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HAYDEN-LEDBETTER MARTHA ET AL: "Induction of apoptosis in B lymphoma cell lines by CytoxB37G, a small modular immunopharmaceutical (SMIP) that binds CD37." BLOOD, vol. 102, no. 11, 16 November 2003 (2003-11-16), page 432a, XP002420825 & 45TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HEMATOLOGY; SAN DIEGO, CA, USA; DECEMBER 06-09, 2003 ISSN: 0006-4971

Fortsættes ...

"Trubion Pharmaceuticals Inc. Presents Positive Data On First Pre-Clinical Product Candidates At American Society of Hematology (ASH)" BIOSPACE, [Online] 8 December 2003 (2003-12-08), pages 1-3, XP002420826 San Diego Retrieved from the Internet: URL:http://www.biospace.com/news_story.asp?StoryID=14573420&full=1> [retrieved on 2007-02-15] & LEANDRO M J ET AL: "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" ANNALS OF THE RHEUMATIC DISEASES, BRITISH MEDICAL ASSOCIATION, LONDON, GB, vol. 61, no. 10, October 2002 (2002-10), pages 883-888, XP002348932 ISSN: 0003-4967

DAVIES JAMIE: "American society of hematology-45th annual meeting and exposition: hematological malignancies." IDRUGS : THE INVESTIGATIONAL DRUGS JOURNAL JAN 2004, vol. 7, no. 1, January 2004 (2004-01), pages 1-3, XP002420827 ISSN: 1369-7056

LEANDRO M J ET AL: "Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion" ANNALS OF THE RHEUMATIC DISEASES, BRITISH MEDICAL ASSOCIATION, LONDON, GB, vol. 61, no. 10, October 2002 (2002-10), pages 883-888, XP002348932 ISSN: 0003-4967

PRESS O W ET AL: "TREATMENT OF REFRACTORY NON-HODGKIN'S LYMPHOMA WITH RADIOLABELED MB-1 (ANTI-CD37) ANTIBODY" JOURNAL OF CLINICAL ONCOLOGY, GRUNE AND STRATTON, NEW YORK, NY, US, vol. 7, no. 8, August 1989 (1989-08), pages 1027-1038, XP002936241 ISSN: 0732-183X

PRESS O W ET AL: "RADIOLABELED-ANTIBODY THERAPY OF B-CELL LYMPHOMA WITH AUTOLOGOUS BONE MARROW SUPPORT" NEW ENGLAND JOURNAL OF MEDICINE, THE, MASSACHUSETTS MEDICAL SOCIETY, WALTHAM, MA, US, vol. 329, no. 17, 21 October 1993 (1993-10-21), pages 1219-1224, XP008075157 ISSN: 0028-4793

BARONE D ET AL: "Prolonged depletion of circulating B cells in cynomolgus monkeys after a single dose of TRU-015, a novel CD20 directed therapeutic" ANNALS OF THE RHEUMATIC DISEASES, vol. 64, no. Suppl. 3, July 2005 (2005-07), pages 159-160, XP008075207 & ANNUAL EUROPEAN CONGRESS OF RHEUMATOLOGY; VIENNA, AUSTRIA; JUNE 08 11, 2005 ISSN: 0003-4967

ZHAO XIAOBIN B ET AL: "Novel anti-CD37 small modular immunopharmaceutical (SMIP) induces B-cell-specific, caspase-independent apoptosis in human CLL cells." BLOOD, vol. 104, no. 11, Part 1, November 2004 (2004-11), page 689A, XP002420828 & 46TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY; SAN DIEGO, CA, USA; DECEMBER 04 -07, 2004 ISSN: 0006-4971

D. S. BARONE, C. NILSSON, J. LEDBETTER, M. HAYDEN-LEDBETTER AND K. MOHLER: "Efficacy of SMIP-016, a novel CD37-directed biologic therapy, in human NHL tumor xenograft models" JOURNAL OF CLINICAL ONCOLOGY, [Online] vol. 24, no. 18s, 20 June 2006 (2006-06-20), page 1, XP002420829 USA Retrieved from the Internet: URL:http://meeting.jco.org/cgi/gca?sendit=Get+All+Checked+Abstract%28s%29&SEARCHID=1&FULLTEXT=smip&VOLUME=24&ISSUE=18_suppl&FI RSTINDEX=0&hits=10&RESULTFORMAT=&gca=ascomtg%3B24%2F18_suppl%2F2565> [retrieved on 2007-02-16]

REFF M E ET AL: "Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 83, no. 2, 15 January 1994 (1994-01-15), pages 435-445, XP002112549 ISSN: 0006-4971

BUCHSBAUM D J ET AL: "Therapy with unlabeled and 131I-labeled pan-B-cell monoclonal antibodies in nude mice bearing Raji Burkitt's lymphoma xenografts." CANCER RESEARCH 1 DEC 1992, vol. 52, no. 23, 1 December 1992 (1992-12-01), pages 6476-6481, XP008075262 ISSN: 0008-5472

RADER C ET AL: "A phage display approach for rapid antibody humanization: Designed combinatorial V gene libraries", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES (PNAS), NATIONAL ACADEMY OF SCIENCE, US LNKD- DOI:10.1073/PNAS.95.15.8910, vol. 95, no. 15, 21 July 1998 (1998-07-21), pages 8910-8915, XP002182776, ISSN: 0027-8424

LAMMINMÄKI U ET AL: "Crystal structure of a recombinant anti-estradiol Fab fragment in complex with 17beta-estradiol.", THE JOURNAL OF BIOLOGICAL CHEMISTRY 28 SEP 2001 LNKD- PUBMED:11451948, vol. 276, no. 39, 28 September 2001 (2001-09-28), pages 36687-36694, ISSN: 0021-9258

PADLAN ET AL: "Anatomy of the antibody molecule", MOLECULAR IMMUNOLOGY, PERGAMON, GB LNKD- DOI:10.1016/0161-5890(94)90001-9, vol. 31, no. 3, 1 February 1994 (1994-02-01), pages 169-217, XP023681629, ISSN: 0161-5890 [retrieved on 1994-02-01]

Description**FIELD OF THE INVENTION**

5 [0001] The present invention generally provides the use of CD37-specific binding molecules in methods for B-cell reduction in an individual. In particular, the invention provides the use of CD-37 specific binding molecules in combination with CD20-specific binding molecules, in some instances a synergistic combination, in methods of B-cell reduction. The invention further provides materials and their use in methods for treatment of aberrant B-cell activity.

BACKGROUND OF THE INVENTION

10 [0002] 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
15 substance or pathogen.

[0003] 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,
20 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
25 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.

[0004] 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
30 (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.

[0005] 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
35 on normal antibody-producing (slg+)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
40 all malignancies of B-cell origin are positive for CD37 expression, including CLL, NHL, and hairy cell leukemia [Moore et al., J. 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
45 influencing antigen presentation via complex formation with MHC class II molecules. See Knobloch et al., Mol. Cell. Biol., 20(15):5363-5369 (2000). CD37 also seems to play a role in TCR signaling. See Van Spriël et al., J. Immunol., 172: 2953-2961 (2004).

[0006] 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
50 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.

[0007] One antibody to CD37 has been labeled with ¹³¹I 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.,
55 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).

[0008] 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).

[0009] 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)].

[0010] 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 non-specific 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.

[0011] 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)].

[0012] 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.

[0013] 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 Waldenström's macroglobulinemia [Trean et al., *Immunother.*, 24:272-279 (2000)], where patients showed increased hematocrit (HCT) and platelet (PLT) counts after 4 infusions of rituximab.

[0014] 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).

[0015] 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.

[0016] 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.

[0017] 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.

[0018] 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).

[0019] 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.

[0020] 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.

[0021] 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)].

[0022] 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)].

[0023] 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.

[0024] 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.

[0025] 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. 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.

[0026] 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 uses of the present invention described and claimed herein provide such improved uses as well as other advantages.

[0027] In a first aspect of the present invention there is provided a humanized CD37-specific binding protein for use in treating a B-cell cancer or an autoimmune disease, wherein the humanized CD37-specific binding protein comprises:

(a) human light chain framework regions, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 61, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO:64, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO:66; and

(b) human heavy chain framework regions, a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO:63, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO:65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 67 or 68 characterized in that the humanized CD-37 specific binding protein is to be used in combination with a CD20-specific binding protein.

[0028] In a second aspect of the present invention there is provided a CD20-specific binding protein for use in treating a B-cell cancer or an autoimmune disease characterized in that the CD20-specific binding protein is to be used in combination with a humanized CD37-specific binding protein for use in treating a B-cell cancer or an autoimmune disease, wherein the humanized CD37-specific binding protein comprises:

(a) human light chain framework regions, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 61, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO:64, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO:66; and

(b) human heavy chain framework regions, a heavy chain CDR1 comprising an amino acid sequence of SEQ ID NO:63, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO:65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 67 or 68..

[0029] In a third aspect there is provided an article of manufacture comprising a CD20-specific binding protein and a humanized CD37-specific binding protein for use in treating a B-cell cancer or an autoimmune disease, wherein the humanized CD37-specific binding protein comprises:

(a) human light chain framework regions, a light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 61, a light chain CDR2 comprising an amino acid sequence of SEQ ID NO:64, and a light chain CDR3 comprising an amino acid sequence of SEQ ID NO:66; and

(b) human heavy chain framework regions, a heavy chain CDR1 comprising an amino acid sequence of SEQ ID

NO:63, a heavy chain CDR2 comprising an amino acid sequence of SEQ ID NO:65, and a heavy chain CDR3 comprising an amino acid sequence of SEQ ID NO: 67 or 68.

[0030] Preferably, in all aspects of the invention, the light chain CDR1, CDR2, and CDR3 of the humanized CD37-specific binding protein consists of the amino acid sequences of SEQ ID NOS: 61, 64 and 66, respectively; and wherein the heavy chain CDR1, CDR2, and CDR3 consists of the amino acid sequences of SEQ ID NOS: 63, 65 and 67 respectively.

[0031] Preferably, in all aspects of the invention, the light chain CDR1, CDR2, and CDR3 of the humanized CD37-specific binding protein consists of the amino acid sequences of SEQ ID NOS: 61, 64 and 66, respectively; and wherein the heavy chain CDR1, CDR2, and CDR3 consists of the amino acid sequences of SEQ ID NOS: 63, 65 and 68 respectively.

[0032] Preferably, in all aspects of the invention, the human light chain framework region is selected from SEQ ID NOS:170-172, 175, 179,182, 184-188, 191, 194-198, 203 and 205-210; or the human heavy chain framework region is selected from SEQ ID NOS:140, 141, 143-147, 150, 151, 154-163, 168 and 169.

[0033] Preferably, in all aspects of the invention, the first human light chain framework region (FR1) is selected from SEQ ID NOS:170-172, 175 and 177-181; the human FR2 of the light chain framework region is selected from SEQ ID NOS: 182, 184-188 and 191; the human FR3 of the light chain is selected from SEQ ID NOS:194-198, 203 and 205; and the human FR4 of the light chain is selected from SEQ ID NOS:206-210; or

the first human heavy chain framework region (FR1) is selected from SEQ ID NOS:140, 141 and 143-146; the human FR2 of the heavy is selected from SEQ ID NOS:147, 150 and 151; the human FR3 of the heavy chain is selected from SEQ ID NOS:154-160; and the human FR4 of the heavy chain is selected from SEQ ID NOS:161-163, 168 and 169.

[0034] Preferably, in all aspects of the invention, the binding protein comprises a single chain Fv (scFv) polypeptide, an antibody or a binding fragment thereof, or an immunoglobulin fusion protein that comprises, from amino to carboxy terminus, (i) a binding domain having immunoglobulin light and heavy chain variable regions separated by a linker peptide, (ii) an immunoglobulin hinge region polypeptide having either zero, one or two cysteine residues, and (iii) immunoglobulin CH2 and CH3 domains. Further preferably, the immunoglobulin hinge region polypeptide can be an IgG1, IgGA or IgE hinge region polypeptide or a mutant IgG1. Advantageously, the binding protein is an immunoglobulin fusion protein of comprising a light chain variable region and a heavy chain variable region;

the light chain variable region is attached to the heavy chain variable region by a linker peptide comprising (Gly₄SER)_n, wherein n is 1, 2, 3, 4, 5, or 6; and

the linked light and heavy chain variable regions are connected to an IgG1 effector domain via a hinge comprised of an amino acid sequence selected from the group consisting of SEQ ID NO:90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 12, 115, 116, 118, 120, 122, 124, 126, or 127.

[0035] Preferably, in all aspects of the invention, the humanized CD37-specific binding protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 48 and 52.

[0036] Preferably, in all aspects of the invention, the CD20-specific binding protein is a CD20-specific monoclonal antibody, preferably Rituximab.

[0037] Advantageously, in all aspects of the invention the CD20-specific binding protein is an immunoglobulin fusion protein that is CD20-specific and comprises, from amino to carboxy terminus, (i) a binding domain having immunoglobulin light and heavy chain variable regions separated by a linker peptide, (ii) an immunoglobulin hinge region polypeptide having either zero, one or two cysteine residues, and (iii) immunoglobulin CH2 and CH3 domain. Further preferably, the immunoglobulin hinge region polypeptide can be an IgG1, IgGA or IgE hinge region polypeptide or a mutant IgG1.

[0038] Preferably, in all aspects of the invention, the method further comprises the use of chemotherapeutic agent, a radiotherapeutic agent, a cytokine, a chemokine or a growth factor.

[0039] Preferably, in all aspects of the invention, the B-cell cancer is Hodgkin's disease, non-Hodgkin's lymphoma, central nervous system lymphomas, acute lymphoblastic leukemia, chronic lymphocytic leukemia, hairy cell leukemia, chronic myeloblastic leukemia, multiple myeloma, small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extra-osseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated 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, or post-transplant lymphoproliferative disorder.

[0040] Advantageously, in all aspects of the invention the autoimmune disease is rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, Crohn's disease, Sjogren's syndrome, Grave's disease, type I diabetes mellitus, psoriasis, immune thrombocytopenic purpura, pemphigus, idiopathic inflammatory myopathy, myasthenis gravis, or Waldenstrom's macroglobinemia.

[0041] Preferably, in all aspects of the invention, the CD37-specific binding protein and the CD20-specific binding protein are administered sequentially within about a 24-hour period or are administered simultaneously.

[0042] In some uses 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.

[0043] The present disclosure 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 uses 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 disclosure provides methods of treating individuals having, or suspected of having, a disease associated with aberrant B-cell activity. Related aspects of the disclosure 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.

[0044] "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.

[0045] "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.

[0046] 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 uses of the invention, each in an effective dose.

[0047] "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.

[0048] In one exemplary aspect, an individual treated by methods of the disclosure 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 disclosure 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.

[0049] 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 myeloblastic 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 plas-

macytoma, 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 lymphomas, Burkitt lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] Crohn's disease may be characterized by antibodies against neutrophil antigens, i.e., the "perinuclear anti-neutrophil antibody" (pANCA), and *Saccharomyces cerevisiae*, i.e. the "anti-*Saccharomyces cerevisiae* 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.

[0054] 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 autoan-

tibodies 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.

[0055] 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.

[0056] 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.

[0057] Sjogren's syndrome is an autoimmune disease characterized by destruction of the body's moisture-producing glands.

[0058] Immune thrombocytopenic purpura (ITP) is caused by autoantibodies that bind to blood platelets and cause their destruction.

[0059] 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.

[0060] 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,

[0061] 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).

[0062] 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.

[0063] 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.

[0064] 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 for the uses of the invention is a small modular immunopharmaceutical (SMIP™).

[0065] 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 K_a 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).

[0066] 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.

[0067] 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.

[0068] 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, Moldenhauer, 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).

[0069] 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 (Ghetle et al.); US Appln No. US2002/0041847A1, (Goldenberg, D.); US Appln no. US2003/0026801A1 (Weiner and Hartmann); WO02/102312 (Engleman, E.). 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.).

[0070] Rituximab has been approved for human clinical use as Rituxan®. Rituxan® is considered to be a CD20-specific binding molecule of the invention.

[0071] 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. 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.

[0072] 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 joiner in a binding molecule. Exemplary linkers are linkers based on the Gly₄Ser linker motif, such as (Gly₄Ser)_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.

[0073] The disclosure 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: 48, and 52. 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.

[0074] In one embodiment, the disclosure 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 disclosure 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 (GYNMN).

[0075] 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 (NIDPYYGGTTYNRKFKG).

[0076] 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 (QHSDNPWT). 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 (SVGPFDY). The disclosure 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).

[0077] In another aspect, the disclosure 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 disclosure 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.

[0078] In still another aspect, the disclosure 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 disclosure 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.

[0079] The disclosure 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 disclosure includes a humanized CD37-specific SMIP polypeptide, wherein FR2 of the light chain comprises the amino acid sequence of SEQ ID NO: 72 (WYQKPGQAPRLLIY). In a further embodiment, the disclosure 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 disclosure includes a humanized CD37-specific SMIP polypeptide, wherein FR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 74 (GIPARFSGSGSGTDFTLTISLEPEDFAVYYC). In yet another embodiment, the disclosure includes a humanized CD37-specific SMIP polypeptide, wherein FR3 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 75 (QVTISADKSISTAYLQWSSSLKASDTAMYCAR). In yet another embodiment, the disclosure 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 disclosure includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 77 (WGQGTLLTVSS). In yet another embodiment, the disclosure includes a humanized CD37-specific SMIP polypeptide, wherein FR4 of the heavy chain comprises the amino acid sequence of SEQ ID NO: 78 (WGQGTLLTVSS).

[0080] The disclosure 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 disclosure 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.

[0081] In addition, the disclosure 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 disclosure 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.

[0082] The disclosure 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.

[0083] The disclosure also includes processes of producing the polypeptides described herein, comprising culturing the host cells under suitable conditions to express the polypeptide, and optionally isolating the polypeptides from the culture.

[0084] In yet another aspect, the invention includes compositions comprising the humanized CD37-specific SMIP polypeptides of the invention and a pharmaceutically acceptable carrier.

[0085] The disclosure 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.

[0086] In yet another aspect, the disclosure 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.

[0087] The disclosure 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.

[0088] In another aspect, the disclosure 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.

[0089] The disclosure 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.

[0090] In still a further aspect, the disclosure 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 (RASQSVSYLA). 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 (IYPGDS DTRYSPSFQG) and 139 (RIDPSDSYTNYSFQG).

[0091] The disclosure also includes a humanized CD37-specific SMIP polypeptide, wherein CDR3 of the light chain comprises the amino acid sequence of SEQ ID NO: 220 (QHSDNPWT). In another embodiment, the disclosure 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 (SVGP-FDS), 215 (SVGPFDV), 216 (SVGPFDH), 217 (SVGPFDV), 218 (SVGPFDI) and 219 (SVGPFDL).

[0092] In still a further aspect, the disclosure includes CD37-specific SMIP polypeptides with alternative framework regions. In one aspect, the disclosure 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 disclosure 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

disclosure 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 disclosure 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 disclosure 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 disclosure 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 further aspect, the disclosure 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 disclosure 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.

[0093] Exemplary CD37-specific SMIPs useful in the disclosure 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; G28-1 scFv 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 WlgACH2 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 hlgE 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 hlgAH WlgACH2 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.

[0094] Exemplary CD20-specific SMIPs useful in the disclosure 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-Illama IgG1, comprising the llama IgG1 hinge and CH2CH3 regions, 2H7 scFv-Illama IgG2, comprising the llama IgG2 hinge and CH2CH3 regions; 2H7 scFv-Illama IgG3, comprising the llama IgG3 hinge and CH2CH3 regions.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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 VHL11 S 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 mlgE CH2 CH3 CH4, comprises mouse IgE regions; 2H7 scFv VH L11S mlgAH 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 L11 S (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 L11 S (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 an 18 amino acid truncation at the carboxy end.

[0099] A binding molecule of the disclosure 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.

[0100] 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.

[0101] In one aspect of the uses 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.

[0102] In addition, compounds may form solvates with water or common organic solvents. Such solvates are contemplated as well.

[0103] 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, intramuscular, 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.

[0104] Pharmaceutical compositions of the present invention containing binding molecules used in accordance with 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.

[0105] 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

[0106] 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, hydroxypropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents
5 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
10 aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate.

[0107] 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
15 compensate.

[0108] 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.

[0109] 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,
25 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.

[0110] 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,
35 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.

[0111] 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 and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be desirable to
45 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.

[0112] 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).

[0113] 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
55 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.

[0114] In one aspect, uses of the invention include a step of administration of a binding molecule composition.

[0115] Uses 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.

[0116] 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.

[0117] 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.

[0118] Binding molecule compositions for use in 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.

[0119] 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.

[0120] 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 neurotrophic factor, ciliary neurotrophic factor receptor α , cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil chemotactic factor 2 α , cytokine-induced neutrophil chemotactic factor 2 β , 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 neurotrophic factor receptor α 1, glial cell line-derived neurotrophic factor receptor α 2, 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 β 1, transforming growth factor β 1.2, transforming growth factor β 2, transforming growth factor β 3, transforming growth factor β 5, latent transforming growth factor β 1, 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.

[0121] 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 thiophosphoramidate (thiotepa), and hexamethylmelamine (HMM, altretamine)); alkyl sulfonates (e.g., busulfan); 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'-deoxycorformycin (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-bromodeoxyuridine, 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 procabazine.

[0122] Non-limiting examples of chemotherapeutic agents, radiotherapeutic agents and other active and ancillary agents are also shown in Table 1.

TABLE 1

Alkylating agents	Natural products
Nitrogen mustards	Antimitotic drugs
mechlorethamine	Taxanes
cyclophosphamide	
ifosfamide	
melphalan	
chlorambucil	
Nitrosoureas	paclitaxel
carmustine (BCNU)	Vinca alkaloids
lomustine (CCNU)	vinblastine (VLB)
semustine (methyl-CCNU)	vincristine
Ethylenimine/Methyl-melamine	vinorelbine
triethylenemelamine (TEM)	Taxotere® (docetaxel)
triethylene thiophosphoramidate (thiotepa)	estramustine
hexamethylmelamine (HMM, altretamine)	estramustine phosphate
Alkyl sulfonates	Epipodophylotoxins
	etoposide
	teniposide
	Antibiotics
busulfan	actinomycin D
	daunomycin (rubido-mycin)
	doxorubicin (adria-mycin)

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(continued)

	Alkylating agents	Natural products
5	Nitrogen mustards	Antimitotic drugs
	Triazines	mitoxantroneidarubicin
	dacarbazine (DTIC)	bleomycin
10	Antimetabolites	splicamycin (mithramycin)
	Folic Acid analogs	mitomycin
	methotrexate	dactinomycin
	Trimetrexate	aphidicolin
15	Pemetrexed (Multi-targeted antifolate)	Enzymes
		L-asparaginase
		L-arginase
	Pyrimidine analogs	Radiosensitizers
20	5-fluorouracil	metronidazole
	fluorodeoxyuridine	misonidazole
	gemcitabine	desmethylmisonidazole
	cytosine arabinoside (AraC, cytarabine)	pimonidazole
25	5-azacytidine	etanidazole
	2,2'-difluorodeoxy-cytidine	nimorazole
		RSU 1069
		EO9
30	topotecan	mitoxantrone
	irinotecan	
	Purine analogs	RB 6145
	6-mercaptopurine	SR4233
35	6-thioguanine	nicotinamide
	azathioprine	5-bromodeoxyuridine
	2'-deoxycoformycin (pentostatin)	5-iododeoxyuridine
		bromodeoxycytidine
40	erythrohydroxynonyl-adenine (EHNA) fludarabine phosphate	Miscellaneous agents
	2-chlorodeoxyadenosine (cladribine, 2-CdA)	Platinum coordination complexes
		cisplatin
		Carboplatin
45	Type I Topoisomerase Inhibitors	oxaliplatin
	camptothecin	Anthracedione
		Substituted urea
	Biological response modifiers	hydroxyurea
50	G-CSF	
	GM-CSF	Methylhydrazine derivatives
		N-methylhydrazine (MIH)
	Differentiation Agents	procarbazine
55	retinoic acid derivatives	
		Adrenocortical suppressant

(continued)

	Alkylating agents	Natural products
	Nitrogen mustards	Antimitotic drugs
5	Hormones and antagonists	mitotane (<i>o,p'</i> -DDD)
	Adrenocorticosteroids/ antagonists	ainoglutethimide
	prednisone and equivalents	
	dexamethasone	Cytokines
10	ainoglutethimide	interferon (α , β , γ)
	Progestins	interleukin-2
	hydroxyprogesterone caproate	Photosensitizers
15	medroxyprogesterone acetate	hematoporphyrin derivatives
	megestrol acetate	Photofrin®
	Estrogens	benzoporphyrin derivatives
	diethylstilbestrol	Npe6
20	ethynyl estradiol/ equivalents	tin etioporphyrin (SnET2)
	Antiestrogen	pheoboride-a
	tamoxifen	bacteriochlorophyll-a
25		naphthalocyanines
	Androgens	phthalocyanines
	testosterone propionate	zinc phthalocyanines
	fluoxymesterone/equivalents	Radiation
30	Antiandrogens	X-ray
	flutamide	ultraviolet light
	gonadotropin-releasing	gamma radiation
35	hormone analogs	visible light
	leuprolide	infrared radiation
	Nonsteroidal antiandrogens	microwave radiation
40	flutamide	

[0123] 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.

[0124] 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 propoxyphene 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.

[0125] 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.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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 cerevisiae antibody (ASCA) in individuals affected by inflammatory bowel disease.

[0134] 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).

[0135] 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).

[0136] 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).

[0137] 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.

[0138] 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.

[0139] 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.

[0140] 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)

[0141] 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.

[0142] 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.

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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.

[0147] 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.

[0148] It will also be apparent that dosing may be modified if traditional therapeutics are administered in combination with therapeutics of the invention.

[0149] 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 method 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.

[0150] The present disclosure also comprises articles of manufacture.

[0151] 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.

[0152] The present disclosure 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

[0153]

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.

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

Figure 3A demonstrates FITC C1q binding to TRU-016 molecular forms incubated with Ramos B Cells in normal

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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.

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.

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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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.

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

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.

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

Figure 27 shows that TRU-016 effectively reduced relative cell viability in cells treated with fludarabine, thereby

potentiating the cytotoxic effect of fludarabine alone.

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

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

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).

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

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

EXAMPLES

[0154] 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

[0155] 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.

[0156] 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. 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.

[0157] 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.

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[0158] 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.

[0159] 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.

[0160] 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.

[0161] The recombinant TRU-016 was eluted from the column with 1000mM 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.

[0162] 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).

[0163] 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).

[0164] 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 FACs-Calibur instrument using Cell Quest software. Cells were gated for B lymphocytes or non-B lymphocytes by CD19 staining.

[0165] 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 scFvlg. Thus, the staining pattern of the G28-1 murine monoclonal antibody is preserved with TRU-016 (Figure 1C).

[0166] 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

[0167] Experiments were performed to identify the CD37 epitope bound by TRU-016 and other previously described CD37-specific antibodies.

[0168] 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.

[0169] 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 μ l) was added to kit tubes with a stirbar, and 1 M NaHCO₃ (adjusted to pH 8.5 with 6N NaOH), was added to a final concentration of 0.1 M. 50 μ l DMSO was added to 370 μ g 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 μ l of FITC to add: [μ l 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)].

[0170] 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 μ l 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 ratios 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.

[0171] 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.

[0172] FITC labeled antibody conjugates were titrated from 10 ng/mL to 10 μ g/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).

[0173] 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.

[0174] For competitive binding assays, 2.5×10^5 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 μ g/mL in the presence of unconjugated anti-CD37 MAb at the indicated concentrations (2.5, 1.25, 0.6, or 0.3 μ g/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 $\frac{1}{2}$ 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).

[0175] For FACs cross blocking assays, 2.5×10^5 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 μ g/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 μ g/ml, resulting in a dilution of the unlabelled reagents to 3.3 μ g/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).

[0176] 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 4×10^6 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 μ l 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).

[0177] 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).

[0178] 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

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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

[0179] 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.

[0180] C1 q 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 5×10^5 /well in 100 μ l. 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 μ l to each well for a final concentration of 10, 5, 2.5, or 1.25 % human serum. Fifty μ l 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.

[0181] 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 μ l of PBS. Fifty μ l 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).

[0182] 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.

[0183] 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, 3×10^5 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 μ g/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).

[0184] 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 C1 q binding (Figure 3B).

[0185] 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 μ g/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 μ g/mL in cold PBS. Cells with PI were incubated in the dark at RT for 15 minutes and analyzed by flow cytometry.

[0186] 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

[0187] 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).

[0188] 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).

[0189] 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 multimer 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.

[0190] 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 4×10^6 cells/mL and then plated at 50 μ L/well (2×10^5 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 μ L 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).

[0191] To determine the CDC activity of SEC fractions, cells were suspended at 5×10^5 cells/well in 75 μ L IMDM. TRU 016 SEC fractions (75 μ L) 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 μ g/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).

[0192] To determine the ADCC activity of SEC fractions, BJAB, Ramos, and Daudi lymphoblastoid B cells (10^7) cells were labeled with 500 μ Ci/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 (2×10^4 cells/well). The PBMCs were then added at (5×10^5 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 20 μ g/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% CO₂ prior to harvesting and counting. CPM released was measured on a Packard TopCounNXT from 50 μ L 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).

[0193] 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.

[0194] 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

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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.

[0195] 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 20 µg/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

[0196] 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.

[0197] 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.

[0198] 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 aspgly3-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.

[0199] 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

[0200] 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% CO₂ in Iscoves (Gibco) complete media with 10% FBS at 3 X 10⁵ 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 Pharmingen 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 1X10⁶ 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).

[0201] -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.

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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 GMA
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

[0202] 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 μ g/mL TRU-016 while that of TRU-015 was also 0.5 μ g/ml The concentration of rituxan was usually 0.04-0.06 μ g/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.

[0203] Cells were suspended in Iscoves (#12440-053, Gibco/Invitrogen, Grand Island, NY) at 5x10E5 cells/well in 75 μ l. TRU-016 (75 μ l), 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 μ l 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 μ l staining media with 5 μ l 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).

[0204] 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 C1 q from the reactions results in the elimination of all CDC activity observed.

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[0205] 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.

[0206] Figure 8 shows another CDC experiment where the sample reagents were mixed at the following ratios: 0.5 μ /mL TRU-015, 0.5 μ g/mL TRU-016, and 0.06 μ g/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.

[0207] 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 μ g/mL from 0.5 μ g/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

[0208] 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.

[0209] BJAB, Ramos, and Daudi lymphoblastoid B cells (10E7) cells were labeled with 500 μ Ci/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 μ l/well for the indicated final concentrations. The ^{51} Cr labeled BJAB were added to the plates at 50 μ l/well (2x10E4 cells/well). The PBMCs were then added to the plates at 100 μ l/well (3x10E5 cells/well) for a final ratio of 15:1 effectors (PBMC):target (BJAB).

[0210] 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 1 μ g/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% CO₂ prior to harvesting and counting. Fifty μ l 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.

[0211] 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.

[0212] 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.

[0213] Thus, TRU-016 can enhance CD20-specific SMIP or CD20-specific antibody ADCC mediated killing of B cells.

[0214] 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 μ g/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).

[0215] 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 $\mu\text{g/mL}$ or 2 $\mu\text{g/mL}$ TRU-016. The concentration of Rituxan was usually 0.04-0.06 $\mu\text{g/mL}$ 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.

[0216] Figure 18B shows a graph of the percentage of live cells (PI negative) observed over the titration range indicated (0.06-0.5 $\mu\text{g/mL}$) when Rituxan is used alone or in combination with TRU-016 at 2.5 $\mu\text{g/mL}$. 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).

[0217] 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 $\mu\text{g/mL}$ for TRU-016, and Rituxan concentrations ranging from 0.06 $\mu\text{g/mL}$ to 0.5 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$ from 0.5 $\mu\text{g/mL}$ did not significantly increase the CDC mediated killing in these experiments (data not shown).

[0218] 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

[0219] 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).

[0220] 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 (10 $\mu\text{g/mL}$). 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.

[0221] 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 $\mu\text{g/mL}$ TRU-016 protein. Because apoptosis was reduced using the dimer form of TRU-016, 20 $\mu\text{g/mL}$ 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.

[0222] 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.

[0223] Human PBMCs were isolated from whole blood by LSM density centrifugation. Cells were incubated for 48 or

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72 hours with 1 $\mu\text{g/mL}$ 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 $\mu\text{L/mL}$) for 24 and 72 hours.

[0224] 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.

[0225] 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 1 C) 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

[0226] 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 (5×10^6) 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 $5.0 \times 10^7/\text{mL}$. 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 = $152\text{-}296 \text{ mm}^3$) were used. Some mean tumor volumes ranging from $237\text{-}251 \text{ mm}^3$ 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), $200 \mu\text{g}/\text{mouse}$; rituxan, $200 \mu\text{g}/\text{mouse}$, or human IgG (control) at 200 or $400 \mu\text{g}/\text{mouse}$ as single reagents, or as the following combinations of reagents: Rituxan + TRU-016 at $100 \mu\text{g}$ each per mouse; or Rituxan + TRU-016 at $200 \mu\text{g}$ 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.

[0227] Similar studies were also performed using smaller tumors, with mice sorted into groups with smaller mean tumor volume ranging between $153\text{-}158 \text{ mm}^3$, 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).

[0228] Figure 21 graphs the efficacy of TRU-016 compared to hulgG, rituxan, and the combinations at $100 \mu\text{g}$ and $200 \mu\text{g}$ 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 (hulgG). 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 ($100 \mu\text{g}$ each), the combination therapy was not as effective as each single reagent at a higher dose.

[0229] 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

[0230] 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 (5×10^6) 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 mm³ were used. 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 GPC dimer peak, 200 μg/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.

[0231] Figure 22A shows the efficacy of TRU-016 compared to hulgG 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 (hulgG). Figure 22B graphs the survival curves for the different treatment groups, comparing hulgG, 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

[0232] 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.

[0233] 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 mulg-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.

[0234] 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

[0235] 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

[0236] 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.

[0237] Ramos and Daudi tumor cells were separately grown and (5×10^6) 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 infected intravenously (IV) at days 0, 2, 4, 6, and 8 with one of the following reagents: rituximab, 200 $\mu\text{g}/\text{mouse}$; TRU-016, 200 $\mu\text{g}/\text{mouse}$; rituximab + TRU-016 at 100 or 200 $\mu\text{g}/\text{mouse}$; or human IgG (control), 400 $\mu\text{g}/\text{mouse}$. 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).

[0238] 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 HulgG control-treated mice. Pooled data from 3 experiments demonstrated that TRU-016 and rituximab combination therapy resulted in a statistically significant 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 200 μg 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
HulgG	0	10

[0239] 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
HulgG	0	15

[0240] 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 HulgG 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

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

[0241] 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.

[0242] Cells were treated with TRU-016 at dosages ranging from 0.1-100 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$; 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 $1 \times 10^6/\text{mL}$. Media was changed the night before each study to assure fresh cells being used.

[0243] 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 $\mu\text{g/mL}$) 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 1×10^7 cells/mL (0.1 mL) were then added to the 0.9 mL media in each well to make a final density of 1×10^6 cells/mL. For Raji cells, the final cell density was 5×10^4 cells/mL. Thus, the cell suspension used was 5×10^5 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.

[0244] 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. 50 μL 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.

[0245] The apoptosis of CLL cells after incubation with antibodies was measured using annexin V-FITC/propidium iodide (PI) staining with FACS analysis. 5×10^5 cells in 200 μL 1x binding buffer (BD Pharmingen) were stained with 5 μL annexin V (BD Pharmingen) and 5 μL PI (BD Pharmingen), and kept in the dark at room temperature for 15 minutes before suspension with 300 μL 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.

[0246] 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: $\text{Ca}/\text{Ca}_b + \text{Cb}/\text{Cb}_a = \text{Cl}$, where Ca and Cb are the concentration of drug A and drug B alone, respectively, to produce a desired effect (e.g. 50%

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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.

[0247] 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

[0248] 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 cytotoxicity in cells resistant to rituximab.

[0249] Rituximab-resistant cells (1X10⁶ 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 cytotoxicity was measured by annexin/PI staining and cell viability (percent) was calculated relative to control cells (cells treated with herceptin).

[0250] 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 cytotoxicity 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 CDC19+ Primary CLL B Cells

[0251] To determine how TRU-016 induces signal transduction in B cells, experiments were performed to examine the effect of TRU-016 on tyrosine phosphorylation.

[0252] Freshly isolated CD19+ cells (~50-100x10⁶) from CLL patients were suspended at a concentration of 5X10⁶/ml PBS. Cells were then incubated for 10 minutes at 37°C, 5% CO₂, with control, trastuzumab (herceptin), or TRU-016 at a final concentration of 5 µg/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 (25 µg/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 1 ml of RIPA lysis buffer with protease and phosphatase inhibitors (10mM Tris, pH 7.4, 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 bicinchoninic acid (BCA) method (Pierce, Rockford, IL). The control and treated protein samples (50 µg 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).

[0253] 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 phosphorylation pathway.

Example 18

Humanized TRU-016 Molecules

[0254] 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. 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.

[0255] 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.

[0256] "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)).

[0257] 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)).

[0258] 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)).

[0259] 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).

[0260] 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.

[0261] 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 choose 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 Gly₄Ser ((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 <i>Linker</i> (TG-SS)	15aa gly4ser	SSC-P	7	8
019003	Vk3:VH5-51 <i>VH V11S</i>	15aa gly4ser	SSC-P	9	10

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(continued)

Construct No.	Description	Linker	Hinge	DNA SEQ ID NO:	AA SEQ ID NO:
019004	Vk3:VH5-51 VK3, <i>cdr1</i> (E → Q)	15aa gly4ser	SSC-P	11	12
019005	Vk3:VH5-51 VK3, <i>cdr1</i> (N → S)	15aa gly4ser	SSC-P	13	14
019006	Vk3:VH5-51 VK3, <i>cdr1</i> (T → A)	15aa gly4ser	SSC-P	15	16
019010	mVk:VH5-5a	15aa gly4ser	SSC-P	17	18
019011	Vk3:mVH (<i>linker G-S mutation</i>)	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 (<i>019011 with 2H7 Leader</i>)	15aa gly4ser	SSC-P	25	26
019021	mVH:Vk3	15aa gly4ser	SSC-P	27	28
019023	Vk3:mVH (<i>fixed 019011 GS4 mutation</i>)	15aa gly4ser	SSC-P	29	30
019024	Vk3:mVH (<i>fixed 019011 GS4 mutation</i>)	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 VH3-13L11S FW1	15aa gly4ser	SSC-P	41	42
019035	Vk3:VH5 VH3-13L11S 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 gly4ser	CPPCP	83	84
019008				85	86
019009				87	88

[0262] 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).

[0263] 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.

[0264] 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.

[0265] 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)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatgtccaccgtgccca	89
ccc(p)-hlgG1 (AA)	EPKSCDKTHTCPPCP	90
scc(p)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatgtccaccgtgccca	91
scc(p)-hlgG1 (AA)	EPKSSDKTHTCPPCP	92
scc(s)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatgtccaccgtgctca	93
scc(s)-hlgG1 (AA)	EPKSSDKTHTCPPCS	94
scs(s)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatgtccaccgagctc a	95
scs(s)-hlgG1 (AA)	EPKSSDKTHTCPPSS	96
sss(p)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgagccc a	97
sss(p)-hlgG1 (AA)	EPKSSDKTHTSPPSP	98
sss(s)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgagctca	99
sss(s)-hlgG1 (AA)	EPKSSDKTHTSPPSS	100
csc(p)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgtgccca	101
csc(p)-hlgG1 (AA)	EPKSCDKTHTSPPCP	102
csc(s)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgtgctca	103
csc(s)-hlgG1 (AA)	EPKSCDKTHTSPPCS	104
ssc(p)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgtgccca	105
ssc(p)-hlgG1 (AA)	EPKSSDKTHTSPPCP	106
scs(s)-hlgG1 (DNA)	gagcccaaatcttctgacaaaactcacacatctccaccgtgctca	107
scs(s)-hlgG1 (AA)	EPKSSDKTHTSPPCS	108
css(p)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgagccc a	109
css(p)-hlgG1 (AA)	EPKSCDKTHTSPPSP	110
css(s)-hlgG1 (DNA)	gagcccaaatcttgtgacaaaactcacacatctccaccgagctca	111
css(s)-hlgG1 (AA)	EPKSCDKTHTSPPSS	112
scs(s)-hlgG1	gagcccaaatcttgtgacaaaactcacacatgtccaccgagctc a	113
(DNA)		
scs(s)-hlgG1 (AA)	EPKSSDKTHTCPPSS	114
hlgA1	VPSTPPTSPSTPPTSPSPS	115

(continued)

Hinge description	DNA or Amino Acid Sequence	SEQ ID NO:
hlgA2	VPPPPP	116
hlgG3 (DNA)	gagctcaaaactcctctcggggatagacgacccatacgtgtccccgc tgtctgaaccgaagtcctgcgatacgccctccgcatgtccacggt gcccagagcccaaatcatgcgatacgccccaccgtgtccccgc tgtctgaaccgaagtcctgcgatacgccccaccaccatgtccaaga tgccca	117
hlgG3 (AA)	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCP EPKSCDTPPPCPRCPEPKSCDTPPPCPRCP	118
IgG315hsc (DNA)	gagcccaaatctctgacacacctccccatgccacggtgcccc	119
IgG315hsc (AA)	EPKSSDTPPPCPRCP	120
IgG315hcss (DNA)	gagcccaaatctctgacacacctccccatccccacggtcccca	121
IgG315hcss (AA)	EPKSCDTPPPSPRSP	122
IgG315hsss (DNA)	gagcccaaatctctgacacacctccccatccccacggtcccca	123
IgG315hsss (AA)	EPKSSDTPPPSPRSP	124
IgG3hI5csc (DNA)	gagcccaaatctctgacacacctccccatccccacggtgcccc	125
IgG3hI5csc (AA)	EPKSCDTPPPSPRCP	126
hlgD	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

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(continued)

	FR3	
5	VH1 RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR	154
	VH1 RVTITADESTSTAYMELSSLRSED TAVYYCAR	155
	VH1 RVTITADKSTSTAYMELSSLRSED TAVYYCAR	156
	VH1 RVTITADTSTD TAYMELSSLRSED TAVYYCAT	157
10	VH5 QVTISADKSISTAYLQWSSLKASDTAMY YCAR	158
	VH5 HVTISADKSISTAYLQWSSLKASDTAMY YCAR	159
	VH7 RFVFSLDTSVSTAYLQISSLKAEDTAVYYCAR	160
15	FR4	
	WGQGLTVTVSS	161
	WGRGLTVTVSS	162
	WGQGMTVTVSS	163
20	WGQGMTVTVSS	164
	WGQGLTVTVSS	165
	WGQGLTVTVSS	166
25	WGQGLTVTVSS	167
	WGQGTTVTVSS	168
	WGKGTTVTVSS	169
	Human VK Framework Regions for anti-CD37 Humanization	
30	FR1	
	VK3 EIVMTQSPATLSVSPGERATLSC	170
	VK3 EIVLTQSPATLSLSPGERATLSC	171
35	VK1 DIQMTQSPSSLSASVGDRVITIC	172
	VK1 DIQMTQSPSSLSASVGDRVITIC	173
	VK1 DIQMTQSPSSLSASVGDRVITIC	174
	VK1 NIQMTQSPSAMSASVGDRVITIC	175
40	VK1 DIQMTQSPSSLSASVGDRVITIC	176
	VK1 AIQLTQSPSSLSASVGDRVITIC	177
	VK1 DIQLTQSPSFLSASVGDRVITIC	178
45	VK1 AIRMTQSPFSLSASVGDRVITIC	179
	VK1 AIQMTQSPSSLSASVGDRVITIC	180
	VK1 DIQMTQSPSTLSASVGDRVITIC	181
50	FR2	
	VK3 WYQQKPGQAPRLLIY	182
	VK3 WYQQKPGQAPRLLIY	183
55	VK1 WYQQKPGKAPKLLIY	184

(continued)

	FR2	
5	VK1 WYQQKPGKVPKLLIY	185
	VK1 WYQQKPGKAPKRLIY	186
	VK1 WFQQKPGKVPKHLLIY	187
	VK1 WFQQKPGKAPKSLIY	188
10	VK1 WYQQKPGKAPKLLIY	189
	VK1 WYQQKPGKAPKLLIY	190
	VK1 WYQQKPAKAPKLFYIY	191
15	VK1 WYQQKPGKAPKLLIY	192
	VK1 WYQQKPGKAPKLLIY	193
	FR3	
	VK3 GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC	194
20	VK3 GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC	195
	VK1 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	196
	VK1 GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	197
25	VK1 GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	198
	VK1 GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	199
	VK1 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	200
	VK1 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	201
30	VK1 GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	202
	VK1 GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC	203
	VK1 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	204
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	FGQGTKVEIK	206
	FGQGTKLEIK	207
40	FGPGTKVDIK	208
	FGGGTKVEIK	209
	FGQGTRLEIK	210

[0266] 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

[0267]

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
019049	79	aagctgccgcatggaagccccagcgcagcttcttctcctgctactctggctcccag ataccaccggagaaattgtgtgacacagctctccagccaccctgtcttgtctccaggcga aagagccaccctctcctgccgagcaagtgaaatgttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctatttgcaaaaaccttagcagaagg aattccagccagggtcagtggcagtggaatccgggacagactcactctcaccatcagca gcctagagcctgaagattttgcagtttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaagggtggcgggtggcggcggtgggtggat ctggaggagggtgggaccggtgaggtgcagctggtgcagctcggagcagaggtgaaaa agcccgagagctctgaagatttctgtaaggatccggttactcattcactggctacaa tatgaactgggtgcgccagatgcccggaaggcctcagtgagtgaggcaatattgatc cttattatgggtgactacctacaaccggaagttcaaggggcagggtcactatctccgccga caagtccatcagcaccgctacctgcaatggagcagcctgaaggcctcggacaccgc catgtattactgtgcacgctcagtcggcccttctgacctctggggcagaggcaccctggtc actgtctcctctgatcaggagcccaaatcttctgacaaaactcacacatctccaccgtgcc cagcacctgaactcctgggtggaccgtcagcttctcttccccccaaaacccaaggac accctcatgatctccggaccctgaggtcacatgcgtggtggtggacgtgagccacga agaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaag acaaagccgaggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcacc gtctgcaccaggactggctgaatggcaaggagtacaagtgcaagggtctcaacaaa gccctccagccccatcgagaaaaccatctccaaagccaaagggcagccccgaga accacaggtgtacaccctgcccccatccgggatgagctgaccaagaaccaggtcag cctgacctgctggtcaaaggcttctatccaagcgacatcgccgtggagtgggagagca atggggcagccggagacaactacaagaccacgctcccgctgctggactccgacggct ccttcttctctacagcaagctcaccgtggacaagagcagggtggcagaggggaacgt cttctcatgctcgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctc cctgtctccgggtaaatgatctaga
019049	80	MEAPAQLFLLLLWLDPDTTGEIVLTQSPATLSLSPGERATLSCR ASENVVSYLAWYQQKPGQAPRLIYFAKTLAEGIPARFSGSGS GTDFTLTISSELPEDFAVYYCQHHSDNPWTFGQGTVKVEIKGGG GSGGGGSGGGGTGEVQLVQSGAEVKKPGESLKISCKGSGYS FTGYNMNWVRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQ VTISADKSISTAYLQWSSLKASDTAMYYCARSVGPFDLWGRGT LVTVSSDQEPKSSDKTHTSPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHLEHNHYTQKSLSLSPGK

(continued)

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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(continued)

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
019008	85	aagcttgccgcatggaagccccagctcagcttctctctctgctactctggctcccag ataccaccggagaaattgtgtgacacagctcagccaccctgtcttgtctccaggcga aagagccaccctctctgccaacaagtgaaaatgtttacagctacttagcctggtacca acagaaacctggccaggctcctaggctcctcatctatttgcaaaaaccttagcagaagg aatccagccagggtcagtgccagtggaatccgggacagacttcacttcaccatcagca gcctagagcctgaagatttgcagttattactgtcaacatcattccgataatccgtggacat tcggccaagggaccaaggtggaaatcaaaggtggcgggtgctcgggcgggtggtgat ctggaggagggtgggaccgggtgaggtgcagctgggtgcagctcggagcagaggtgaaaa agcccgagagctctgaagatttctgtaagggatccgggtactcatctcactggctacaa tatgaactgggtgcgccagatgccgggaaaggcctggagtggaatgggcaatattgat ccttattatggtggtactacctacaaccggaagttcaagggccaggtcactatctccgccg acaagtccatcagcaccgcctacctgcaatggagcagcctgaaggcctcggacaccg ccatgtattactgtgcacgctcagtcggccctatggactactggggccgcccaccctgg tcactgtctctctgatcaggagcccaaatctctgacaaaactcacacatctccaccgtg cccagcacctgaactctgggtggaccgtcagctctctctctcccccaaaaccaagg acaccctcatgatctcccgacccctgaggtcacatgcgtggtggtggacgtgagccac gaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgcc agacaaagccgcccggaggagcagtacaacagcacgtaccgtgtggtcagcgtctc accgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggctccaac aaagccctcccagcccccacgagaaaaccatctcaaaggccaaagggcagcccg agaaccacaggtgtacaccctgccccatcccggtgagctgaccaagaaccaggt cagcctgacctgctggtcaaaggcttctatccaagcgacatcccggtggagtgaggaga gcaatgggcagccggagaaactacaagaccacgcctcccggtgctggactccgac ggctccttctctctacagcaagctcaccgtggacaagagcaggtggcagcagggga acgtcttctcatgctccgtgatgatgaggtctgcacaaccactacacgcagaagagc ctctccctgtctccgggtaaatga

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(continued)

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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(continued)

Construct #	SEQ ID NO:	DNA or Amino Acid Sequence
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SEQUENCE LISTING

[0268]

<110> Grosmaire et al.

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

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<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

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 Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu
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 His His Ser Asp Asn Pro Trp Thr Phe Gly Gly Gly Thr Glu Leu Glu
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5 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
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 15 ttctcttaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1440
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 20 ccgggtaaat gatctaga 1518

<210> 4

<211> 499

25 <212> PRT

<213> Artificial sequence

<220>

30 <223> TRU-015 polypeptide

<400> 4

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45

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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

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	Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Ser		
	130					135					140						
5	Gln	Ala	Tyr	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Ser	Val	Arg	Pro	Gly	Ala	
	145				150					155					160		
10	Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	
					165					170					175		
15	Asn	Met	His	Trp	Val	Lys	Gln	Thr	Pro	Arg	Gln	Gly	Leu	Glu	Trp	Ile	
				180					185					190			
20	Gly	Ala	Ile	Tyr	Pro	Gly	Asn	Gly	Asp	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	
			195					200					205				
25	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	
	210						215					220					
30	Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Phe	Cys	
	225					230					235					240	
35	Ala	Arg	Val	Val	Tyr	Tyr	Ser	Asn	Ser	Tyr	Trp	Tyr	Phe	Asp	Val	Trp	
					245					250					255		
40	Gly	Thr	Gly	Thr	Thr	Val	Thr	Val	Ser	Asp	Gln	Glu	Pro	Lys	Ser	Cys	
				260					265					270			
45	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Cys	Ser	Ala	Pro	Glu	Leu	Leu	Gly	
			275					280					285				
50	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	
	290						295					300					
55	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	
	305					310					315					320	
60	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	
				325					330						335		
65	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	
				340					345					350			
70	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	
			355					360					365				
75	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	
	370						375					380					

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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

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ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240
aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300
gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
gggaccaagg tggaaatcaa aggtggcggg 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

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 cgctcagtcg gccctatgga ctactggggc cgcggcacc tggtcactgt ctctctgat 780
 5 caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840
 ctgggtggac cgtcagtctt cctcttcccc ccaaaacca aggacaccct catgatctcc 900
 cggacccctg aggtcacatg cgtgggtggg gacgtgagcc acgaagaccc tgaggtcaag 960
 10 ttcaactggg acgtggacgg cgtggagggt cataatgcc agacaaagcc gcgggaggag 1020
 cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080
 aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140
 15 accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 1200
 cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaagg cttctatcca 1260
 agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg 1320
 20 cctcccgtgc tggactccga cggtccttc ttcctctaca gcaagctcac cgtggacaag 1380
 agcagggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac 1440
 25 cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 6

<211> 493

<212> P12T

30 <213> Artificial sequence

<220>

<223> Humanized TRU-016

35 <400> 6

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 40 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30
 45 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
 55 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

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

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

10 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

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

20 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

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

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

35 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

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

45 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

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

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

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

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

10 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

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

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

25 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

30 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

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ctctcttgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

ggccaggctc ctaggctcct catctatctt gcaaaaacct tagcagaagg aattccagcc 240

50 aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420

55 gggagctctg aggtgcagct ggtgcagctt ggagcagagg tgaaaaagcc cggagagtct 480

ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540

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 5 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 cgctcagtcg gccctatgga ctactggggc cgccggcacc tggtcactgt ctccctctgat 780
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 15 cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080
 aatggcaagg agtacaagt caaggtctcc aacaaagccc tcccagccc catcgagaaa 1140
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 20 cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaaagg cttctatcca 1260
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 25 cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag 1380
 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgagg tctgcacaac 1440
 cactacacgc agaagagcct ctccctgtct ccgggtaa at ga 1482

30 <210> 8
 <211> 493
 <212> PRT
 <213> Artificial sequence

35 <220>
 <223> Humanized TRU-016

<400> 8

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 45 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
 50 Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 50 55 60
 55 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80

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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95
 5 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
 100 105 110
 10 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
 15 Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
 145 150 155 160
 20 Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
 165 170 175
 25 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
 30 Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
 210 215 220
 35 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
 40 Val Ser Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser
 260 265 270
 45 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
 50 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 305 310 315 320
 55 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 325 330 335

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Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 340 345 350
 5 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 355 360 365
 10 Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 370 375 380
 15 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
 20 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 420 425 430
 25 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
 30 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 465 470 475 480
 35 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

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 aggttcagtg gcagtggtac cgggacagac ttactctca ccatcagcag cctagagcct 300
 10 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtggtggatc tggaggaggt 420
 15 gggaccggtg aggtgcagct ggtgcagtct ggagcagagt cgaaaaagcc cggagagtct 480
 ctgaagattt cctgtaaggg atccgggttac tcattcactg gctacaatat gaactgggtg 540
 20 cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600
 actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660
 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 25 cgctcagtcg gccctatgga ctactggggc cggggcaccc tggtcactgt ctctctgat 780
 caggagccca aatcttctga caaaactcac acatctccac cgtgcccagc acctgaactc 840
 ctgggtggac cgtcagtctt cctcttccc ccaaaacca aggacacct catgatctcc 900
 30 cggacccttg aggtcacatg cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag 960
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 35 aatggcaagg agtacaagt caaggtctcc aacaaagccc tccagcccc catcgagaaa 1140
 accatctcca aagccaaagg gcagccccga gaaccacagg tgtacacct gcccccattc 1200
 cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaagg cttctatcca 1260
 40 agcgacatcg ccgtggagt ggagagcaat gggcagccgg agaacaacta caagaccag 1320
 cctcccgctg tggactccga cggctcctt ttctctaca gcaagctcac cgtggacaag 1380
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 45 cactacacgc agaagagcct ctccctgtct ccgggtaaat ga 1482

<210> 10

<211> 493

<212> PRT

<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 10

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Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
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5 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
20 25 30

10 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

15

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

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

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 325 330 335
 5 Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 340 345 350
 10 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
 15 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 385 390 395 400
 20 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 405 410 415
 25 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
 30 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
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 35 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

40 <210> 11
 <211> 1482
 <212> DNA
 <213> Artificial sequence

45 <220>
 <223> Humanized TRU-016

<400> 11

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 5 ctctcctgcc gaacaagtca aaatgtttac agctacttag cctggtacca acagaaacct 180
 ggccaggctc ctaggctcct catctatctt gcaaaaacct tagcagaagg aattccagcc 240
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 10

gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 15 gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420
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 25 cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggctactgt ctctctgat 780
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 30 cggacccctg aggtcacatg cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag 960
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 cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag 1380
 45 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcagaggc tctgcacaac 1440
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50 <210> 12
 <211> 493
 <212> PRT
 <213> Artificial sequence

55 <220>
 <223> Humanized TRU-016

<400> 12

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

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

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

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

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

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

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

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

20 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

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

30 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

35 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
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40 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

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ctctcctgcc gaacaagtga aagtgtttac agctacttag cctggtacca acagaaacct 180

5 gggcaggctc ctaggctcct catctatctt gcaaaaacct tagcagaagg aattccagcc 240
 aggttcagtg gcagtggatc cgggacagac ttactctca ccatcagcag cctagagcct 300
 10 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 gggaccaagg tggaaatcaa aggtggcggt ggctcgggcg gtgggtggatc tggaggaggt 420
 gggaccggtg aggtgcagct ggtgcagtct ggagcagagg tgaaaaagcc cggagagtct 480
 15 ctgaagattt cctgtaaggg atccgggttac tcattcactg gctacaatat gaactgggtg 540
 cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatgggtgg 600
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 ctgggtggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc 900
 cggacccctg aggtcacatg cgtgggtggg gacgtgagcc acgaagacc tgaggtcaag 960
 25 ttcaactggt acgtggacgg cgtggagggt cataatgcca agacaaagcc gcgggaggag 1020
 cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tctgcacca ggactggctg 1080
 aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140
 30 accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc 1200
 cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tgggtcaaagg cttctatcca 1260
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 35 cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag 1380
 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac 1440
 40 cactacacgc agaagagcct ctccctgtct ccgggtaaata 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
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Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30

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

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

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 290 295 300
 5 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 305 310 315 320
 10 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
 15 Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 355 360 365
 20 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
 25 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 405 410 415
 30 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 420 425 430
 35 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
 40 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 465 470 475 480
 45 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

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 5 ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240
 aggttcagtg gcagtggatc cgggacagac ttactctca ccatcagcag cctagagcct 300
 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 10 gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420
 gggaccggtg aggtgcagct ggtgcagctt ggagcagagg tgaaaaagcc cggagagtct 480
 ctgaagattt cctgtaaggg atccggttac tcattcactg gctacaatat gaactgggtg 540
 15 cgccagatgc ccgggaaagg cctcgagtgg atgggcaata ttgatcctta ttatggtggt 600
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 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 20 cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctctctgat 780
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 25 ctgggtggac cgtcagctct cctcttcccc ccaaaaccca aggacaccct catgatctcc 900
 cggacccttg aggtcacatg cgtggtggtg gacgtgagcc acgaagacc tgaggtcaag 960
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 30 cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg 1080
 aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa 1140
 accatctcca aagccaaagg gcagccccga gaaccacagg tgtacacgct gccccatcc 1200
 35 cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaagg cttctatcca 1260
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 cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag 1380
 40 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac 1440
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<210> 16

<211> 493

<212> PRT

<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 16

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

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

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

10 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

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

20 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

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

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

35 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

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

45 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

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

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

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

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

10 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

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

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

25 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

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

35 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

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

45 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

50

<210> 17

<211> 1479

<212> DNA

<213> Artificial sequence

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<220>

<223> Humanized TRU-016

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<400> 17

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 ggaaaatctc ctcagctcct ggtctctttt gcaaaaacct tagcagaagg tgtgccatca 240
 10 aggttcagtg gcagtggatc aggcacacag ttttctctga agatcagcag cctgcagcct 300
 gaagattctg gaagttattt ctgtcaacat cattccgata atccgtggac gttcggtgga 360
 ggacccgaac tggagatcaa aggtggcggg ggctcgggag gtggtgggtc ggggtggcggc 420
 15 ggagctagcg aggtgcagct ggtgcagctt ggagcagagg tgaaaaagcc cggagagtct 480
 ctgaggattt cctgtaaggg atccgggttac tcattcactg gctacaatat gaactgggtg 540
 20 cgccagatgc ccgggaaagg cctggagtggt atgggcaata ttgatcctta ttatggtggt 600
 actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660
 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 25 cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctctcagagc 780
 gagcccaa atctctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg 840
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 40 gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320
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 45 tacacgcaga agagcctctc cctgtctccg ggtaaatga 1479

<210> 18

<211> 492

50 <212> PRT

<213> Artificial sequence

<220>

<223> Humanized TRU-016

55

<400> 18

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

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Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
 260 265 270
 5 Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 275 280 285
 10 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
 15 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 325 330 335
 20 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
 25 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 30 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 385 390 395 400
 35 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
 40 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 435 440 445
 45 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
 50 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 485 490

55 <210> 19
 <211> 1479
 <212> DNA
 <213> Artificial sequence

EP 1 912 675 B1

<220>

<223> Humanized TRU-016

<400> 19

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10 ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct      180
ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc      240
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15 gaagattttt cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa      360
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20 gtgaagattt cctgcaaggc ttctggttac tcattcactg gctacaatat gaactgggtg      540
aagcagaata atggaaagag ccttgagtgg attggaaata ttgatcctta ttatgggtgg      600
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25 acagcctaca tgcagctcaa gagtctgaca tctgaggact ctgcagtcta ttactgtgca      720
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30 gagcccaaat cttctgacaa aactcacaca tctccaccgt gcccagcacc tgaactcctg      840
ggtggaccgt cagtcttcct cttcccccca aaaccaagg acaccctcat gatctcccgg      900
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35 aactggtacg tggacggcgt ggaggtgcat aatgccaaaga caaagccgcg ggaggagcag      1020
tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat      1080
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gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct      1320
45 cccgtgctgg actccgacgg ctcttcttct ctctacagca agctcaccgt ggacaagagc      1380
aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac      1440
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50

<210> 20

<211> 492

<212> PRT

<213> Artificial sequence

55

<220>

<223> Humanized TRU-016

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<400> 20

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 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95
 25 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
 30 Gly Gly Gly Ser Gly Gly Ser Gly Ser Gly Gly Gly Gly Ala Ser Ala
 130 135 140
 35 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
 40 Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser Leu Glu Trp Ile Gly
 180 185 190
 45 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
 50 Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
 225 230 235 240
 55

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Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
 245 250 255
 5 Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro
 260 265 270
 10 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
 15 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 305 310 315 320
 20 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
 25 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 355 360 365
 30 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 35 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
 40 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 420 425 430
 45 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
 50 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 465 470 475 480
 55 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 485 490

<210> 21

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

5 <220>
 <223> Humanized TRU-016

<400> 21

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   ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct      180
15 ggccagggtc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc      240
   aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct      300
   gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa      360
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   ggagctagcc aggtgcagct ggtggagtct ggtggaggcg tggccagcc tgggaggtcc      480
25 ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc      540
   cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt      600
   actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc      660
30 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca      720
   cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctctcagagc      780
   gagcccaaat cttctgacaa aactcacaca tctccaccgt gccagcacc tgaactcctg      840
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   cccgtgctgg actccgacgg ctctctcttc ctctacagca agctcaccgt ggacaagagc      1380
50 aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac      1440
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55 <210> 22
 <211> 492
 <212> PRT

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<213> Artificial sequence

<220>

<223> Humanized TRU-016

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<400> 22

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 15 Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
 35 40 45
 20 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
 25 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95
 30 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
 100 105 110
 35 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
 40 Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
 145 150 155 160
 45 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
 50 Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
 195 200 205
 55 Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
 210 215 220

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

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

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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

5 <210> 23
<211> 1503
<212> DNA
<213> Artificial sequence

10 <220>
<223> Humanized TRU-016

<400> 23

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aaccggaagt tcaagggcaa ggccacattg actgtagaca aatcctccag cacagcctac 300
atgcagctca agagtctgac atctgaggac tctgcagtct attactgtgc aagatcggtc 360
25 ggccctatgg actactgggg tcaaggaacc tcagtcaccg tctcttctgg tggcgggtggc 420
tcggggcggtg gtgggtcggtg tggcggcgga tcaggaggag gcgggagtgc tagcgaaatt 480
gtgttgacac agtctccagc caccctgtct ttgtctccag gcgaaagagc caccctctcc 540
30 tgccgaacaa gtgaaaatgt ttacagctac ttagcctggt accaacagaa acctggccag 600
gctcctaggc tcctcatcta ttttgcaaaa acctagcag aaggaattcc agccagggttc 660
35 agtggcagtg gatccgggac agacttcact ctcacatca gcagcctaga gcctgaagat 720
tttgagttt attactgtca acatcattcc gataatccgt ggacattcgg ccaagggacc 780
aaggtggaaa tcaaaggctc gagcgagccc aaatcttctg acaaaactca cacatctcca 840
40 ccgtgcccag cacctgaact cctgggtgga ccgtcagtct tcctcttccc cccaaaaccc 900
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cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020
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gtcctgcacc aggactggct gaatggcaag gagtacaagt gcaaggcttc caacaaagcc 1140
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50 gtgtacaccc tgccccatc ccgggatgag ctgaccaaga accagggtcag cctgacctgc 1260
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gagaacaact acaagaccac gctccccgtg ctggactccg acggctcctt cttcctctac 1380
55 agcaagctca ccgtggacaa gagcaggtgg cagcagggga acgtcttctc atgctccgtg 1440
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tga

1503

<210> 24

5 <211> 500

<212> PRT

<213> Artificial sequence

<220>

10 <223> Humanized TRU-016

<400> 24

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 Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser
 35 40 45
 25 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
 30 65 70 75 80
 Asn Arg Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser
 85 90 95
 35 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
 40 115 120 125
 Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
 45 130 135 140
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Glu Ile
 145 150 155 160
 50 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
 55 180 185 190
 Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Phe
 195 200 205

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	Ala	Lys	Thr	Leu	Ala	Glu	Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	
	210						215					220					
5	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	
10					245					250					255		
	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Gly	Ser	Ser	Glu	Pro	Lys	Ser	
				260					265					270			
15	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	
20		290					295					300					
	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	
25	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	
30				340					345					350			
	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	
35			355					360					365				
	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	
		370					375					380					
40	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	
45					405					410					415		
	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	
				420					425					430			
50	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	
55		450					455					460					

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

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

10 Ser Pro Gly Lys
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<210> 25

<211> 1488

<212> DNA

15 <213> Artificial sequence

<220>

<223> Humanized TRU-016

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 25 agagccaccc tctcctgcgc aacaagtga aatgtttaca gctacttagc ctggtaccaa 180
 cagaaacctg gccaggtccc taggtccttc atctattttg caaaaacctt agcagaagga 240
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 30 ctagagcctg aagattttgc agtttattac tgtcaacatc attccgataa tccgtggaca 360
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 35 ggaggaggtg gagctagcgc ggtccagctg cagcagctctg gacctgagtc ggaaaagcct 480
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 aactgggtga agcagaataa tggaaagagc cttgagtggg ttggaaatat tgatccttat 600
 40 tatggtggta ctacctaca cccgaagttc aagggcaagg ccacattgac tgtagacaaa 660
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 55 gagaaaacca tctccaaagc caaagggcag ccccgagAAC cacaggtgta caccctgccc 1200
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 accacgcctc ccgtgctgga ctccgacggc tcctttcttcc tctacagcaa gctcaccgtg 1380
 5 gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg 1440
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<210> 26

10 <211> 495

<212> PRT

<213> Artificial sequence

<220>

15 <223> Humanized TRU-016

<400> 26

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 25 Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr
 35 40 45
 30 Ser Glu Asn Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly
 50 55 60
 35 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
 40 Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln
 100 105 110
 45 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
 50 Ala Ser Ala Val Gln Leu Gln Gln Ser Gly Pro Glu Ser Glu Lys Pro
 145 150 155 160
 55 Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
 165 170 175

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

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

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

15 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

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

25 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

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

35 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

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

45 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

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

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

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

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

10 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

15 <210> 27
 <211> 1503
 <212> DNA
 <213> Artificial sequence

20 <220>
 <223> Humanized TRU-016

<400> 27

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 30 aatggaaaga gccttgagtg gattggaaat attgatcctt attatgggtg tactacctac 240
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 35 ggccctatgg actactgggg tcaaggaacc tcagtcaccg tctcttctgg tggcgggtggc 420
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 45 agtggcagtg gatccgggac agacttctact ctaccatca gcagcctaga gcctgaagat 720
 tttgcagttt attactgtca acatcattcc gataatccgt ggacattcgg ccaagggacc 780
 aagggtggaaa tcaaaggctc gagcgagccc aaatcttctg acaaaactca cacatgccca 840
 50 ccgtgcccag cacctgaact cctgggtgga ccgtcagtct tctcttccc cccaaaaccc 900
 aaggacacc tcattgatct ccggaccct gaggtcacat gcgtgggtgg ggacgtgagc 960
 cacgaagacc ctgaggtcaa gttcaactgg tacgtggacg gcgtggaggt gcataatgcc 1020
 55 aagacaaagc cgcgggagga gcagtacaac agcacgtacc gtgtgggtcag cgtcctcacc 1080
 gtctctgacc aggactggct gaatggcaag gagtacaagt gcaaggctct caacaaagcc 1140

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 5 ctgggtcaaag gcttctatcc aagcgacatc gccgtggagt gggagagcaa tgggcagccg 1320
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15 <210> 28
 <211> 500
 <212> PRT
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20 <220>
 <223> Humanized TRU-016

<400> 28

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 20 25 30
 Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser
 35 35 40 45
 35 Phe Thr Gly Tyr Asn Met Asn Trp Val Lys Gln Asn Asn Gly Lys Ser
 50 55 60
 40 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
 45 Ser Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala
 100 105 110
 50 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
 55 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ser Glu Ile
 145 150 155 160

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Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg
165 170 175

5 Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn Val Tyr Ser Tyr Leu Ala
180 185 190

10 Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Phe
195 200 205

15 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

20 Phe Ala Val Tyr Tyr Cys Gln His His Ser Asp Asn Pro Trp Thr Phe
245 250 255

25 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

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

35 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

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

45 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

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

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

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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 420 425 430
 5
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 435 440 445
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 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
 450 455 460
 15
 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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<210> 29

<211> 1479

<212> DNA

<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 29

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20 <210> 30
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 <212> PRT
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25 <220>
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<400> 30

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35 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
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40 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

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

50 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

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

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

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

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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

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

10

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

15

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
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Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 31

<211> 1479

<212> DNA

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<213> Artificial sequence

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<223> Humanized TRU-016

35

<400> 31

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50 <212> PRT

<213> Artificial sequence

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<223> Humanized TRU-016

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<400> 32

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 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20 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
 25
 30
 35
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 45
 50
 55

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

5 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Ala
 130 135 140

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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

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

10 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

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

20 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
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25 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 33

<211> 1479

<212> DNA

<213> Artificial sequence

<220>

<223> Humanized TRU-016

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gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360

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55 cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgacctta ttatgggtgt 600

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<212> PRT

30 <213> Artificial sequence

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 20 25 30
 45 Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
 35 40 45
 50 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
 55 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95

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	Gln	Trp	Ser	Ser	Leu	Lys	Ala	Ser	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Ala	
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50	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
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	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	
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Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 355 360 365
 5 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 10 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 385 390 395 400
 15 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 405 410 415
 20 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 420 425 430
 25 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 435 440 445
 30 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
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 35 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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 40 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 35

<211> 1479

<212> DNA

<213> Artificial sequence

<220>

<223> Humanized TRU-016

<400> 35

EP 1 912 675 B1

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<210> 36

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50 <212> PRT

<213> Artificial sequence

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<223> Humanized TRU-016

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<400> 36

EP 1 912 675 B1

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
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 5 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20
 25
 30
 35
 40
 45
 50
 55

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

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

10 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

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

20 Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Tyr Asn
 165 170 175

25 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

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

35 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

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

45 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

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

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

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Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 340 345 350
 5 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 355 360 365
 10 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 15 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 385 390 395 400
 20 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 405 410 415
 25 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 420 425 430
 30 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 435 440 445
 35 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 450 455 460
 40 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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 45 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 485 490
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 <211> 1476
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> Humanized TRU-016
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 ctctcctgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180
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 aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300
 55 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 gggaccaagg tggaaatcaa aggtggcgggt ggctcgggcg gtggtggatc tggaggaggt 420

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5
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 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
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 acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960
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 gatgagctga ccaagaacca ggtcagcctg acctgctgg tcaaaggctt ctatccaagc 1260
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 aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440
 tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

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<210> 38
 <211> 492
 <212> PRT
 <213> Artificial sequence

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<220>
 <223> Humanized TRU-016
 <400> 38

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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

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

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

10 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

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

20 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

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

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

35 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

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

45 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

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

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

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Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 325 330 335
 5 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 340 345 350
 10 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
 15 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 385 390 395 400
 20 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
 25 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 435 440 445
 30 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 450 455 460
 35 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

40 <210> 39
 <211> 1476
 <212> DNA
 <213> Artificial sequence

45 <220>
 <223> Humanized TRU-016

<400> 39

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 ctctcttgcc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180
 55 ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240
 aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300

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 gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420
 5 ggggctagcg aggtgcagct ggtggagtct ggtggaggct tggtecagcc tggagggtcc 480
 ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540
 cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600
 10 actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660
 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 cgctcagtcg gccctatgga ctactggggc cgcggcacc tggtcactgt ctccctcagc 780
 15 gagcccaaatt cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840
 ggtggaccgt cagtcttcct cttcccccca aaaccaagg acaccctcat gatctcccgg 900
 20 acccctgagg tcacatgcgt ggtgggtggac gtgagccacg aagaccctga ggtcaagttc 960
 aactggtacg tggacggcgt ggaggtgcat aatgccaaaga caaagccgcg ggaggagcag 1020
 tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080
 25 ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagcccccat cgagaaaacc 1140
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 gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260
 30 gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct 1320
 cccgtgctgg actccgacgg ctctttcttc ctctacagca agctcaccgt ggacaagagc 1380
 aggtggcagc aggggaacgt cttctcatgc tccgtgatgc atgaggctct gcacaaccac 1440
 35 tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 40

<211> 492

40 <212> PRT

<213> Artificial sequence

<220>

<223> Humanized TRU-016

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<400> 40

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

50

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

55

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

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

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

10 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

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

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

25 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

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

35 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

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

45 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

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

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

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Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 305 310 315 320
 5 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 325 330 335
 10 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
 15 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 20 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
 25 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 420 425 430
 30 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
 35 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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 40 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

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ctctctgccc gaacaagtga aaatgtttac agctacttag cctggtacca acagaaacct 180

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 aggttcagtg gcagtggatc cgggacagac ttactctca ccatcagcag cctagagcct 300
 5 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420
 ggggctagcg aggtgcagct ggtggagtct ggtggaggct ctgtccagcc tggagggtcc 480
 10 ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540
 cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600
 actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660
 15 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
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 acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagttc 960
 25 aactggtacg tggacggcgt ggaggtgcat aatgccaga caaagccgcg ggaggagcag 1020
 tacaacagca cgtaccgtgt ggtcagcgtc ctccaccgtc tgcaccagga ctggctgaat 1080
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 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

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Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Thr Ser Glu Asn
 35 40 45
 5 Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 50 55 60
 10 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
 15 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
 100 105 110
 20 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
 25 Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly Ser
 145 150 155 160
 30 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
 35 Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
 195 200 205
 40 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
 45 Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
 245 250 255
 50 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

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Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 290 295 300
 5
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 305 310 315 320
 10
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 325 330 335
 15
 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
 20
 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 370 375 380
 25
 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
 30
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 420 425 430
 35
 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
 40
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 465 470 475 480
 45
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 485 490

<210> 43

<211> 1476

50 <212> DNA

<213> Artificial sequence

<220>

<223> Humanized TRU-016

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<400> 43

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 ctctcctgcc gaacaagtga aaatgtttac agctacttag cctgggtacca acagaaacct 180
 ggccaggctc ctaggetcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240
 10 aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300
 gaagattttg cagtttatta ctgtcaacat cattccgata atccgtggac attcggccaa 360
 gggaccaagg tggaaatcaa aggtggcggg ggctcgggcg gtggtggatc tggaggaggt 420
 15 ggggctagcg aggtgcagct ggtggagtct ggtggaggct ctgtccagcc tggagggtcc 480
 ctgagactct cctgtgcagc ctctggattc accttcagtg gctacaatat gaactgggtc 540
 cgccagatgc ccgggaaagg cctggagtgg atgggcaata ttgatcctta ttatggtggt 600
 20 actacctaca accggaagtt caagggccag gtcactatct ccgccgacaa gtccatcagc 660
 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 cgctcagtcg gccctatgga ctactggggc cgcggcacc ttggtcactgt ctccctcagc 780
 25 gagcccaaat cttctgacaa aactcacaca tgcccaccgt gcccagcacc tgaactcctg 840
 ggtggaccgt cagtcttctt cttcccccca aaaccaagg acaccctcat gatctcccg 900
 30 acccctgagg tcacatgcgt ggtggtggac gtgagccacg aagaccctga ggtcaagtcc 960
 aactggtacg tggacggcgt ggaggtgcat aatgccaaaga caaagcccg ggaggagcag 1020
 tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga ctggctgaat 1080
 35 ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc cagcccccat cgagaaaacc 1140
 atctccaaag ccaaagggca gccccgagaa ccacaggtgt acaccctgcc cccatcccg 1200
 gatgagctga ccaagaacca ggtcagcctg acctgcctgg tcaaaggctt ctatccaagc 1260
 40 gacatcgccg tggagtggga gagcaatggg cagccggaga acaactacaa gaccagcct 1320
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 45 tacacgcaga agagcctctc cctgtctccg ggtaaa 1476

<210> 44

<211> 492

50 <212> PRT

<213> Artificial sequence

<220>

<223> Humanized TRU-016

55

<400> 44

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

5

10

15

20

25

30

35

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45

50

55

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

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

10 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

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

20 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

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

30 Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Pro Gly Gly Ser
 145 150 155 160

35 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

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

45 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

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

55 Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
 260 265 270

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

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

10 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

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

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

25 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

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

35 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

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

45 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
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Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 45

<211> 1482

<212> DNA

<213> Artificial sequence

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<220>

<223> Humanized TRU-016

<400> 45

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 ctctcctgcc gagcaagtca aagtgtttac agctacttag cctgggtacca acagaaacct 180
 ggccaggctc ctaggctcct catctatttt gcaaaaacct tagcagaagg aattccagcc 240
 10 aggttcagtg gcagtggatc cgggacagac ttcactctca ccatcagcag cctagagcct 300
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 accgcctacc tgcaatggag cagcctgaag gcctcggaca ccgccatgta ttactgtgca 720
 25 cgctcagtcg gccctatgga ctactggggc cgcggcaccc tggtcactgt ctctctgat 780
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 45 agcaggtggc agcaggggaa cgtcttctca tgctccgtga tgc¹atgaggc tctgcacaac 1440
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<210> 46

50 <211> 493

<212> PRT

<213> Artificial sequence

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55 <223> Humanized TRU-016

<400> 46

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 20 25 30
 10 Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
 35 40 45
 15 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
 20 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95
 25 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
 30 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
 130 135 140
 35 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
 45 Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
 195 200 205
 50 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
 55 Arg Ser Val Gly Pro Met Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
 245 250 255

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

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

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

15 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

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

25 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

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

35 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

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

45 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

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

<210> 47

55 <211> 1500

<212> DNA

<213> Artificial sequence

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<220>

<223> Humanized TRU-016

<400> 47

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gaaagagcca cctctctctg ccgagcaagt gaaaatgttt acagctactt agcctgggtac 180

caacagaaac ctggccaggc tcttaggctc ctcattctatt ttgcaaaaac cttagcagaa 240

ggaattccag ccagggttcag tggcagtgga tccgggacag acttcactct caccatcagc 300

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agcctagagc ctgaagattt tgcagtttat tactgtcaac atcattccga taatccgtgg 360

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tctggaggag gtgggaccgg tgaggtgcag ctggtgcagt ctggagcaga ggtgaaaaag 480

20

cccggagagt ctctgaagat ttctgttaag ggatccggtt actcattcac tggctacaat 540

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cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 1020

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ggctttctatc caagcgacat cgcgtggag tgggagagca atgggcagcc ggagaacaac 1320

tacaagacca cgcctcccgt gctggactcc gacggctcct tcttctctca cagcaagctc 1380

accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1440

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<210> 48

<211> 493

<212> PRT

<213> Artificial sequence

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<223> Humanized TRU-016

<400> 48

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 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
 85 90 95
 25 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
 30 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly Glu
 130 135 140
 35 Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu Ser
 145 150 155 160
 40 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
 45 Asn Ile Asp Pro Tyr Tyr Gly Gly Thr Thr Tyr Asn Arg Lys Phe Lys
 195 200 205
 50 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
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	Arg	Ser	Val	Gly	Pro	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	
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				260					265					270			
10	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					
15	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
	305					310					315					320	
20	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
					325					330					335		
25	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				
30	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
		370					375					380					
35	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
	385					390					395					400	
40	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			
45	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					
50	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	
	465					470					475					480	
55	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys				
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<210> 49
 <211> 356
 <212> DNA
 <213> Artificial sequence

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<220>
 <223> Humanized TRU-016

<400> 49

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cagatgcccg ggaaaggcct ggagtggatg ggcaatattg atccttatta tgggtgtact      180
acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc      240
gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc      300
tcagtcggcc ctatggacgt ctggggccaa ggcaccactg tcactgtctc ctcgag      356

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45

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EP 1 912 675 B1

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 1 5 10 15
 5 Asp Thr Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser
 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20 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
 25 Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
 115 120 125
 30 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Thr Gly
 130 135 140
 35 <210> 51
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 <212> DNA
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 <223> Humanized TRU-016
 <400> 51
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 50
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EP 1 912 675 B1

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 ggaattccag ccagggttcag tggcagtggg tccgggacag acttcactct caccatcagc 300
 10 agcctagagc ctgaagattt tgcagtttat tactgtcaac atcattccga taatccgtgg 360
 acattcggcc aagggacca ggtggaaatc aaaggtggcg gtggctcggg cgggtggtgga 420
 tctggaggag gtgggaccgg tgaggtgcag ctggtgcagt ctggagcaga ggtgaaaaag 480
 15 cccggagagt ctctgaagat ttctgtaag ggatccggtt actcattcac tggctacaat 540
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 20 aagtccatca gcaccgcta cctgcaatgg agcagcctga aggcctcgga caccgcatg 720
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<213> Artificial sequence

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<223> Humanized TRU-016

<400> 52

EP 1 912 675 B1

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 20 25 30
 10 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
 15 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
 65 70 75 80
 20 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
 25 -----
 Asp Asn Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Gly
 115 120 125
 30 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
 35 Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Gly Tyr Asn
 165 170 175
 40 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
 45 Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr Leu
 210 215 220
 50
 55

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Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys Ala
 225 230 235 240
 5 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
 10 260 265 270
 Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
 275 280 285
 15 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
 20 305 310 315 320
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 25 325 330 335
 Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 340 345 350
 30 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
 35 370 375 380
 Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 385 390 395 400
 40 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
 45 420 425 430
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 435 440 445
 50 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 450 455 460
 55 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 465 470 475 480

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

5 <210> 53
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 <212> DNA
 <213> Artificial sequence

10 <220>
 <223> Humanized TRU-016

<400> 53

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 cagatgcccc ggaaaggcct ggagtggatg ggcaatattg atccttatta tgggtggtact 180
 20 acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240
 gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300
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35 <400> 54

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45

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EP 1 912 675 B1

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
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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
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<210> 55

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<212> DNA

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<400> 55

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cagatgcccc ggaaaggcct ggagtggatg ggcaatattg atccttatta tgggtgtact 180
acctacaacc ggaagttcaa gggccaggtc actatctccg ccgacaagtc catcagcacc 240
gcctacctgc aatggagcag cctgaaggcc tcggacaccg ccatgtatta ctgtgcgcgc 300
tcagtcggcc cttttcagca ctggggccaa ggcaccctcg tcactgtctc ctgcag 356

<210> 56

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EP 1 912 675 B1

<220>

<223> Humanized TRU-016

<400> 56

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

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

15

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

20

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

25

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

30

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

35

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

<210> 57

<211> 356

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<212> DNA

<213> Artificial sequence

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<400> 57

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55

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 20 25 30

20

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

25

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

30

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

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Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
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 35 Val Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
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 Arg Leu Leu Ile Tyr Phe Ala Lys Thr Leu Ala Glu Gly Ile Pro Ala
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 85 90 95
 45 Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His His Ser
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EP 1 912 675 B1

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EP 1 912 675 B1

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EP 1 912 675 B1

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Patentkrav

1. Humaniseret CD37-specifikt bindingsprotein til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom, hvor det humaniserede CD37-specifikke bindingsprotein omfatter:

- 5 (a) human let kædelæseramme-regioner, en let kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:61, en let kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:64, og en let kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO:66; og
- 10 (b) human tung kædelæseramme-regioner, en tung kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:63, en tung kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:65, og en tung kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO: 67 eller 68

kendetegnet ved at det humaniserede CD-37 specifikke bindingsprotein skal
15 anvendes i kombination med et CD20-specifikt bindingsprotein.

2. CD20-specifikt bindingsprotein til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom **kendetegnet ved at** det CD20-specifikke bindingsprotein skal anvendes i kombination med humaniseret CD37-specifikt bindingsprotein til
20 anvendelse i behandling af B-cellecancer eller en autoimmun sygdom, hvor det humaniserede CD37-specifikke bindingsprotein omfatter:

- 25 (a) human let kædelæseramme-regioner, en let kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:61, en let kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:64, og en let kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO:66; og
- (b) human tung kædelæseramme-regioner, en tung kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:63, en tung kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:65, og en tung kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO:67 eller
30 68.

- 3.** Fremstillingsgenstand omfattende et CD20-specifikt bindingsprotein og et humaniseret CD37-specifikt bindingsprotein til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom, hvor det humaniserede CD37-specifikke
5 bindingsprotein omfatter:

- (a) human let kædelæseramme-regioner, en let kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:61, en let kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:64, og en let kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO:66; og
- 10 (b) human tung kædelæseramme-regioner, en tung kæde CDR1 omfattende en aminosyresekvens ifølge SEQ ID NO:63, en tung kæde CDR2 omfattende en aminosyresekvens ifølge SEQ ID NO:65, og en tung kæde CDR3 omfattende en aminosyresekvens ifølge SEQ ID NO: 67 eller 68.
- 15 **4.** Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge kravene 1, 2 eller 3, hvor den lette kæde CDR1, CDR2, og CDR3 af humaniseret CD37-specifikt bindingsprotein består af aminosyresekvenser ifølge henholdsvis SEQ ID NO: 61, 64 og 66; og
20 hvor den tunge kæde CDR1, CDR2, og CDR3 består af aminosyresekvenser ifølge henholdsvis SEQ ID NO: 63, 65 og 67.

- 5.** Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-
25 celledcancer eller en autoimmun sygdom ifølge kravene 1, 2 eller 3, hvor den lette kæde CDR1, CDR2, og CDR3 af humaniseret CD37-specifikt bindingsprotein består af aminosyresekvenser ifølge henholdsvis SEQ ID NO: 61, 64 og 66; og hvor den tunge kæde CDR1, CDR2, og CDR3 består af aminosyresekvenser ifølge henholdsvis SEQ ID NO: 63, 65 og 68.

30

- 6.** Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsartiklen til anvendelse i behandling af B-

cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, omfattende:

en human let kædelæseramme-region valgt fra SEQ ID NO:170-172, 175, 179,182, 184-188, 191, 194-198, 203 og 205-210; eller

- 5 en human tung kædelæseramme-region valgt fra SEQ ID NO:140, 141, 143-147, 150, 151, 154-163, 168 og 169.

7. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsartiklen til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de

- 10 foregående krav, omfattende:

en første human let kædelæseramme-region (FR1) valgt fra SEQ ID NO:170-172, 175 og 177-181; en human FR2 af den lette kæde læseramme-region valgt fra SEQ ID NO: 182, 184-188 og 191; en human FR3 af den lette kæde valgt fra SEQ ID NO:194-198, 203 og 205; og en

15 human FR4 af den lette kæde valgt fra SEQ ID NO:206-210; eller

en første human tung kædelæseramme-region (FR1) valgt fra SEQ ID NO:140, 141 og 143-146; en human FR2 af den tunge kæde valgt fra SEQ ID NO:147, 150 og 151; en human FR3 af den tunge kæde valgt fra SEQ ID NO:154-160; og en human FR4 af den tunge kæde valgt fra SEQ ID

20 NO:161-163, 168 og 169.

8. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsartiklen til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav,

- 25 hvor bindingsproteinet omfatter et enkelt kæde Fv (scFv)-polypeptid, et antistof eller et bindingsfragment deraf, eller et immunoglobulinfusionsprotein der omfatter, fra amino- til carboxyterminus, (i) et bindingsdomæne med immunoglobulin let kæde- og tung kæde-variable regioner adskilt af et linkerpeptid, (ii) et immunoglobulin-forbindelsesled-region-polypeptid med enten
- 30 nul, én eller to cysteingrupper, og (iii) immunoglobulin CH2- og CH3-domæner.

9. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge krav 8, hvor immunoglobulinforbindelsesled-region-polypeptidet kan være et IgG1-, IgGA- eller 5 IgE-forbindelsesled-region-polypeptid eller en mutant IgG1.

10. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af kravene 8 10 eller 9, hvor:

bindingsproteinet er et immunoglobulinfusionsprotein ifølge krav 8 omfattende en let kæde variabel region og en tung kæde variabel region;

den let kæde variable region er forbundet til den tung kæde variable region med et linkerpeptid omfattende (Gly₄SER)_n, hvor n er 1, 2, 3, 4, 5, eller 6; 15 og

de linkede let kæde- og tung kæde-variable regioner er forbundet til et IgG1-effektordomæne via et forbindelsesled bestående af en aminosyresekvens valgt fra gruppen bestående af SEQ ID NO: 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 115, 116, 118, 120, 122, 124, 20 126, eller 127.

11. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, hvor det humaniserede CD37-specifikke bindingsprotein 25 omfatter en aminosyresekvens valgt fra gruppen bestående af SEQ ID NO: 48 og 52.

12. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B- 30 celledcancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, hvor det CD20-specifikke bindingsprotein er et CD20-specifikt monoklonalt antistof.

13. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, hvor det CD20-specifikke monoklonale antistof er Rituximab.

5

14. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af kravene 1 til 11, hvor det CD20-specifikke bindingsprotein er et

10 immunoglobulinfusionsprotein der er CD20-specifikt og omfatter, fra amino- til carboxyterminus, (i) et bindingsdomæne med immunoglobulin let kæde- og tung kæde-variable regioner adskilt af et linkerpeptid, (ii) et immunoglobulin-forbindelsesled-region-polypeptid med enten nul, én eller to cysteingroupper, og (iii) immunoglobulin CH2- og CH3-domæne.

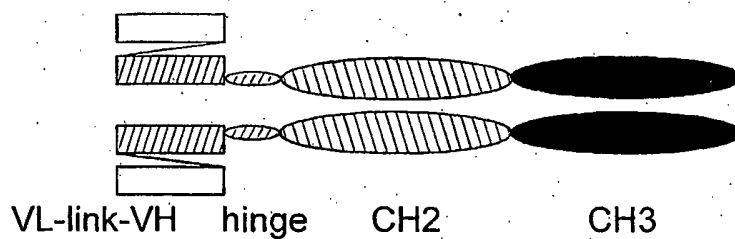
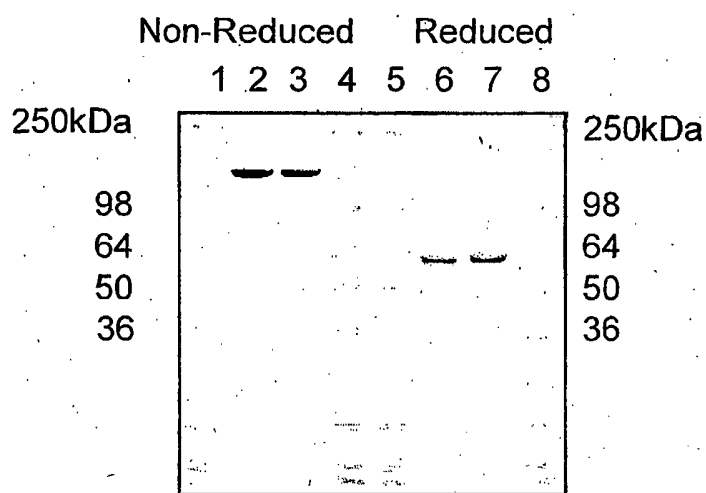
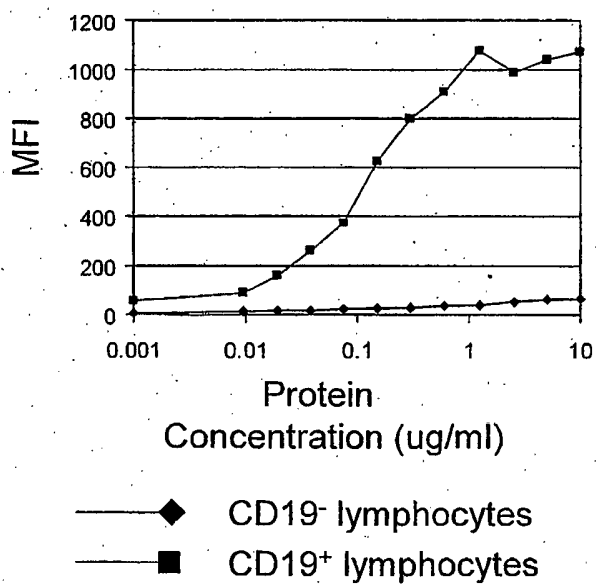
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15. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge krav 14, hvor immunoglobulinforbindelsesled-region-polypeptid kan være et IgG1-, IgGA- eller
20 IgE-forbindelsesled-region-polypeptid eller en mutant IgG1.

16. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i en fremgangsmåde til behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som
25 helst af de foregående krav, hvor fremgangsmåden yderligere omfatter anvendelsen af kemoterapeutisk middel, et radioterapeutisk middel, en cytokin, en kemokin eller en vækstfaktor.

17. Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke
30 bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, hvor B-cellecanceren er Hodgkin's sygdom, ikke-Hodgkin's lymfom, centralnervesystem-lymfomer, akut lymfoblast-leukæmi, kronisk lymfocytisk leukæmi, hårcelleleukæmi, kronisk myoblastleukæmi, multipel
35 myelom, lille lymfocytisk lymfom, B-celle-prolymfocytisk leukæmi,

- lymfoplasmacytisk lymfom, milt-marginal-zone-lymfom, plasmacellelymfom, enkeltstående plasmacytom af knogle, ekstraossøst plasmacytom, ekstra-knude-marginal-zone-B-celle-lymfom fra slimhinde-associeret lymfoidt væv, knude-marginal-zone-B-celle-lymfom, follikulær lymfom, kappe-cellelymfom, diffus stor
- 5 B-celle-lymfom, mediastinal (thymisk) stor B-celle-lymfom, intravaskulær stor B-celle-lymfom, primary effusion lymfom, Burkitt lymfom/leukæmi, B-celle-proliferationer fra usikkert malignt potentiale, lymfomatoid granulomatosis, eller post-transplantat lymfoproliferative sygdomme.
- 10 **18.** Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af kravene 1 til 16, hvor den autoimmune sygdom er rheumatoid arthritis, multipel sklerose, systemisk lupus erythematosus, Crohn's sygdom, Sjogren's syndrom, Grave's
- 15 sygdom, type I diabetes mellitus, psoriasis, immun thrombocytopenisk purpura, pemphigus, idiopatisk inflammatorisk myopati, myasthenis gravis, eller Waldenstorm's macroglobinæmi.
- 19.** Humaniseret CD37-specifikt bindingsprotein eller det CD20-specifikke
- 20 bindingsprotein eller fremstillingsgenstanden til anvendelse i behandling af B-cellecancer eller en autoimmun sygdom ifølge et hvilket som helst af de foregående krav, hvor det CD37-specifikke bindingsprotein og det CD20-specifikke bindingsprotein administreres sekventielt inden for ca. en 24-timers periode eller administreres simultant.

**FIG. 1A****FIG. 1B****FIG. 1C**

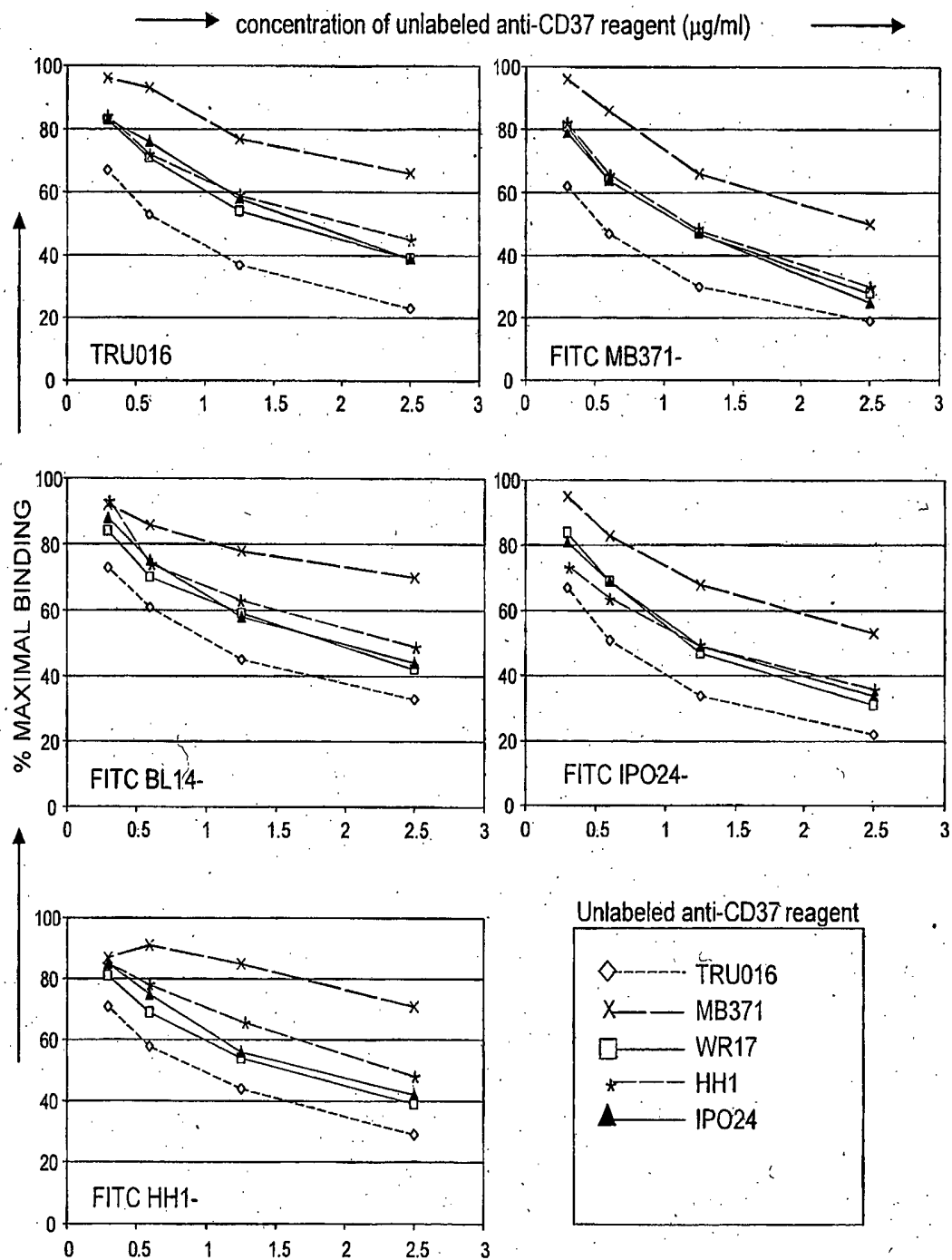


FIG. 2

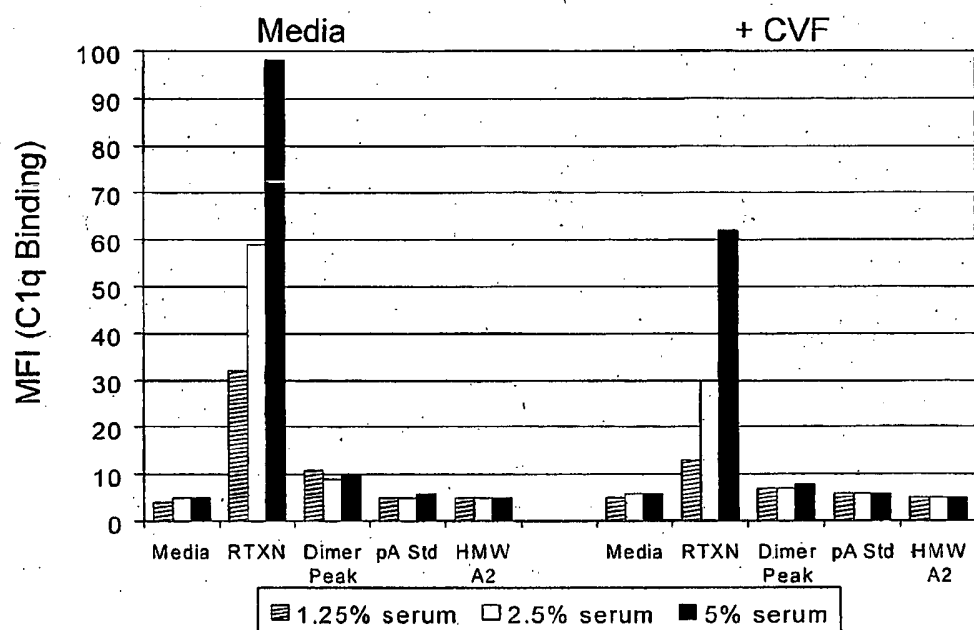


FIG. 3A

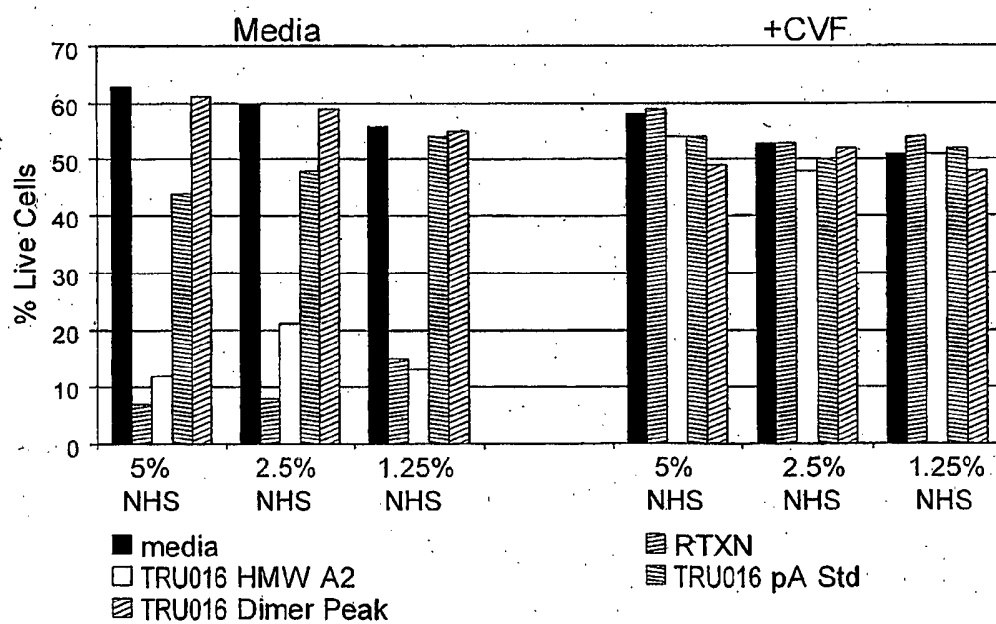
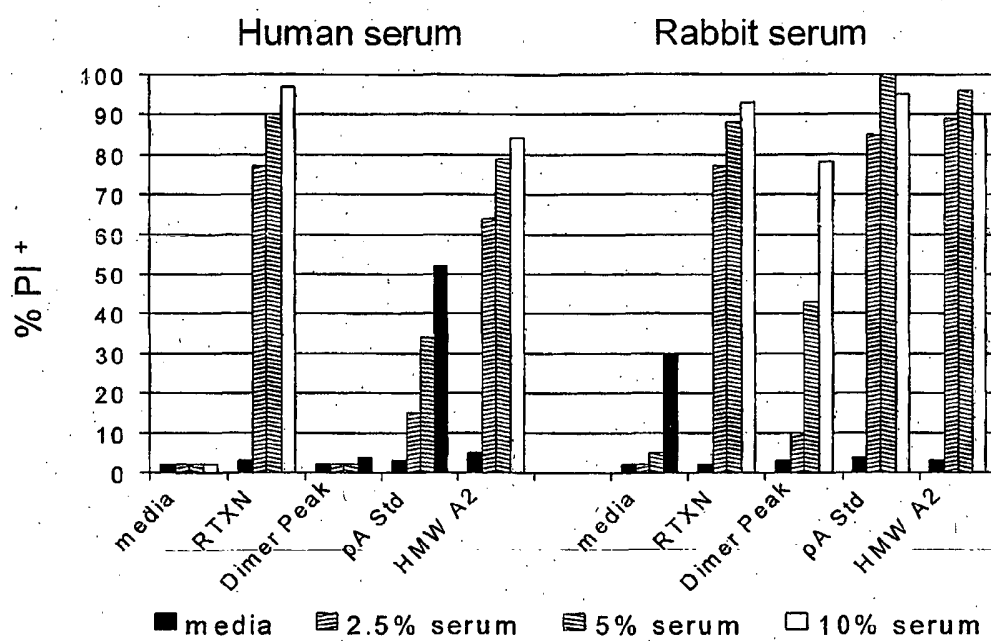


FIG. 3B

**FIG. 3C**

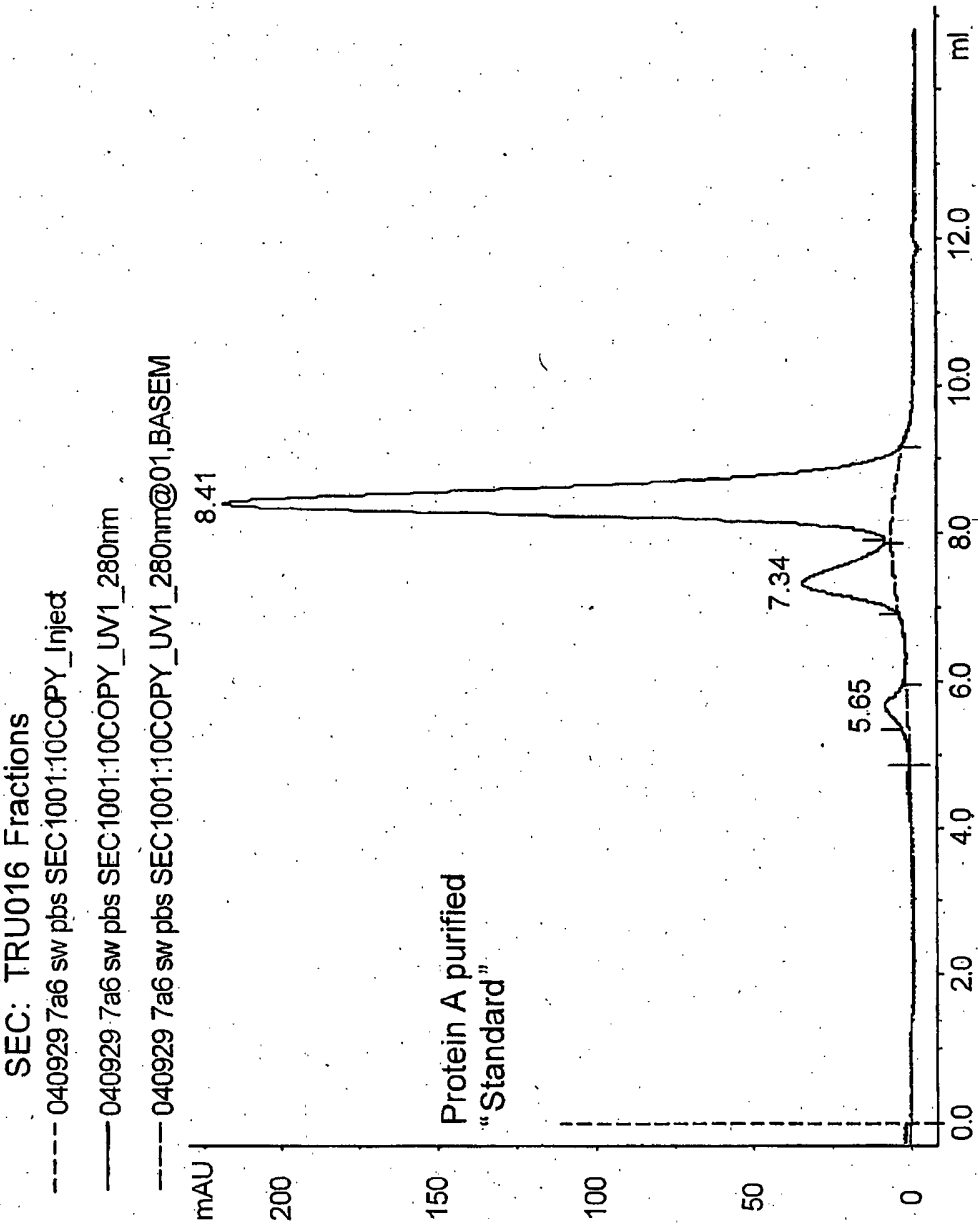


FIG. 4A

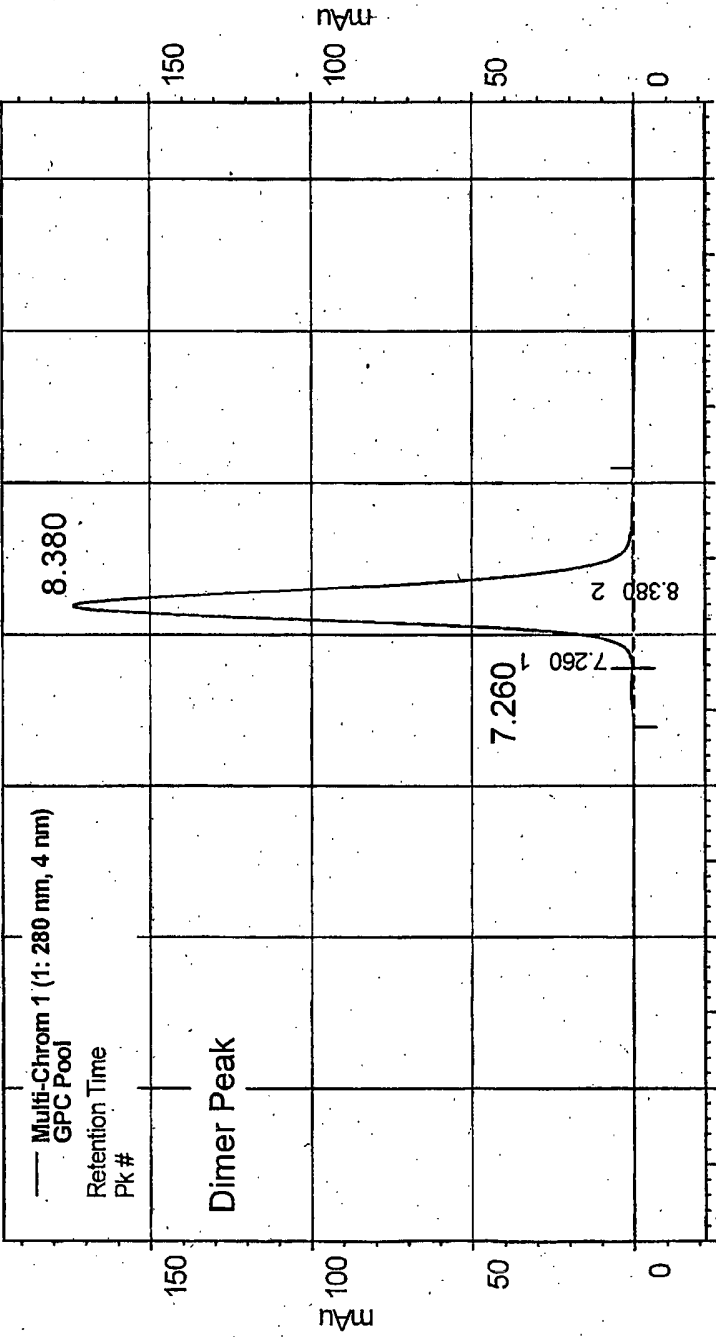


FIG. 4B

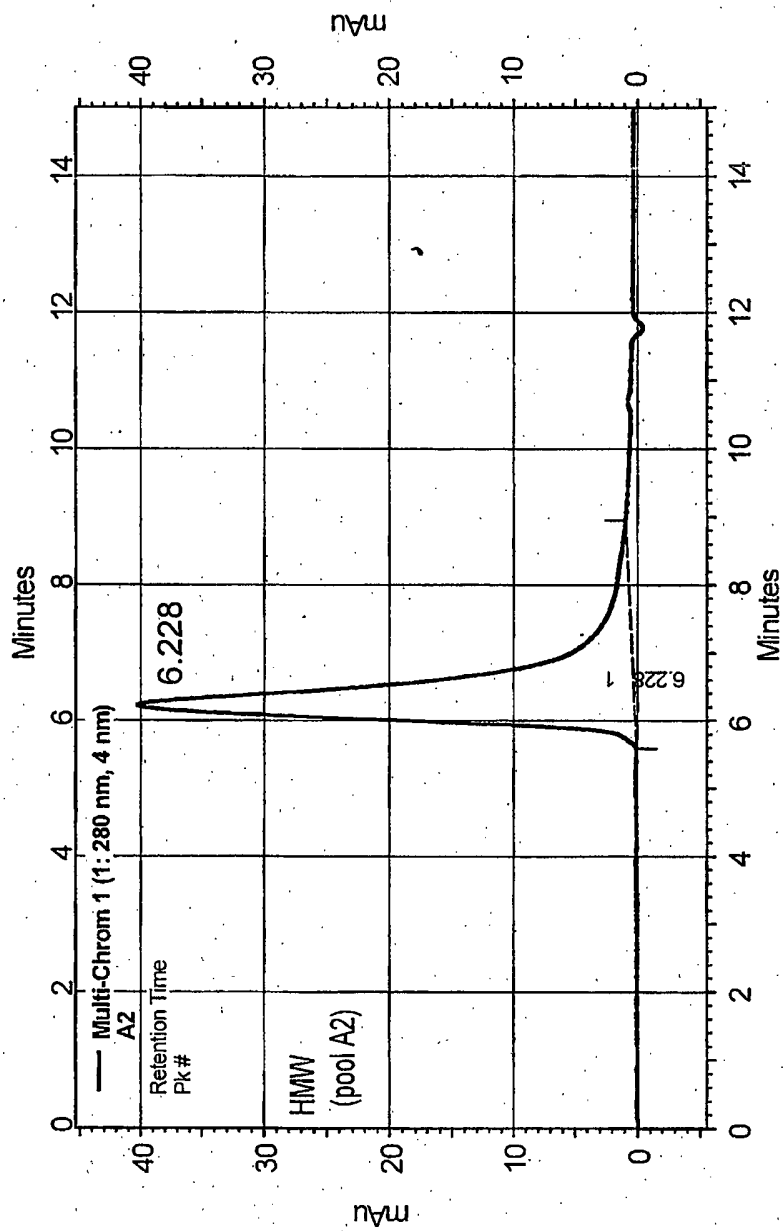
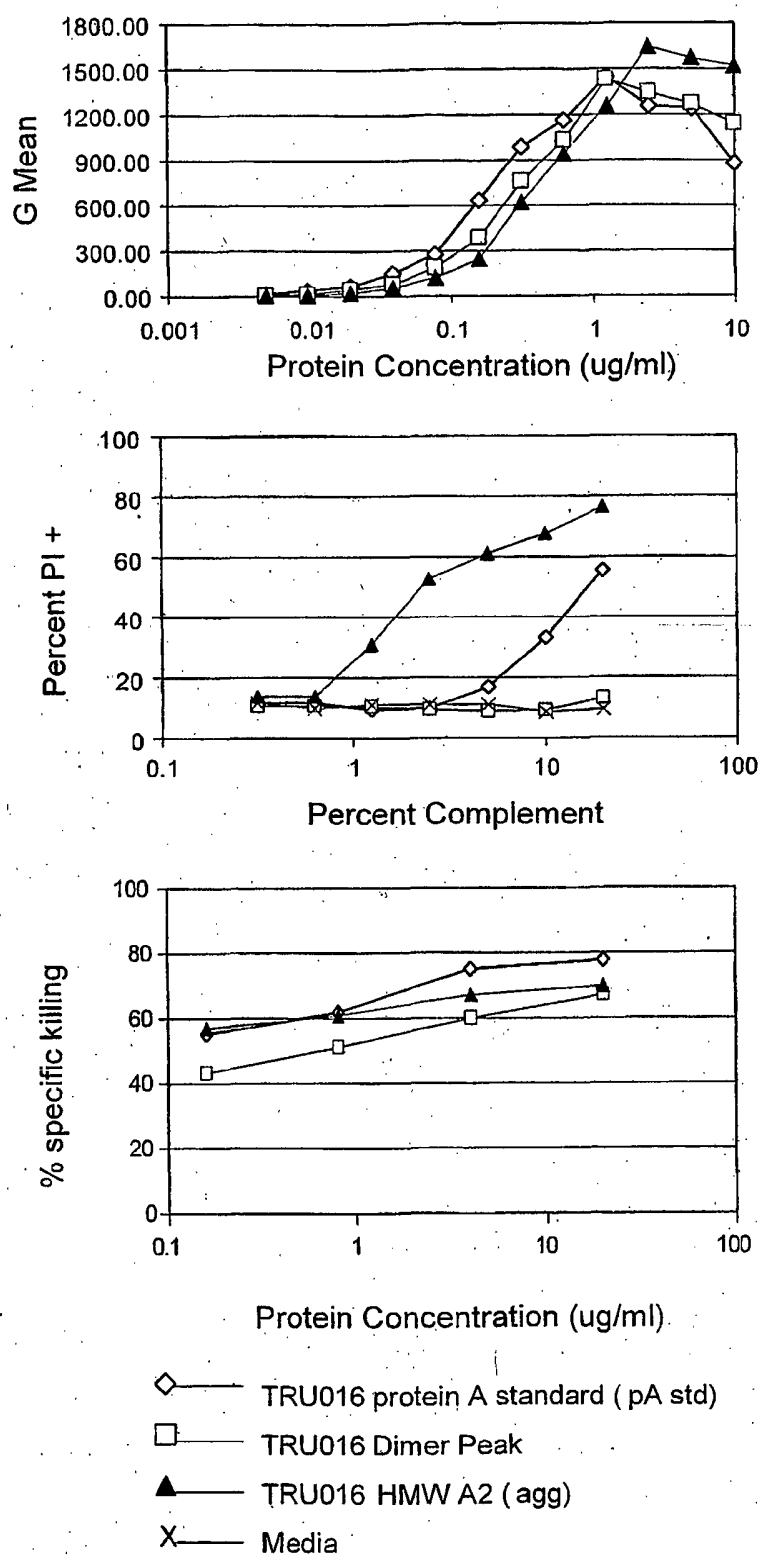


FIG. 4C

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**FIG. 5**

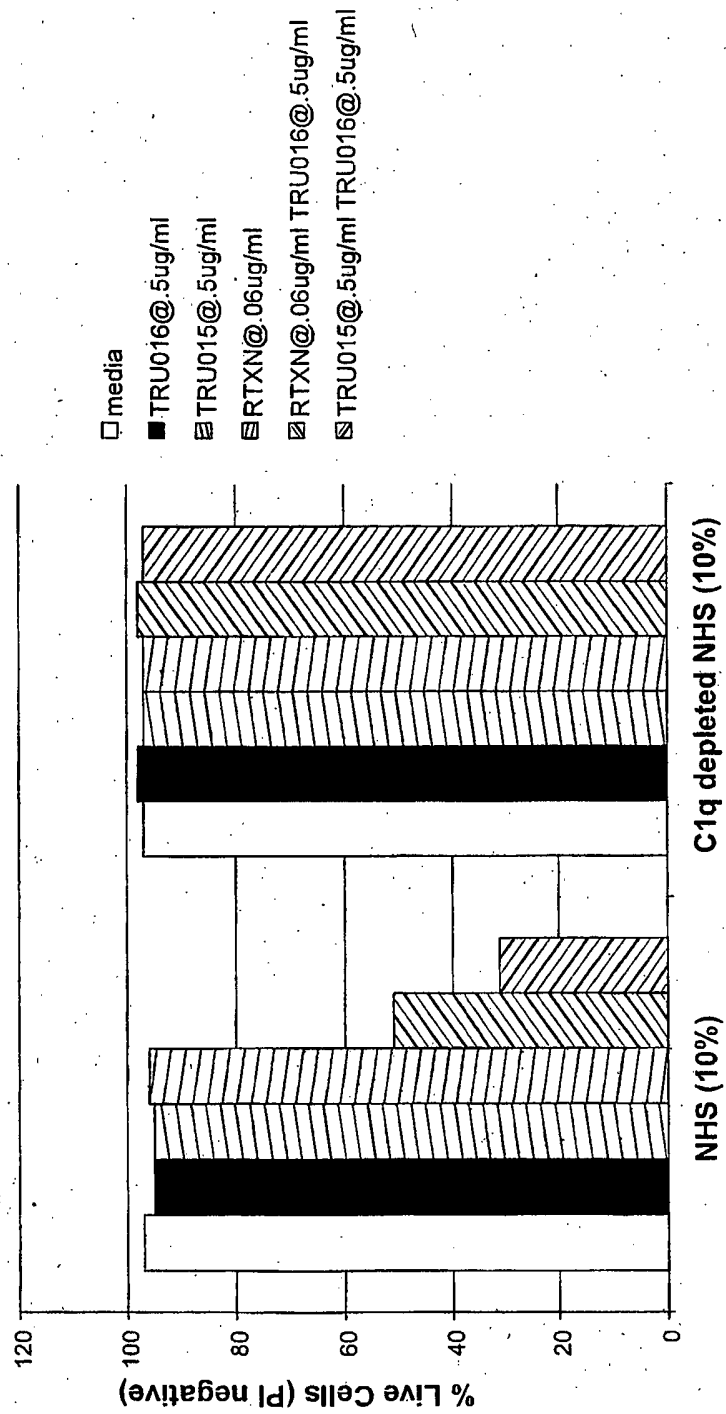
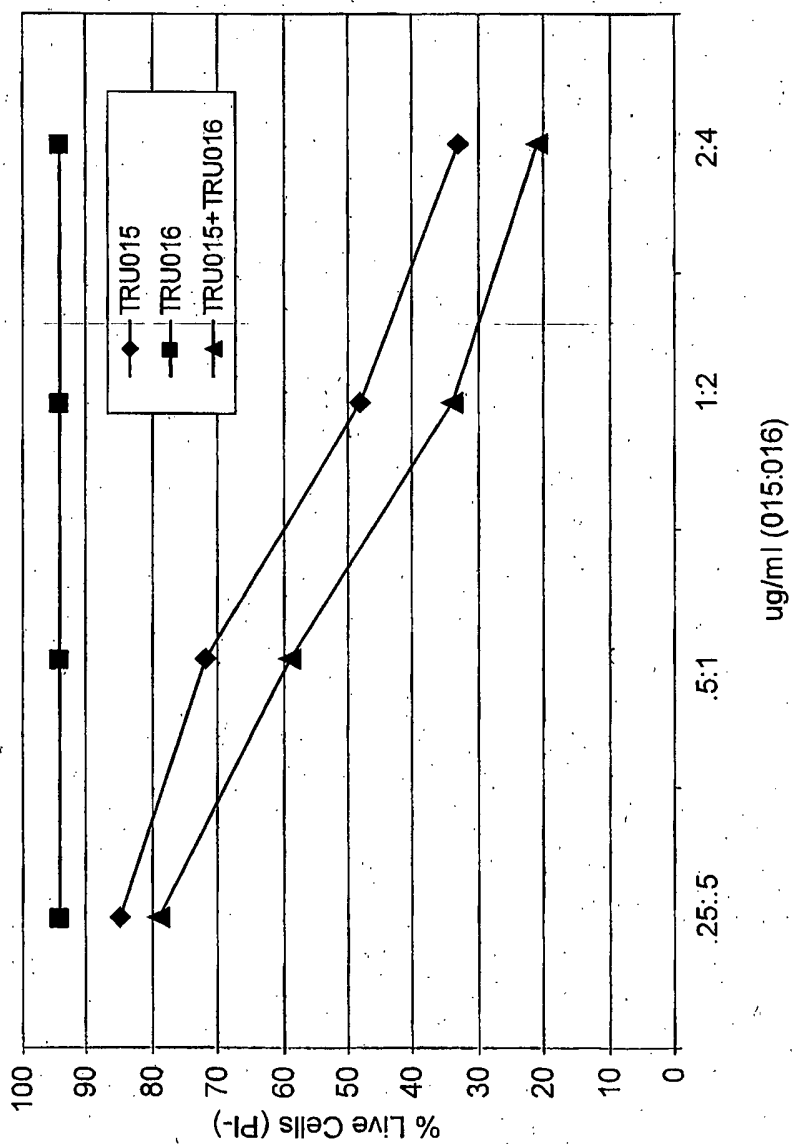


FIG. 6

**FIG. 7**

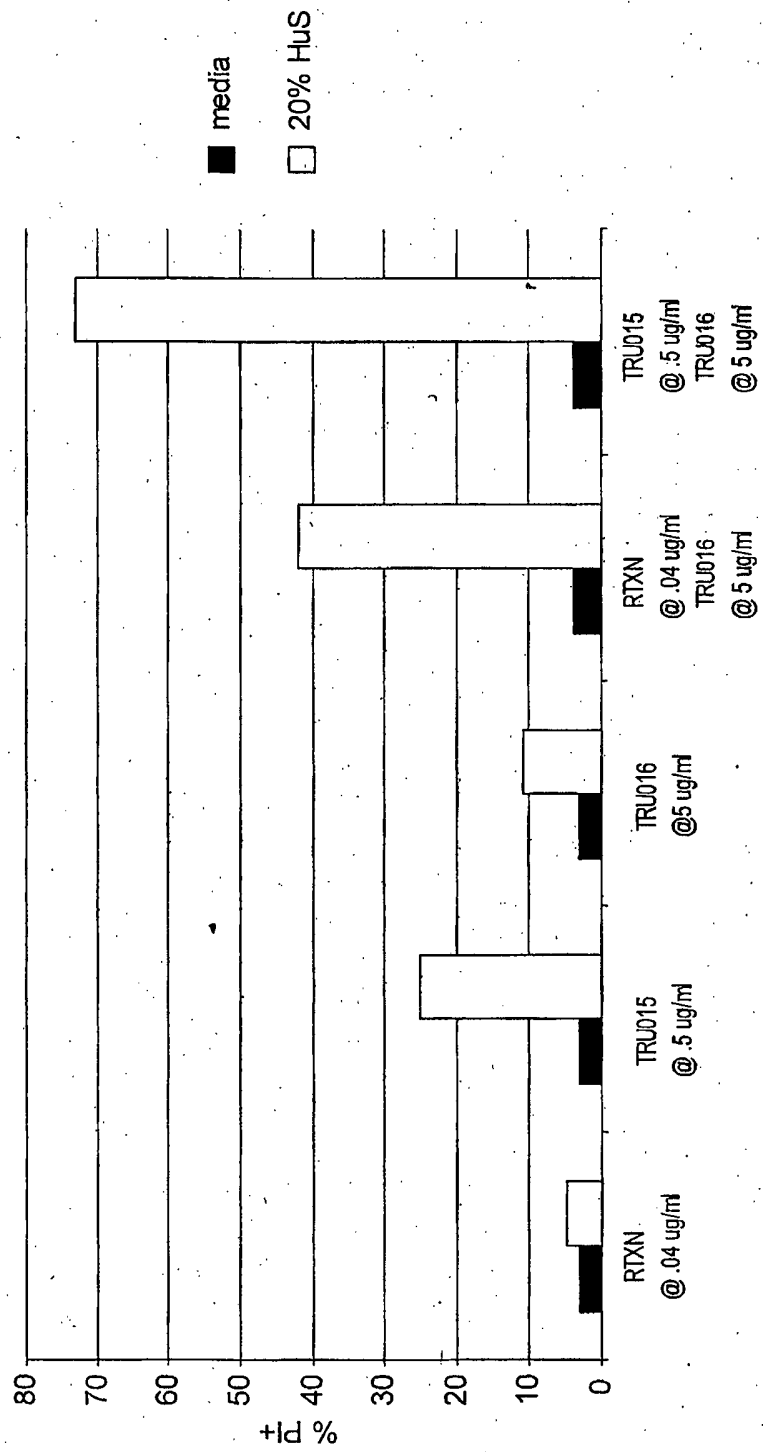


FIG. 8

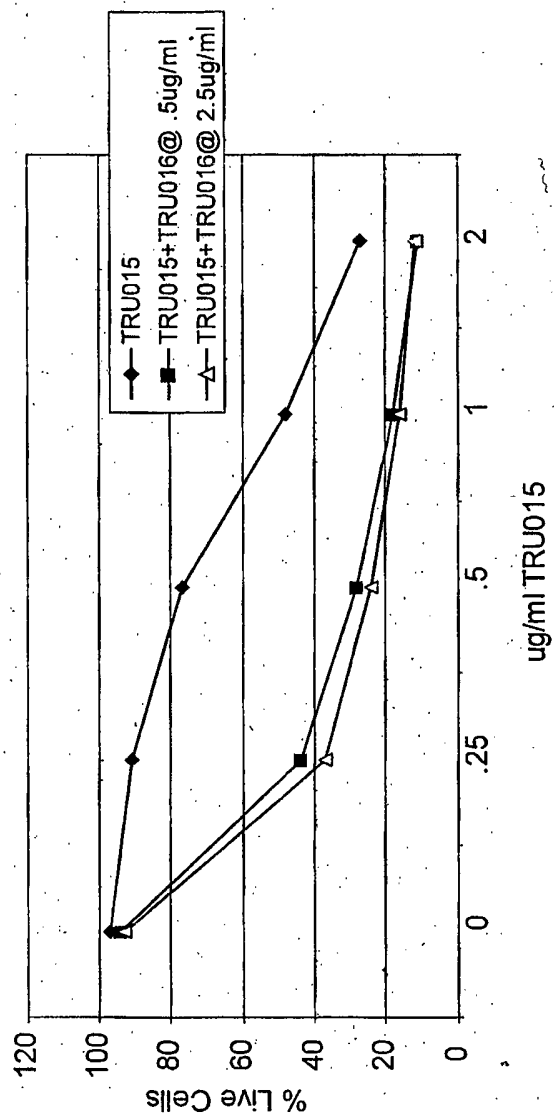


FIG. 9

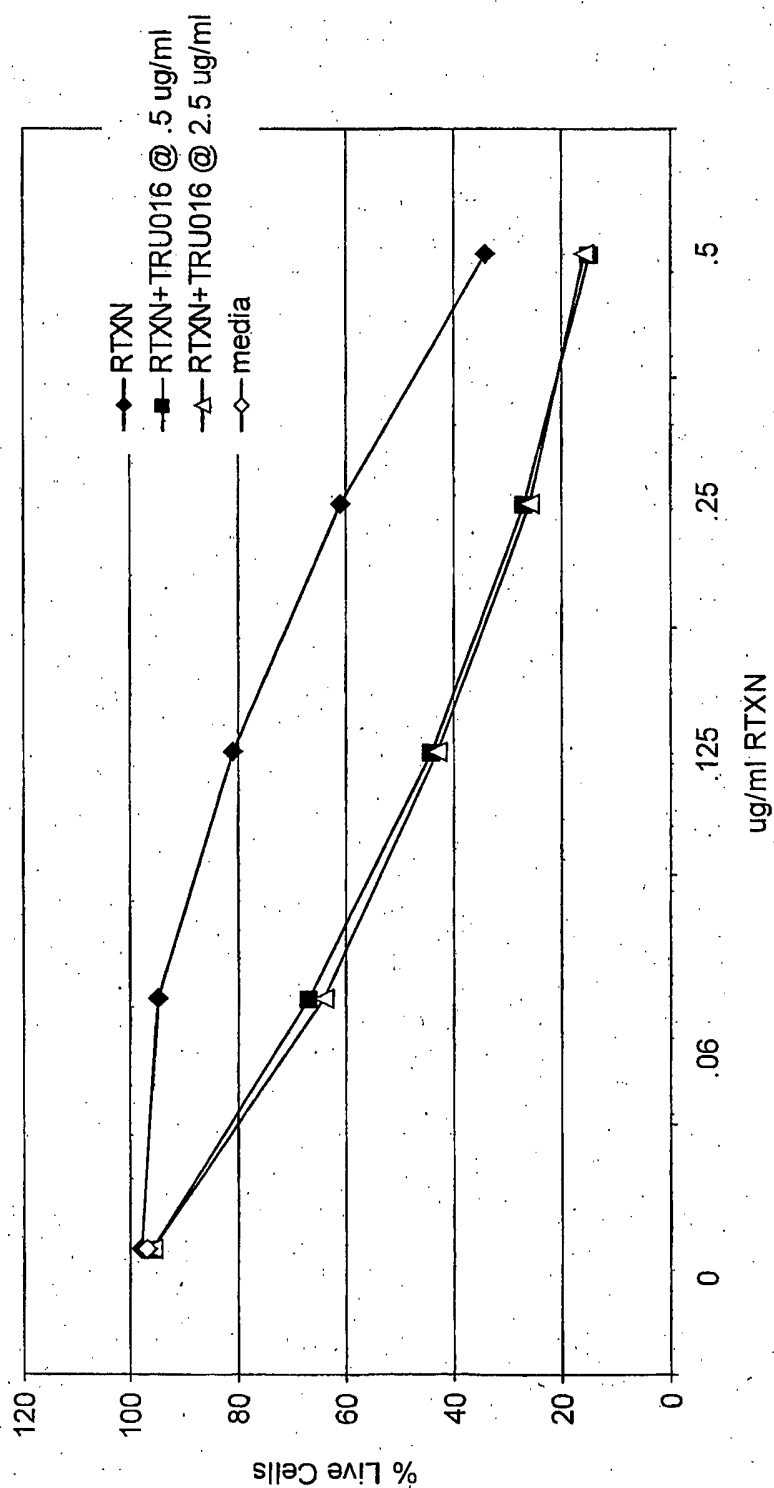


FIG. 10

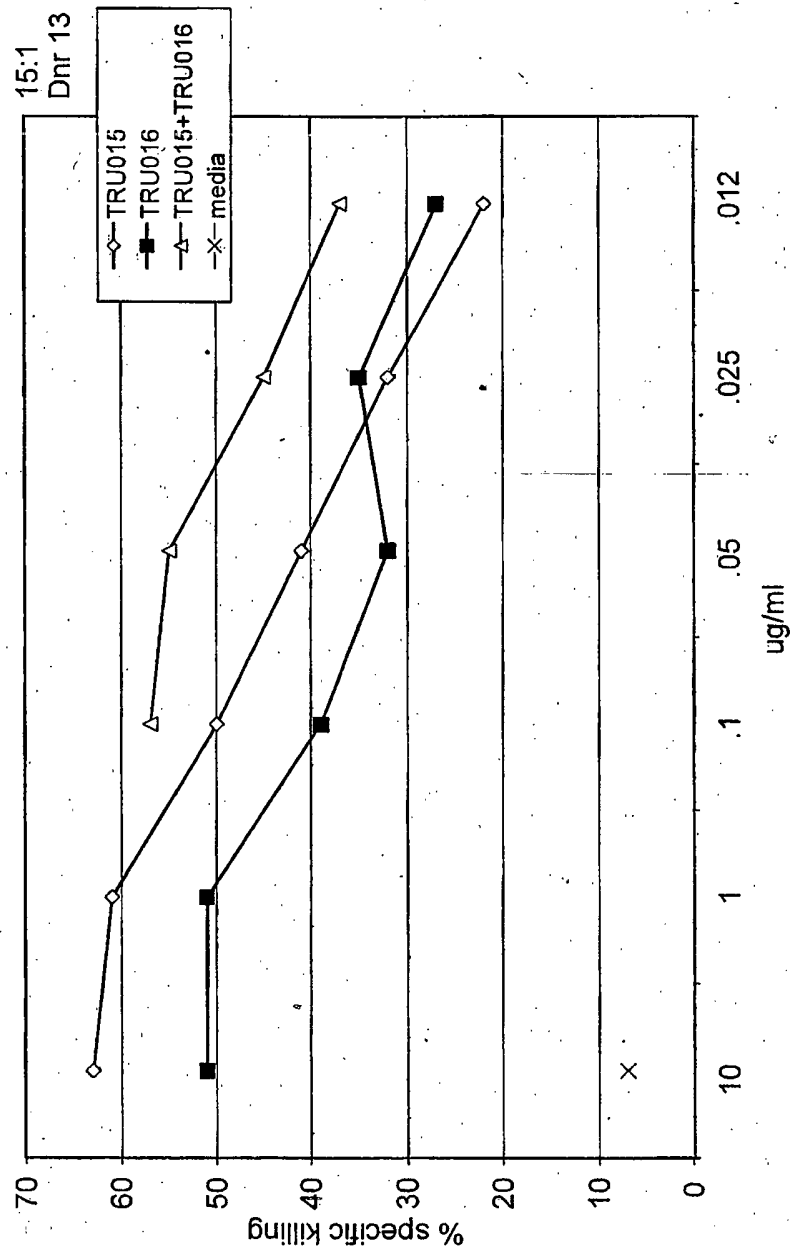


FIG. 11

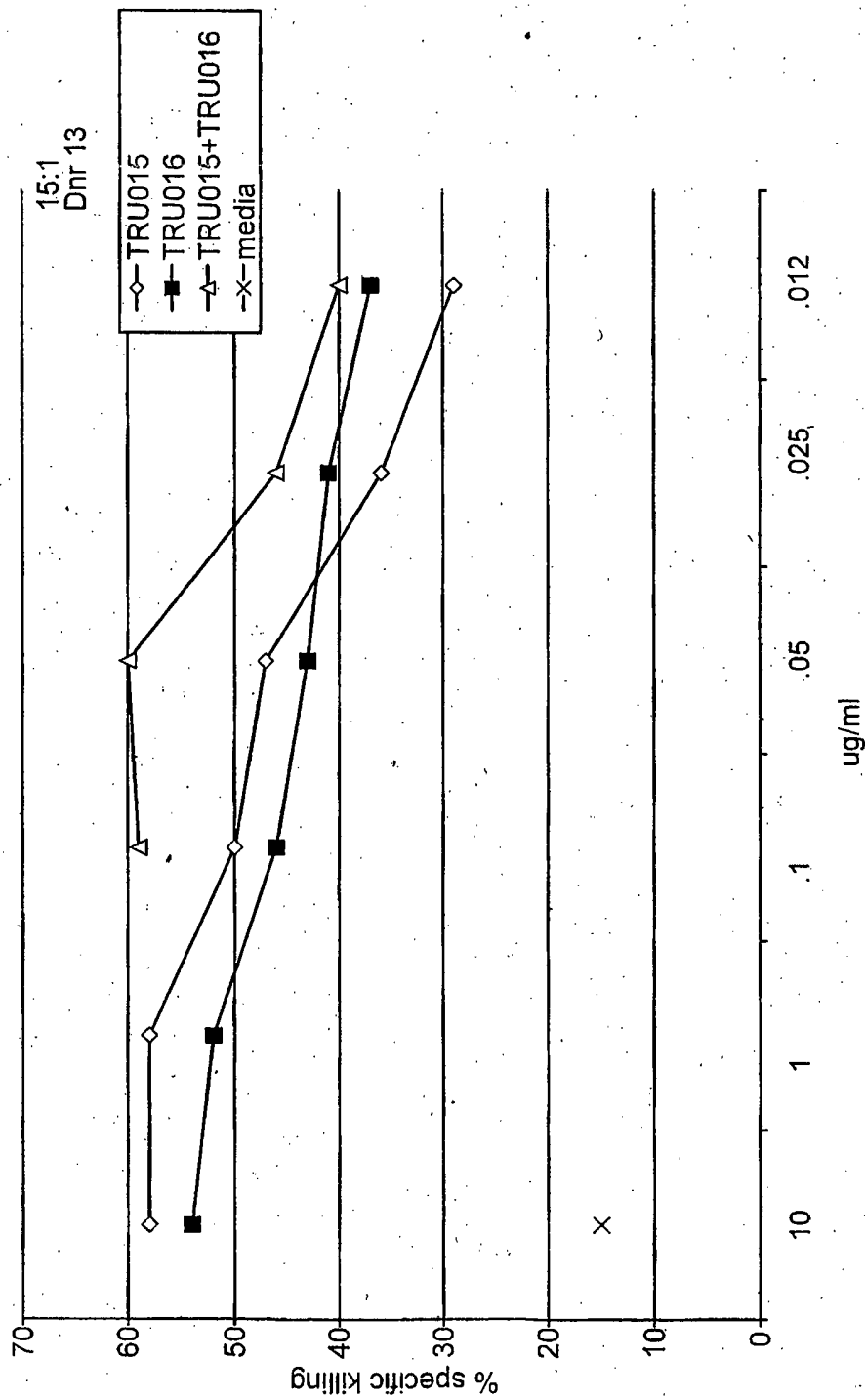


FIG. 12

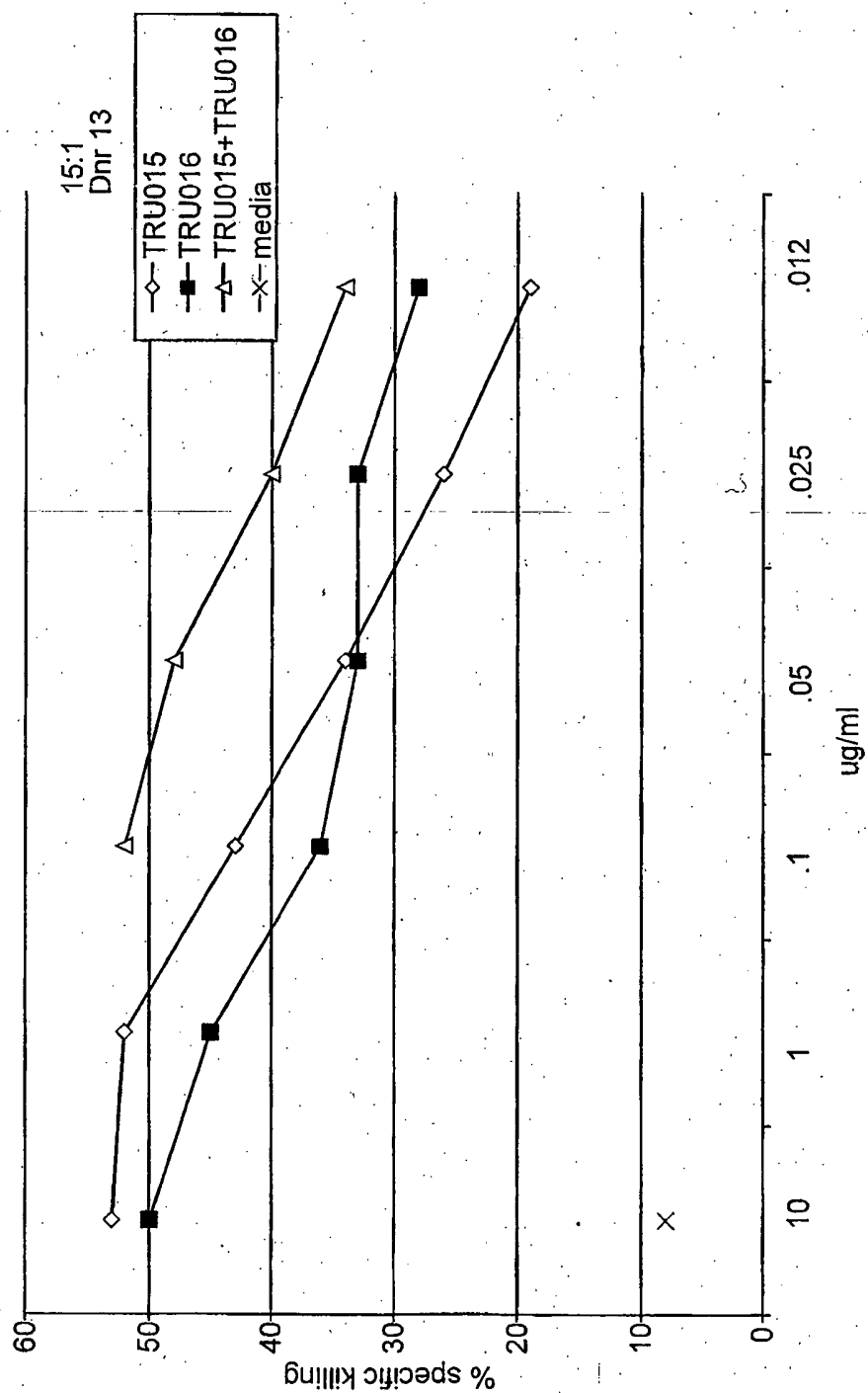


FIG. 13

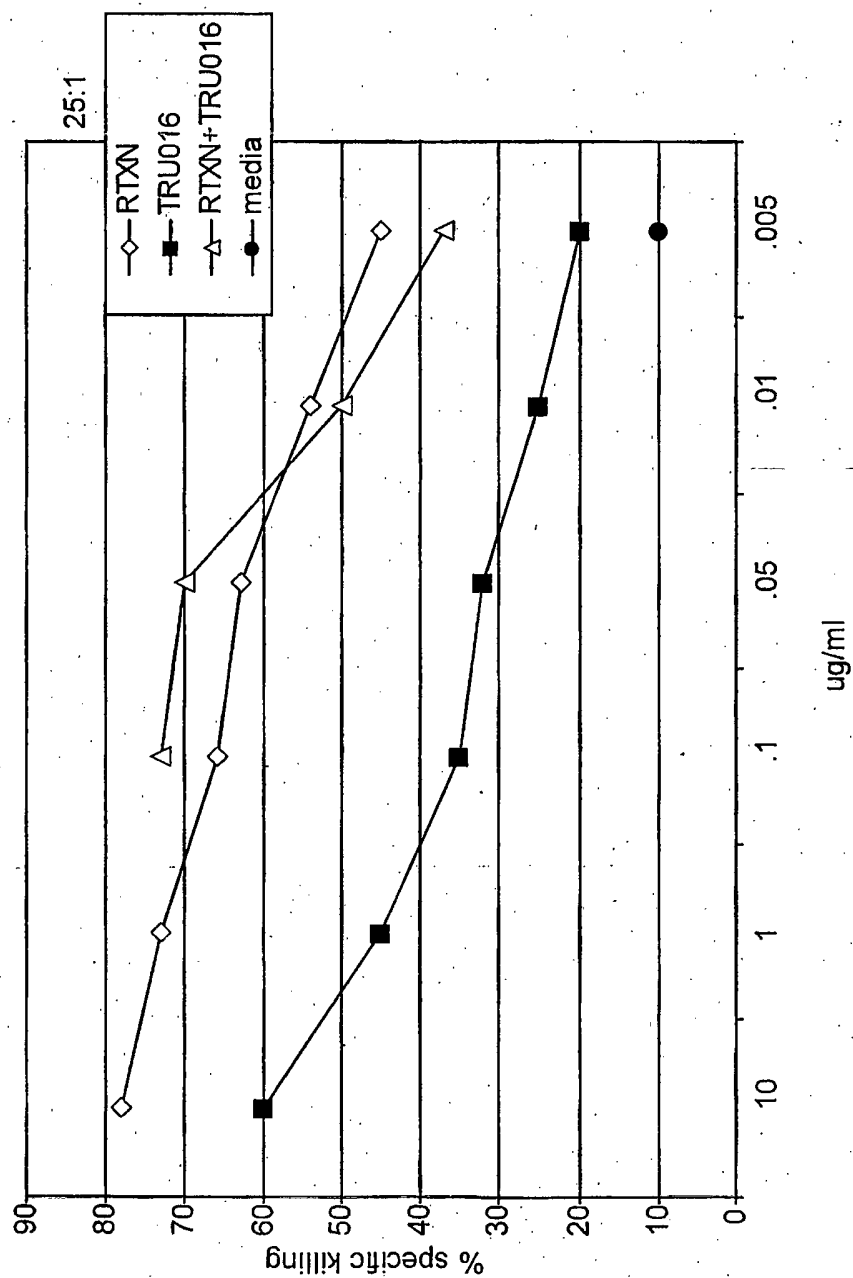


FIG. 14

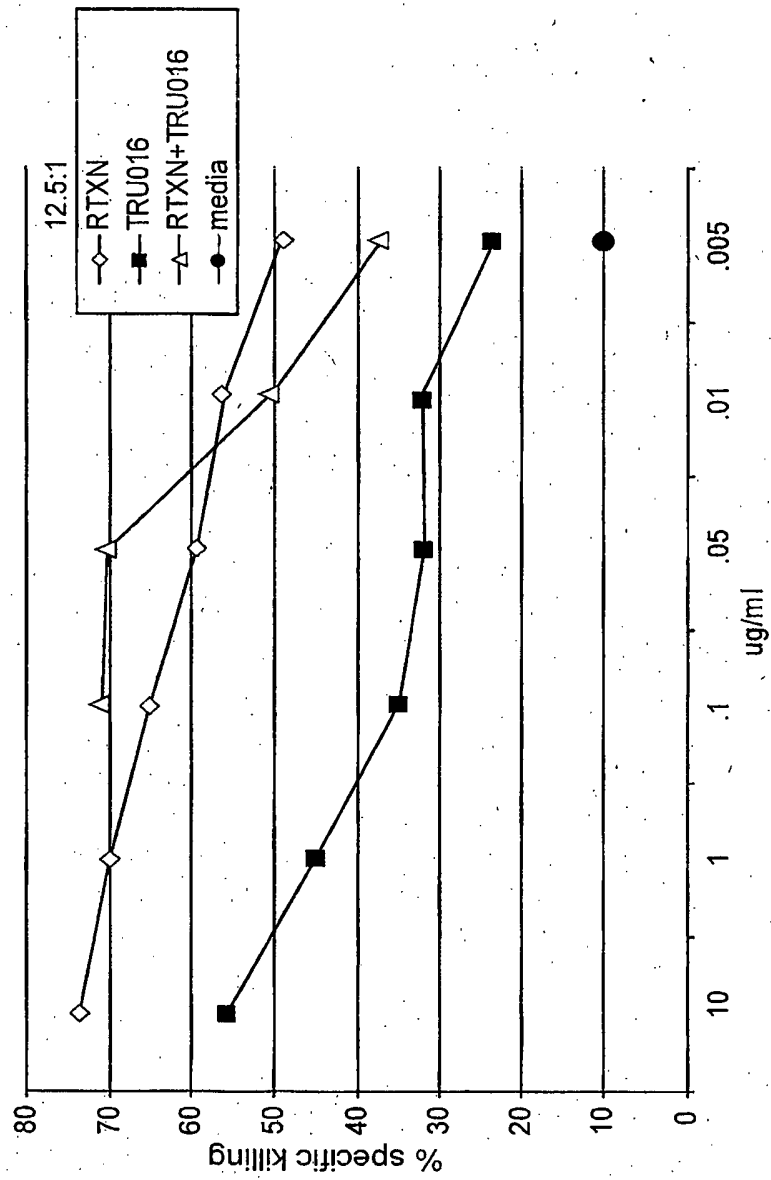


FIG. 15.

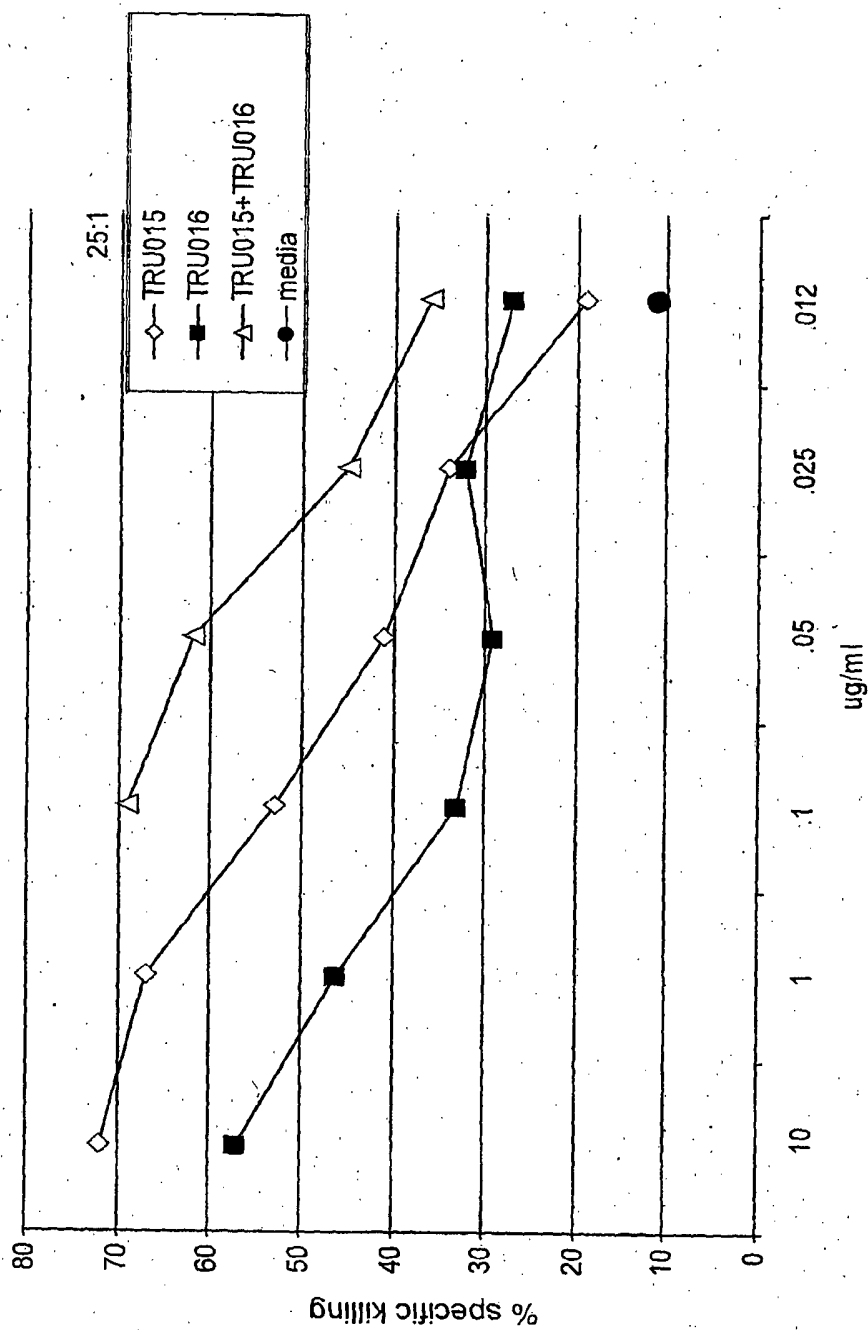


FIG. 16

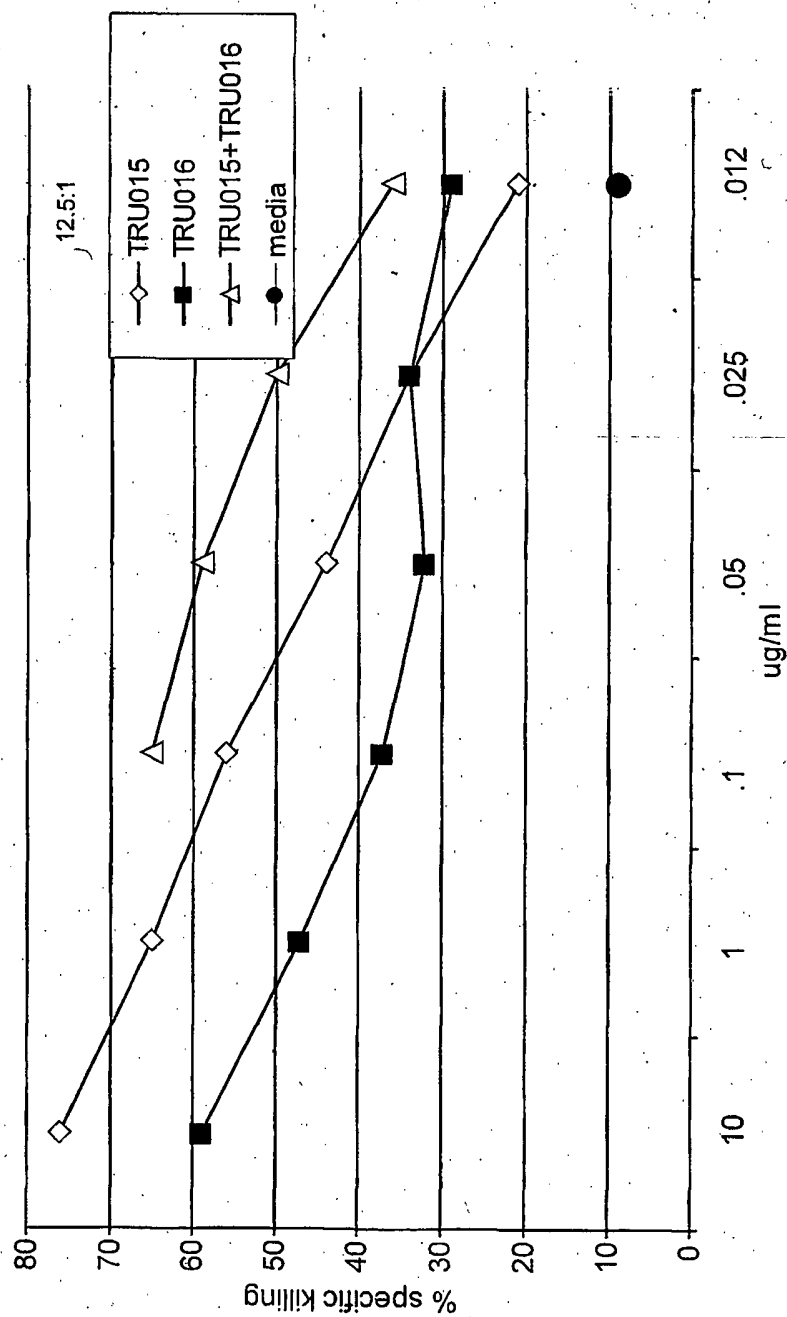
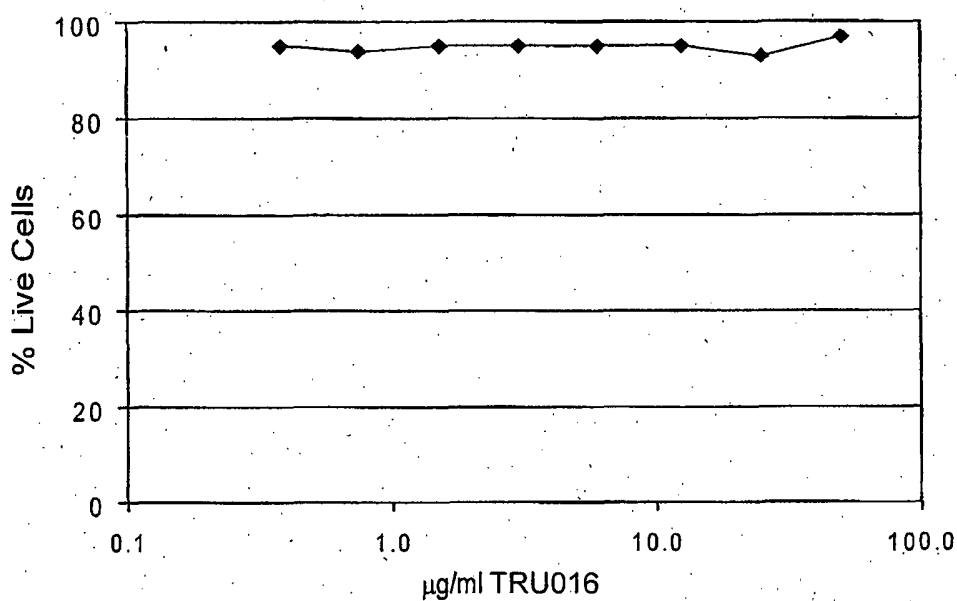
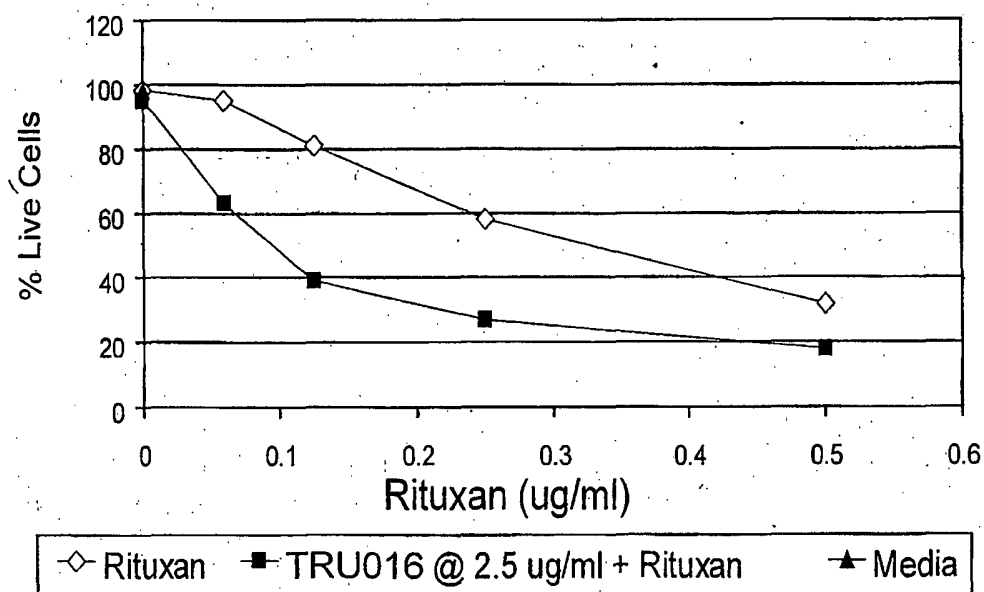
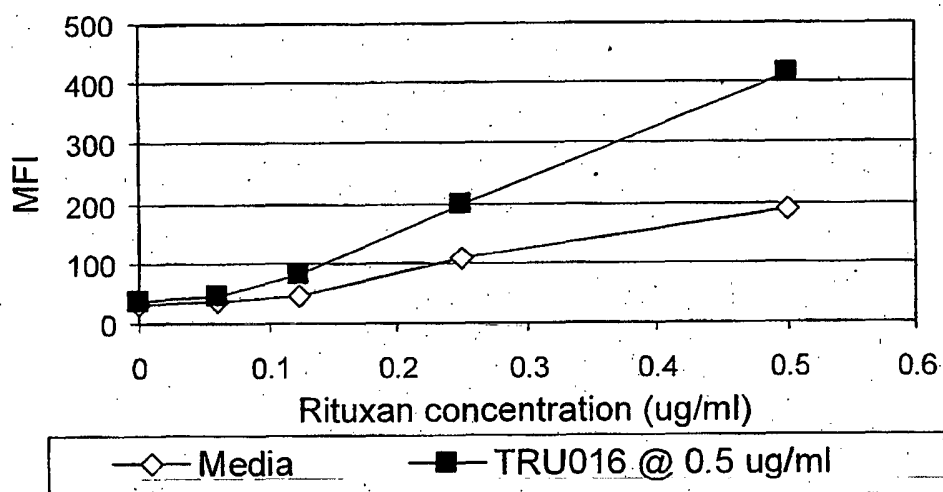
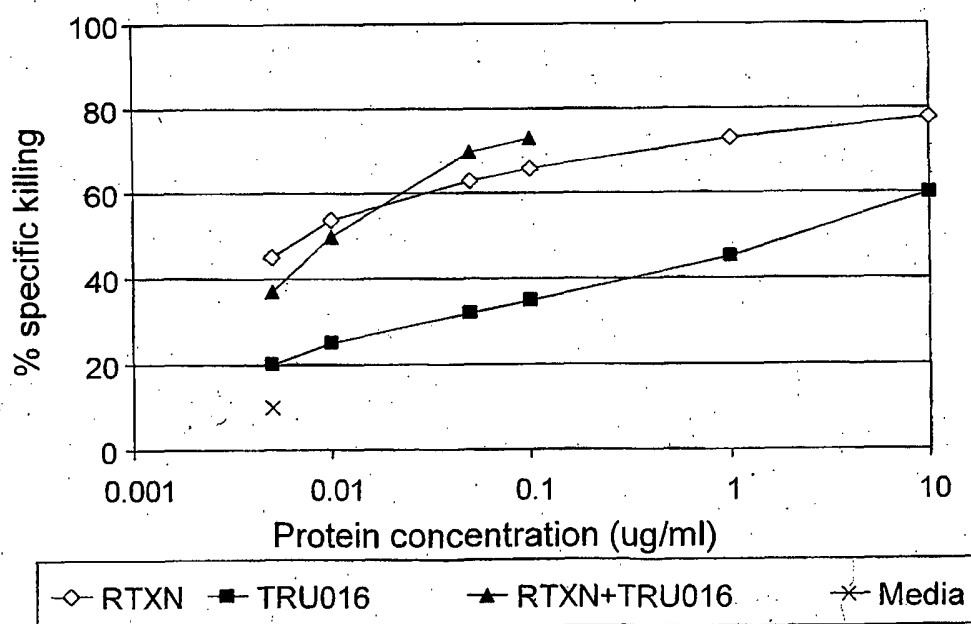
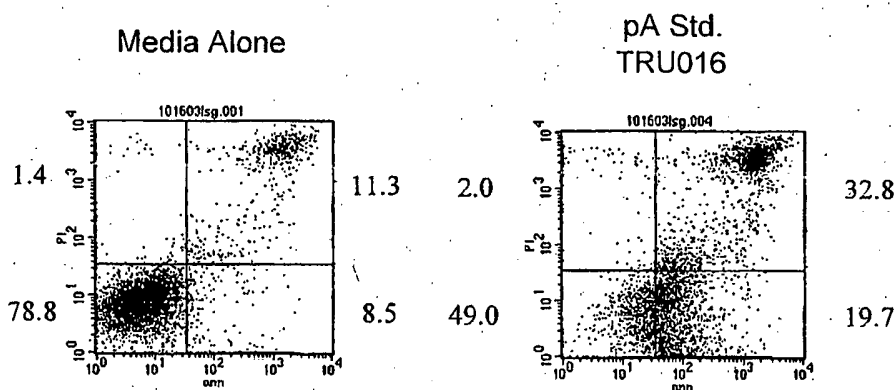
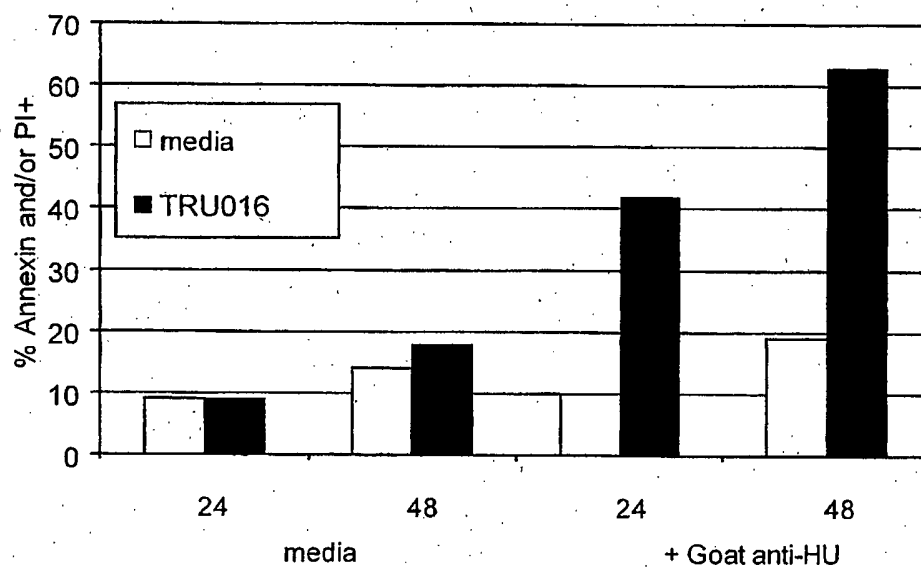


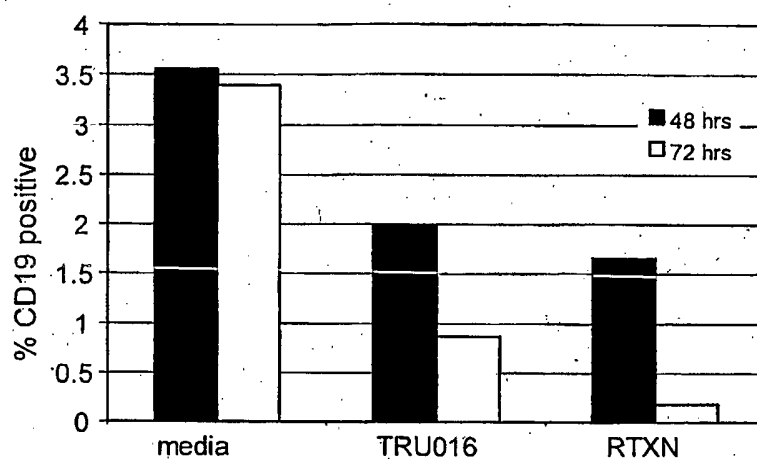
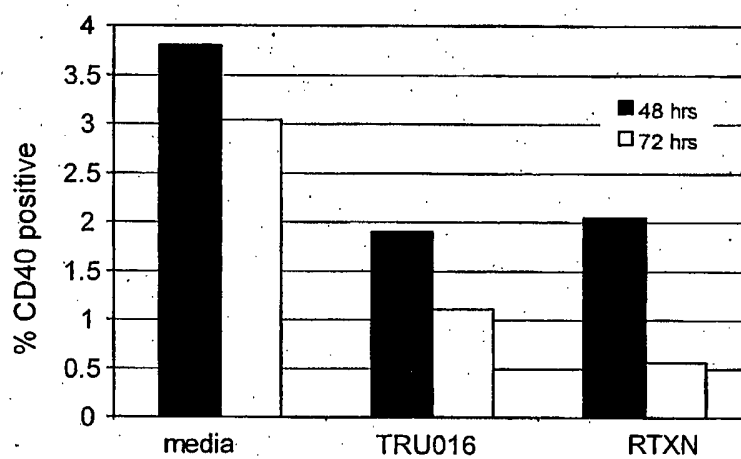
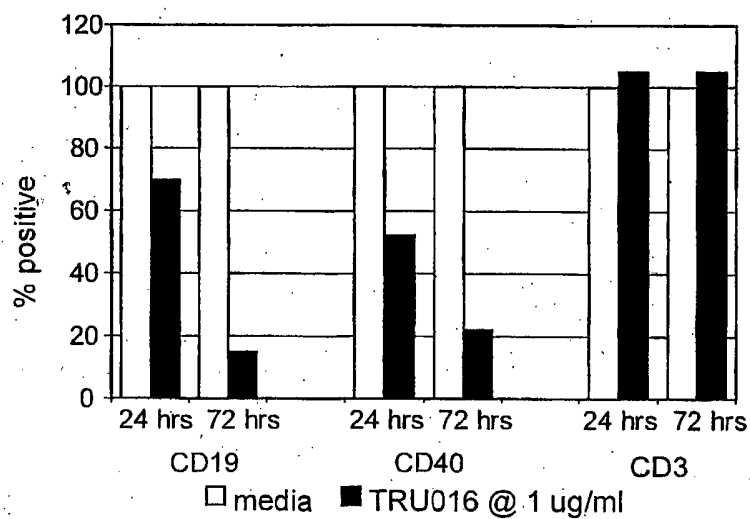
FIG. 17

**FIG. 18A****FIG. 18B**

**FIG. 18C****FIG. 18D**

**FIG. 19A****FIG. 19B**

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**FIG. 20A****FIG. 20B****FIG. 20C**

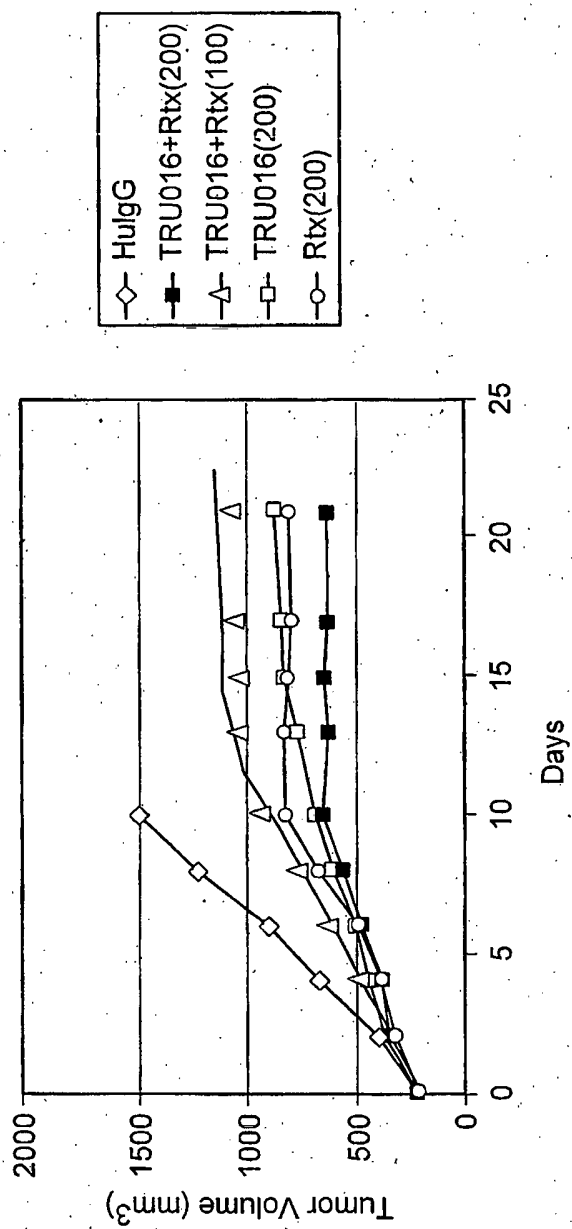


FIG. 21

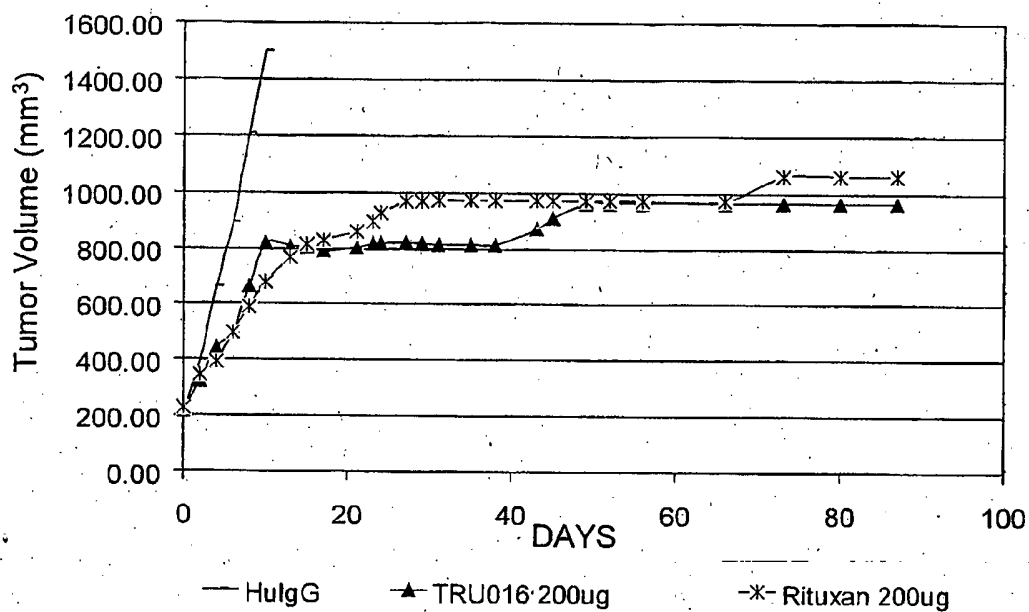
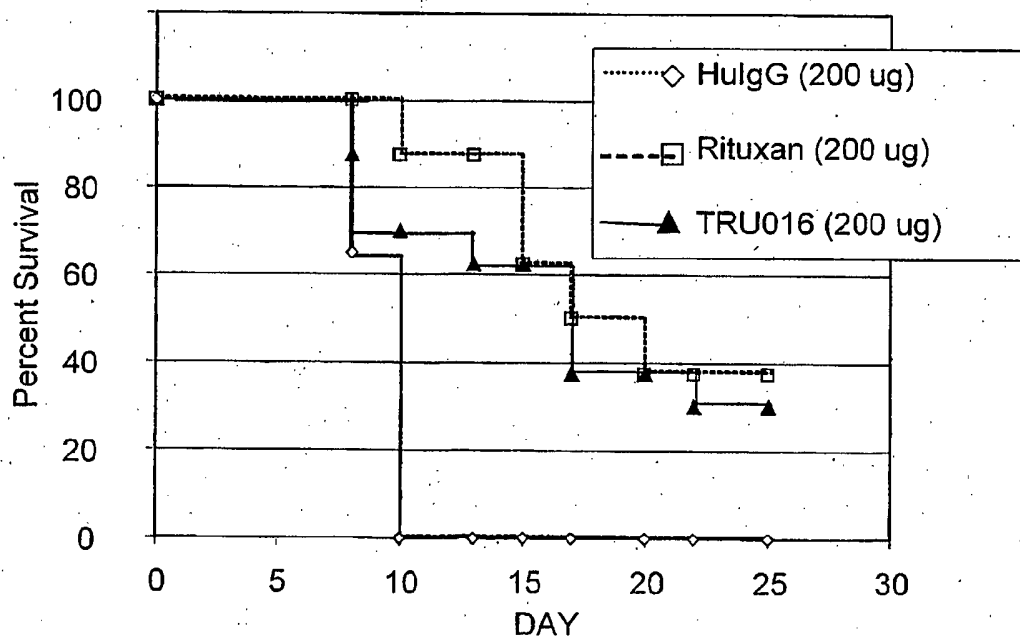
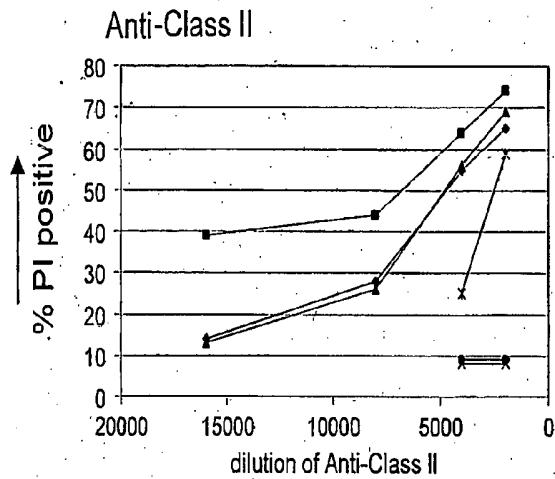
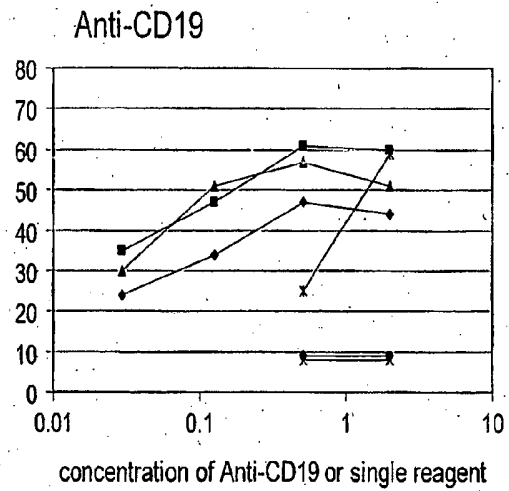
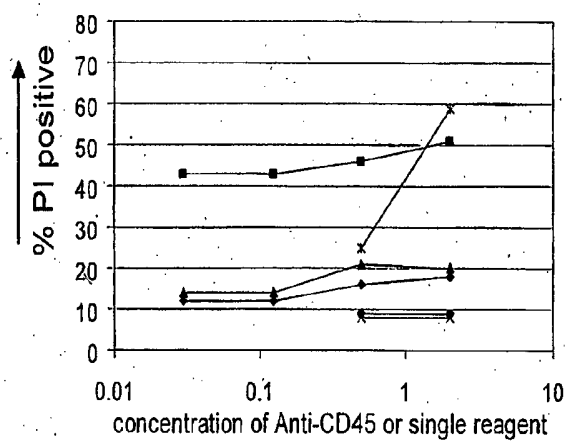
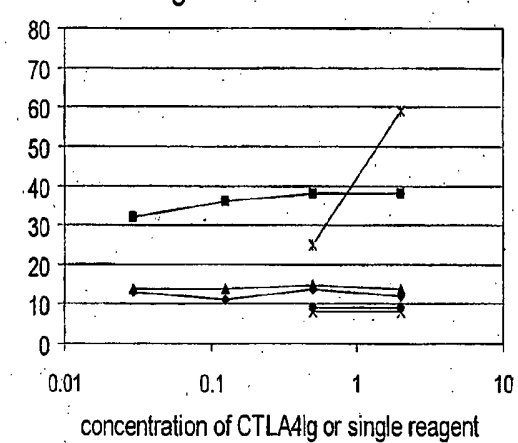
**FIG. 22A****FIG. 22B**

FIG. 23A**FIG. 23B****C. Anti-CD45****D. CTLA4Ig****FIG. 23C**

- ◆— A, B, C, or D + Media
- A, B, C, or D + Rituxan (0.05 mg/ml)
- ▲— A, B, C, or D + TRU016 (2 µg/ml)
- X— Media alone
- *— Rituxan alone (2 and 0.5 µg/ml)
- *— TRU016 alone (2 and 0.5 µg/ml)

FIG. 23D

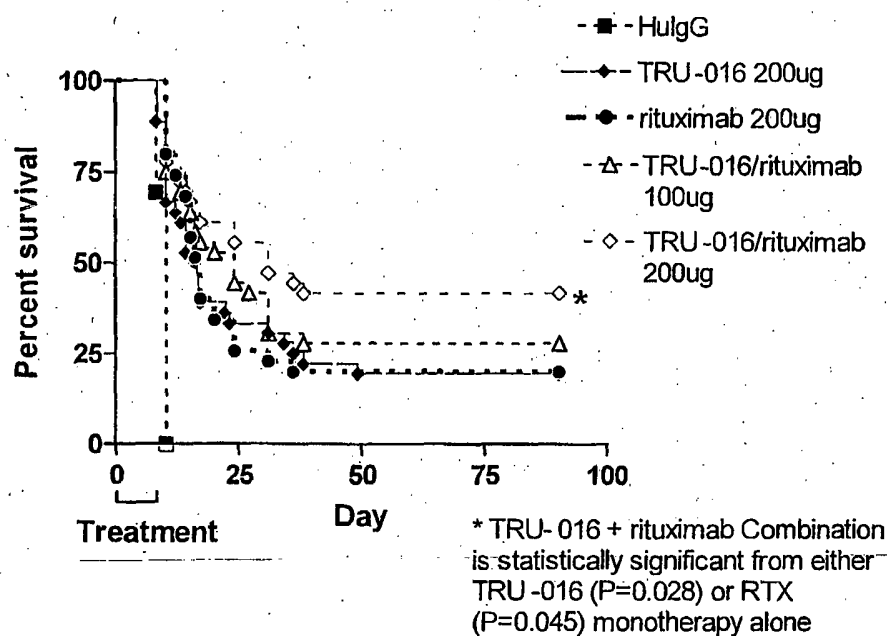


FIG. 24

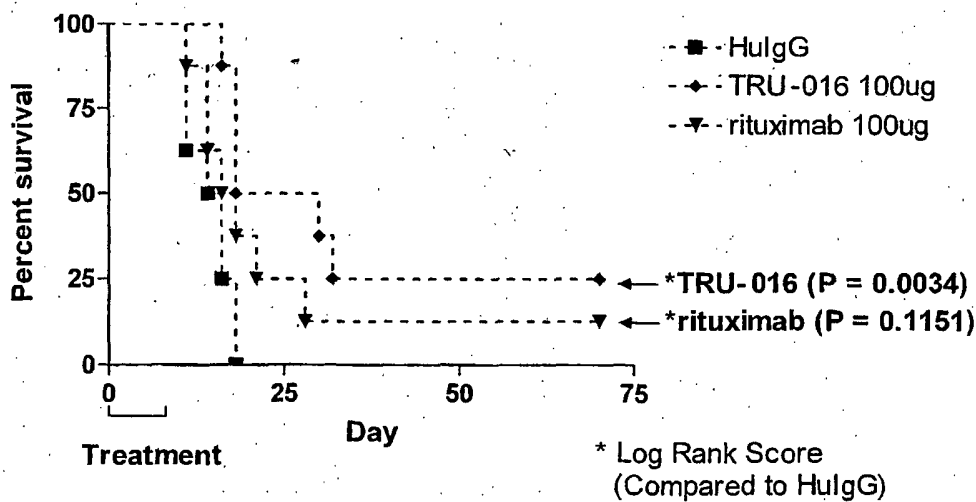
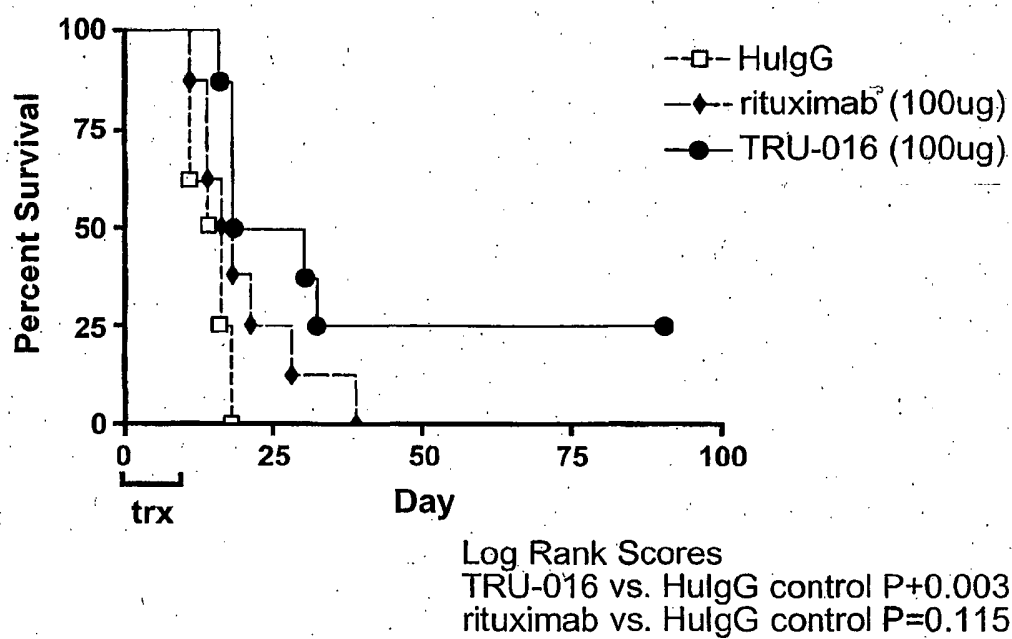
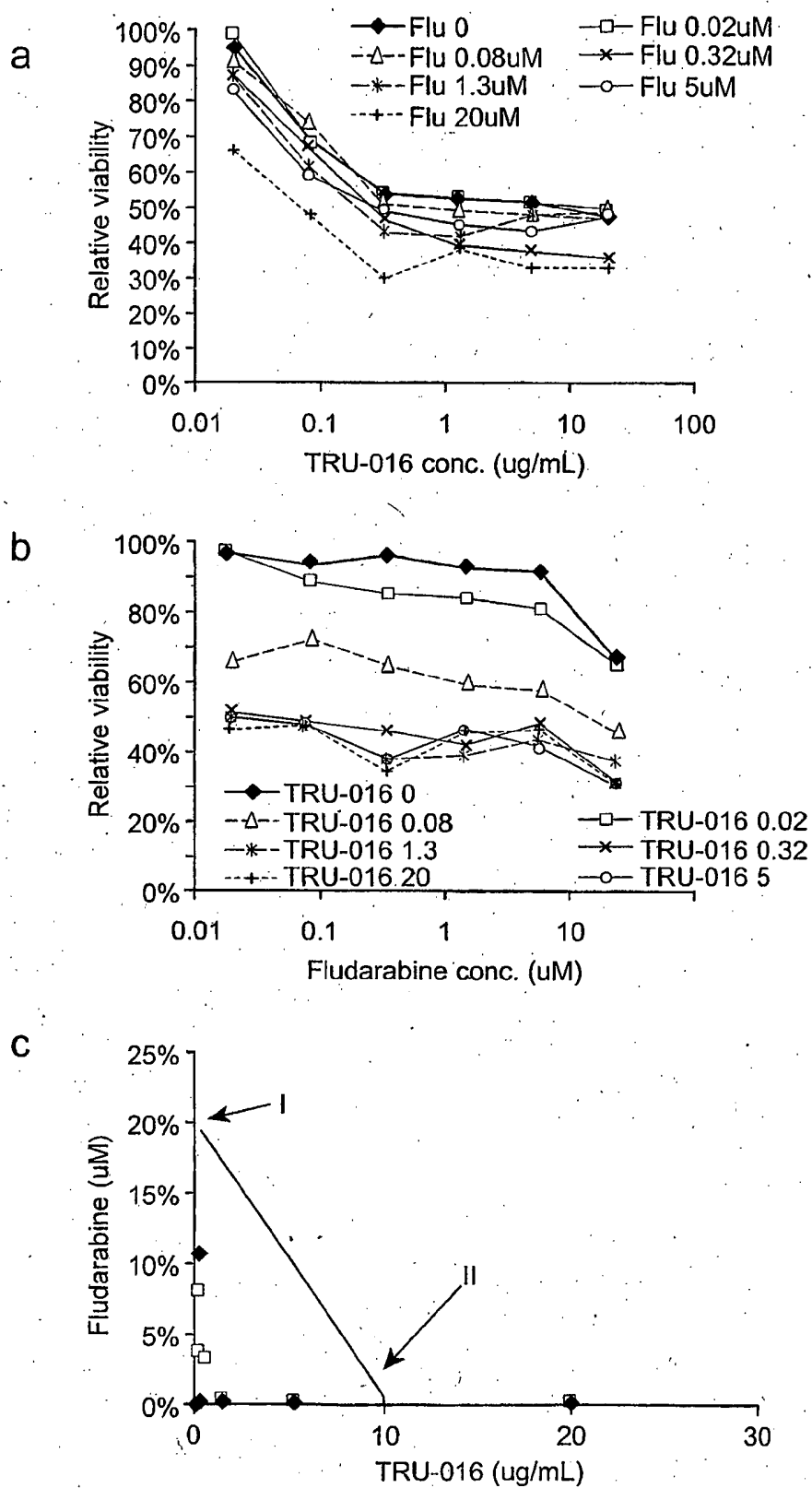
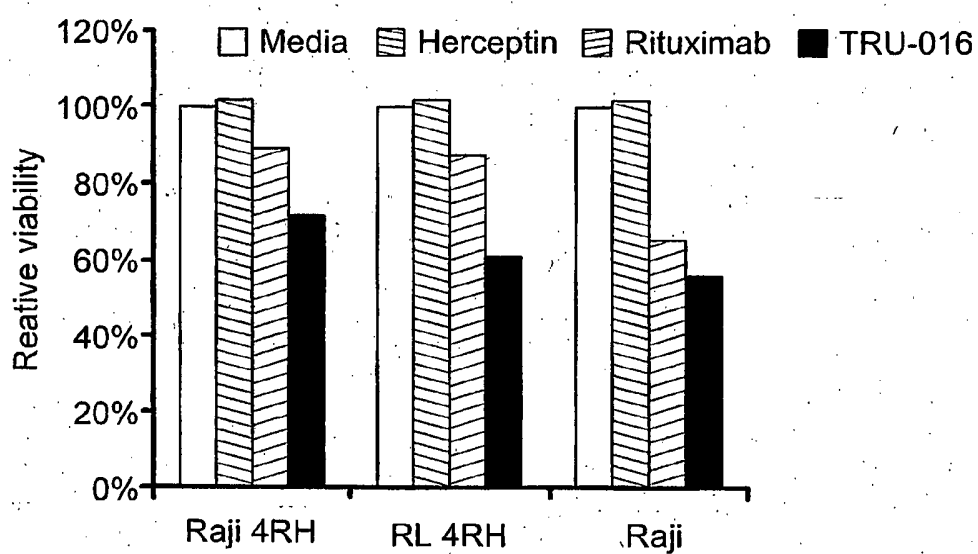


FIG. 25

**FIG. 26**

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**FIG. 27**

**FIG. 28**

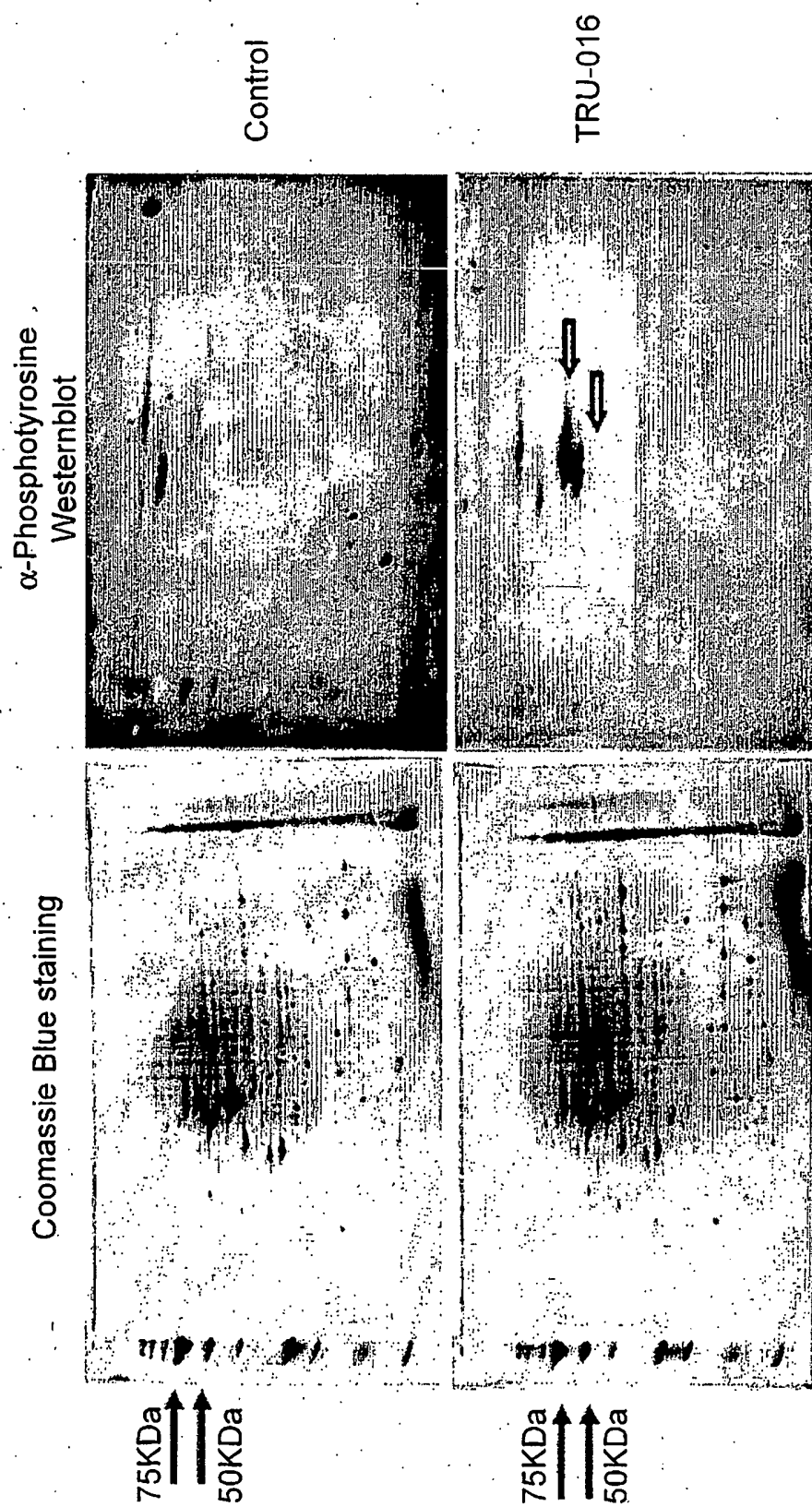


FIG. 29

		L-FR1		L-CDR1		L-FR2	
	1						49
016_-_G28-1	DIQMTQSPASLSASVGETVTITC			RTSENVYSYLA		WYQQKQKGKSPQLLV	
H016_-_019001	EIVLTQSPATLSLSPGERATLSC			RTSENVYSYLA		WYQQKPGQAPRLTIY	
Consensus	I	TQSPA	LS	S	GE	T C	RTSENVYSYLA WYQQK G P LL
		L-CDR2		L-FR3		L-CDR3	
	50						97
016_-_G28-1	FAKTLAE	GVPSRFS	SGSGSGTQFSLKISSLPEDSGSYFC			QHHSNPNWT	
H016_-_019001	FAKTLAE	GIPARFS	SGSGSGTDFLTISSLEPEDFAVYYC			QHHSNPNWT	
Consensus	FAKTLAE	G P	RFSGSGSGT F L	ISSL	PED	Y C	QHHSNPNWT
		L-FR4	(G4S)3 linker			H-FR1	
	98						30
016_-_G28-1	FGGGTELEIK	GGGGSGGGSGGGGSS		AVQLQQSGPESEKPGASVKISCKASGYSFT			
H016_-_019001	FGQGTKVEIK	GGGGSGGGSGGGGTG		EVQLVQSGAEVKKPGESLKISCKSGYSFT			
Consensus	FG GT	EIK	GGGGSGGGSGGGG	VQL	QSG E	KPG S	KISCK SGYSFT
		H-CDR1		H-FR2		H-CDR2	
	31						76
016_-_G28-1	GYNMN	WVKQNGKSL	EWIG	NIDPYYGGTTYNRKFKG		KATLTVDKSSS	
H016_-_019001	GYNMN	WVRQMPGKGL	EWIG	NIDPYYGGTTYNRKFKG		QVTISADKSSS	
Consensus	GYNMN	WV Q	GK LEW G	NIDPYYGGTTYNRKFKG		T DKS S	
		H-FR3		H-CDR3		H-FR4	
	77						113
016_-_G28-1	TAYMLKSLTSEDS	SAVYYCAR	SVGPM DY	WGQGT	SVTVSS		
H016_-_019001	TAYLQWSSLKASD	TAMYYCAR	SVGPM DY	WGRGT	LVTVSS		
Consensus	TAY Q	SL	D A YYCAR	SVGPM DY	WG GT	VTVSS	

FIG. 30A

	1	50
H016_-_019001	EIVLTQSPATLSLSPGERATLSCRTSENVVSYLAWYQQKPGQAPRLIYF	
H016_-_019008	EIVLTQSPATLSLSPGERATLSCRTSENVVSYLAWYQQKPGQAPRLIYF	
H016_-_019009	EIVLTQSPATLSLSPGERATLSCRTSENVVSYLAWYQQKPGQAPRLIYF	
	51	100
H016_-_019001	AKTLAEGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHHSNPNWTFGQ	
H016_-_019008	AKTLAEGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHHSNPNWTFGQ	
H016_-_019009	AKTLAEGIPARFSGSGSGTDFTLTISSELEPEDFAVYYCQHHSNPNWTFGQ	
	101	150
H016_-_019001	GTKVEIKGGGSGGGGSGGGGTGEVQLVQSGAEVKKPGESLKISCKGSGY	
H016_-_019008	GTKVEIKGGGSGGGGSGGGGASEVQLVQSGAESKKPGESLKISCKGSGY	
H016_-_019009	GTKVEIKGGGSGGGGSGGGGASEVQLVQSGAESKKPGESLRISCKGSGY	
	151	200
H016_-_019001	SFTGYNMNWRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQVTISADKSI	
H016_-_019008	SFTGYNMNWRQMPGKGLEWMGNIDPYYGGTTYNRKFKGQVTISADKSI	
H016_-_019009	SFTGYNMNWRQMPGKGLEWMGNIDPYYGGTTYNRKFKGHVTISADKSI	
	201	239
H016_-_019001	TAYLQWSSLKASDTAMYYCARSVGPMQDYWGRGTLVTVSS	
H016_-_019008	TAYLQWSSLKASDTAMYYCARSVGPMQDYWGRGTLVTVSS	
H016_-_019009	TAYLQWSSLKASDTAMYYCARSVGPMQDYWGRGTLVTVSS	

FIG. 30B

Amino Acid Sequence

[illegible]

ellgpgsvflfpkpkdlmistrpevtcvwdvshedpevkfnwyvdgvevhnaktipreeqynstyrvsvlilhqdwlngkeyckvsnkalpapietkiskakgqprepqvyltpsrdeitknqvsitclvkgfy

rpdsiaivewesngapennyktpvp/dsdgdflyskltvdksnwqggnvfscsvmhealthhyqksispgk
rpdsiaivewesngapennyktpvp/dsdgdflyskltvdksnwqggnvfscsvmhealthhyqksispgk*
rpdsiaivewesngapennyktpvp/dsdgdflyskltvdksnwqggnvfscsvmhealthhyqksispgk*

FIG. 31

FIG. 32A

```

LIGHT CHAIN .....FR1..... CDR1 .....FR2.....
019041 EIVLTQSPATLSLSPGERATLSC RASENVYSYLA WYQQKPGQAPRLLIY
019044 EIVLTQSPATLSLSPGERATLSC RASENVYSYLA WYQQKPGQAPRLLIY
019001 EIVLTQSPATLSLSPGERATLSC RTSENVYSYLA WYQQKPGQAPRLLIY

      CDR2 .....FR3..... CDR3 .....FR4...
019041 FAKTLAE GIPARFSGSGSCTDFTLTISSELEPEDFAVYYC QHHSNPNWT FGQGTKVEIK
019044 FAKTLAE GIPARFSGSGSCTDFTLTISSELEPEDFAVYYC QHHSNPNWT FGQGTKVEIK
019001 FAKTLAE GIPARFSGSGSCTDFTLTISSELEPEDFAVYYC QHHSNPNWT FGQGTKVEIK

HEAVY CHAIN .....FR1..... CDR1 .....FR2.....
019041 EVQLVQSGAEVKKPGESLKISCKGSGYSFT GYNMN WVRQMPGKGLEWMG
019044 EVQLVQSGAEVKKPGESLKISCKGSGYSFT GYNMN WVRQMPGKGLEWMG
019001 EVQLVQSGAEVKKPGESLKISCKGSGYSFT GYNMN WVRQMPGKGLEWMG

      CDR2 .....FR3.....
019041 NIDPYYGGTTYNRKFKG QVTISADKSISTAYLQWSSLKASDTAMYYCAR
019044 NIDPYYGGTTYNRKFKG QVTISADKSISTAYLQWSSLKASDTAMYYCAR
019001 NIDPYYGGTTYNRKFKG QVTISADKSISTAYLQWSSLKASDTAMYYCAR

      CDR3 .....FR4...
019041 SVGPFDY WGQGTLLVTVSS
019044 SVGPFDS WGQGTLLVTVSS
019001 SVGPM DY WGRGTLLVTVSS

```

CLUSTAL W (1.83) multiple sequence alignment

```

019041 -----ATGGAAGCCCCAGCGCAGCTTCTCTTCTCTGCTACTCTGGCTCCCA 48
019044 -----ATGGAAGCCCCAGCGCAGCTTCTCTTCTCTGCTACTCTGGCTCCCA 48
019001 AAGCTTGCGCCCATGGAAGCCCCAGCTCAGCTTCTCTTCTCTGCTACTCTGGCTCCCA 60

```



FIG. 32B



019041 GATACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGC 108
019044 GATACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGC 108
019001 GATACCACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGC 120

019041 GAAAGAGCCACCCTCTCCTGCCGAGCAAGTGAAAATGTTTACAGCTACTTAGCCTGGTAC 168
019044 GAAAGAGCCACCCTCTCCTGCCGAGCAAGTGAAAATGTTTACAGCTACTTAGCCTGGTAC 168
019001 GAAAGAGCCACCCTCTCCTGCCGAGCAAGTGAAAATGTTTACAGCTACTTAGCCTGGTAC 180

019041 CAACAGAAACCTGGCCAGGCTCCTAGGCTCCTCATCTATTTTGCAAAAACCTTAGCAGAA 228
019044 CAACAGAAACCTGGCCAGGCTCCTAGGCTCCTCATCTATTTTGCAAAAACCTTAGCAGAA 228
019001 CAACAGAAACCTGGCCAGGCTCCTAGGCTCCTCATCTATTTTGCAAAAACCTTAGCAGAA 240

019041 GGAATTCAGCCAGGTTTCACTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGC 288
019044 GGAATTCAGCCAGGTTTCACTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGC 288
019001 GGAATTCAGCCAGGTTTCACTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGC 300

019041 AGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAACATCATTCGGATAATCCGTGG 348
019044 AGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAACATCATTCGGATAATCCGTGG 348
019001 AGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCAACATCATTCGGATAATCCGTGG 360

019041 ACATTCCGCCAAGGGACCAAGGTGGAATCAAAGGTGGCGGCGGCTCGGGCGGTGGTGA 408
019044 ACATTCCGCCAAGGGACCAAGGTGGAATCAAAGGTGGCGGCGGCTCGGGCGGTGGTGA 408
019001 ACATTCCGCCAAGGGACCAAGGTGGAATCAAAGGTGGCGGCGGCTCGGGCGGTGGTGA 420

019041 TCTGGAGGAGGTGGGACCGGTGAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAG 468
019044 TCTGGAGGAGGTGGGACCGGTGAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAG 468
019001 TCTGGAGGAGGTGGGACCGGTGAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAAAAG 480

019041 CCCGGAGAGTCTCTGAAGATTTCTGTAAAGGGATCCGGTTACTCATTCAGTGGCTACAAT 528
019044 CCCGGAGAGTCTCTGAAGATTTCTGTAAAGGGATCCGGTTACTCATTCAGTGGCTACAAT 528
019001 CCCGGAGAGTCTCTGAAGATTTCTGTAAAGGGATCCGGTTACTCATTCAGTGGCTACAAT 540

019041 ATGAAC TGGGTGCGCCAGATGCCCGGGAAGGCCCTCGAGTGGATGGGCAATATTGATCCT 588
019044 ATGAAC TGGGTGCGCCAGATGCCCGGGAAGGCCCTCGAGTGGATGGGCAATATTGATCCT 588
019001 ATGAAC TGGGTGCGCCAGATGCCCGGGAAGGCCCTCGAGTGGATGGGCAATATTGATCCT 600

019041 TATTATGGTGGTACTACCTACAACCGGAAGTTCAAGGGCCAGGTCACTATCTCCGCCGAC 648



FIG. 32C



019044 TATTATGGTGGTACTACCTACAACCGGAAGTCAAGGGCCAGGTCACTATCTCCGCCGAC 648
019001 TATTATGGTGGTACTACCTACAACCGGAAGTCAAGGGCCAGGTCACTATCTCCGCCGAC 660

019041 AAGTCCATCAGCACCGCCTACCTGCAATGGAGCAGCCTGAAGGCCTCGGACACCGCCATG 708
019044 AAGTCCATCAGCACCGCCTACCTGCAATGGAGCAGCCTGAAGGCCTCGGACACCGCCATG 708
019001 AAGTCCATCAGCACCGCCTACCTGCAATGGAGCAGCCTGAAGGCCTCGGACACCGCCATG 720

019041 TATTACTGTGCACGCTCAGTCGGCCCTTTTCGACTACTGGGGCCAGGGCACCCCTGGTCACT 768
019044 TATTACTGTGCACGCTCAGTCGGCCCTTTTCGACTACTGGGGCCAGGGCACCCCTGGTCACT 768
019001 TATTACTGTGCACGCTCAGTCGGCCCTATGGACTACTGGGGCCAGGGCACCCCTGGTCACT 780

019041 GTCTCCTCTGATCAGGAGCCCCAAATCTTCTGACAAAACCTCACACATCTCCACCGTGCCCA 828
019044 GTCTCCTCTGATCAGGAGCCCCAAATCTTCTGACAAAACCTCACACATCTCCACCGTGCCCA 828
019001 GTCTCCTCTGATCAGGAGCCCCAAATCTTCTGACAAAACCTCACACATCTCCACCGTGCCCA 840

019041 GCACCTGAATCCTGGGTGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAGGACACC 888
019044 GCACCTGAATCCTGGGTGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAGGACACC 888
019001 GCACCTGAATCCTGGGTGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAGGACACC 900

019041 CTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGAC 948
019044 CTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGAC 948
019001 CTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGACGTGAGCCACGAAGAC 960

019041 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG 1008
019044 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG 1008
019001 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAG 1020

019041 CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTCTGCAC 1068
019044 CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCTCACCGTCTCTGCAC 1068
019001 CCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCTCACCGTCTCTGCAC 1080

019041 CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC 1128
019044 CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC 1128
019001 CAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCC 1140

019041 CCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC 1188
019044 CCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC 1188
019001 CCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACC 1200





019041 CTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCCTGGTCAAA 1248
019044 CTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCCTGGTCAAA 1248
019001 CTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCCTGGTCAAA 1260

019041 GGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC 1308
019044 GGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC 1308
019001 GGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC 1320

019041 TACAAGACCACGCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTC 1368
019044 TACAAGACCACGCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTC 1368
019001 TACAAGACCACGCCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTC 1380

019041 ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG 1428
019044 ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG 1428
019001 ACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAG 1440

019041 GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA----- 1482
019044 GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA----- 1482
019001 GCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGATCTAGA 1500

FIG. 32D