gave a high MFI that was well maintained over the titration range used, indicating that binding avidity was least affected in this reaction.

[00187] FITC labeled antibody conjugates were titrated from 10 ng/mL to 10 µg/mL in an initial binding study to determine the optimal amounts to use in the blocking studies. The level chosen was just below saturating amounts, and was kept constant in the subsequent assays, while levels of blocking antibody were increased over a 10-fold range. Data were plotted as percent of maximal binding versus concentration of blocking antibody, so that higher levels indicate less efficient blocking, while lower levels indicate more efficient blocking activity. All of the antibodies tested showed blocking activity of the maximal binding observed without unlabeled reagents (Figure 2).

[00188] BJAB-cells, a B lymphoblastoid B-cell line, (courtesy of Ed Clark, University of Washington) were then stained with a panel of various clones of anti-CD37 MAbs, including MB371, BL14, NMN46, IPO24, HH1, WR17, and the TRU-016 SMIP.

[00189] For competitive binding assays, 2.5 X 10^5 BJAB cells were incubated in 96-well V-bottom plates in staining media (PBS with 2% mouse sera) with the FITC-conjugated anti-CD37 MAbs at 1.25 µg/mL in the presence of unconjugated anti-CD37 MAb at the indicated concentrations (2.5, 1.25, 0.6, or 0.3 µg/ml) or staining media for 45 minutes on ice in the dark. Blocking antibodies and FITC labeled antibody conjugates were added to reactions prior to addition of cells. The cells were then washed 2 ½ times

with PBS and fixed with 1% paraformaldehyde (# 19943, USB, Cleveland, Ohio). The cells were analyzed by flow cytometry using a FACsCalibur instrument and CellQuest software (BD Biosciences, San Jose, CA).

[00190] For FACs cross blocking assays, 2.5 X 10^5 BJAB cells were incubated in 96-well V-bottom plates in staining media (PBS with 2% mouse sera) in the presence of unconjugated anti-CD37 MAb at 5 μg/mL staining media for 45 minutes at room temperature in the dark. FITC-conjugated anti-CD37 MAbs were then added to a final concentration of 2 ug/ml, resulting in a dilution of the unlabelled reagents to 3.3 μg/ml. The reactions were then further incubated for 45 minutes at room temperature in the dark. Reactions were washed 2.5 times with PBS and fixed in 1% paraformaldehyde in PBS (#19943, USB, Cleveland, Ohio). Cells were analyzed by flow cytometry on a FACsCalibur instrument using Cell Quest software (BD Biosciences, San Jose, CA).

[00191] For cell binding assays, cells were suspended in PBS (#14040-133, Gibco/Invitrogen, Grand Island NY) containing 2% FBS (#16140-071, Gibco/Invitrogen, Grand Island, NY), (staining media) at a concentration of approximately 4x10^6 cells/mL. Cells were then plated and test samples, diluted in staining media, were then added 1:1 to the final designated concentrations. Reactions were incubated for 45 minutes on ice. Samples were centrifuged and washed 2 times with PBS. FITC goat anti-human IgG (#H10501,CalTag, Burlingame CA) was added at a final dilution of 1:50, and incubated 45 minutes on ice. Samples were centrifuged, washed in PBS, then fixed in 200 μl 1% paraformaldehyde in PBS (#19943, USB, Cleveland, Ohio). Cells were analyzed by flow cytometry on a FACs Calibur instrument using Cell Quest software (BD Biosciences, San Jose, CA).

[00192] Each antibody showed dose dependent inhibition of binding, indicating that all the molecules tested bind to an identical or closely related epitope. A different potency for inhibition of binding was observed for each antibody. TRU-016 SMIP had the highest level of blocking activity of all molecules tested, while HH1 gave an intermediate level of blocking activity,

and WR17, IPO24 blocked better than MB371; but showed less effective blocking than the other two unlabeled molecules (Figure 2).

[00193] In addition to analysis of blocking activity, a similar series of experiments was performed in which various CD37 targeted antibodies were tested for their ability to compete with one another for binding to the CD37 receptor. The results from these experiments, like results obtained in the blocking studies for all the molecules tested, indicated that the various CD37 targeted antibodies and TRU-016 have the same or closely overlapping epitopes.

Example 3

TRU-016 is Deficient in Binding C1q and Activating the Classical Complement Activation Pathway

[00194] Experiments were performed to explore why the TRU-016 dimer peak fails to mediate significant levels of complement dependent killing of B cell targets. One possibility was that TRU-016 dimer shows reduced binding to components of the complement cascade relative to normal human IgG1 antibody. Thus, experiments were performed to determine if TRU-016 activates the classical complement activation pathway by looking for TRU-016 binding to C1q. C1q, is a subunit of the C1 enzyme complex that activates the serum complement system, and is the recognition component of the classical complement activation pathway.

[00195] C1q binding studies were performed as previously described (Cragg et al., *Blood* 2004, 103:2738-2743). Briefly, Ramos B-cells in Iscoves media (#12440-053, Gibco/Invitrogen, Grand Island, NY) with no serum were plated in 96-well V bottom plates at 5 X 10^5/well in 100 µl. Cells were incubated with reagents for 15 minutes at 37°C, and normal human serum (NHS, #A113, Quidel Corp., San Diego, CA) diluted in Iscoves was then added at a volume of 50 µl to each well for a final concentration of 10, 5, 2.5, or 1.25 % human serum. Fifty µl of media was added to the control well. For cobra venom factor (CVF) experiments, CVF was added to human serum

complement samples at 20 Units CVF/mL of serum for 90 minutes at 37°C prior to addition of serum to complement assays, and the dilution of serum by CVF accounted for when making sample dilutions.

[00196] The cells plus complement source were incubated for an additional 5 minutes at 37°C, and washed 2 times with cold PBS (#14040-133, Gibco/Invitrogen, Grand Island, NY) via centrifugation and resuspended in 100 µl of PBS. Fifty µl from each well was transferred to a second plate for second step control staining. Both plates were stained for 15 minutes in the dark on ice with either FITC sheep anti-HU C1q (#C7850-06A, US Biological, Swampscott, Mass) or FITC Sheep IgG (#11904-56P, US Biological, Swampscott, Mass). Samples were washed, resuspended in cold PBS, and read immediately on a FACsCalibur flow cytometer and analyzed with Cell Quest software (Becton Dickinson, San Jose, CA).

[00197] FITC C1q does not bind well to any subfractions of SEC purified TRU-016, although the higher molecular weight (HMW) or A2 aggregate fraction does show more binding than the other forms (Figure 3A). In contrast, Rituxan showed a significant level of C1q binding, particularly at lower levels of NHS. The presence of CVF failed to completely block this binding, although the MFI levels are reduced significantly compared to media alone.

[00198] CDC assays were then performed to compare the ability of the different subfractions of the TRU-016 purified forms and Rituxan to mediate cell killing in the presence or absence of CVF and human serum complement (Figure 3B). CDC assays were performed using propidium iodide staining to discriminate between live and dead cells after incubations of target cells with antibody, fusion proteins, ascites fluid, TRU-016 molecular forms, or media, and a source of complement such as human serum. Briefly, 3 x 10^5 Ramos B-cells were pre-incubated with test reagents for 30-45 minutes at 37°C prior to addition of complement. The prebound samples were centrifuged, washed, and resuspended in Iscoves with human serum (# A113, Quidel, San Diego, CA) at the indicated concentrations, then incubated for 90 minutes at 37°C. Samples were washed and propidium iodide (# P-16063, Molecular Probes,

Eugene, OR) was added to a final concentration of 0.5 μg/mL in PBS. The cells were incubated with the propidium iodide for 15 minutes at room temperature in the dark and then analyzed by flow cytometry on a FACsCalibur instrument with CellQuest software (Becton Dickinson).

[00199] Cell killing mediated by both the A2 fraction of TRU-016 and Rituxan was significantly reduced in the presence of CVF despite its failure to completely block C1q binding (Figure 3B).

[00200] Human and rabbit complement were then compared for their CDC activity in the presence of the TRU-016. The CDC activity of TRU-016 molecular forms incubated with Ramos B cells and human or rabbit complement was measured (Figure 3C). Ramos B cells were added to wells in serum free media. Rituxan or the dimer, HMW A2, or pA fractions of TRU-016 were added to cells to give a final concentration of 10 μg/ml, and incubated for 15 minutes at 37°C, prior to washing 1.5X in serum free media and addition of normal human serum (NHS) or rabbit complement (Pelfreez) at 10, 5, or 2.5 %. Cells plus complement source were incubated 90 minutes at 37°C. Cells were washed once with cold PBS and propidium iodide (Molecular Probes #P3566) added to a final concentration of 0.5 μg/mL in cold PBS. Cells with PI were incubated in the dark at RT for 15 minutes and analyzed by flow cytometry.

[00201] The origin of the complement fraction affects the CDC results obtained (Figure 3C). Rabbit complement mediated higher levels of CDC than human complement in the presence of TRU-016 molecular forms. Interestingly, the dimer form of the TRU-016 mediated good CDC using rabbit complement, but very low CDC activity in the presence of human complement.

Example 4

Activity and Binding of TRU-016 Multimers

[00202] Experiments were performed to examine the biological activity of multimeric forms of TRU-016 (TRU-016 multimers) in solution. First, to

determine the size of TRU-016 fusion protein in solution, protein A purified material was analyzed by SEC HPLC and revealed that TRU-016 exists in multiple forms in solution (Figure 4).

[00203] HPLC size exclusion chromatography (SEC) traces were obtained from GPC purification of TRU-016, plotting absorbance versus retention time for the different fractions collected (Figure 4). TRU-016 was purified from cell culture supernatants initially by affinity chromatography using Protein A Sepharose. The recombinant molecule was eluted from the column with 100mM glycine, pH 3.5. 10mL fractions of the eluted product were recovered and the eluted product was then brought to pH 5.0 with 20% of the eluted volume of 0.5 M 2-(N-Morpholino)ethanesulfonic acid (MES) pH6.0. The eluate was prepared for GPC purification by concentration of the sample to approximately 25mg/mL TRU-016 and then filter sterilized in preparation for GPC purification. Size exclusion chromatography was performed on a GE Healthcare AKTA Explorer 100 Air apparatus, using a GE healthcare XK column and Superdex 200 preparative grade (GE Healthcare).

[00204] The HMW or A2 pools exhibited a retention time of approximately 6.23 minutes, while the most prominent form showed a retention time of 8.38 minutes. The reference standard used here (pA standard or std) is protein A purified material containing both dimers and HMW mulitimer forms, as shown in the first panel of Figure 4. The most prominent form, migrating at a retention time of 8.38 minutes, most likely corresponds to the dimer molecule seen on non-reduced SDS-PAGE, and several minor forms most likely correspond to multimers that associate through non-covalent interactions as they are not evident on nonreducing SDS-PAGE. To separate these different forms of TRU-016, material obtained from protein A sepharose affinity chromatography of culture supernatants was further purified by GPC and HPLC fractionation to isolate the dimer form (identified as "dimers" or "dimer peak") from higher molecular weight multimers (identified as HMW or A2 agg fraction). Each of these three subfractions was then analyzed separately for functional activity *in vitro* using binding, ADCC, and CDC assays.

[00205] To explore whether the fractions isolated from SEC showed different binding properties, each fraction of TRU 016 SEC was tested for binding to Ramos cells. To determine the binding properties of SEC fractions, cells were suspended in staining media at a concentration of approximately $4x10^6$ cells/mL and then plated at 50 µl/well ($2x10^5$ cells/well) in staining media. Serial dilutions of SEC fractions were then added to sequential wells, incubated for 45 minutes, washed, and binding activity was detected using FITC goat anti-human IgG. Samples were fixed in 200 µl 1% paraformaldehyde in PBS. Cells were analyzed by flow cytometry on a FACsCalibur instrument using Cell Quest software (BD Biosciences, San Jose, CA) (Figure 5A).

[00206] To determine the CDC activity of SEC fractions, cells were suspended at $5x10^5$ cells/well in 75 µl IMDM. TRU 016 SEC fractions (75 µl) were added to the cells at twice the concentrations indicated. Binding reactions were allowed to proceed for 45 minutes prior to centrifugation and washing in serum free Iscoves. Cells were resuspended in Iscoves with human serum (#A113, Quidel, San Diego, CA) at the indicated concentrations. The cells were incubated 60 minutes at 37°C, washed, and resuspended in staining media with 0.5 µg/mL propidium iodide (PI, #P-16063, Molecular Probes, Eugene OR). Samples were incubated 15 minutes at room temperature in the dark prior to analysis by flow cytometry using a FACsCalibur and CellQuest software (Becton Dickinson) (Figure 5B).

[00207] To determine the ADCC activity of SEC fractions, BJAB, Ramos, and Daudi lymphoblastoid B cells (10^7) cells were labeled with 500 µCi/mL ^{51}Cr sodium chromate for 2 hours at 37°C in IMDM/10%FBS. PBMCs were isolated from heparinized, human whole blood by fractionation over Lymphocyte Separation Media (LSM, ICN Biomedical) gradients. Reagent samples were added to RPMI media with 10% FBS and five serial dilutions for each reagent were prepared. For combinations, the reagents were premixed and diluted prior to addition to the wells. The ^{51}Cr labeled BJAB were added at ($2x10^4$ cells/well). The PBMCs were then added at ($5x10^5$ cells/well) for a final ratio of 25:1 effectors (PBMC):targets (BJAB). Reactions were set up in

quadruplicate wells of a 96 well plate. TRU-016 SEC fractions were added to wells at a final concentration ranging from 10 ng/mL to 20μg/mL as indicated on the graphs. Each data series plots a different SEC fraction at the titration ranges described. Reactions were allowed to proceed for 6 hours at 37°C in 5% CO_2 prior to harvesting and counting. CPM released was measured on a Packard TopCounNXT from 50 μl dried culture supernatant. Percent specific killing was calculated by subtracting (cpm [mean of quadruplicate samples] of sample – cpm spontaneous release)/ (cpm maximal release-cpm spontaneous release) x100 (Figure 5C).

[00208] Figure 5A shows the titration curves of the different SEC fractions binding to Ramos cells. All of the fractionated molecules bound to CD37 with similar binding curves except at the highest concentrations tested, where the HMW material exhibited better binding (higher fluorescence intensity) than the pA standard and the dimer peak forms.

[00209] Experiments were also performed to determine if the TRU 016 SEC fractions exhibited different levels of functional activity such as CDC and ADCC mediated target cell killing. The graph shown in Figure 5B indicates that only the purified HMW multimer fraction mediated significant levels of CDC activity against Ramos B cells using human complement. The pA standard exhibited some CDC activity at higher concentrations, while the dimer peak form showed very little or no CDC activity at all concentrations tested.

[00210] ADCC assays were performed on serial dilutions of various TRU-016 size fractions using labeled BJAB B cells as targets and human PBMC as effector cells. TRU 016 SEC fractions were present in wells at a final concentration ranging from 10 ng/mL to 20μg/mL as indicated in the graph shown in Figure 5C. Each data series plotted a different SEC fraction at the titration ranges described. Data were plotted as % specific killing versus protein concentration. All of the SEC subfractions, including the pA standard, HMW or A2 fraction, and dimer peak, mediated potent, dose-dependent ADCC against BJAB target cells. Similar results were also obtained using Ramos cells as labeled targets (data not shown).

Example 5

Production of a CD20-specific Binding Molecule

[00211] CD20-specific SMIPs are described in co-owned US Patent Publications 2003/133939, 2003/0118592 and 2005/0136049. Production of an exemplary CD20-specific SMIP, TRU-015, is described below.

[00212] TRU-015 is a recombinant (murine/human) single chain protein that binds to the CD20 antigen. The binding domain was based on a publicly available human CD20 antibody sequence. The binding domain is connected to the effector domain, the CH2 and CH3 domains of human IgG1, through a modified CSS hinge region. TRU-015 exists as a dimer in solution and the dimer has a theoretical molecular weight of approximately 106,000 daltons. The nucleotide sequence encoding TRU-015 and the amino acid sequence of TRU-015 are respectively set out in SEQ ID NOs: 3 and 4.

[00213] Referring to the amino acid sequence set out in SEQ ID NO: 4, TRU-015 comprises the 2e12 leader peptide cloning sequence from amino acids 1-23; the 2H7 murine anti-human CD20 light chain variable region with a lysine to serine (VHL11S) amino acid substitution at residue 11 in the variable region, which is reflected at position 34; an asp-gly3-ser-(gly4ser)2 linker beginning at residue 129, with the linker having an additional serine at the end to incorporate the SacI restriction site for cassette shuffling; the 2H7 murine anti-human CD20 heavy chain variable region, which lacks a serine residue at the end of the heavy chain region, i.e., changed from VTVSS to VTVS; a human IgG1 Fc domain, including a modified hinge region comprising a (CSS) sequence, and wild type CH2 and CH3 domains.

[00214] The CHO cells that produce TRU-015 were cultured in a bioreactor using proprietary media. TRU-015 was purified using a series of chromatography and filtration steps including a virus reduction filter. The material was then concentrated and formulated with 20 mM sodium phosphate and 240 mM sucrose, with a resulting pH of 6.0. The composition

is filtered before filling into glass vials at a concentration of 10 mg/mL. Each glass vial contained 5 mL of TRU-015 (50 mg/vial).

Example 6

Combinations of TRU-016 with TRU-015 or Rituxan

Synergistically Increase Apoptosis in B cells

[00215] Experiments examining the effect of B cell targeted SMIPS on B cell line apoptosis were performed. Each SMIP was tested individually and then in combination. Samples were analyzed at both 24 and 48 hours after initiation of incubation reactions. Annexin/PI Analysis was performed as follows: BJAB (courtesy of Ed Clark, University of Washington), Ramos (ATCC# CRL-1596), and Daudi cells were incubated 24 or 48 hours at 37°C in 5% CO_2 in Iscoves (Gibco) complete media with 10% FBS at 3 X 10^5 cells/mL and 20 μg/mL SMIP protein. In addition, 20 μg/mL goat anti-human IgG was added to reactions in order to cross link reagents on the cell surface. Cells were then stained with Annexin V-FITC and propidium iodide using the BD Pharmigen Apoptosis Detection Kit I (#556547), and processed according to kit instructions. Briefly, cells were washed twice with cold PBS and resuspended in "binding buffer" at 1X10^6 cells/mL. One hundred microliters of the cells in binding buffer were then stained with 5 μL of Annexin V-FITC and 5 μL of propidium iodide. The cells were gently vortexed and incubated in the dark at room temperature for 15 minutes. Four hundred microliters of binding buffer was then added to each sample. They were then read and analyzed on a FACsCalibur (Becton Dickinson) instrument using Cell Quest software (Becton Dickinson).

[00216] Table 2 below shows that in the presence of crosslinking, treatment with TRU-016 had a more significant effect on apoptosis of cell lines than TRU-015 alone, although both molecules when used alone do induce some apoptosis. The increase varies depending on the cell line.

TABLE 2

Bjab	Annexin V Positive	
No SMIP	17.5	
CD20 SMIP	27	
CD37 SMIP	30.6	
CD19 SMIP	29.1	
CD20+CD37 SMIP	41	
CD20+CD19 SMIP	37.1	
CD37+CD19 SMIP	35.3	
		plus GAM
Ramos	AnnexinV Positive	AnnexinV positive
cells alone	3	3.3
CD20 MAb	1.4	3.1
CD37 Mab	18.3	8.7
CD19 MAb	3.7	3.1
CD40 MAb	3.9	8.3
CD20+CD37	32.3	35.7
CD20+CD19	5	10.5
CD20+CD40	5.7	19.4
CD19+CD37	26.9	50
CD19+CD40	8.2	18.4

Example 7

Combinations of TRU-016 with CD20-specific Antibodies

or SMIPs Synergistically Increase CDC

[00217] Experiments were performed to determine the CDC activity of combinations of TRU-016 with CD20-specific antibodies or SMIPS against B cells. The amount of reagents chosen for combination experiments was 0.5 μg/mL TRU-016 while that of TRU-015 was also 0.5 μg/ml. The concentration of rituxan was usually 0.04-0.06 μg/mL because of its higher activity in single reagent CDC experiments. In some experiments, the concentration of CD20 reagent was held constant at a suboptimal concentration, while the concentration of TRU-016 was varied to explore the minimal levels of CD37 directed reagent required to observe augmentation effects on CDC.

[00218] Cells were suspended in Iscoves (#12440-053, Gibco/Invitrogen, Grand Island, NY) at 5x10E5 cells/well in 75 μl. TRU-016 (75 μl), TRU-015, rituxan, or combinations of these reagents were added to the cells at twice the concentrations indicated. Binding reactions were allowed to proceed for 45 minutes prior to centrifugation and washing in serum free Iscoves. Cells were resuspended in Iscoves with human serum (#A113, Quidel, San Diego, CA) at the indicated concentrations. The cells were incubated 60 minutes at 37°C.

Cells were washed by centrifugation and resuspended in 125 µl PBS with 2% FBS (#16140-071, Gibco, Invitrogen, Grand Island, NY), staining media. The cells were transferred to FACS cluster tubes (#4410, CoStar, Corning, NY) and 125 µl staining media with 5 µl propidium iodide (PI, #P-16063, Molecular Probes, Eugene OR) was added. Samples were incubated 15 minutes at room temperature in the dark prior to analysis by flow cytometry using a FACsCalibur and CellQuest software (Becton Dickinson).

[00219] Figure 6 shows that at suboptimal concentrations for killing as a single agent, TRU-015 and rituxan exhibit high levels of CDC activity when combined with TRU-016. TRU-016 alone fails to mediate CDC unless aggregates are present. Depletion of C1q from the reactions results in the elimination of all CDC activity observed.

[00220] Figure 7 shows a combination experiment performed on DHL-4 B cells. Addition of TRU-016 to the CDC reactions results in a downward shift to the TRU-015 killing curve, demonstrating more effective killing at each concentration tested even though TRU-016 exhibits little or no activity alone.

[00221] Figure 8 shows another CDC experiment where the sample reagents were mixed at the following ratios: 0.5 µ/mL TRU-015, 0.5 µg/mL TRU-016, and 0.06 µg/mL rituxan. Again, the single agents are used at suboptimal concentrations in order to see augmentation effects in the presence of TRU-016. For both TRU-015 and rituxan, TRU-016 enhances the level of CDC killing when added to the assays.

[00222] Figures 9 and 10 show graphical representations of the data for CDC assays where the concentration of TRU-015 or rituxan was kept constant and TRU-016 concentration was increased. Again, CDC activity was greater when TRU-016 was added to the reactions, but increasing the concentration of TRU-016 to 2.5 µg/mL from 0.5 µg/mL did not significantly increase the CDC-mediated killing in these experiments.

Example 8

TRU-016 Augments the ADCC and the CDC Activity

of CD20-Specific Antibodies and SMIPs

[00223] Experiments were performed to determine if combinations of TRU-016 SMIP with CD20-specific antibodies or SMIPs could augment ADCC and CDC activity against B cell targets.

[00224] BJAB, Ramos, and Daudi lymphoblastoid B cells (10E7) cells were labeled with 500 μCi/mL ^{51}Cr sodium chromate for 2 hours at 37°C in IMDM/10%FBS. The labeled BJAB cells were washed three times in RPMI/10% FBS and resuspended at 4x10E5 cells/mL in RPMI. Heparinized, human whole blood was obtained from anonymous, in-house donors and PBMC isolated by fractionation over Lymphocyte Separation Media (LSM, ICN Biomedical) gradients. Buffy coats were harvested and washed twice in RPMI/10% FBS prior to resuspension in RPMI/10% FBS at a final concentration of 3x10E6 cells/ml. Cells were counted by trypan blue exclusion using a hemacytometer prior to use in subsequent assays. Reagent samples were added to RPMI media with 10% FBS at 4 times the final concentration and five serial dilutions for each reagent were prepared. For combinations, the reagents were premixed and diluted prior to addition to the wells. These reagents were then added to 96 well U bottom plates at 50 μl/well for the indicated final concentrations. The ^{51}Cr labeled BJAB were added to the plates at 50 μl/well (2x10E4 cells/well). The PBMCs were then added to the plates at 100 μl/well (3x10E5 cells/well) for a final ratio of 15:1 effectors (PBMC):target (BJAB).

[00225] Effectors and targets were added to media alone to measure background killing. The ^{51}Cr labeled BJAB were added to media alone to measure spontaneous release of ^{51}Cr and to media with 5% NP40 (#28324, Pierce, Rockford, IL) to measure maximal release of ^{51}Cr. Reactions were set up in quadruplicate wells of a 96-well plate. SMIPs were added to wells at a final concentration ranging from 12 ng/mL to 10μg/mL as indicated on the graphs. For SMIP combinations, the reagents were mixed prior to addition to the wells. Each data series plots a different single SMIP or combination at the

titration ranges described. Reactions were allowed to proceed for 6 hours at 37°C in 5% CO_2 prior to harvesting and counting. Fifty µl of the supernatant from each well was then transferred to a Luma Plate 96 (#6006633, Perkin Elmer, Boston, Mass) and dried overnight at room temperature. CPM released was measured on a Packard TopCounNXT. Percent specific killing was calculated by subtracting (cpm {mean of quadruplicate samples} of sample – cpm spontaneous release)/(cpm maximal release-cpm spontaneous release) x100.

[00226] Data were plotted as % specific killing versus SMIP concentration. The effector to target ratio is indicated on each figure, and the target cell line was also indicated. Figures 11, 12, and 13 show data for experiments on different cell lines (BJAB, Daudi, and Ramos) where the same donor was used.

[00227] In Figures 14 and 15 (rituxan + TRU-016) and Figures 16 and 17 (TRU-015 + TRU-016) data is presented for experiments in which the target cell line used was BJAB. The specific killing observed for each combination was greater than either single reagent alone at the same concentration, indicating that the CD20 and CD37 targeted SMIPs augment the killing mediated by the other, although the augmentation effect is not completely additive.

[00228] Thus, TRU-016 can enhance CD20-specific SMIP or CD20-specific antibody ADCC mediated killing of B cells.

[00229] Initial experiments to explore the effects of combinations of TRU-016 with CD20-directed antibodies were designed to determine the relative amounts of each reagent to use so that CDC synergy could be detectable. Ramos cells were suspended in IMDM, and TRU-016, Rituxan, or combinations of these reagents were added to the cells to the final concentrations indicated in Figure 18. Binding reactions were allowed to proceed for 45 minutes prior to centrifugation and washing in serum free Iscoves. Cells were resuspended in Iscoves with 10% NHS. The cells were incubated 60 minutes at 37°C. In experiments shown in Figure 18A-C, cells were washed by centrifugation and resuspended in staining media containing

0.5 μg/mL propidium iodide (PI, #P-16063, Molecular Probes, Eugene OR). Samples were incubated 15 minutes at room temperature in the dark prior to analysis by flow cytometry using a FACsCalibur and CellQuest software (Becton Dickinson).

[00230] The more highly purified TRU-016 dimer peak is a poor mediator of CDC when used alone, as shown in Figure 18A by the flat dose-response curve even at high concentrations. Because CD20 directed reagents were efficient inducers of CDC activity, non saturating amounts of the CD20 directed reagents were desirable in combination experiments, so that synergy between the reagents could be detected. From these initial studies, the usual amount of reagent chosen for combination experiments was 0.5 μg/mL or 2 μg/mL TRU-016. The concentration of Rituxan was usually 0.04-0.06 μg/mL because of its higher activity in single reagent CDC experiments. In some experiments, the concentration of CD20 reagent was held constant at a suboptimal concentration, while the concentration of TRU 016 was varied to explore the minimal levels of CD37 directed reagent required to observe augmentation effects on CDC. Thus, TRU-016 alone fails to mediate CDC unless aggregates are present.

[00231] Figure 18B shows a graph of the percentage of live cells (PI negative) observed over the titration range indicated (0.06-0.5 μg/ml) when Rituxan is used alone or in combination with TRU-016 at 2.5 μg/ml. Rituxan, when used at a range of suboptimal doses for killing as a single agent, exhibits higher levels of CDC activity at each concentration when combined with TRU-016 (Figure 18B). Depletion of C1q from the reactions results in the elimination of all CDC activity observed (Figure 3B).

[00232] In Figure 18C, samples were also incubated with FITC anti-C1q for 45 minutes on ice prior to analysis by flow cytometry. Lymphocyte gating was on compromised cells. The percentage of cells in this gate increased with increasing Rituxan concentration, and the relative MFI for this population of cells was graphed. Figure 18C shows the results of a CDC experiment where the sample reagents were mixed at the following ratios: 0.5 μg/mL for TRU-016, and Rituxan concentrations ranging from 0.06 μg/mL to 0.5 μg/mL, and

cells stained with PI prior to flow cytometry. The results show a dose dependent increase in MFI with increasing doses of Rituxan. The addition of TRU-016 dimer forms resulted in an additional increase in the MFI at each concentration of Rituxan. A similar series of CDC assays were performed, keeping the concentration of Rituxan constant and increasing the TRU-016 concentration. Again, CDC activity was greater when TRU-016 was added to the Rituxan reactions, but increasing the concentration of TRU-016 to 2.5 μg/mL from 0.5 μg/mL did not significantly increase the CDC mediated killing in these experiments (data not shown).

[00233] Rituxan and TRU-016 proteins used alone and in combination with one another were compared for their ADCC activity *in vitro* using a similar concentration range as that used for the CDC assays. Figure 18D shows the results of an ADCC assay with labeled Ramos cell targets and human PBMC effector cells at an effector to target ratio of 25:1, using TRU-016 or Rituxan, alone and in combination with one another over the concentration ranges indicated. Similar data were obtained at an effector:target ratio of 12.5:1. Both the TRU-016 dimer form and Rituxan mediate significant levels of ADCC against Ramos cells expressing the CD20 and CD37 target antigens; however, the combination of the two reagents does not result in significant augmentation in the level of killing.

Example 9

TRU-016 Induces Apoptosis in B cells

[00234] Experiments examining the effect of TRU-016 on B cell line apoptosis were performed. Initial assays of the effects on apoptosis of TRU-016 molecules targeted to different B cell receptors were performed using protein A purified material that still contained higher order aggregates. After 24 hour treatment with CD37 antibodies or engineered TRU-016 molecules, similar patterns of increased apoptosis were observed in multiple experiments using annexin V positive cell percentages as a measure of apoptotic activity and both Ramos and BJAB cells as binding targets (data not shown).

[00235] Figure 19A demonstrate that apoptosis is significantly increased after incubation of B cell lines with unfractionated TRU-016. Figure 19A shows a dot plot of Annexin V-PI staining of Ramos cells after incubation for 24 hours with the TRU-016 (10μg/mL). The % of annexin V-PI double positive cells increased from 11.3 % of the total population to 32.8%, and the % of annexin V positive-PI negative cells increased from 8.5% to 19.7%, indicating that apoptosis is induced after exposure to TRU-016. Similar data were obtained whether Ramos or BJAB cells were used as the binding targets in these assays.

[00236] Further experiments examining the effect of TRU-016 on B cell line apoptosis were performed using the more highly purified dimer form of TRU-016 (Figure 19B). Samples were analyzed at both 24 and 48 hours after initiation of incubation reactions. Annexin/PI analysis was performed on several cell types using 20 μg/mL TRU-016 protein. Because apoptosis was reduced using the dimer form of TRU-016, 20 μg/mL goat anti-human IgG was added to reactions in order to cross link reagents on the cell surface. Cells were then stained with Annexin V-FITC and propidium iodide. The data shown in Figure 19B demonstrates that the TRU-016 dimer peak induces apoptosis of Daudi cells after 24-48 hours, but that the presence of a crosslinking agent such as anti-human IgG results in a significant increase in the level of CD37 targeted apoptosis.

[00237] Experiments were also performed to determine the effect of TRU-016 on normal human B cells in culture using human PBMCs. Figures 20A and 20B shows results from one such experiment, with columnar graphs of the percentage of CD19 or CD40 positive lymphocytes (B cells) present in PBMC cultures treated for 48-72 hours with media alone, TRU-016, or Rituxan.

[00238] Human PBMCs were isolated from whole blood by LSM density centrifugation. Cells were incubated for 48 or 72 hours with 1 μg/mL of Rituxan or TRU-016. A portion of the incubation reaction was harvested at 48 hours and again at 72 hours after initiation of the experiment. PBMCs were washed and incubated with FITC anti-CD19, FITC anti-CD40, or FITC anti-CD3 for 45 minutes on ice. The percentage of total lymphocytes staining with these reagents was then tabulated and compared to PBMC samples incubated under similar conditions but without test reagents, and stained as for the treated samples. Figures 20A and B show columnar graphs of the fraction of the total lymphocyte population (%) which give a positive FACs signal after 48 and 72 hours with the indicated reagents. Figure 20C shows a composite graph from a similar experiment, showing the percent reduction from the original number of lymphocytes expressing the indicated CD antigen (i.e. CD19, CD40 or CD3 positive) after incubation of PBMCs with TRU-016 (at 1 μg/ml) for 24 and 72 hours.

[00239] In the presence of crosslinking, treatment with the TRU-016 dimer form or Rituxan resulted in a reduction in the percentage of B lymphocytes in PBMC cultures as measured by positive staining for CD19 and CD40. Although the percentage of B lymphocytes in culture was low at the outset of the experiment, coculture with Rituxan or TRU-016 decreased the number of CD19 and CD40 positive lymphocytes in the PBMC culture by approximately 1.5-2 fold after 48 hours, and by more than 3 fold after 72 hours. This general pattern of B cell depletion after 48-72 hours was reproducible in all normal PBMC cultures tested, regardless of the initial starting percentage of B lymphocytes in these cultures, which ranged from approximately 3% to as much as 7% of the total lymphocytes, depending on the sample.

[00240] Figure 20C shows a columnar graph of the percentage depletion of B lymphocytes compared to T lymphocytes in short term PBMC cultures incubated with TRU-016 for 24 to 72 hours. These data indicate that the TRU-016 is capable of specific depletion of CD37 positive B lymphocytes from normal peripheral blood cultures, and that the low level of binding by TRU-016 to non-B lymphocytes (Figure 1C) is insufficient to mediate significant depletion of these lymphocytes from the cell population.

Example 10

Combinations of TRU-016 and Rituximab Synergistically Reduce Tumor Volume in a Murine Tumor Xenograft Model

[00241] Mouse tumor xenograft studies exploring combination therapies were performed using nude mice (Harlan) and Ramos or Daudi human tumor lines. Ramos or Daudi tumor cells were grown in T150 flasks in IMDM/10% FBS until they reached 80% confluency. Five million ($5x10^6$) cells were used as a tumor inoculum per mouse. Cells were injected subcutaneously in the right flank using PBS in a total volume of 0.1 mL or $5.0x10^7$/mL. Nude mice were allowed to develop tumors and sorted into groups based on tumor size/volume. For each treatment group, 12 mice with a mean tumor volume of approximately 222 mm^3 (range =152-296mm^3) were used. Some mean tumor volumes ranging from 237-251 mm^3 were also used. Animals were injected intravenously (IV) at days 0, 2, 4, 6, and 8 with one of the following reagents: TRU-016 GPC POI (peak of interest), 200µg/mouse; rituxan, 200 µg/mouse, or human IgG (control) at 200 or 400 µg/mouse as single reagents, or as the following combinations of reagents: Rituxan + TRU-016 at 100 µg each per mouse; or Rituxan + TRU-016 at 200 µg each per mouse. Tumor volume was measured daily with calipers until completion of the experiment (sacrifice or regression). Tumor volume as a function of treatment time was plotted for each animal and results were also averaged within each group.

[00242] Similar studies were also performed using smaller tumors, with mice sorted into groups with smaller mean tumor volume ranging between

153-158 mm^3, and with larger tumors but using Daudi cells rather than Ramos cells. These studies were performed in an AAALAC accredited animal facility and animal use program in accordance with guidelines from an Institutional Animal Care and Use Committee (IACUC).

[00243] Figure 21 graphs the efficacy of TRU-016 compared to huIgG, rituxan, and the combinations at 100 μg and 200 μg each averaged over each group of 12 animals. Tumor volume was plotted as a function of time after treatment with the IV injection(s). The average tumor volume after treatment with TRU-016 was smaller than that observed using the negative control (huIgG). When % survival or % tumor free animals were graphed, the higher dose combination therapy exhibited higher anti-tumor activity in this *in vivo* tumor model. However, at the lower dose (100 μg each), the combination therapy was not as effective as each single reagent at a higher dose.

[00244] These data indicate that TRU-016 therapy, when used in combination with rituxan at the appropriate doses, will have greater efficacy in treating patient tumors than rituxan therapy alone.

Example 11

TRU-016 Reduces Tumor Volume and Increases Survival in a Murine Tumor Xenograft Model

[00245] Mouse tumor xenograft studies were performed using nude mice (Harlan) and Ramos or Daudi human tumor lines. Three different studies were performed based on tumor type and tumor size at the time of treatment with the TRU-016 or other test reagent. Ramos or Daudi tumor cells were grown and ($5x10^6$) cells were injected subcutaneously in the right flank to inoculate each treated mouse with the tumor. Nude mice were allowed to develop tumors and sorted into groups based on tumor size/volume. In the first study, for each treatment group, 12 mice with a mean tumor volume of 155-237 mm^3 were used. Animals were injected intravenously (IV) at days 0, 2, 4, 6, and 8 with one of the following reagents: Rituximab, 200μg/mouse; TRU-016 GPC dimer peak, 200μg/mouse; or human IgG (control), 400

μg/mouse. Tumor volume was measured daily with calipers until completion of the experiment (sacrifice or regression). Tumor volume as a function of treatment time was plotted for each animal and results were also averaged within each group. Group averages were shown in Figure 22A, while Figure 22B shows a comparison of the percent survival data for each group of mice as a function of time.

[00246] Figure 22A shows the efficacy of TRU-016 compared to huIgG and Rituxan in the Ramos tumor model, averaged over each group of 12 animals. Tumor volume was plotted as a function of time after treatment with the IV injection(s). The average tumor volume after treatment with the TRU-016 was smaller than that observed using the negative control (huIgG). Figure 22B graphs the survival curves for the different treatment groups, comparing huIgG, Rituxan, and TRU-016. Administration of TRU-016, utilizing the more demanding Ramos tumor model with increased baseline tumor volume, resulted in an inhibition of tumor growth rate relative to human IgG (data not shown). Administration of TRU-016 to mice with the smaller Ramos tumors resulted in both an inhibition of tumor growth and increased median survival times.

Example 12

TRU-016 Does Not Affect the CDC Activity of Other B Cell Surface Receptors

[00247] To determine whether the TRU-016 molecule augments the level of CDC activity resulting from treatment with antibodies to other B cell surface receptors, in addition to CD20, such as MHCII, CD19, CD80/86, and CD40, a panel of experiments was performed similar to those just described for CD20-CD37 directed combinations.

[00248] Ramos cells were added to wells in Iscoves complete media with 10% FBS. The MAbs (reagent B: HD37-anti CD19, reagent C: 9.4-anti-CD45), fusion protein (reagent D: CTLA-4 muIg-IgG2a, Ancell #501-820), and ascites fluid (reagent A: HB10a-anti-MHCII), were added at the indicated dilutions (see Figure 23) and duplicate reactions were set up with and without

Rituximab (at 0.05 μg/ml) or TRU-016 (at 2 μg/ml) added. Reactions were incubated for 30 minutes at 37°C. The cells were washed and NHS was added to a final concentration of 10% in serum free media. Cells were incubated for 90 minutes at 37°C with the complement source. The cells were washed; propidium iodide was added to a final concentration of 0.5 μg/mL in PBS; the cells were incubated in the dark at room temperature for 15 minutes; and then cells were assayed by flow cytometry. Each graph in panels A-D of Figure 23 plots the %PI positive cells over the titration ranges indicated.

[00249] In general, the data indicate that there was not a significant difference in the level of CDC activity when antibodies directed to these receptors were used alone or in combination with the TRU-016 (Figure 23A-D). There may be a slight increase in CDC levels for the CD19 and CD45 directed reagents when used with TRU-016 at suboptimal concentrations. However, the differences in CDC levels are not nearly as significant as those observed for the CD20-CD37 combination. In addition to the augmentation of CDC when CD20 and CD37 directed reagents are used in combination, there appears to be augmentation in the level of killing observed using combinations of anti-classII (HB10a), anti-CD19, anti-CD45 (9.4) or CTLA4Ig with Rituxan at the suboptimal dose.

Example 13

TRU-016 Does Not Augment the CDC Activity of Other Targeted Receptors, Including MHCII, CD19, CD80/86, and CD40

[00250] To determine whether the TRU-016 molecule augments the level of CDC activity resulting from treatment with antibodies to other B cell surface receptors, in addition to CD20, a panel of experiments was performed similar to those described for CD20-CD37 directed combinations (see Example 8). The results of these experiments are shown in Figure 23. In general, there was not a significant difference in the level of CDC activity when antibodies directed to these receptors were used alone or in combination with the TRU-016. CDC levels slightly increased in response to

CD19 and CD45 directed reagents when used with TRU-016 at suboptimal concentrations. However, the differences in CDC levels were not nearly as significant as those observed for the CD20-CD37 combination (see Example 8). In addition to the augmentation of CDC when CD20 and CD37 directed reagents are used in combination, there appeared to be augmentation in the level of killing observed using combinations of anti-MHCII (HB10a), anti-CD19, anti-CD45 (9.4) or CTLA4Ig with Rituxan at the suboptimal dose.

Example 14

TRU-016 Increases Survival in a Murine Tumor Xenograft Model

[00251] Mouse tumor xenograft studies beyond those described in Example 11 were performed to examine the efficacy of TRU-016 in increasing long-term survival using nude mice (Harlan) and either Ramos or Daudi human tumor cell lines.

[00252] Ramos and Daudi tumor cells were separately grown and ($5x10^6$) cells were injected subcutaneously in the right flank of mice to initiate the formation of mouse tumor xenografts. After tumor development, mice were sorted into groups based on tumor size/volume (day 0). Animals were injected intravenously (IV) at days 0, 2, 4, 6, and 8 with one of the following reagents: rituximab, 200 μg/mouse; TRU-016, 200 μg/mouse; rituximab + TRU-016 at 100 or 200 μg/mouse; or human IgG (control), 400 μg/mouse. Tumor volume was blindly measured three times weekly with calipers until completion of the experiment (sacrifice or regression). Tumor volume as a function of treatment time was plotted for each animal and results were averaged within each group. Figure 24 shows the percent survival of mice with Ramos tumors (up to 90 days) after treatment with TRU-016, rituximab, or a combination thereof. The combination treatment with TRU-016 + rituximab significantly increased median survival time versus treatment with single agent therapy alone. Figures 25 and 26 show the percent survival of mice with Daudi tumors (up to 90 days) after treatment with TRU-016 or rituximab. Treatment with TRU-016 increased median survival time in

established Daudi tumors (Figure 25). TRU-016 was more effective than rituximab in maintaining survival in mice with Daudi tumors (Figure 26).

[00253] Administration of TRU-016 as a single agent in mice with established Ramos tumors demonstrated an inhibition of tumor growth and improved survival times equivalent to rituximab administered as a single agent, and was superior to HuIgG control-treated mice. Pooled data from 3 experiments demonstrated that TRU-016 and rituximab combination therapy resulted in a statistically significantly improvement in survival time compared to TRU-016 ($p= 0.028$) or rituximab ($p=0.045$) monotherapies. Complete tumor regressions were also enhanced for the TRU-016 and rituximab combination groups. Forty-two percent of the TRU-016 + rituximab 200μg combination group were able to achieve long-term complete regression of their tumors compared to a 20% tumor regression rate in mice treated with either TRU-016 or rituximab alone (see Table 3 and Figure 24).

TABLE 3. Survival after Treatment in Established Ramos Tumors

	Percentage of Tumor-Free Mice at Day 90	**Median Survival Time (Days)**
TRU-016 + rituximab (200 μg)	42	31
TRU-016 + rituximab (100 μg)	25	24
TRU-016 (200 μg)	20	16

Rituximab (200 μg)	20	17
HuIgG	0	10

[00254] Reduction in tumor growth and improved survival time were found after TRU-016 treatment in the Daudi tumor xenograft model (see Table 4 and Figures 25 and 26). TRU-016 administration significantly enhanced survival time compared to the control group. An increase in percentage of tumor-free mice was also observed with SMIP-016 treatment in this model compared to both control and rituximab groups.

TABLE 4. Survival after Treatment in Established Daudi Tumors

	Percentage of Tumor Free Mice at Day 90	**Median Survival Time (Days)**
TRU-016 (100 μg)	25	24
Rituximab (100 μg)	0	17
HuIgG	0	15

[00255] Treatment with a CD37-directed SMIP (TRU-016) is as effective as rituximab monotherapy in reducing tumor volume and increasing survival time in the Ramos tumor xenograft model. TRU-016 + rituximab combination therapy demonstrated enhanced benefit in reducing tumor volume and significantly improving survival time compared to either rituximab or TRU-016 monotherapy in the Ramos tumor xenograft model. In the Daudi xenograft model, TRU-016-treated mice demonstrated a statistically significant increase in median survival time compared to HuIgG controls. Treatment with rituximab did not extend survival times compared to control mice. These data highlight the efficacy of a CD37-directed therapy in these NHL xenograft models.

Example 15

TRU-016 Potentiates Fludarabine-Induced Cell Death in CLL Cells *In Vitro*

[00256] Fludarabine is a chemotherapy drug used in the treatment of hematological malignancies. Fludarabine is a purine analog that inhibits DNA synthesis by interfering with ribonucleotide reductase and DNA polymerase. Fludarabine is active against both dividing and resting cells. Fludarabine is highly effective in the treatment of chronic lymphocytic leukemia (CLL), producing higher response rates than alkylating agents such as chlorambucil alone (Rai et al., N. Engl. J. Med. 343:1750-1757, 2000). Fludarabine is used in various combinations with cyclophosphamide, mitoxantrone, dexamethasone and rituximab in the treatment of indolent lymphoma and non-Hodgkins lymphoma. However, resistance to fludarabine has also been observed in treatment. Fludarabine induces caspase-dependent apoptosis in CLL cells, and apoptosis mediated by TRU-016 appears to be independent of caspase activation. The present study examined the effect of TRU-016 with fludarabine on CLL cells.

[00257] Cells were treated with TRU-016 at dosages ranging from 0.1-100 μg/mL and with fludarabine at dosages ranging from 0-20 μM (see Figure 27). TRU-016 was provided by Trubion Pharmaceuticals (Seattle, WA). Fludarabine (F-araA) was purchased from SIGMA (St. Louis, MO). RPMI 1640 media was purchased from Invitrogen (Carlsbad, CA). Fluorescein isothiocyanate (FITC)-labeled annexin V, and propidium iodide (PI) were purchased from BD Pharmingen, San Diego, CA. [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO). B-CLL cells were isolated immediately following donation using ficoll density gradient centrifugation (Ficoll-Paque Plus, Amersham Biosciences, Piscataway, NJ). Isolated mononuclear cells were incubated in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT), 2 mM L-glutamine (Invitrogen, Carlsbad, CA), and penicillin (100U/mL)/streptomycin (100 μg/ml; Sigma-Aldrich, St. Louis) at 37°C in an atmosphere of 5% CO_2. Freshly isolated B-CLL cells were used for all the experiments described herein except for the surface staining. For those samples with less than 90% B cells,

negative selection was applied to deplete non-B cells using B cell Isolation Kit II (Miltenyi Biotec, Auburn, CA) or by "Rosette-Sep" kit from Stem Cell Technologies (Vancouver, British Columbia, Canada) according to the manufacture suggested protocol. Raji (Human Burkitt's lymphoma cell line) cell line was purchased from ATCC and maintained in RPMI 1640 media containing 10% FBS, supplemented with penicillin, streptomycin and glutamine. Cells were split 1:3 when the cell density reached $1x10^6$/mL. Media was changed the night before each study to assure fresh cells being used.

[00258] Cells were treated *in vitro* as described herein. 1:4 serial dilution of fludarabine (44, 11, 2.8, 0.7, 0.17 and 0.04 µM) was prepared in a 6-well plate by transferring 2 mL of drug-containing media to the next well containing 6 mL blank media. In a separate 6-well plate, 1:4 serial dilution of TRU-016 (44, 11, 2.8, 0.7, 0.17, and 0.04 µg/ml) in media was prepared using the same dilution method. From each of the plates, 0.45 mL media was transferred to a designed well in a 48-well plate to make a mixed drug solution in media (0.9 mL total in each well). Suspended CLL cells in media at a density of $1x10^7$ cells/mL (0.1mL) were then added to the 0.9 mL media in each well to make a final density of $1x10^6$ cells/mL. For Raji cells, the final cell density was $5x10^4$ cells/mL. Thus, the cell suspension used was $5x10^5$ cells/mL. For the MTT assays, drug serial dilutions were prepared in 96-well plates, and transferred to other 96-well plates for incubation with cells. The total volume for incubation is 200 µL (90 µL of fludarabine solution, 90 µL of TRU-016 solution, and 20 µL cell suspension). Cell viability was assessed using MTT assays at 48 hr, and apoptosis was measured using Annexin V/PI at 24 hr.

[00259] MTT assays were performed to measure cell viability as described herein. Briefly, 10^6 CLL cells were seeded to 96-well plates. Cells were incubated for 48 hours. 50µl of MTT working solution (2mg/ml, prepared from 5mg/mL MTT reagent mixed with RPMI 1640 2:3 v/v) was added to each well, and the cells were incubated for 8 hours. Plates were centrifuged and supernatant was removed and dissolved in 100 µl lysis solution. Samples

were measured with a plate reader at O.D.540. Cell viability was expressed as the percentage of viability compared with media control.

[00260] The apoptosis of CLL cells after incubation with antibodies was measured using annexin V-FITC/propidium iodide (PI) staining with FACS analysis. $5x10^5$ cells in 200µl 1x binding buffer (BD Pharmingen) were stained with 5µL annexin V (BD Pharmingen) and 5µL PI (BD Pharmingen), and kept in the dark at room temperature for 15 minutes before suspension with 300µl 1x buffer and analyzed by flow cytometry. Cells without staining, cells stained only with Annexin V, and cells stained only with PI were prepared. For all flow cytometry experiments, FACS analysis was performed using a Beckman-Coulter EPICS XL cytometer (Beckman-Coulter, Miami, FL). Fluorophores were excited at 488nm. FITC-fluorescence was measured with FL1, while PI and PE fluorescence was measured with FL3. System II software package (Beckman-Coulter) was applied to analyze the data. The counted cell number was set at 10,000 for each sample.

[00261] A synergistic effect was determined by use of the isobologram method. To identify synergy, the effect of a drug combination was compared to the effect of each drug alone. This is based on the equation: Ca/Ca,b + Cb/Cb,a = CI, where Ca and Cb are the concentration of drug A and drug B alone, respectively, to produce a desired effect (e.g. 50% cell death). Ca,b and Cb,a are the concentrations of drug A and drug B in a combination, respectively, to produce the same effect. CI is the combination index. The concentrations of fludarabine and TRU-016, which elicit 50% death (IC50) were determined and are shown in Figure 27C [IC50 of Fludarabine (I) and IC50 of TRU-016 (II)]. The straight line between these two points on the axes is the line of additive effect. Subsequently, different combinations of fludarabine and TRU-016 that achieve 50% cell death were also determined from the viability study and plotted to the same graph. When points fall below the additivity line, synergy is indicated. When points rise above the line, antagonism is indicated. When points are on the line, additivity is indicated.

[00262] Figure 27 shows that TRU-016 effectively reduced relative cell viability in cells treated with fludarabine, thereby potentiating the cytotoxic

effect of fludarabine alone. Thus, this study provides evidence that TRU-016 can be co-administered with fludarabine, resulting in increased effectiveness (i.e., synergistic reduction of CLL cells) in the treatment of hematological malignancies.

Example 16

TRU-016 Induces Direct Cytotoxicity in Rituximab-Resistant Cells

[00263] As disclosed herein, rituximab is a monoclonal antibody used in the treatment of NHL, FCC, MCL, DLCL, SLL, and CLL. The present study was undertaken to determine the efficacy of TRU-016 in inducing direct cytotoxicty in cells resistant to rituximab.

[00264] Rituximab-resistant cells ($1X10^6$ cells) (Raji 4RH and RL 4RH, supplied by Dr. Myron S. Czuczman, Roswell Park Cancer Institute, Buffalo, NY) were treated with herceptin (10 μg/mL), rituximab (10 μg/mL), or TRU-016 (5 μg/mL) in the presence of a five-fold excess of goat anti-human IgG for 24 hours. Direct cytoxicity was measured by annexin/PI staining and cell viability (percent) was calculated relative to control cells (cells treated with herceptin).

[00265] TRU-016 induced greater cell toxicity than rituximab in rituximab-resistant cell lines (see Figure 28). Thus, TRU-016 is an effective agent for inducing cytoxicity in rituximab-resistant cells, making it useful as a therapeutic in diseases characterized by or involving rituximab-resistant cells, such as some B cells.

Example 17

TRU-016 Induces Tyrosine Phosphorylation in CD19+ Primary CLL B Cells

[00266] To determine how TRU-016 induces signal transduction in B cells, experiments were performed to examine the effect of TRU-016 on tyrosine phosphorylation.

[00267] Freshly isolated CD19+ cells (~50-100x106) from CLL patients were suspended at a concentration of $5X10^6$/ml PBS. Cells were then incubated for 10 minutes at 37°C, 5% $C0_2$, with control, trastuzumab (herceptin), or TRU-016 at a final concentration of 5ug/ml. Cells were spun down, supernatant was removed, and cells were resuspended in fresh PBS of initial volume. Goat anti-human Fc fragment specific crosslinker (25ug/ml) was added and cells were incubated for an additional 5 minutes. Cells were again spun down, supernatant was removed, and cells were lysed in 1ml of RIPA lysis buffer with protease and phosphatase inhibitors (10mM Tris, ph7.4, 150mM NaCl,1% Triton X-100,1% deoxycholic acid, 0.1% SDS and 5mM EDTA all final concentrations. Sigma protease inhibitor cocktail cat# P-8340; Sigma phosphatase inhibitor cocktail: serine/threonine phosphatase inhibitor cocktail cat# P-2850; and tyrosine phosphatase inhibitor cat# P-5726;PMSF (100mM) were all used. The inhibitors were added to the lysis buffer immediately prior to use at a 1:100 dilution. Protein concentration in the lysates was quantified by the bicin choninic acid (BCA) method (Pierce, Rockford, IL). The control and treated protein samples (50ug total protein) were separated by two-dimensional gel electrophoresis (pH Range 3-10) (1st Dimension) and 10% SDS-PAGE (2nd Dimension). The protein was transferred to 0.2 Nm nitrocellulose membranes (Schleicher & Schuell, Keene, NH) and subjected to immunoblot analysis using anti-phosphotyrosine antibody clone 4G10 (Upstate Biotechnology), using standard protocol. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG was used as a secondary antibody. Detection of the phosphoprotein was made with chemiluminescent substrate (SuperSignal, Pierce Inc. Rockford, IL).

[00268] TRU-016 induced tyrosine phosphorylation in CD19+ primary CLL B cells, as shown by two-dimensional gel analysis (see Figure 29). Thus, these experiments show that one way that TRU-016 acts is via a tyrosine phosphorlylation pathway.

Example 18

Humanized TRU-016 Molecules

[00269] As set out in Example 1, CD37-specific SMIPs (such as TRU-016) are described in co-owned U.S. Application No. 10/627,556 and U.S. Patent Application Publication Nos. 2003/133939, 2003/0118592 and 2005/0136049. Those descriptions are incorporated by reference herein. An exemplary CD37-specific SMIP, TRU-016 polypeptide (SEQ ID NO: 2), was produced and described therein. The present example provides humanized TRU-016 SMIPs.

[00270] Humanized antibodies are known in the art and are discussed in United States Patent Application Publication No. 2006/0153837. The present application uses the techniques involved in antibody humanization (discussed below) to humanize SMIPs, and particularly to humanize TRU-016.

[00271] "Humanization" is expected to result in an antibody that is less immunogenic, with complete retention of the antigen-binding properties of the original molecule. In order to retain all of the antigen-binding properties of the original antibody, the structure of its antigen binding site should be reproduced in the "humanized" version. This can be achieved by grafting only the nonhuman CDRs onto human variable framework domains and constant regions, with or without retention of critical framework residues (Jones et al, *Nature* 321:522 (1986); Verhoeyen et al, *Science* 239:1539 (1988)) or by recombining the entire nonhuman variable domains (to preserve ligand-binding properties), but "cloaking" them with a human-like surface through judicious replacement of exposed residues (to reduce antigenicity) (Padlan, *Molec. Immunol.* 28:489 (1991)).

[00272] Essentially, humanization by CDR grafting involves recombining only the CDRs of a non-human antibody onto a human variable region framework and a human constant region. Theoretically, this should substantially reduce or eliminate immunogenicity (except if allotypic or idiotypic differences exist). However, it has been reported that some framework residues of the original antibody also may need to be preserved

(Reichmann et al, Nature, 332:323 (1988); Queen et al, Proc. Natl. Acad. Sci. USA, 86:10,029 (1989)).

[00273] The framework residues that need to be preserved are amenable to identification through computer modeling. Alternatively, critical framework residues may potentially be identified by comparing known antigen-binding site structures (Padlan, Molec. Immun., 31(3):169-217 (1994)), incorporated herein by reference.

[00274] The residues that potentially affect antigen binding fall into several groups. The first group comprises residues that are contiguous with the antigen site surface, which could therefore make direct contact with antigens. These residues include the amino-terminal residues and those adjacent to the CDRs. The second group includes residues that could alter the structure or relative alignment of the CDRs, either by contacting the CDRs or another peptide chain in the antibody. The third group comprises amino acids with buried side chains that could influence the structural integrity of the variable domains. The residues in these groups are usually found in the same positions (Padlan, 1994, *supra*) although their positions as identified may differ depending on the numbering system (see Kabat et al, "Sequences of proteins of immunological interest, 5th ed., Pub. No. 91-3242, U.S. Dept. Health & Human Services, NIH, Bethesda, Md., 1991).

[00275] Although the present invention is directed to the humanization of SMIPs and not antibodies, knowledge about humanized antibodies in the art is applicable to the SMIPs according to the invention. Some examples of humanized TRU-016 molecules are set out in Table 5 below.

[00276] To make humanized TRU-016 constructs of the invention, the mouse framework regions of TRU-016 were aligned to human VH1 and VH5 framework residues for the heavy chain and VK1 and VK3 for the light chain. Best matches were analyzed for framework compatibility with the CDRs of the mouse variable regions. Although there were several equally compatible combinations to chose from, we had previous success using the VK3 (X01668), VH5-51(Z12373) combination, so the humanized anti-CD37 SMIPs were designed using these human frameworks joined by a 15aa Gly_4Ser

((g4s)3) scFv linker. The VK3 construct was constructed with JK1 as a preferred FR4 match and the VH5 was constructed with JH2 coding for FR4, as with previously-described constructs. SMIPs were constructed *de novo* using overlapping oligonucleotide PCR. Full-length products were cloned into the SMIP expression vector in frame with the human IgG1 hinge, CH2, and CH3. These clones were sequence verified, transfected into COS-7 cells and 3-day conditioned media tested for binding to the B-cell lymphoma line, Ramos. In order to increase humanization, changes were incorporated into CDR1 of the light chain at positions L25, L27 and L28 and were well tolerated, showing equal binding activity with the original humanized molecule 019001. Further DNA constructs were made in a similar fashion to alter the CDR3 of the VH region by incorporating germline amino acids, H100-H102, encoded by various human JH regions. Constructs were examined for expression level and degree of binding to CD37 on Ramos cells.

TABLE 5. Humanized TRU-016 Constructs

Construct No.	Description	Linker	Hinge	DNA SEQ ID NO:	AA SEQ ID NO:
019001	Vk3:VH5-51	15aa gly4ser	SSC-P	5	6
019002	Vk3:VH5-51 *Linker (TG-SS)*	15aa gly4ser	SSC-P	7	8
019003	Vk3:VH5-51 *VH V11S*	15aa gly4ser	SSC-P	9	10
019004	Vk3:VH5-51 *VK3,cdr1 (E →Q)*	15aa gly4ser	SSC-P	11	12
019005	Vk3:VH5-51 *VK3,cdr1 (N → S)*	15aa gly4ser	SSC-P	13	14
019006	Vk3:VH5-51 *VK3,cdr1 (T → A)*	15aa gly4ser	SSC-P	15	16
019010	mVk:VH5-5a	15aa gly4ser	SSC-P	17	18
019011	Vk3:mVH *(linker G-S mutation)*	15aa gly4ser	SSC-P	19	20

019017	Vk3:VH5 *VH3 FW1*	15aa gly4ser	SSC-P	21	22
019018	mVH:Vk3	15aa gly4ser	SSC-P	23	24
019019	Vk3:mVH *(019011 with 2H7 Leader)*	15aa gly4ser	SSC-P	25	26
019021	mVH:Vk3	15aa gly4ser	SSC-P	27	28
019023	Vk3:mVH *(fixed 019011 GS4 mutation)*	15aa gly4ser	SSC-P	29	30
019024	Vk3:mVH *(fixed 019011 GS4 mutation)*	15aa gly4ser	SSC-P	31	32
019025	Vk3:VH5 *VH3 FW1*	15aa gly4ser	SSC-P	33	34
019026	Vk3:VH5 *VH3 FW1*	15aa gly4ser	SSC-P	35	36
019032	Vk3:VH5 *VH3-13 FW1*	15aa gly4ser	SSC-P	37	38
019033	Vk3:VH5 *VH3-13 FW1*	15aa gly4ser	SSC-P	39	40
019034	Vk3:VH5 *VH3-13 L11S FW1*	15aa gly4ser	SSC-P	41	42
019035	Vk3:VH5 *VH3-13 L11S FW1*	15aa gly4ser	SSC-P	43	44
019037	Vk3(CDR-L1 changes):VH5	15aa gly4ser	SSC-P	45	46
019041	019006 - CDR-H3 JH4	15aa gly4ser	SSC-P	47	48
019043	019006 - CDR-H3 JH6	15aa gly4ser	SSC-P	49	50
019044	019006 - CDR-H3 JH5a	15aa gly4ser	SSC-P	51	52
019045	019006 - CDR-H3 JH5b	15aa gly4ser	SSC-P	53	54
019046	019006 - CDR-H3 JH1	15aa gly4ser	SSC-P	55	56
019047	019006 - CDR-H3 JH3a	15aa gly4ser	SSC-P	57	58
019048	019006 - CDR-H3 JH3b	15aa gly4ser	SSC-P	59	60
019049	019006 - CDR-H3 JH2	15aa gly4ser	SSC-P	79	80
019050	019006 - CDR-H2 changes	15aa gly4ser	SSC-P	81	82
019051	019044	20aa	CPPCP	83	84

		gly4ser			
019008				85	86
019009				87	88

[00277] The amino acid consensus sequence of humanized TRU-016 construct no. 019001 (SEQ ID NO: 6; H016-019001) and non-humanized TRU-016 (SEQ ID NO: 2; 016-G28-1) is shown with Kabat numbering in Figure 30A. Figure 30 B shows the amino acid sequence alignments of humanized TRU-016 construct nos. 019001 (SEQ ID NO: 6), 019008 (SEQ ID NO: 86), and 019009 (SEQ ID NO: 88).

[00278] DNA and amino acid sequence alignments of three humanized constructs of TRU-016 (019001, 019041, and 019044), demonstrating high CD37-specific binding to Ramos B cells are shown in Figure 31.

[00279] FASTA formatted DNA and amino acid sequence alignments of the same three humanized constructs of TRU-016 (019001, 019041, and 019044) are shown in Figure 32.

[00280] Additional hinge regions (Table 6) and framework regions (Table 7) that may be used in the humanized TRU-016 molecules of the invention are provided below.

TABLE 6. Hinge Regions for Humanized TRU-016 SMIPs

Hinge description	DNA or Amino Acid Sequence	SEQ ID NO:
ccc(p)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatgtccaccgtgccca	89
ccc(p)-hIgG1 (AA	EPKSCDKTHTCPPCP	90
scc(p)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatgtccaccgtgccca	91
scc(p)-hIgG1 (AA)	EPKSSDKTHTCPPCP	92
scc(s)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatgtccaccgtgctca	93
scc(s)-	EPKSSDKTHTCPPCS	94

hIgG1 (AA)		
scs(s)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatgtccaccgagctca	95
scs(s)-hIgG1 (AA)	EPKSSDKTHTCPPSS	96
sss(p)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgagcccca	97
sss(p)-hIgG1 (AA)	EPKSSDKTHTSPPSP	98
sss(s)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgagctca	99
sss(s)-hIgG1 (AA)	EPKSSDKTHTSPPSS	100
csc(p)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgtgccca	101
csc(p)-hIgG1 (AA)	EPKSCDKTHTSPPCP	102
csc(s)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgtgctca	103
csc(s)-hIgG1 (AA)	EPKSCDKTHTSPPCS	104
ssc(p)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgtgccca	105
ssc(p)-hIgG1 (AA)	EPKSSDKTHTSPPCP	106
scs(s)-hIgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgtgctca	107
scs(s)-hIgG1 (AA)	EPKSSDKTHTSPPCS	108
css(p)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgagcccca	109
css(p)-hIgG1 (AA)	EPKSCDKTHTSPPSP	110
css(s)-hIgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgagctca	111
css(s)-hIgG1 (AA)	EPKSCDKTHTSPPSS	112
scs(s)-hIgG1	gagcccaaatcttgtgacaaaactcacacatgtccaccgagctca	113

(DNA)		
scs(s)-hIgG1 (AA)	EPKSSDKTHTCPPSS	114
hIgA1	VPSTPPTPSPSTPPTPSPS	115
hIgA2	VPPPPP	116
hIgG3 (DNA)	gagctcaaaactcctctcggggatacgacccatacgtgtccccgctgtcctgaaccgaagtcctgcgatacgcctccgccatgtccacggtgcccagagcccaaatcatgcgatacgcccccaccgtgtccccgctgtcctgaaccaaagtcatgcgataccccaccaccatgtccaagatgccca	117
hIgG3 (AA)	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCP EPKSCDTPPPCPRCPEPKSCDTPPPCPRCP	118
IgG315hscc (DNA)	gagcccaaatcttctgacacacctcccccatgcccacggtgcccc	119
IgG315hscc (AA)	EPKSSDTPPPCPRCP	120
IgG315hcss (DNA)	gagcccaaatcttgtgacacacctcccccatccccacggtcccca	121
IgG315hcss (AA)	EPKSCDTPPPSPRSP	122
IgG315hsss (DNA)	gagcccaaatcttctgacacacctcccccatccccacggtcccca	123
IgG315hsss (AA)	EPKSSDTPPPSPRSP	124
IgG3hl5csc (DNA)	gagcccaaatcttgtgacacacctcccccatccccacggtgccca	125
IgG3hl5csc (AA)	EPKSCDTPPPSPRCP	126
hIgD	ESPKAQASSVPTAQPQAEGSLAKATTAPATTR NTGRGGEEKKKEKEKEEQEERETKTP	127

TABLE 7. Framework Regions for Humanized TRU-016 SMIPs

V-region	Human VH Framework Regions for anti-CD37 Humanization	SEQ ID NO:
	FR1	
VH1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	140
VH1	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS	141
VH1	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS	142
VH1	EVQLVQSGAEVKKPGATVKISCKVSGYTFT	143
VH5	EVQLVQSGAEVKKPGESLKISCKGSGYSFT	144
VH5	EVQLVQSGAEVKKPGESLRISCKGSGYSFT	145
VH7	QVQLVQSGSELKKPGASVKVSCKASGYTFT	146
	FR2	
VH1	WVRQAPGQGLEWMG	147
VH1	WVRQAPGQGLEWMG	148

VH1	WVRQAPGQGLEWMG	149
VH1	WVQQAPGKGLEWMG	150
VH5	WVRQMPGKGLEWMG	151
VH5	WVRQMPGKGLEWMG	152
VH7	WVRQAPGQGLEWMG	153
	FR3	
VH1	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR	154
VH1	RVTITADESTSTAYMELSSLRSEDTAVYYCAR	155
VH1	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR	156
VH1	RVTITADTSTDTAYMELSSLRSEDTAVYYCAT	157
VH5	QVTISADKSISTAYLQWSSLKASDTAMYYCAR	158
VH5	HVTISADKSISTAYLQWSSLKASDTAMYYCAR	159
VH7	RFVFSLDTSVSTAYLQISSLKAEDTAVYYCAR	160
	FR4	
	WGQGTLVTVSS	161
	WGRGTLVTVSS	162
	WGQGTMVTVSS	163
	WGQGTMVTVSS	164
	WGQGTLVTVSS	165
	WGQGTLVTVSS	166
	WGQGTLVTVSS	167
	WGQGTTVTVSS	168
	WGKGTTVTVSS	169
	Human VK Framework Regions for anti-CD37 Humanization	
	FR1	
VK3	EIVMTQSPATLSVSPGERATLSC	170
VK3	EIVLTQSPATLSLSPGERATLSC	171
VK1	DIQMTQSPSSLSASVGDRVTITC	172
VK1	DIQMTQSPSSLSASVGDRVTITC	173
VK1	DIQMTQSPSSLSASVGDRVTITC	174
VK1	NIQMTQSPSAMSASVGDRVTITC	175
VK1	DIQMTQSPSSLSASVGDRVTITC	176
VK1	AIQLTQSPSSLSASVGDRVTITC	177
VK1	DIQLTQSPSFLSASVGDRVTITC	178
VK1	AIRMTQSPFSLSASVGDRVTITC	179
VK1	AIQMTQSPSSLSASVGDRVTITC	180
VK1	DIQMTQSPSTLSASVGDRVTITC	181
	FR2	
VK3	WYQQKPGQAPRLLIY	182
VK3	WYQQKPGQAPRLLIY	183
VK1	WYQQKPGKAPKLLIY	184
VK1	WYQQKPGKVPKLLIY	185
VK1	WYQQKPGKAPKRLIY	186
VK1	WFQQKPGKVPKHLIY	187
VK1	WFQQKPGKAPKSLIY	188

VK1	WYQQKPGKAPKLLIY	189
VK1	WYQQKPGKAPKLLIY	190
VK1	WYQQKPAKAPKLFIY	191
VK1	WYQQKPGKAPKLLIY	192
VK1	WYQQKPGKAPKLLIY	193
	FR3	
VK3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	194
VK3	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	195
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	196
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	197
VK1	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	198
VK1	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	199
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	200
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	201
VK1	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	202
VK1	GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC	203
VK1	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	204
VK1	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	205
	FR4	
	FGQGTKVEIK	206
	FGQGTKLEIK	207
	FGPGTKVDIK	208
	FGGGTKVEIK	209
	FGQGTRLEIK	210

[00281] Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

DNA and Amino Acid Sequences for SEQ ID NOS: 79-88

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
019049	79	aagcttgccgccatggaagccccagcgcagcttctcttcctcctgctactctggctcccag ataccaccggagaaattgtgttgacacagtctccagccaccctgtctttgtctccaggcga aagagccaccctctcctgccgagcaagtgaaaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctattttgcaaaaaccttagcagaagg aattccagccaggttcagtggcagtggatccgggacagacttcactctcaccatcagca gcctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaaggtggcggtggctcgggcggtggtggat ctggaggaggtgggaccggtgaggtgcagctggtgcagtctggagcagaggtgaaaa agcccggagagtctctgaagatttcctgtaagggatccggttactcattcactggctacaa tatgaactgggtgcgccagatgcccgggaaaggcctcgagtggatgggcaatattgatc cttattatggtggtactacctacaaccggaagttcaagggccaggtcactatctccgccga caagtccatcagcaccgcctacctgcaatggagcagcctgaaggcctcggacaccgc catgtattactgtgcacgctcagtcggccctttcgacctctggggcagaggcaccctggtc actgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtgcc cagcacctgaactcctgggtggaccgtcagtcttcctcttccccccaaaacccaaggac accctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccacga agaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag acaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcacc gtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaa gccctcccagccccccatcgagaaaaccatctccaaagccaaagggcagccccgaga accacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcag cctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagca atgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggct ccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgt cttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc cctgtctccgggtaaatgatctaga

019049	80	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCR ASENVYSYLAWYQQKPGQAPRLLIYFAKTLAEGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQHHSDNPWTFGQGTKVEIKGGG GSGGGGSGGGGTGEVQLVQSGAEVKKPGESLKISCKGSGYS FTGYNMNWVRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQ VTISADKSISTAYLQWSSLKASDTAMYYCARSVGPFDLWGRGT LVTVSSDQEPKSSDKTHTSPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
019050	81	aagcttgccgccatggaagccccagctcagcttctcttcctcctgctactctggctcccag ataccaccggagaaattgtgttgacacagtctccagccaccctgtctttgtctccaggcga aagagccaccctctcctgccgagcaagtgaaaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctattttgcaaaaaccttagcagaagg aattccagccaggttcagtggcagtggatccgggacagacttcactctcaccatcagca gcctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaaggtggcggtggctcgggcggtggtggat ctggaggaggtggggctagcgaggtgcagctggtgcagtctggagcagaggtgaaaa agcccggagagtctctgaagatttcctgtaagggatccggttactcattcactagctacaa tatgaactgggtgcgccagatgcccgggaaaggcctggagtggatgggcaatattgat ccttattatggtggtactaactacgcccagaagttccagggccaggtcactatctccgccg acaagtccatcagcaccgcctacctgcaatggagcagcctgaaggcctcggacaccg ccatgtattactgtgcacgctcagtcggccctatggactactggggccgcggcaccctgg tcactgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtg cccagcacctgaactcctgggtggaccgtcagtcttcctcttcccccaaaacccaagg acaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccac gaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca agacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctc accgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac aaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccg agaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggt cagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgggaga gcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggga acgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga

019050	82	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCR ASENVYSYLAWYQQKPGQAPRLLIYFAKTLAEGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQHHSDNPWTFGQGTKVEIKGGG GSGGGGSGGGGASEVQLVQSGAEVKKPGESLKISCKGSGYS FTSYNMNWVRQMPGKGLEWMGNIDPYYGGTNYAQKFQGQ VTISADKSISTAYLQWSSLKASDTAMYYCARSVGPMDYWGRG TLVTVSSDQEPKSSDKTHTSPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
019051	83	aagcttgccgccatggaagccccagcgcagcttctcttcctcctgctactctggctcccag ataccaccggagaaattgtgttgacacagtctccagccaccctgtctttgtctccaggcga aagagccaccctctcctgccgagcaagtgagaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctattttgcaaaaaccttagcagaagg gattccagccagattcagtggcagtggttccgggacagacttcactctcaccatcagcag cctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacatt cggccaagggaccaaggtggaaatcaaaggtggcggtggctcgggcggtggtggat ctggaggaggtgggagcggaggaggagctagcgaggtgcagctggtgcagtctgga gcagaggtgaaaaagcccggagagtctctgaagatttcctgtaagggatccggttactc attcactggctacaatatgaactgggtgcgccagatgcccgggaaaggcctcgaatgg atgggcaatattgatccttattatggtggtactacctacaaccggaagttcaagggccagg tcactatctccgccgacaagtccatcagcaccgcctacctgcaaggagcagcctgaag gcctcggacaccgccatgtattactgtgcacgctcagtcggccctttcgactcctggggcc agggcaccctggtcactgtctcgagttgtccaccgtgcccagcacctgaactcctgggtg gaccgtcagtcttcctcttcccccccaaaacccaaggacaccctcatgatctcccggaccc ctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaa ctggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggaggagc agtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctg aatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgag aaaaccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctgcc cccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaagg cttctatccaagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaa ctacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctc accgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatg aggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatgactc taga

019051	84	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCR ASENVYSYLAWYQQKPGQAPRLLIYFAKTLAEGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQHHSDNPWTFGQGTKVEIKGGG GSGGGGSGGGGSGGGASEVQLVQSGAEVKKPGESLKISCKG SGYSFTGYNMNWVRQMPGKGLEWMGNIDPYYGGTTYNRKF KGQVTISADKSISTAYLQWSSLKASDTAMYYCARSVGPFDSW GQGTLVTVSSCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
019008	85	aagcttgccgccatggaagccccagctcagcttctcttcctcctgctactctggctcccag ataccaccggagaaattgtgttgacacagtctccagccaccctgtctttgtctccaggcga aagagccaccctctcctgccgaacaagtgaaaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctattttgcaaaaaccttagcagaagg aattccagccaggttcagtggcagtggatccgggacagacttcactctcaccatcagca gcctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaaggtggcggtggctcgggcggtggtggat ctggaggaggtgggaccggtgaggtgcagctggtgcagtctggagcagaggtgaaaa agcccggagagtctctgaagatttcctgtaagggatccggttactcattcactggctacaa tatgaactgggtgcgccagatgcccgggaaaggcctggagtggatgggcaatattgat ccttattatggtggtactacctacaaccggaagttcaagggccaggtcactatctccgccg acaagtccatcagcaccgcctacctgcaatggagcagcctgaaggcctcggacaccg ccatgtattactgtgcacgctcagtcggccctatggactactggggccgcggcaccctgg tcactgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtg cccagcacctgaactcctgggtggaccgtcagtcttcctcttcccccaaaacccaagg acaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggacgtgagccac gaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca agacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctc accgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac aaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccg agaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggt cagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgggaga gcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggga acgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga

019008	86	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCR TSENVYSYLAWYQQKPGQAPRLLIYFAKTLAEGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQHHSDNPWTFGQGTKVEIKGGG GSGGGGSGGGGASEVQLVQSGAEVKKPGESLKISCKGSGYS FTGYNMNWVRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQV TISADKSISTAYLQWSSLKASDTAMYYCARSVGPMDYWGRGT LVTVSSDQEPKSSDKTHTSPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK
019009	87	aagcttgccgccatggaagccccagctcagcttctcttcctcctgctactctggctcccag ataccaccggtgaaattgtgttgacacagtctccagccaccctgtctttgtctccaggcga aagagccaccctctcctgccgaacaagtgaaaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctattttgcaaaaaccttagcagaagg aattccagccaggttcagtggcagtggatccgggacagacttcactctcaccatcagca gcctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaaggtggcggtggctcgggcggtggtggat ctggaggaggtggggctagcgaggtgcagctggtgcagtctggagcagaggtgaaaa agcccggagagtctctgaggatttcctgtaagggatccggttactcattcactggctacaa tatgaactgggtgcgccagatgcccgggaaaggcctggagtggatgggcaatattgat ccttattatggtggtactacctacaaccggaagttcaagggccaggtcactatctccgccg acaagtccatcagcaccgcctacctgcaatggagcagcctgaaggcctcggacaccg ccatgtattactgtgcacgctcagtcggccctatggactactggggccgcggcaccctgg tcactgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtg cccagcacctgaactcctgggtggaccgtcagtcttcctcttcccccaaaacccaagg acaccctcatgatctcccggaccccctgaggtcacatgcgtggtggtggacgtgagccac gaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcca agacaaagccgcgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctc accgtcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggtctccaac aaagccctcccagccccccatcgagaaaaccatctccaaagccaaagggcagccccg agaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggt cagcctgacctgcctggtcaaaggcttctatccaagcgacatcgccgtggagtgggaga gcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgac ggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggcagcagggga acgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga

019009	88	MEAPAQLLFLLLLWLPDTTGEIVLTQSPATLSLSPGERATLSCR TSENVYSYLAWYQQKPGQAPRLLIYFAKTLAEGIPARFSGSGS GTDFTLTISSLEPEDFAVYYCQHHSDNPWTFGQGTKVEIKGGG GSGGGGSGGGGASEVQLVQSGAEVKKPGESLRISCKGSGYS FTGYNMNWVRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQV TISADKSISTAYLQWSSLKASDTAMYYCARSVGPMDYWGRGT LVTVSSDQEPKSSDKTHTSPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK

WHAT IS CLAIMED IS:

1. A method of reducing B-cells comprising exposing B-cells to a synergistic combination of one or more CD37-specific binding molecules and one or more CD20-specific binding molecules.

2. A method of reducing B-cells comprising exposing B-cells to a synergistic combination of one or more CD37-specific small, modular immunopharmaceuticals (SMIPs) and one or more CD20-specific antibodies.

3. The method of claim 2 wherein said CD37-specific SMIP is TRU-016.

4. The method of claim 2 wherein said CD20-specific antibody is rituxan.

5. The method of claim 2 wherein said CD37-specific SMIP is TRU-016 and said CD20-specific antibody is rituxan.

6. A method of reducing B-cells comprising exposing B-cells to a synergistic combination of CD37-specific SMIPs and CD20-specific SMIPs.

7. The method of claim 6 wherein said CD37-specific SMIP is TRU-016.

8. The method of claim 6 wherein said CD20-specific antibody is TRU-015.

9. The method of claim 6 wherein said CD37-specific SMIP is TRU-016 and said CD20-specific antibody is TRU-015.

10. The method of claim 1, 2, or 6 wherein B cell reduction by complement-dependent cytoxicity is synergistically increased by treatment with the combination.

11. A method of treating a individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to a individual a synergistic combination of one or more CD37-specific binding molecules and one or more CD20-specific binding molecules.

12. A method of treating a individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to a individual a synergistic combination of one or more CD37-specific SMIPs and one or more CD20-specific antibodies.

13. A method of treating a individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to a individual a synergistic combination of one or more CD37-specific SMIP and one or more CD20-specific SMIPs.

14. The method of claim 11, 12 or 13 wherein the disease is selected from the group consisting of a B-cell lymphoma, a B-cell leukemia, a B-cell myeloma, a disease characterized by autoantibody production and a disease characterized by inappropriate T-cell stimulation associated a B-cell pathway.

15. The method of claim 14 wherein the disorder characterized by autoantibody production is selected from the group consisting of idiopathic inflammatory myopathy, rheumatoid arthritis, myasthenia gravis, Grave's

disease, type I diabetes mellitus, multiple sclerosis, an autoimmune disease, dermatomyositis, polymyositis and Waldenstrom's macroglobinemia.

16. The method of claim 14 wherein the combination is administered in conjunction with a second agent.

17. The method of claim 16 wherein the second agent is a cytokine, a chemokine, a growth factor, a chemotherapeutic agent, or a radiotherapeutic agent.

18. The method of claim 16 wherein the second agent is an immunosuppressive agent.

19. The method of claim 12 or 13 wherein a SMIP is administered in a dose range of about 0.01 to about 50 mg/kg.

20. The method of claim 19 wherein a SMIP is administered in a dose range of about 0.015 to about 30 mg/kg.

21. The method of claim 20 wherein a SMIP is administered in a dose of about 0.015, about 0.05, about 0.15, about 0.5, about 1.5, about 5, about 15 or about 30 mg/kg.

22. The method of claim 11, 12 or 13 wherein the binding molecules of the combination are administered sequentially within about a 24-hour period or are administered simultaneously.

23. The method of claim 11 wherein the binding molecules have affinities of about 0.5 to about 10 nM.

24. The method of claim 11 wherein the binding molecules have half lives in circulation of about 7 to about 30 days.

25. An article of manufacture comprising a CD37-specific binding molecule and a CD20-specific binding molecule and a label indicating a method according to claim 11.

26. An article of manufacture comprising a CD37-specific SMIP and a CD20-specific antibody and a label indicating a method according to claim 12.

27. An article of manufacture comprising a CD37-specific SMIP and a CD20-specific SMIP and a label indicating a method according to claim 13.

28. A method of reducing B-cells comprising exposing B-cells to one or more CD37-specific binding molecules.

29. A method of reducing B-cells comprising exposing B-cells to a combination of one or more CD37-specific small, modular immunopharmaceuticals (SMIPs) and one or more CD37-specific antibodies.

30. The method of claim 29 wherein said CD37-specific SMIP is TRU-016.

31. The method of claim 29 wherein the CD37-specific antibody is selected from the group consisting of: G28-1, MB371, BL14, NMN46, IPO24, HH1, and WR17.

32. The method of claim 28 or 29 wherein B cell reduction by complement-dependent cytoxicity is increased by treatment with the combination.

33. A method of treating an individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to the individual one or more CD37-specific binding molecules.

34. A method of treating an individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to the individual one or more CD37-specific SMIPs.

35. A method of treating an individual having or suspected of having a disease associated with aberrant B-cell activity, comprising administering to the individual a combination of one or more CD37-specific SMIP and one or more CD37-specific antibodies.

36. The method of claim 33, 34, or 35 wherein the disease is selected from the group consisting of a B-cell lymphoma, a B-cell leukemia, a B-cell myeloma, a disease characterized by autoantibody production, and a disease characterized by inappropriate T-cell stimulation associated with a B-cell pathway.

37. The method of claim 36 wherein the disease characterized by autoantibody production is selected from the group consisting of idiopathic inflammatory myopathy, rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis, an autoimmune disease, dermatomyositis, polymyositis and Waldenstrom's macroglobinemia.

38. The method of claim 35 wherein the combination is administered in conjunction with a second agent.

39. The method of claim 38 wherein the second agent is a cytokine, a chemokine, a growth factor, a chemotherapeutic agent, or a radiotherapeutic agent.

40. The method of claim 38 wherein the second agent is an immunosuppressive agent.

41. The method of claim 34 or 35 wherein the SMIP is administered in a dose range of about 0.01 mg/kg to about 50 mg/kg.

42. The method of claim 41 wherein the SMIP is administered in a dose range of about 0.015 mg/kg to about 30 mg/kg.

43. The method of claim 42 wherein the SMIP is administered in a dose of about 0.015 mg/kg, about 0.05 mg/kg, about 0.15 mg/kg, about 0.5 mg/kg, about 1.5 mg/kg, about 5 mg/kg, about 15 mg/kg or about 30 mg/kg.

44. The method of claim 33, 34, or 35 wherein the binding molecules of the combination are administered sequentially within about a 24-hour period or are administered simultaneously.

45. The method of claim 33 wherein the binding molecules have affinities of about 0.5 to about 10 nM.

46. The method of claim 33 wherein the binding molecules have half lives in circulation of about 7 to about 30 days.

47. An article of manufacture comprising a CD37-specific binding molecule and a label indicating a method according to claim 33.

48. An article of manufacture comprising a CD37-specific SMIP and a label indicating a method according to claim 34.

49. An article of manufacture comprising a CD37-specific SMIP and a CD37-specific antibody and a label indicating a method according to claim 35.

50. A humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37.

51. A humanized CD37-specific SMIP polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 80, 82, 84, 86, and 88.

52. The humanized CD37-specific SMIP polypeptide of claim 50 or 51 comprising at least one amino acid modification in a complementarity-determining region (CDR) selected from the group consisting of: light chain CDR1, heavy chain CDR1, light chain CDR2, heavy chain CDR2, light chain CDR3, and heavy chain CDR3.

53. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR1 of the light chain comprises the amino acid sequence of SEQ ID NO: 61 (RASENVYSYLA).

54. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR1 of the light chain comprises the amino acid sequence of SEQ ID NO: 62 (RTSENVYSYLA).

55. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR1 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 63 (GYMNM).

56. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR2 of the light chain comprises the amino acid sequence of SEQ ID NO: 64 (FAKTLAE).

57. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR2 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 65 (NIDPYYGGTTTYNRKFKG).

58. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 66 (QHHSDNPWT).

59. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 67 (SVGPFDY).

60. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 68 (SVGPFDS).

61. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 69 (SVGPMDY).

62. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least one of the light chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 61, 62, 64, and 66.

63. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least two sequences of the light chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 61, 62, 64, and 66.

64. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least three sequences of the light chain CDR amino acid sequences selected from the group consisting of SEQ ID NO: 61, 62, 64, and 66.

65. The humanized CD37-specific SMIP polypeptide of claim 50 comprising:

(a) a light chain CDR1 amino acid sequence of SEQ ID NO: 61 or 62, or a variant thereof in which one or two amino acids of SEQ ID NO: 61 or 62 has been changed;

(b) a light chain CDR2 amino acid sequence of SEQ ID NO: 64, or a variant thereof in which one or two amino acids of SEQ ID NO: 64 has been changed; and

(c) a light chain CDR3 amino acid sequence of SEQ ID NO: 66, or a variant thereof in which one or two amino acids of SEQ ID NO: 66 has been changed.

66. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least one of the heavy chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 63, 65, and 67-69.

67. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least two sequences of the heavy chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 63, 65, and 67-69.

68. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least three sequences of the heavy chain CDR amino acid sequences selected from the group consisting of SEQ ID NOS: 63, 65, and 67-69.

69. The humanized CD37-specific SMIP polypeptide of claim 50 comprising:

(a) a heavy chain CDR1 amino acid sequence of SEQ ID NO: 63, or a variant thereof in which one or two amino acids of SEQ ID NO: 63 has been changed;

(b) a heavy chain CDR2 amino acid sequence of SEQ ID NO: 65, or a variant thereof in which one or two amino acids of SEQ ID NO: 65 has been changed; and

(c) a heavy chain CDR3 amino acid sequence selected from the group consisting of SEQ ID NOS: 67-69, or a variant thereof in which one or two amino acids of any one of SEQ ID NOS: 67-69 has been changed.

70. The humanized CD37-specific SMIP polypeptide of claim 50 or 51 comprising at least one amino acid modification in a framework region (FR) selected from the group consisting of: light chain FR1, heavy chain FR1, light chain FR2, heavy chain FR2, light chain FR3, heavy chain FR3, light chain FR4, and heavy chain FR4.

71. The humanized CD37-specific SMIP polypeptide of claim 50, wherein the first framework region (FR1) of the light chain comprises the amino acid sequence of SEQ ID NO: 70 (EIVLTQSPATLSLSPGERATLSC).

72. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR1 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 71 (EVQLVQSGAEVKKPGESLKISCKGSGYSFT).

73. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR2 of the light chain comprises the amino acid sequence of SEQ ID NO: 72 (WYQQKPGQAPRLLIY).

74. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR2 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 73 (WVRQMPGKGLEWMG).

75. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 74 (GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC).

76. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 75 (QVTISADKSISTAYLQWSSLKASDTAMYYCAR).

77. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR4 of the light chain comprises the amino acid sequence of SEQ ID NO: 76 (FGQGTKVEIK).

78. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 77 (WGQGTLVTVSS).

79. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 78 (WGRGTLVTVSS).

80. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least one sequence of the light chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 70, 72, 74, and 76.

81. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least two sequences of the light chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 70, 72, 74, and 76.

82. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least three sequences of the light chain FR amino acid

sequences selected from the group consisting of SEQ ID NOS: 70, 72, 74, and 76.

83. The humanized CD37-specific SMIP polypeptide of claim 50 comprising:

(a) a light chain FR1 amino acid sequence of SEQ ID NO: 70, or a variant thereof in which one or two amino acids of SEQ ID NO: 70 have been changed;

(b) a light chain FR2 amino acid sequence of SEQ ID NO: 72, or a variant thereof in which one or two amino acids of SEQ ID NO: 72 have been changed;

(c) a light chain FR3 amino acid sequence of SEQ ID NO: 74, or a variant thereof in which one or two amino acids of SEQ ID NO: 74 have been changed; and

(d) a light chain FR4 amino acid sequence of SEQ ID NO: 76, or a variant thereof in which one or two amino acids of SEQ ID NO: 76 have been changed.

84. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least one sequence of the heavy chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 71, 73, 75, 77, and 78.

85. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least two sequences of the heavy chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 71, 73, 75, 77, and 78.

86. The humanized CD37-specific SMIP polypeptide of claim 50 comprising at least three sequences of the heavy chain FR amino acid sequences selected from the group consisting of SEQ ID NOS: 71, 73, 75, 77, and 78.

87. The humanized CD37-specific SMIP polypeptide of claim 50 comprising:

(a) a heavy chain FR1 amino acid sequence of SEQ ID NO: 71, or a variant thereof in which one or two amino acids of SEQ ID NO: 71 has been changed;

(b) a heavy chain FR2 amino acid sequence of SEQ ID NO: 73, or a variant thereof in which one or two amino acids of SEQ ID NO: 73 has been changed;

(c) a heavy chain FR3 amino acid sequence of SEQ ID NO: 75, or a variant thereof in which one or two amino acids of SEQ ID NO: 75 has been changed; and

(d) a heavy chain FR4 amino acid sequence of SEQ ID NO: 77 or 78, or a variant thereof in which one or two amino acids of SEQ ID NO: 77 or 78 have been changed.

88. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37.

89. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 79, 81, 83, 85, and 87.

90. A vector comprising the nucleic acid molecule of claim 88 or 89.

91. A host cell comprising the vector of claim 90.

92. A process of producing a polypeptide comprising culturing the host cell of claim 91 under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

93. A composition comprising the humanized CD37-specific SMIP polypeptide of any one of claims 50-87 and a pharmaceutically acceptable carrier.

94. The method of any one of claims 2, 6, 10-29, and 34-49 wherein the CD37-specific SMIP or CD37-specific binding molecule is humanized and exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2.

95. The method of any one of claims 2, 6, 10-29, and 34-49 wherein the CD37-specific SMIP or CD37-specific binding molecule comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 80, 82, 84, 86, and 88.

96. A kit for reducing B-cells comprising:

(a) the composition of claim 93; and

(b) a protocol for using the kit to reduce B cells.

97. The kit of claim 96 further comprising a CD20-specific binding molecule.

98. The kit of claim 96 or 97 wherein the CD20-specific binding molecule is TRU-015 or a humanized TRU-015.

99. A humanized CD37-specific SMIP polypeptide comprising a CDR1, a CDR2, and a CDR3, that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2.

100. The polypeptide of claim 99 further comprising a human framework domain separating each of CDR1, CDR2, and CDR3.

101. A humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37 and comprises a hinge region polypeptide sequence selected from the group consisting of SEQ ID NOS: 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 115, 116, 118, 120, 122, 124, 126 and 127.

102. A humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein the humanized CD37-specific SMIP polypeptide binds CD37 and comprises a hinge region polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 90, 92, 94, 96, 98, 100, 102, 104, 106, 108 110, 112, 114, 115, 116, 118, 120, 122, 124, 126 and 127.

103. A humanized CD37-specific SMIP polypeptide that exhibits at least 80 percent identity to the polypeptide set forth in SEQ ID NO: 2, wherein

the humanized CD37-specific SMIP polypeptide binds CD37 and comprises a linker comprising $(Gly_4Ser)_n$, wherein n is 1, 2, 3, 4, 5, or 6.

104. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR1 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 128 (RTSQNVYSYLA), 129 (RTSESVYSYLA), 130 (RASQSVYSYLA), 131 (RASQSVSSYLA) and 132 (RASQSVSYYLA).

105. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR1 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 133 (SYMNM) and 134 (SYWIG).

106. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR2 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 135 (AASSLQS), 136 (GASTRAT) and 137 (DASNRAT).

107. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR2 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 138 (IIYPGDSDTRYSPSFQG) and 139 (RIDPSDSYTNYSPSFQG).

108. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 220 (QHHSDNPWT).

109. The humanized CD37-specific SMIP polypeptide of claim 50, wherein CDR3 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 211 (SVGPMDY), 212 (SVGPFDY), 213 (SVGPMDV), 214 (SVGPFDS), 215 (SVGPFDP), 216 (SVGPFQH), 217 (SVGPFDV), 218 (SVGPFDI) and 219 (SVGPFDL).

110. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR1 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 170-181.

111. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR1 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 140-146.

112. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR2 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 182-193.

113. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR2 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 147-153.

114. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR3 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 194-205.

115. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR3 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 154-160.

116. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR4 of the light chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 206-210.

117. The humanized CD37-specific SMIP polypeptide of claim 50, wherein FR4 of the heavy chain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 161-169.

SEQUENCE LISTING

<110> Grosmaire et al.

<120> B-Cell Reduction Using CD37-Specific and CD20-Specific Binding Molecules

<130> 30906/41324PCT

<150> US 60/702,499
<151> 2005-07-25

<150> US 60/800,595
<151> 2006-05-16

<160> 78

<170> PatentIn version 3.3

<210> 1
<211> 1510
<212> DNA
<213> Artificial sequence

<220>
<223> TRU-016 polynucleotide

<400> 1
aagcttgccg ccatggattt tcaagtgcag attttcagct tcctgctaat cagtgcttca 60

gtcataattg ccagaggagt cgacatccag atgactcagt ctccagcctc cctatctgca 120

tctgtgggag agactgtcac catcacatgt cgaacaagtg aaaatgttta cagttatttg 180

gcttggtatc agcagaaaca gggaaaatct cctcagctcc tggtctcttt tgcaaaaacc 240

ttagcagaag gtgtgccatc aaggttcagt ggcagtggat caggcacaca gttttctctg 300

aagatcagca gcctgcagcc tgaagattct ggaagttatt tctgtcaaca tcattccgat 360

aatccgtgga cgttcggtgg aggcaccgaa ctggagatca aaggtggcgg tggctcgggc 420

ggtggtgggt cgggtggcgg cggatcgtca gcggtccagc tgcagcagtc tggacctgag 480

tcggaaaagc ctggcgcttc agtgaagatt tcctgcaagg cttctggtta ctcattcact 540

ggctacaata tgaactgggt gaagcagaat aatggaaaga gccttgagtg gattggaaat 600

attgatcctt attatggtgg tactacctac aaccggaagt tcaagggcaa ggccacattg 660

actgtagaca aatcctccag cacagcctac atgcagctca agagtctgac atctgaggac 720

tctgcagtct attactgtgc aagatcggtc ggccctatgg actactgggg tcaaggaacc 780

tcagtcaccg tctcttcaga tctggagccc aaatcttctg acaaaactca cacatctcca 840

ccgtgcccag cacctgaact cttgggtgga ccgtcagtct tcctcttccc cccaaaaccc 900

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 960

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1080

gtccctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1140

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1200

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260

ctggtcaaag gcttctatcc aagcgacatc gccgtggagt gggagagcaa tgggcaaccg 1320

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1380

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 1500

tgagtctaga 1510

<210> 2
<211> 496
<212> PRT
<213> Artificial sequence

<220>
<223> TRU-016 polypeptide

<400> 2

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

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

Ser Leu Ser Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr
35 40 45

Ser Glu Asn Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly
50 55 60

Lys Ser Pro Gln Leu Leu Val Ser Phe Ala Lys Thr Leu Ala Glu Gly
65 70 75 80

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu
85 90 95

Lys Ile Ser Ser Leu Gln Pro Glu Asp Ser Gly Ser Tyr Phe Cys Gln
100 105 110

His His Ser Asp Asn Pro Trp Thr Phe Gly Gly Gly Thr Glu Leu Glu
115 120 125

Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ser Ser Ala Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro
145 150 155 160

Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
165 170 175

Gly Tyr Asn Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu
180 185 190

Trp Ile Gly Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg
195 200 205

Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr
210 215 220

Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
225 230 235 240

Tyr Cys Ala Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr
245 250 255

Ser Val Thr Val Ser Ser Asp Leu Glu Pro Lys Ser Ser Asp Lys Thr
260 265 270

His Thr Ser Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
275 280 285

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
290 295 300

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
305 310 315 320

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
325 330 335

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
340 345 350

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
355 360 365

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
370 375 380

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
385 390 395 400

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
405 410 415

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
420 425 430

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
435 440 445

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
450 455 460

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
465 470 475 480

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490 495

<210> 3
<211> 1518
<212> DNA
<213> Artificial sequence

<220>
<223> TRU-015 polynucleotide

<400> 3
aagcttgccg ccatggattt tcaagtgcag attttcagct cctgctaat cagtgcttca 60

gtcataatgt ccagaggaca aattgttctc tcccagtctc cagcaatcct gtctgcatct 120

ccaggggaga aggtcacaat gacttgcagg gccagctcaa gtgtaagtta catgcactgg 180

taccagcaga agccaggatc ctccccaaa ccctggattt atgccccatc caacctggct 240

tctggagtcc ctgctcgctt cagtggcagt gggtctggga cctcttactc tctcacaatc 300

agcagagtgg aggctgaaga tgctgccact tattactgcc agcagtggag ttttaaccca 360

cccacgttcg gtgctgggac caagctggag ctgaaagatg gcggtggctc gggcggtggt 420

ggatctggag gaggtgggag ctctcaggct tatctacagc agtctggggc tgagtcggtg 480

aggcctgggg cctcagtgaa gatgtcctgc aaggcttctg gctacacatt taccagttac 540

aatatgcact gggtaaagca gacacctaga cagggcctgg aatggattgg agctatttat 600

ccaggaaatg gtgatacttc ctacaatcag aagttcaagg gcaaggccac actgactgta 660

gacaaatcct ccagcacagc ctacatgcag ctcagcagcc tgacatctga agactctgcg 720

gtctatttct gtgcaagagt ggtgtactat agtaactctt actggtactt cgatgtctgg 780

ggcacaggga ccacggtcac cgtctctgat caggagccca aatcttgtga caaaactcac 840

acatctccac cgtgctcagc acctgaactc ctgggtggac cgtcagtctt cctcttcccc 900

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 960

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 1020

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 1080

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 1140

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 1200

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 1260

ctgacctgcc tggtcaaagg cttctatcca agcgacatcg ccgtggagtg ggagagcaat 1320

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1380

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1440

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1500

ccgggtaaat gatctaga 1518

<210> 4
<211> 499
<212> PRT
<213> Artificial sequence

<220>
<223> TRU-015 polypeptide

<400> 4

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Ser Gln Ser Pro Ala Ile
20 25 30

Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser
35 40 45

Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser
50 55 60

Pro Lys Pro Trp Ile Tyr Ala Pro Ser Asn Leu Ala Ser Gly Val Pro
65 70 75 80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
85 90 95

Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
100 105 110

Ser Phe Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
115 120 125

Asp Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
130 135 140

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Ser Val Arg Pro Gly Ala
145 150 155 160

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
165 170 175

Asn Met His Trp Val Lys Gln Thr Pro Arg Gln Gly Leu Glu Trp Ile
180 185 190

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
195 200 205

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
210 215 220

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
225 230 235 240

Ala Arg Val Val Tyr Tyr Ser Asn Ser Tyr Trp Tyr Phe Asp Val Trp
245 250 255

Gly Thr Gly Thr Thr Val Thr Val Ser Asp Gln Glu Pro Lys Ser Cys
260 265 270

Asp Lys Thr His Thr Ser Pro Pro Cys Ser Ala Pro Glu Leu Leu Gly
275 280 285

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
290 295 300

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
305 310 315 320

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
325 330 335

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
340 345 350

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
355 360 365

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
370 375 380

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
385 390 395 400

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
405 410 415

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
420 425 430

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
435 440 445

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
450 455 460

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
465 470 475 480

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
485 490 495

Pro Gly Lys

<210> 5
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 5
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata tccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 6
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 6

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
275 280 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
290 295 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305 310 315 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
325 330 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
340 345 350

```
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        355                 360                 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    370                 375                 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385                 390                 395                 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                405                 410                 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            420                 425                 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        435                 440                 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    450                 455                 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465                 470                 475                 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 7
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 7

```
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga     60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc    120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct    180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc    240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct    300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa    360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt    420

gggagctctg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct    480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg    540
```

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 8
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 8

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
275 280 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
290 295 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305 310 315 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
325 330 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
340 345 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
355 360 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
370 375 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385 390 395 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
405 410 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
420 425 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
435 440 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
450 455 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465 470 475 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 9
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 9
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagt cgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggaccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 10
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 10

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Ser Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
275 280 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
290 295 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305 310 315 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
325 330 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
340 345 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
355 360 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
370 375 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385 390 395 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
405 410 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
420 425 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
435 440 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
450 455 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465 470 475 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 11
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 11
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtca aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 12
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 12

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Gln Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
275 280 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
290 295 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305 310 315 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
325 330 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
340 345 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
355 360 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
370 375 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385 390 395 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
405 410 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
420 425 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
435 440 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
450 455 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465 470 475 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 13
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 13
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aagtgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 14
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 14

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Ser
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
275 280 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
290 295 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305 310 315 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
325 330 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
340 345 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
355 360 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
370 375 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385 390 395 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
405 410 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
420 425 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
435 440 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
450 455 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465 470 475 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 15
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 15
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gagcaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gcccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 16
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 16

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
260 265 270

```
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
        275                 280                 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    290                 295                 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305                 310                 315                 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                325                 330                 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            340                 345                 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        355                 360                 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    370                 375                 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385                 390                 395                 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                405                 410                 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            420                 425                 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        435                 440                 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    450                 455                 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465                 470                 475                 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 17
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 17
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga gactgtcacc 120

atcacatgtc gaacaagtga aaatgtttac agttatttgg cttggtatca gcagaaacag 180

ggaaaatctc ctcagctcct ggtctctttt gcaaaaacct tagcagaagg tgtgccatca 240

aggttcagtg gcagtggatc aggcacacag ttttctctga agatcagcag cctgcagcct 300

gaagattctg gaagttattt ctgtcaacat cattccgata atccgtggac gttcggtgga 360

ggcaccgaac tggagatcaa aggtggcggt ggctcgggcg gtggtgggtc gggtggcggc 420

ggagctagcg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaggattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagcccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 18
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 18

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro

1 5 10 15

Asp Thr Thr Gly Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
20 25 30

Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro
50 55 60

Gln Leu Leu Val Ser Phe Ala Lys Thr Leu Ala Glu Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Ser
85 90 95

Ser Leu Gln Pro Glu Asp Ser Gly Ser Tyr Phe Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gly Gly Thr Glu Leu Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 19
<211> 1479
<212> DNA

<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 19
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata tccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtagtggatc tggaggaggt 420

ggagctagcg cggtccagct gcagcagtct ggacctgagt cggaaaagcc tggcgcttca 480

gtgaagattt cctgcaaggc ttctggttac tcattcactg gctacaatat gaactgggtg 540

aagcagaata atggaaagag ccttgagtgg attggaaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggcaag gccacattga ctgtagacaa atcctccagc 660

acagcctaca tgcagctcaa gagtctgaca tctgaggact ctgcagtcta ttactgtgca 720

agatcggtcg gccctatgga ctactggggt caaggaacct cagtcaccgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 20
<211> 492
<212> PRT
<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 20

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Ser Gly Ser Gly Gly Gly Gly Ala Ser Ala
130 135 140

Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro Gly Ala Ser
145 150 155 160

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu Trp Ile Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met
210 215 220

Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 21
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 21
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggagctagcc aggtgcagct ggtggagtct ggtggaggcg tggtccagcc tgggaggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccca cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 22
<211> 492
<212> PRT

<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 22

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
130 135 140

Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
145 150 155 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 23
<211> 1503
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 23
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gcggtccagc tgcagcagtc tggacctgag tcggaaaagc ctggcgcttc agtgaagatt 120

tcctgcaagg cttctggtta ctcattcact ggctacaata tgaactgggt gaagcagaat 180

aatggaaaga gccttgagtg gattggaaat attgatcctt attatggtgg tactacctac 240

aaccggaagt tcaagggcaa ggccacattg actgtagaca aatcctccag cacagcctac 300

atgcagctca agagtctgac atctgaggac tctgcagtct attactgtgc aagatcggtc 360

ggccctatgg actactgggg tcaaggaacc tcagtcaccg tctcttctgg tggcggtggc 420

tcgggcggtg gtgggtcggg tggcggcgga tcaggaggag gcgggagtgc tagcgaaatt 480

gtgttgacac agtctccagc caccctgtct ttgtctccag gcgaaagagc caccctctcc 540

tgccgaacaa gtgaaaatgt ttacagctac ttagcctggt accaacagaa acctggccag 600

gctcctaggc tcctcatcta ttttgcaaaa accttagcag aaggaattcc agccaggttc 660

agtggcagtg gatccgggac agacttcact ctcaccatca gcagcctaga gcctgaagat 720

tttgcagttt attactgtca acatcattcc gataatccgt ggacattcgg ccaagggacc 780

aaggtggaaa tcaaaggctc gagcgagccc aaatcttctg acaaaactca cacatctcca 840

ccgtgcccag cacctgaact cctgggtgga ccgtcagtct tcctcttccc cccaaaaccc 900

aaggacaccc tcatgatctc ccggacccct gaggtcacat gcgtggtggt ggacgtgagc 960

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1080

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1140

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1200

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260

ctggtcaaag gcttctatcc aagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1380

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 1500

<210> 24
<211> 500
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 24

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Ala Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu
20 25 30

Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser
35 40 45

Phe Thr Gly Tyr Asn Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser
50 55 60

Leu Glu Trp Ile Gly Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr
65 70 75 80

Asn Arg Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser
85 90 95

Ser Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala
100 105 110

Val Tyr Tyr Cys Ala Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln
115 120 125

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Glu Ile
145 150 155 160

Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg
165 170 175

Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn Val Tyr Ser Tyr Leu Ala
180 185 190

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Phe
195 200 205

Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
210 215 220

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp
225 230 235 240

Phe Ala Val Tyr Tyr Cys Gln His His Ser Asp Asn Pro Trp Thr Phe
245 250 255

Gly Gln Gly Thr Lys Val Glu Ile Lys Gly Ser Ser Glu Pro Lys Ser
260 265 270

Ser Asp Lys Thr His Thr Ser Pro Pro Cys Pro Ala Pro Glu Leu Leu
275 280 285

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
290 295 300

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
305 310 315 320

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
325 330 335

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
340 345 350

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
355 360 365

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
370 375 380

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
385 390 395 400

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
405 410 415

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
420 425 430

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
435 440 445

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
450 455 460

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
465 470 475 480

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
485 490 495

Ser Pro Gly Lys
500

<210> 25
<211> 1488
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 25
atggattttc aagtgcagat tttcagcttc ctgctaatca gtgcttcagt cataattgcc 60

agaggagtcg aaattgtgtt gacacagtct ccagccaccc tgtctttgtc tccaggcgaa 120

agagccaccc tctcctgccg aacaagtgaa aatgtttaca gctacttagc ctggtaccaa 180

cagaaacctg gccaggctcc taggctcctc atctattttg caaaaacctt agcagaagga 240

attccagcca ggttcagtgg cagtggatcc gggacagact tcactctcac catcagcagc 300

ctagagcctg aagattttgc agtttattac tgtcaacatc attccgataa tccgtggaca 360

ttcggccaag ggaccaaggt ggaaatcaaa ggtggcggtg gctcgggcgg tggtggatct 420

ggaggaggtg gagctagcgc ggtccagctg cagcagtctg gacctgagtc ggaaaagcct 480

ggcgcttcag tgaagatttc ctgcaaggct tctggttact cattcactgg ctacaatatg 540

aactgggtga agcagaataa tggaaagagc cttgagtgga ttggaaatat tgatccttat 600

tatggtggta ctacctacaa ccggaagttc aagggcaagg ccacattgac tgtagacaaa 660

tcctccagca cagcctacat gcagctcaag agtctgacat ctgaggactc tgcagtctat 720

tactgtgcaa gatcggtcgg ccctatggac tactggggtc aaggaacctc agtcaccgtc 780

tcctcgagcg agcccaaatc ttctgacaaa actcacacat ctccaccgtg cccagcacct 840

gaactcctgg gtggaccgtc agtcttcctc ttccccccaa aacccaagga caccctcatg 900

atctcccgga ccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag 960

gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg 1020

gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac 1080

tggctgaatg gcaaggagta caagtgcaag gtctccaaca aagccctccc agcccccatc 1140

gagaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc 1200

ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggcttc 1260

tatccaagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag 1320

accacgcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcaa gctcaccgtg 1380

gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg 1440

cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtaaatga 1488

<210> 26
<211> 495
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 26

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1 5 10 15

Val Ile Ile Ala Arg Gly Val Glu Ile Val Leu Thr Gln Ser Pro Ala
20 25 30

Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr
35 40 45

Ser Glu Asn Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
50 55 60

Gln Ala Pro Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly
65 70 75 80

Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
85 90 95

Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln
100 105 110

His His Ser Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu
115 120 125

Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
130 135 140

Ala Ser Ala Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro
145 150 155 160

Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
165 170 175

Gly Tyr Asn Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu
180 185 190

Trp Ile Gly Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg
195 200 205

Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr
210 215 220

Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
225 230 235 240

Tyr Cys Ala Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr
245 250 255

Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His
260 265 270

Thr Ser Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
275 280 285

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
290 295 300

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
305 310 315 320

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
325 330 335

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
340 345 350

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
355 360 365

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
370 375 380

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
385 390 395 400

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
405 410 415

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
420 425 430

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
435 440 445

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
450 455 460

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
465 470 475 480

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490 495

<210> 27
<211> 1503
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 27
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gcggtccagc tgcagcagtc tggacctgag tcggaaaagc ctggcgcttc agtgaagatt 120

tcctgcaagg cttctggtta ctcattcact ggctacaata tgaactgggt gaagcagaat 180

aatggaaaga gccttgagtg gattggaaat attgatcctt attatggtgg tactacctac 240

aaccggaagt tcaagggcaa ggccacattg actgtagaca aatcctccag cacagcctac 300

atgcagctca agagtctgac atctgaggac tctgcagtct attactgtgc aagatcggtc 360

ggccctatgg actactgggg tcaaggaacc tcagtcaccg tctcttctgg tggcggtggc 420

tcgggcggtg gtgggtcggg tggcggcgga tcaggaggag gcgggagtgc tagcgaaatt 480

gtgttgacac agtctccagc caccctgtct ttgtctccag gcgaaagagc caccctctcc 540

tgccgaacaa gtgaaaatgt ttacagctac ttagcctggt accaacagaa acctggccag 600

gctcctaggc tcctcatcta ttttgcaaaa accttagcag aaggaattcc agccaggttc 660

agtggcagtg gatccgggac agacttcact ctcaccatca gcagcctaga gcctgaagat 720

tttgcagttt attactgtca acatcattcc gataatccgt ggacattcgg ccaagggacc 780

aaggtggaaa tcaaaggctc gagcgagccc aaatcttctg acaaaactca cacatgccca 840

ccgtgcccag cacctgaact cctgggtgga ccgtcagtct tcctcttccc cccaaaaccc 900

aaggacaccc tcatgatctc ccggaccccc gaggtcacat gcgtggtggt ggacgtgagc 960

cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020

aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtggtcag cgtcctcacc 1080

gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggtctc caacaaagcc 1140

ctcccagccc ccatcgagaa aaccatctcc aaagccaaag ggcagccccg agaaccacag 1200

gtgtacaccc tgcccccatc ccgggatgag ctgaccaaga accaggtcag cctgacctgc 1260

ctggtcaaag gcttctatcc aagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320

gagaacaact acaagaccac gcctcccgtg ctggactccg acggctcctt cttcctctac 1380

agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440

atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa 1500

tga 1503

<210> 28
<211> 500
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 28

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Ala Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu
20 25 30

Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser
35 40 45

Phe Thr Gly Tyr Asn Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser
50 55 60

Leu Glu Trp Ile Gly Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr
65 70 75 80

Asn Arg Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser
85 90 95

Ser Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala
100 105 110

Val Tyr Tyr Cys Ala Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln
115 120 125

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Glu Ile
145 150 155 160

Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg
165 170 175

Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn Val Tyr Ser Tyr Leu Ala
180 185 190

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Phe
195 200 205

Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
210 215 220

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp
225 230 235 240

Phe Ala Val Tyr Tyr Cys Gln His His Ser Asp Asn Pro Trp Thr Phe
245 250 255

Gly Gln Gly Thr Lys Val Glu Ile Lys Gly Ser Ser Glu Pro Lys Ser
260 265 270

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
275 280 285

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
290 295 300

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
305 310 315 320

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
325 330 335

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
340 345 350

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
355 360 365

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
370 375 380

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
385 390 395 400

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
405 410 415

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
420 425 430

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
435 440 445

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
450 455 460

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
465 470 475 480

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
485 490 495

Ser Pro Gly Lys
500

<210> 29
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 29
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata tccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggagctagcg cggtccagct gcagcagtct ggacctgagt cggaaaagcc tggcgcttca 480

gtgaagattt cctgcaaggc ttctggttac tcattcactg gctacaatat gaactgggtg 540

aagcagaata atggaaagag ccttgagtgg attggaaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggcaag gccacattga ctgtagacaa atcctccagc 660

acagcctaca tgcagctcaa gagtctgaca tctgaggact ctgcagtcta ttactgtgca 720

agatcggtcg gccctatgga ctactggggt caaggaacct cagtcaccgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 30
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 30

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

```
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Ala
    130                 135                 140

Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro Gly Ala Ser
145                 150                 155                 160

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
                165                 170                 175

Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu Trp Ile Gly
            180                 185                 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
        195                 200                 205

Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met
    210                 215                 220

Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
225                 230                 235                 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
                245                 250                 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
            260                 265                 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
        275                 280                 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
    290                 295                 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305                 310                 315                 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
                325                 330                 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
            340                 345                 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
        355                 360                 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
    370                 375                 380
```

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 31
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 31
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggagctagcg cggtccagct gcagcagtct ggacctgagt cggaaaagcc tggcgcttca 480

gtgaagattt cctgcaaggc ttctggttac tcattcactg gctacaatat gaactgggtg 540

aagcagaata atggaaagag ccttgagtgg attggaaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggcaag gccacattga ctgtagacaa atcctccagc 660

acagcctaca tgcagctcaa gagtctgaca tctgaggact ctgcagtcta ttactgtgca 720

agatcggtcg gccctatgga ctactggggt caaggaacct cagtcaccgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttcccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 32
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 32

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Ala
130 135 140

Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro Gly Ala Ser
145 150 155 160

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu Trp Ile Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met
210 215 220

Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

```
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
    370                 375                 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385                 390                 395                 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                405                 410                 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
            420                 425                 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
        435                 440                 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
    450                 455                 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465                 470                 475                 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 33
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 33

```
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt    60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc   120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct   180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc   240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct   300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa   360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt   420

ggagctagcc aggtgcagct ggtggagtct ggtggaggcg tggtccagcc tgggaggtcc   480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc   540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt   600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc   660
```

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttcccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 34
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 34

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
130 135 140

Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
145 150 155 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 35
<211> 1479
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 35
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata tccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggagctagcc aggtgcagct ggtggagtct ggtggaggcg tggtccagcc tgggaggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 36
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 36

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
130 135 140

Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
145 150 155 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 37
<211> 1476
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 37
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggggctagcg aggtgcagct ggtggagtct ggtggaggct tggtccagcc tggagggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccсat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 38
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 38

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Glu
130 135 140

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
145 150 155 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
260 265 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 39
<211> 1476
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 39
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggggctagcg aggtgcagct ggtggagtct ggtggaggct tggtccagcc tggagggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccca aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 40
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 40

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
35 40 45

```
Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Glu
    130                 135                 140

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
145                 150                 155                 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
                165                 170                 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
            180                 185                 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
        195                 200                 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
    210                 215                 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225                 230                 235                 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
                245                 250                 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
            260                 265                 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
        275                 280                 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
    290                 295                 300
```

```
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305                 310                 315                 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
                325                 330                 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
            340                 345                 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
        355                 360                 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
    370                 375                 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385                 390                 395                 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                405                 410                 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
            420                 425                 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
        435                 440                 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
    450                 455                 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465                 470                 475                 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 41
<211> 1476
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 41
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggggctagcg aggtgcagct ggtggagtct ggtggaggct ctgtccagcc tggagggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccсat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 42
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 42

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

```
Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
        35                  40                  45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Glu
    130                 135                 140

Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly Ser
145                 150                 155                 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
                165                 170                 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
            180                 185                 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
        195                 200                 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
    210                 215                 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225                 230                 235                 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
                245                 250                 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
            260                 265                 270

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
        275                 280                 285
```

```
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
    290                 295                 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305                 310                 315                 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
                325                 330                 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
            340                 345                 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
        355                 360                 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
    370                 375                 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385                 390                 395                 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                405                 410                 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
            420                 425                 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
        435                 440                 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
    450                 455                 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465                 470                 475                 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 43
<211> 1476
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 43
atggaagcac cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

ggggctagcg aggtgcagct ggtggagtct ggtggaggct ctgtccagcc tggagggtcc 480

ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540

cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctcgagc 780

gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840

ggtggaccgt cagtcttcct cttccccccа aaacccaagg acaccctcat gatctcccgg 900

acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960

aactggtacg tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag 1020

tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080

ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagccccсat cgagaaaacc 1140

atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccgg 1200

gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260

gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt ggacaagagc 1380

aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440

tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 44
<211> 492
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 44

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

```
Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
            20                  25                  30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
        35                  40                  45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Glu
    130                 135                 140

Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly Ser
145                 150                 155                 160

Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
                165                 170                 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
            180                 185                 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
        195                 200                 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
    210                 215                 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225                 230                 235                 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
                245                 250                 255

Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
            260                 265                 270
```

```
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
        275                 280                 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
    290                 295                 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305                 310                 315                 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
                325                 330                 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
            340                 345                 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
        355                 360                 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
    370                 375                 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385                 390                 395                 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
                405                 410                 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
            420                 425                 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
        435                 440                 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
    450                 455                 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465                 470                 475                 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 45
<211> 1482
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 45
atggaagccc cagctcagct tctcttcctc ctgctactct ggctcccaga taccaccgga 60

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccaggcga aagagccacc 120

ctctcctgcc gagcaagtca aagtgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240

aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420

gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600

actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660

accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720

cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctcctctgat 780

caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840

ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900

cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagaccc tgaggtcaag 960

ttcaactggt acgtggacgg cgtggaggtg cataatgcca agacaaagcc gcgggaggag 1020

cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080

aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140

accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 1200

cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtcaaagg cttctatcca 1260

agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320

cctcccgtgc tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag 1380

agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcatgaggc tctgcacaac 1440

cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 46
<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 46

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro

1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225 230 235 240

Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
245 250 255

```
Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
            260                 265                 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
        275                 280                 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    290                 295                 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305                 310                 315                 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                325                 330                 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            340                 345                 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        355                 360                 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    370                 375                 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385                 390                 395                 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                405                 410                 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            420                 425                 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        435                 440                 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    450                 455                 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465                 470                 475                 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 47
<211> 1500
<212> DNA

<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 47
aagcttgccg ccatggaagc cccagcgcag cttctcttcc tcctgctact ctggctccca 60

gataccaccg gagaaattgt gttgacacag tctccagcca ccctgtcttt gtctccaggc 120

gaaagagcca ccctctcctg ccgagcaagt gaaaatgttt acagctactt agcctggtac 180

caacagaaac ctggccaggc tcctaggctc ctcatctatt ttgcaaaaac cttagcagaa 240

ggaattccag ccaggttcag tggcagtgga tccgggacag acttcactct caccatcagc 300

agcctagagc ctgaagattt tgcagtttat tactgtcaac atcattccga taatccgtgg 360

acattcggcc aagggaccaa ggtggaaatc aaaggtggcg gcggctcggg cggtggtgga 420

tctggaggag gtgggaccgg tgaggtgcag ctggtgcagt ctggagcaga ggtgaaaaag 480

cccggagagt ctctgaagat ttcctgtaag ggatccggtt actcattcac tggctacaat 540

atgaactggg tgcgccagat gcccgggaaa ggcctcgagt ggatgggcaa tattgatcct 600

tattatggtg gtactaccta caaccggaag ttcaagggcc aggtcactat ctccgccgac 660

aagtccatca gcaccgccta cctgcaatgg agcagcctga aggcctcgga caccgccatg 720

tattactgtg cacgctcagt cggccctttc gactactggg gccagggcac cctggtcact 780

gtctcctctg atcaggagcc caaatcttct gacaaaactc acacatctcc accgtgccca 840

gcacctgaac tcctgggtgg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc 900

ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac 960

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 1020

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 1080

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc 1140

cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 1200

ctgccccat cccgggatga gctgaccaag aaccaggtca gcctgacctg cctggtcaaa 1260

ggcttctatc caagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 1320

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta cagcaagctc 1380

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1440

gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa atgatctaga 1500

<210> 48
<211> 493
<212> PRT
<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 48

```
Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1               5                  10                  15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
            20                  25                  30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
        35                  40                  45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
    130                 135                 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145                 150                 155                 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
                165                 170                 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
            180                 185                 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
        195                 200                 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
    210                 215                 220

Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225                 230                 235                 240
```

```
Arg Ser Val Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
                245                 250                 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
            260                 265                 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
        275                 280                 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    290                 295                 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305                 310                 315                 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                325                 330                 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            340                 345                 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        355                 360                 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    370                 375                 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385                 390                 395                 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                405                 410                 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            420                 425                 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        435                 440                 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    450                 455                 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465                 470                 475                 480

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
```

<210> 49
<211> 356
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 49
gctagcgagg tgcagctggt gcagtctgga gcagaggtga aaaagcccgg agagtctctg 60

aagatttcct gtaagggatc cggttactca ttcactggct acaatatgaa ctgggtgcgc 120

cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tggtggtact 180

acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240

gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300

tcagtcggcc ctatggacgt ctggggccaa ggcaccactg tcactgtctc ctcgag 356

<210> 50
<211> 143
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 50

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
130 135 140

<210> 51
<211> 1381
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 51
aagcttgccg ccatggaagc cccagcgcag cttctcttcc tcctgctact ctggctccca 60

gataccaccg gagaaattgt gttgacacag tctccagcca ccctgtcttt gtctccaggc 120

gaaagagcca ccctctcctg ccgagcaagt gaaaatgttt acagctactt agcctggtac 180

caacagaaac ctggccaggc tcctaggctc ctcatctatt ttgcaaaaac cttagcagaa 240

ggaattccag ccaggttcag tggcagtgga tccgggacag acttcactct caccatcagc 300

agcctagagc ctgaagattt tgcagtttat tactgtcaac atcattccga taatccgtgg 360

acattcggcc aagggaccaa ggtggaaatc aaaggtggcg gtggctcggg cggtggtgga 420

tctggaggag gtgggaccgg tgaggtgcag ctggtgcagt ctggagcaga ggtgaaaaag 480

cccggagagt ctctgaagat ttcctgtaag ggatccggtt actcattcac tggctacaat 540

atgaactggg tgcgccagat gcccgggaaa ggcctcgagt ggatgggcaa tattgatcct 600

tattatggtg gtactaccta caaccggaag ttcaagggcc aggtcactat ctccgccgac 660

aagtccatca gcaccgccta cctgcaatgg agcagcctga aggcctcgga caccgccatg 720

tattactgtg cacgctcagt cggccctttc gactcctggg gccagggcac cctggtcact 780

gtctcctctg atcaggagcc caaatcttct gacaaaactc acacatctcc accgtgccca 840

gcacctgaac tcctgggtgg accgtcagtc ttcctcttcc ccccaaaacc caaggacacc 900

ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac 960

cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 1020

ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 1080

caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccagcc 1140

cccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 1200

ctgccccat cccgggatga gctgaccaag aaccaggtca gcctgacctg cctggtcaaa 1260

ggcttctatc caagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 1320

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttcctcta cagcaagctc 1380

a 1381

<210> 52

<211> 493
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 52

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
130 135 140

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
145 150 155 160

Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
165 170 175

Met Asn Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
180 185 190

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
195 200 205

Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
210 215 220

```
Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
225                 230                 235                 240

Arg Ser Val Gly Pro Phe Asp Ser Trp Gly Gln Gly Thr Leu Val Thr
                245                 250                 255

Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
            260                 265                 270

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
        275                 280                 285

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
    290                 295                 300

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
305                 310                 315                 320

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
                325                 330                 335

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
            340                 345                 350

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
        355                 360                 365

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
    370                 375                 380

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
385                 390                 395                 400

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                405                 410                 415

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
            420                 425                 430

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
        435                 440                 445

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
    450                 455                 460

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
465                 470                 475                 480
```

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 53
<211> 356
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 53
gctagcgagg tgcagctggt gcagtctgga gcagaggtga aaaagcccgg agagtctctg 60

aagatttcct gtaagggatc cggttactca ttcactggct acaatatgaa ctgggtgcgc 120

cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tggtggtact 180

acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240

gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300

tcagtcggcc cttttgaccc ctggggccaa ggcaccctgg tcactgtctc ctcgag 356

<210> 54
<211> 143
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 54

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
130 135 140

<210> 55
<211> 356
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 55
gctagcgagg tgcagctggt gcagtctgga gcagaggtga aaaagcccgg agagtctctg 60

aagatttcct gtaagggatc cggttactca ttcactggct acaatatgaa ctgggtgcgc 120

cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tggtggtact 180

acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240

gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300

tcagtcggcc cttttcagca ctggggccaa ggcaccctcg tcactgtctc ctcgag 356

<210> 56
<211> 143
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 56

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
100 105 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
115 120 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
130 135 140

<210> 57
<211> 356
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 57
gctagcgagg tgcagctggt gcagtctgga gcagaggtga aaaagcccgg agagtctctg 60

aagatttcct gtaagggatc cggttactca ttcactggct acaatatgaa ctgggtgcgc 120

cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tggtggtact 180

acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240

gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300

tcagtcggcc cttttgacgt ctggggccaa ggcaccatgg tcactgtctc ctcgag 356

<210> 58
<211> 143
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 58

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
35 40 45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
50 55 60

Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65 70 75 80

```
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
    130                 135                 140
```

<210> 59
<211> 356
<212> DNA
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 59

```
gctagcgagg tgcagctggt gcagtctgga gcagaggtga aaaagcccgg agagtctctg     60

aagatttcct gtaagggatc cggttactca ttcactggct acaatatgaa ctgggtgcgc    120

cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tggtggtact    180

acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc    240

gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc    300

tcagtcggcc cttttgacat ctggggccaa ggcaccatgg tcactgtctc ctcgag        356
```

<210> 60
<211> 143
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 60

```
Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1               5                   10                  15

Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
            20                  25                  30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Glu Asn
        35                  40                  45

Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
    50                  55                  60
```

```
Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
                85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
            100                 105                 110

Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
    130                 135                 140
```

<210> 61
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 61

```
Arg Ala Ser Glu Asn Val Tyr Ser Tyr Leu Ala
1               5                   10
```

<210> 62
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 62

```
Arg Thr Ser Glu Asn Val Tyr Ser Tyr Leu Ala
1               5                   10
```

<210> 63
<211> 5
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 63

```
Gly Tyr Met Asn Met
1               5
```

<210> 64
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 64

Phe Ala Lys Thr Leu Ala Glu
1 5

<210> 65
<211> 18
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 65

Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Thr Tyr Asn Arg Lys Phe
1 5 10 15

Lys Gly

<210> 66
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 66

Gln His His Ser Asp Asn Pro Trp Thr
1 5

<210> 67
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 67

Ser Val Gly Pro Phe Asp Tyr
1 5

<210> 68
<211> 7
<212> PRT

<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 68

Ser Val Gly Pro Phe Asp Ser
1 5

<210> 69
<211> 7
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 69

Ser Val Gly Pro Met Asp Tyr
1 5

<210> 70
<211> 23
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 70

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys
20

<210> 71
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 71

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

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr
20 25 30

<210> 72
<211> 15

<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 72

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
1 5 10 15

<210> 73
<211> 14
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 73

Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met Gly
1 5 10

<210> 74
<211> 32
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 74

Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys
20 25 30

<210> 75
<211> 32
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 75

Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu Gln
1 5 10 15

Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala Arg
20 25 30

<210> 76

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

<220>
<223> Humanized TRU-016

<400> 76

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 77
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 77

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 78
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> Humanized TRU-016

<400> 78

Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser
1 5 10