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METHODS AND COMPOSITIONS FOR USE IN TREATMENT OF PATIENTS WITH AUTOANTIBODY POSITIVE DISEASE

Background of the Invention

[0001] Neutrokine-alpha protein (SEQ ID NO:2) is a member of the TNF family of ligands that shares amino acid sequence identity to APRIL (28.7%, SEQ ID NO:4), TNFα (16.2%), and lymphotoxin-α (LTα) (14.1%) (Moore, *et al.*, (1999) Science 285:260-263). Neutrokine-alpha is known in the scientific and patent literature under many names, including B lymphocyte Stimulator (BLyS), B cell activating factor (BAFF), TNF- and ApoL-related leukocyte expressed ligand -1 (TALL-1). (Moore, *et al.*, (1999) *Science* 285:260-263; Schneider *et al.*, (1999) *J. Exp. Med.* 189:1747-1756; and Khare *et al.*, (2000) *Proc. Natl. Acad. Sci.* 97:3370-3375). The official nomenclature for Neutrokine-alpha is Tumor Necrosis Factor (ligand) Super Family member 13B (TNFSF13b). The full length Neutrokine-alpha gene encodes a 285 amino acid polypeptide that has a transmembrane spanning domain between amino acids 47 and 73 preceded by a non-hydrophobic sequence characteristic of type II membrane bound proteins. Like other members of the TNF family, Neutrokine-alpha functions as a trimeric protein. Upon expression of Neutrokine-alpha at the surface of the cell, the extracellular domain is cleaved off at amino acid 134 to release a biologically active trimer.

[0002] Neutrokine-alpha is known to bind to three different receptors from the Tumor Necrosis Factor Receptor Super Family. These are transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790, SEQ ID NO:6), B cell activating factor receptor, B-cell maturation antigen (BCMA, GenBank accession number NP_001183 SEQ ID NO:8) and (BAFF-R, GenBank Accession Number NP_443177 SEQ ID NO:10). (Gross, *et al.*, (2000) *Nature* 404:995-999; Thompson *et al.*, (2001) *Science* 293:2108-2111; and Yan *et al.*, (2000) *Nature Immunol.* 1:252-256) Expression of the receptors is largely restricted to B lymphocytes (Moore, *et al.*, (1999) *Science* 285:260-263). The bulk of Neutrokine-alpha's effects are believed to be mediated by BAFF-R because of marked defects in the B cell compartments of mice deficient in Neutrokine-alpha expression or BAFF-R expression that are not apparent in TACI or BCMA deficient mice. (Schieman, *et al.*, (2001) *Science* 292:2111-2114; Gross *et al.*, (2001) *Immunity* 15:289-302; and Yan *et al.*, (2000) *Nature Immunol.* 1:252-256).

[0003] When Neutrokine-alpha protein was assayed in *in vitro* and *in vivo*, it was shown that Neutrokine-alpha promotes B cell proliferation, differentiation and survival. Additionally, Neutrokine-alpha was shown to have some effect on T cells as well. (MacKay *et al.*, (1999) *J. Exp. Med.* 190:1697-1710; Huard *et al.*, (2001) *J. Immunol.* 167:6225-6231; Huard *et al.*, (2004) *Int. Immunol.* 16:467-475; Ng et al., (2004) *J. Immunol.* 173:807-817). Mice that were engineered to transgenically overexpress Neutrokine-alpha had increased numbers of peripheral B cells and increased serum immunoglobulin concentrations. Additionally, Neutrokine-alpha transgenic mice presented with an autoimmune phenotype akin to that seen in human systemic lupus erythematosus including the development of autoantibodies and symptoms associated with glomerulonephritis. (Moore, *et al.*, (1999) *Science* 285:260-263; MacKay, *et al.*,

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(1999) *J. Exp. Med.* 192:129-135). Later studies showed that levels of Neutrokine-alpha in serum and/or synovial fluid were also upregulated in patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and Sjogren's Syndrome. (Cheema *et al.*, (2001) *Arthritis Rheum.* 44:1313-1319; Groom *et al.*, (2002) *J. Clin. Invest.* 109:59-68; Mariette *et al.*, (2003) *Ann. Rheum. Dis* 62:168-171). Accordingly, there is widespread belief in the scientific community that antagonists of Neutrokine-alpha have therapeutic potential in the treatment of autoimmune diseases.

[0004] Systemic lupus erythematosus (SLE or "lupus") is an autoimmune disease whose symptoms are extremely heterogeneous. The current standard for diagnosing a patient with SLE contains 11 criteria: (1) malar "butterfly" rash, (2) discoid rash, (3) photosensitivity, (4) oral ulcers, (5) arthritis, (6) serositis, (7) renal disorder, (8) neurologic disorder, (9) hematologic disorder, (10) immunologic disorder, and (11) presence of anti-nuclear antibody. These criteria are explained in more detail in Tan *et al.*, (1982) *Arthritis Rheum.* 25:1271-1277; and Hochberg *et al., Arthritis Rheum.* (1997) 40:1725, which are hereby incorporated by reference in their entirety. A person that has any 4 of these eleven criteria can be diagnosed with SLE. Accordingly, individuals having a clinical diagnosis of SLE may have non-overlapping symptoms. Moreover, many of the symptoms of lupus overlap with symptoms in other diseases. For instance, rheumatoid arthritis, polymyositis-dermatomyositis, systemic sclerosis (or scleroderma), Sjogren's syndrome and various forms of vasculitis share symptoms with lupus including one or more of the following characteristics, the presence of autoantibodies, including anti-nuclear antibodies and anti-dsDNA antibodies, joint pain and swelling and skin rashes, and organ involvement. Thus, in practice, it is often difficult to correctly diagnose lupus patients and patients with other similar disease. Additional factors that lead to difficulty in diagnosing lupus disease include the fact that the disease does not develop rapidly; rather, patients gradually accumulate symptoms over time. Additionally, SLE is a disease with variable activity within a patient. Sometimes the disease is quiescent, while at other times patients experience an increase in the number and/or severity of their symptoms, in a "flare" episode. Finally, there is no one laboratory test that will definitively diagnose lupus. Accordingly, there is a need in the art to be able to define subsets of lupus patients with particular symptoms and to make correlations between those subsets of patients and treatments that are more likely to benefit patients in those subsets.

[0005] The present application identifies particular subgroups of patients with autoimmune disease that are more likely to benefit from treatment with immunomodulatory agents.

Summary of the Invention

[0006] In a phase 2 clinical trial, it was found that treatment of lupus patients with an antibody that neutralizes Neutrokine-alpha protein, given as an IV infusion on days 0, 14, 28 and then every four weeks until week 52, significantly ameliorated symptoms associated with lupus in the subset of patients having an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum (see Example 1). Surprisingly, statistically significant improvements in clinical endpoints measuring disease activity (such as reduction in SELENA SLEDAI score, explained in more detail below) were only obtained in a subset of the patients, as opposed to the entire patient population

enrolled in the clinical trial. Thus, the present invention relates to the identification of subgroups of patients that are more likely to respond to treatment with an immunomodulatory agent such as an antagonist of Neutrokine-alpha.

[0007] Accordingly, in one embodiment, the present invention provides a method of treating a patient that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. Immunomodulatory agents are described in more detail below. In a specific embodiment, the immunomodulatory agent is an antagonist of Neutrokine-alpha, including but not limited to an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein or fragment or variant thereof, an antibody that binds a Neutrokine-alpha receptor or antigen binding fragment thereof, a Neutrokine-alpha binding peptide or polypeptide, a Neutrokine-alpha and/or APRIL polypeptide variant (e.g., a dominant negative form of Neutrokine-alpha and/or APRIL). Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, antisense RNAs and short interfering RNAs (siRNAs) that target Neutrokine-alpha, antisense RNAs and short interfering RNAs (siRNAs) that target APRIL, antisense RNAs and short interfering RNAs (siRNAs) that target receptors for Neutrokine-alpha and/or receptors for APRIL. Neutrokine-alpha receptors include, e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790, SEQ ID NO:6), B cell activating factor receptor, B-cell maturation antigen (BCMA, GenBank accession number NP_001183 SEQ ID NO:8) and (BAFF-R, GenBank Accession Number NP_443177 SEQ ID NO:10).

[0008] In another embodiment, the present invention provides a method of treating a patient with an autoimmune disease that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. Examples of autoimmune disease in which one may identify patients with an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum include, but are not limited to, systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome, scleroderma, polymyositis-dermatomyositis, Felty's syndrome, mixed connective tissue disease, Raynaud's syndrome, juvenile chronic arthritis, glomerulonephritis, idiopathic thrombocytopenia purpura and IgA nephropathy.

[0009] In a specific embodiment, the invention provides a method of treating a patient with Sjögren's Syndrome that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of treating a patient with Sjögren's Syndrome that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha.

[0010] In a specific embodiment, the invention provides a method of treating a patient with rheumatoid arthritis that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-

dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of treating a patient with rheumatoid arthritis that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha.

[0011] In a specific embodiment, the invention provides a method of treating a patient with systemic lupus erythematosus (SLE) that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of treating a patient with systemic lupus erythematosus (SLE) that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha. In a specific embodiment, the lupus patient will have a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria (See, for example, Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997), which are hereby incorporated by reference in their entirety).

[0012] The present invention also provides a method of treating a patient comprising making a determination, prior to administration of an immunomodulatory agent, that the patient has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum. The present invention also provides a method of treating a patient comprising making a determination, prior to administration of an antagonist of Neutrokine-alpha, that the patient has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

[0013] In other embodiments, the invention provides a method of treating a lupus patient comprising making a determination, prior to administration of an immunomodulatory agent, that the lupus patient has one or more of the following characteristics: a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria (see, for example, Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997)); a SELENA SLEDAI score $\geq$ 6; depressed C4 complement levels in his/her blood plasma or serum; depressed C3 complement levels in his/her blood plasma or serum; an ANA titer of 1:80 or greater; greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum; is receiving $\geq$ 7.5 milligrams/day of prednisone or other corticosteroid for treatment of lupus-related symptoms; and/or is receiving or had previously received immunosuppressant therapy for treatment of lupus-related symptoms. In a specific embodiment, the determination is made by a medical practitioner on the basis of an evaluation of the patient's medical record. In another specific embodiment, the determination is made by a medical practitioner on the basis of laboratory test results. In a specific embodiment the determination is made by a medical practitioner on the basis of laboratory test results obtained since the patient's last medical treatment (including medical treatments with immunomodulatory agents) for lupus, if any, and prior to commencing medical treatment

comprising administering a therapeutically effective amount of an immunomodulatory agent (including an antagonist of Neutrokine-alpha) as described herein.

[0014] In another embodiment, the present invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent.

[0015] In a specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with Sjögren's Syndrome that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with Sjögren's Syndrome that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha.

[0016] In a specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with rheumatoid arthritis that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with rheumatoid arthritis that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha.

[0017] In a specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with systemic lupus erythematosus (SLE) that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an immunomodulatory agent. In another specific embodiment, the invention provides a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with systemic lupus erythematosus (SLE) that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha. In a specific embodiment, the lupus patient will have a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria (See, for example, Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997), which are hereby incorporated by reference in their entirety).

[0018] In a further embodiment, the invention provides a method of reducing the quantity of corticosteroid administered to a patient that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum is reduced by at least 25% to $\leq$ 7.5 milligrams/day. In a specific embodiment, the corticosteroid is selected from the group consisting of

prednisone, prednisolone, hydrocortisone, methylprednisolone and dexamethasone. In a further specific embodiment, the corticosteroid is prednisone. In another embodiment, a method of reducing the frequency and/or quantity of corticosteroid administered to a patient with an autoimmune disease comprising administering a therapeutically effective amount of an anti-Neutrokine-alpha antibody is provided.

[0019] In another phase 2 clinical trial (Example 3) in which rheumatoid arthritis patients received treatment with an antibody that neutralizes Neutrokine-alpha protein, given as an IV infusion on days 0, 14, 28 and then every four weeks until week 24, treatment was more likely to ameliorate symptoms associated with rheumatoid arthritis in patients that had a DAS28 score greater than 5.1, patients that had not previously received anti-TNF therapy, and/or patients that had rheumatoid factor in his/her blood plasma and/or serum prior to commencing treatment with the antibody that neutralizes Neutrokine-alpha protein. Additional subgroups or rheumatoid arthritis patients that appeared to be more likely to respond to treatment with the antibody that neutralizes Neutrokine-alpha protein included male patients, patients that had anti-CCP (cyclic citrullinated peptide) antibodies in his/her blood plasma and/or serum, patients that received methotrexate concomitantly with the antibody that neutralizes Neutrokine-alpha protein, patients that had previously failed treatment with methotrexate, and/or patients that had previously failed methotrexate therapy and at least one other DMARD therapy.

[0020] In another embodiment, the invention provides a method of determining if a lupus patient is responding to medical treatment comprising determining the patient's SELENA SLEDAI, BILAG and PGA score prior to administration of a medical treatment; administering the medical treatment; and determining the patient's SELENA SLEDAI, BILAG and PGA score following the administration of the medical treatment. In this method, the patient will be considered as having responded to medical treatment if: the patient's SELENA SLEDAI score determined following the administration of the medical treatment is 4 or more points less than the SELENA SLEDAI score prior to the administration of the medical treatment; the patient's BILAG index score determined following the administration of the medical treatment does not include a new BILAG A organ domain score or 2 new BILAG B organ domain scores compared to the BILAG score determined prior to the administration of the medical treatment, and the PGA score determined following the administration of the medical treatment is < 0.3 point higher than the PGA score determined prior to the administration of the medical treatment.

[0021] Accordingly, in one embodiment, the invention provides a method of treating a rheumatoid arthritis patient comprising making a determination, prior to administration of an immunomodulatory agent, that the rheumatoid arthritis patient has one or more of the following characteristics: the patient has not previously received anti-TNF therapy, e.g., Infliximab (also known as Remicade™ Centocor, Inc.), adalimumab (Humira® from Abbott Laboratories) or etanercept (Enbrel®); the patient has rheumatoid factor in his/her blood plasma and/or serum; the patient has measurable anti-CCP (cyclic citrullinated peptide) antibodies in his/her blood plasma and/or serum; the patient has an elevated CRP (C reactive protein) level in his/her blood plasma and/or serum; the patient previously failed treatment with one or more disease-modifying antirheumatic drugs; that patient has a high modified disease activity score (DAS28); the

patient has swollen and tender joints; the patient suffers from morning stiffness; the patient has an increased erythrocyte sedimentation rate (ESR) and/or the patient is male.

[0022] In another embodiment, the invention provides an aqueous pharmaceutical formulation comprising a therapeutically effective amount of an antibody, a buffer in an amount from about 5 mM to about 50 mM, NaCl in an amount from about 150 mM to about 500 mM, a surfactant in an amount from about 0.003% to about 0.05%, with a pH from about 5.5 to about 6.5. In a specific embodiment, the antibody in the above described formulation is an antibody having an IgG1/lambda isotype. In a further embodiment, the antibody in the above-described formulation is a human antibody having an IgG1/lambda isotype, the buffer in the above-described formulation is 10 mM histidine or sodium citrate, the surfactant in the above-described formulation is polysorbate 80 in an amount of 0.01% w/v, the NaCl in the above-described formulation is present in a concentration of about 150mM and the formulation has a pH of 6.0. In other specific embodiments, the above-described formulations are stable at a temperature of about 2-8° C for at least one year. In another embodiment, the antibody in the above-described formulation is present in an amount of 100 mg/ml.

[0023] In a specific embodiment, the invention provides an aqueous pharmaceutical formulation comprising 100 mg/ml IgG1/λ antibody, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl and 0.1 mg/ml polysorbate 80 and wherein the formulation has a pH of 6.0.

Brief Description of the Figures

[0024] The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

[0025] Figure 1 shows the mean percent decrease in SELENA SLEDAI at week 52 in patients that had an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies at baseline. The P-values were determined using a t-test.

Definitions

[0026] In one aspect, the present invention is directed broadly to methods of treating a patient with autoantibody positive disease by administering a therapeutically effective amount of an immunomodulatory agent. A patient with autoantibody positive disease is a patient that has detectable autoantibody titers in one or more biological fluid samples such as blood plasma or serum or synovial fluid.

[0027] Herein, reference to an "immunomodulatory agent" is a reference to the general class of pharmaceutical compounds that can stimulate or inhibit the immune system. The working examples herein describe the successful use of an antagonistic anti-Neutrokine-alpha antibody in the treatment of a subgroup of lupus patients. Thus, the term "immunomodulatory agent" is specifically intended to cover pharmaceutical compounds or molecules that can act as an antagonist of Neutrokine-alpha. Antagonists of Neutrokine-alpha include, but are not limited to, compositions comprising an anti-Neutrokine-alpha antibody or antigen-binding fragments thereof, Neutrokine-alpha receptor proteins or fragments or variants thereof, an antibody that binds a Neutrokine-alpha receptor or antigen binding fragment thereof and

Neutrokine-alpha binding peptide or polypeptides. Neutrokine-alpha receptors include, e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790), BAFF-R (GenBank Accession Number NP_443177) and B-cell maturation antigen (BCMA, GenBank accession number NP_001183). Particularly useful forms of the Neutrokine-alpha receptors include soluble forms of the extracellular domains. Neutrokine-alpha-receptors or fragments or variants thereof and Neutrokine-alpha binding polypeptides may be used as fusion proteins, e.g., Fc or human serum albumin (HSA) fusion proteins. Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, Neutrokine-alpha and/or APRIL polypeptide variants (e.g., dominant negative forms of Neutrokine-alpha and or APRIL). Such Neutrokine-alpha and/or APRIL polypeptide variants may antagonize Neutrokine-alpha function, for example, by interfering with Neutrokine-alpha and/or APRIL homo- or hetero-multimerization. Alternatively, Neutrokine-alpha and/or APRIL polypeptide variants will prevent polypeptides comprising them from binding to and/or signaling through Neutrokine-alpha–receptors such as TACI, BCMA and BAFF-R. Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, antisense RNAs and short interfering RNAs (siRNAs) that target Neutrokine-alpha, antisense RNAs and short interfering RNAs (siRNAs) that target APRIL, antisense RNAs and short interfering RNAs (siRNAs) that target receptors for Neutrokine-alpha and/or receptors for APRIL. Antagonists of Neutrokine-alpha are described in more detail below.

[0028] It is believed that the anti-Neutrokine-alpha antibody works by reducing B cell numbers and/or B cell activity, such as immunoglobulin secretion. Thus, the term "immunomodulatory agent" is also specifically intended to cover B cell modulatory agents and in particular pharmaceutical molecules and compounds that directly or indirectly inhibit or reduce B cell activity (e.g., B cell proliferation, differentiation, survival or immunoglobulin secretion) and or B cell number. In a specific embodiment, a B cell modulatory agent that can be used in conjunction with the methods of the present invention is an agent that reduces the activity or number of total B cells, activated B cells, naïve B cells, memory B cells, plasma B cells, and plasmacytoid B cells, CD19+ B cells and/or CD20+ B cells.

[0029] The immune system is a complex network of interacting cells and cytokines. For example, antigen presenting cells (APCs, such as macrophages and dendritic cells) and T cells, specifically CD4+ T helper (Th) cells, play a role in activating B cells to proliferate and secrete antibodies (including autoantibodies in certain disease settings). Thus, it is possible to inhibit B cell activity by reducing or inhibiting APC or Th cell numbers or activity. Likewise, it is known that there are different types of immune response such as Th1 and Th2 responses. Immunomodulatory agents that may be used in the methods of the invention may promote one type of immune response over another and thereby have beneficial effects in the treatment of patients with autoantibody positive disease. Accordingly, in its broadest sense, the term "immunomodulatory agent" is specifically intended to cover pharamceutical molecules or compounds that stimulate or inhibit the activity or quantity of one or more cells, cell surface molecules (*e.g.*, cell surface signaling molecules) and/or cytokines of the immune system, inlcuding cells, cell surface molecules (*e.g.*, cell surface signaling molecules) and cytokines that are part of the innate and/or

adaptive immune system. Cells of the immune system include, but are not limited to B cells, T cells, dendritic cells, monocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, and natural killer (NK) cells. Cell surface molecues on the surface of cells of the immune system that may be stimulated or inhibited by an immunomodulatory agent include, but are not limited to the CD antigens such as CD20. Cytokines important in the immune system include, but are not limited to, members of the TNF ligand superfamily, including but not limited to Neutrokine-alpha, APRIL and CD40L.

Detailed Description

[0030] In a phase II clinical trial in systemic lupus erythematosus patients, applicants found that treatment of lupus patients with an antibody that neutralizes Neutrokine-alpha protein, given as an IV infusion on days 0, 14, 28 and then every four weeks until week 52, significantly ameliorated symptoms associated with lupus in patients having an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum (see Example 1).

[0031] Accordingly, a specific embodiment of the present invention provides a method of treating a patient with systemic lupus erythematosus that has an ANA titer of $\geq$ 1:80 and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum with an antibody antagonist of Neutrokine-alpha. One of skill in the art would readily understand however, that antibody molecules are but one of a variety of molecules that can act as antagonists of Neutrokine-alpha. Thus, another specific embodiment of the present invention provides a method of treating a patient with systemic lupus erythematosus that has an ANA titer of $\geq$ 1:80 and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum with an antagonist of Neutrokine-alpha.

[0032] Antagonists of Neutrokine-alpha include, but are not limited to, compositions comprising an anti-Neutrokine-alpha antibody or antigen-binding fragments thereof, Neutrokine-alpha receptor proteins or fragments or variants thereof, an antibody that binds a Neutrokine-alpha receptor or antigen binding fragment thereof, or Neutrokine-alpha binding peptide or polypeptides. Neutrokine-alpha receptors include, e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790), BAFF-R (GenBank Accession Number NP_443177) and B-cell maturation antigen (BCMA, GenBank accession number NP_001183. Particularly useful forms of the Neutrokine-alpha receptors include soluble forms of the extracellular domains capable of binding Neutrokine-alpha. Neutrokine-alpha-receptors or fragments or variants thereof and Neutrokine-alpha binding polypeptides may be used as fusion proteins, e.g., Fc or human serum albumin (HSA) fusion proteins. In a specific embodiment a Neutrokine-alpha antagonist is a TACI-Fc protein. One example of a TACI-Fc protein is amino acids 1-154 of SEQ ID NO:6 fused to the Fc region of an IgG1 immunoglobulin molecule. In a specific embodiment a Neutrokine-alpha antagonist is a BAFF-R-Fc protein. One example of a BAFF-R-Fc protein is amino acids 1-70 of SEQ ID NO:10 fused to the Fc region of an IgG1 immunoglobulin molecule. Optionally, amino acid 20 (valine) in BAFF-R is substituted with aspargine and amino acid 27 (leucine) in BAFF-R is substituted with proline. SEQ ID NO:26 shows amino acids 1-70 of BAFF-R with these two amino acid changes.

[0033] Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, Neutrokine-alpha and/or APRIL polypeptide variants (e.g., dominant negative forms of Neutrokine-alpha and or APRIL). Such Neutrokine-alpha and/or APRIL polypeptide variants may antagonize Neutrokine-alpha function, for example, by interfering with Neutrokine-alpha and/or APRIL homo- or hetero-multimerization. In a specific embodiment a Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functiona as a dominant negative. Alternatively, Neutrokine-alpha and/or APRIL polypeptide variants will prevent polypeptides comprising them from binding to and/or signaling through Neutrokine-alpha–receptors such as TACI, BCMA and BAFF-R. Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, antisense RNAs and short interfering RNAs (siRNAs) that target Neutrokine-alpha, antisense RNAs and short interfering RNAs (siRNAs) that target APRIL, antisense RNAs and short interfering RNAs (siRNAs) that target receptors for Neutrokine-alpha and/or receptors for APRIL. Antagonists of Neutrokine-alpha are described in more detail below.

[0034] Similarly, one of skill in the art would appreciate that other immunomodulatory agents, including in particular, molecules that can modulate B cell activities or numbers, may be useful in the present invention. In specific embodiments, a B cell modulatory agent that can be used in conjunction with the methods of the present invention is an agent that directly or indirectly inhibits or reduces B cell activity (e.g., B cell proliferation, differentiation, survival or immunoglobulin secretion) and or B cell number. In another embodiment, B cell modulatory agents that can be used in conjunction with the methods of the present invention are agents that reduce the activity or number of total B cells, activated B cells, naïve B cells, memory B cells, plasma B cells, and plasmacytoid B cells, CD19+ B cells and/or CD20+ B cells. Immunodulatory and B cell modulatory molecules that may be used in the present invention are known to those of skill in the art and are described in more detail below.

[0035] In another embodiment, the invention provides a method of treating a patient that falls within the subset of systemic lupus erythematosus patients that have "active" systemic lupus erythematosus (SLE or "lupus") disease comprising administering a therapeutically effective amount of an antibody antagonist of Neutrokine-alpha. In specific embodiments, the invention provides a method of treating a patient that has previously been diagnosed with lupus and has active lupus, comprising administering a therapeutically effective amount of an antibody antagonist of Neutrokine-alpha. The invention provides a method of treating a patient that falls within the subset of systemic lupus erythematosus patients that have active systemic lupus erythematosus (SLE or "lupus") disease comprising making a determination the patient has "active lupus" prior to administering a therapeutically effective amount of an antibody antagonist of Neutrokine-alpha. In specific embodiments, the invention provides a method of treating a patient that has previously been diagnosed with lupus and has active lupus, comprising making a determination the patient was previously diagnosed with lupus and has active lupus prior to administering a therapeutically effective amount of an antibody antagonist of Neutrokine-alpha.

[0036] In another embodiment, the invention provides a method of treating a patient that falls within the subset of systemic lupus erythematosus patients that have "active" systemic lupus erythematosus (SLE

or "lupus") disease comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein. In specific embodiments, the invention provides a method of treating a patient that has previously been diagnosed with lupus and has active lupus, comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein. The invention provides a method of treating a patient that falls within the subset of systemic lupus erythematosus patients that have active systemic lupus erythematosus disease comprising making a determination the patient has "active lupus" prior to administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein. In specific embodiments, the invention provides a method of treating a patient that has previously been diagnosed with lupus and has active lupus, comprising making a determination the patient was previously diagnosed with lupus and has active lupus prior to administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein.

[0037] In specific embodiments, a patient with active lupus is defined as a patient having a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria (See, for example, Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997), which are hereby incorporated by reference in their entirety).

[0038] In specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 4. SELENA SLEDAI stands for Systemic Lupus Erythematosus Disease Activity Index, as modified by the Safety of Estrogen in Lupus Erythematosus National Assessment trial. SELENA SLEDAI scores are routinely determined by clinicians/physicians using techniques and methodologies known in the art, see, for example, Bombardier, et al., Arthritis Rheum. Jun;35(6):630-40, 1992; and Strand, et al., J Rheumatol. Feb;26(2):490-7, 1999, which are hereby incorporated by reference in their entirety. Briefly, a SELENA SLEDAI score is determined by considering SLE disease activity in 24 categories spread across 9 organ systems. Disease in some organ systems scores is weighted more heavily than disease in other organ systems. In particular, central nervous system and vascular SLE disease activity measures, if present, are assigned 8 points, renal and musculoskeletal SLE disease activity measures, if present, are assigned 4 points, serosal, dermal and immunologic SLE disease activity measures, if present, are assigned 2 points, and constitutional and hematologic SLE disease activity measures, if present, are assigned 1 point. The maximum theoretical SELENA SLEDAI score is 105, but in practice, few patients have scores greater than 45.

[0039] In the standard SELENA SLEDAI scoring system, 4 points are assigned if a subject has a new onset or recent increase in proteinuria greater than 0.5 grams/24 hours. In other words, if the proteinuria value obtained in one 24 hour urine sample is more than 0.5g greater than the value determined for the patient's immediate prior 24 hour urine sample, 4 points will be assigned for proteinuria on the SELENA SLEDAI scale. This is commonly described as an increase or new onset of proteinuria of "> 0.5 g/24 hours." Thus, a under the standard SELENA SLEDAI scoring system, a subject that is assigned 4 points at baseline for proteinuria will have an improving SELENA SLEDAI at a subsequent visit as long as

proteinuria value in the current 24 hour urine sample is not more than 0.5g greater than the proteinuria value determined for the patient's immediate prior 24 hour urine sample. In other words, the patient will have 4 points deducted from their total score even in the face of stable proteinuria or increases in proteinuria ≤ 0.5 g/24 hours. A modification to the SELENA SLEDAI proteinuria scoring rules is described in Example 2. In Example 2, the proteinuria scoring is modified so that 4 points continue to be assigned unless proteinuria determined for the present 24 hour urine sample is more than 0.5 grams lower than the proteinuria value determined for that patient's immediate prior 24 hour urine sample. Further, if there is a new onset of proteinuria or an increase in proteinuria that is > 0.5 g/24 hours, 4 points are assigned. Herein, when the SELENA SLEDAI scale is referred to, scoring for proteinuria may be done according to the standard SELENA SLEDAI scale. Preferably, scoring for proteinuria in the determination of a patient's SELENA SLEDAI score is performed according to the proteinuria scoring system described in Example 2.

[0040] In other specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 5. In additional specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 6. In further specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 7. In additional specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 8. In other specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 9. In other specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 10. In additional specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 11. In additional specific embodiments, a patient with active lupus is defined as a patient having a SELENA SLEDAI score ≥ 12.

[0041] In other embodiments, a patient with active lupus is defined as a patient that has anti-dsDNA antibodies in his/her blood plasma or serum. Anti-dsDNA antibody titers, concentrations or levels can be routinely determined by clinicians/physicians using techniques and methodologies known in the art. One example assay for determining anti-dsDNA antibody titers, concentrations or levels is an enzyme-linked immunosorbent assay (ELISA) based on the specific binding of anti-dsDNA antibodies to immobilized dsDNA, see, for example, Halbert, et al., J Lab Clin Med. 97:97-111, (1981). Another example assay for determining anti-dsDNA antibody titers, concentrations or levels is an indirect immunofluorescence assay based on the specific binding of anti-dsDNA antibodies to the dsDNA of a *Crithidia luciliae* cell, see, for example, Whiteside, et al., Am J Clin Pathol. 72:829-35, (1979). Yet another example assay for determining anti-dsDNA antibody titers, concentrations or levels is the Farr assay based on the specific binding of anti-dsDNA antibodies to radiolabeled dsDNA, followed by precipitation of anti-dsDNA antibody-radiolabeled dsDNA complexes, see for example, Davis, et al., Am J Clin Pathol., 67:374-8, (1977). In specific embodiments, a patient with active lupus is defined as a patient that has greater than or equal to 30 International Units/mL of anti-dsDNA antibodies in his/her blood plasma or serum, wherein an International Unit (IU) is based on the World Heath Organization anti-dsDNA antibody reference preparation, see, for example, Feltkamp, et al., Ann .Rheum. Dis., 47:740-746, (1988). Each of the references referred to in this paragraph is herein incorporated by reference in its entirety.

[0042] In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 40 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum. In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 50 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 60 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 75 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 100 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 125 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 150 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 200 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum In an additional specific embodiment, a patient with active lupus is defined as a patient that has greater than or equal to 300 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum..

[0043] In other embodiments, a patient with active lupus is defined as a patient that has antinuclear antibodies (ANA+) in his/her blood plasma or serum. Antinuclear antibody titer can be routinely determined by clinicians/physicians using techniques and methodologies known in the art. One example assay for determining antinuclear antibody titer is an indirect immunofluorescence assay based on the specific binding of antinuclear antibodies to HEp-2 human epithelial cells, see, for example, Osborn, et al., Arthritis Rheum., 27:1286-9, (1984). In another example assay, antinuclear antibodies concentrations or levels can be determined by using an ELISA based on the specific binding of ANA to immobilized ANA antigens, for example, dsDNA, Ro/SS-A, La/SS-B, Sm, RNP, see, for example, Fenger, et al., Clin Chem., 50:2141-7, (2004). ANA tests are further described in Kavanaugh et al., *Archives of Pathology & Laboratory Medicine (2000)* 124:71-81 and Greidinger, EL and Hoffman, RW, *Laboratory Medicine* (2003) 34:113-117. Each of the references referred to in this paragraph is herein incorporated by reference in its entirety.

[0044] In preferred specific embodiments, a patient with active lupus is defined as a patient that has an ANA titer of 1:80 or greater (i.e., a positive ANA test is obtained when the dilution factor of the patient's blood plasma or serum is 80 or greater). Titers of 1:160, 1:320 and 1:640, for example, are greater than a a titer of 1:80.) In other preferred embodiments, a patient with active lupus is defined as a patient that has an ANA titer of 1:160 or greater. In an additional preferred embodiment, a patient with active lupus is defined as a patient that has an ANA titer of 1:320 or greater. In an additional preferred embodiment, a patient with active lupus is defined as a patient that has an ANA titer of 1:640 or greater. In a specific embodiment the ANA titer is measured using indirect immunofluorescence on HEp-2 cells. In another specific embodiment the ANA titer is measured using an anti-dsDNA ELISA assay.

[0045] In other embodiments, a patient with active lupus is defined as a patient that has detectable autoantibodies, including but not limited to anti-Ro/SS-A antibodies, anti-La/SS-B antibodies, anti-RNP antibodies, anti-cardiolipin (anti-phospholipid), anti-dsDNA antibodies, anti-Sm antibodies. Autoantibody titers, concentrations or levels can be routinely determined by clinicians/physicians using techniques and methodologies known in the art.

[0046] In other embodiments, a patient with active lupus is defined as a patient that has depressed C3 and/or C4 complement levels in his/her blood plasma or serum. One of skill in the art understands that the normal level of C3 and/or C4 may vary depending on the assay used to measure C3 and/or C4. Accordingly, a normal level of plasma or serum C3 complement may be from about 90 milligrams/deciliter to about 180 milligrams/deciliter. In other specific embodiments, a normal level of plasma or serum C3 complement may also range from about 88 milligrams/deciliter to about 206 milligrams/deciliter or from about 88 milligrams/deciliter to about 252 milligrams/deciliter. A normal level of plasma or serum C4 complement may be from about 16 milligrams/deciliter to about 47 milligrams/deciliter. In other specific embodiments, a normal level of plasma or serum C4 complement may also range from about 12 milligrams/deciliter to about 72 milligrams/deciliter or from about 13 milligrams/deciliter to about 75 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C3 complement is defined as less than 90 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C3 complement is defined as less than 88 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C3 complement is defined as less than 85 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C3 complement is defined as less than 80 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C3 complement is defined as less than 75 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 16 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 15 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 14 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 13 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 12 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 11 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 10 milligrams/deciliter. In specific embodiments, a depressed level of plasma or serum C4 complement is defined as less than 9 milligrams/deciliter. Complement levels can be routinely determined by clinicians/physicians using techniques and methodologies known in the art, e.g., using a radial immunodiffusion assay.

[0047] In other embodiments, a patient with active lupus is defined as a patient that has any one or more of the following characteristics: a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria (see, for example, Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997)); a SELENA SLEDAI score ≥ 6; depressed C4 complement levels in his/her blood plasma or serum; depressed C3 complement levels in his/her blood

plasma or serum; an ANA titer of 1:80 or greater; greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum; is receiving ≥7.5 milligrams/day of prednisone or other corticosteroid for treatment of lupus-related symptoms; and/or is receiving or had previously received immunosuppressant therapy for treatment of lupus-related symptoms.

[0048] In other embodiments, a patient with active lupus is defined as a patient that has any one or more of the following characteristics: a clinical diagnosis of SLE according to the American College of Rheumatology (ACR) criteria; a SELENA SLEDAI score ≥8; depressed C4 complement levels in his/her blood plasma or serum; depressed C3 complement levels in his/her blood plasma or serum; an ANA titer of 1:80 or greater; greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum; is receiving up to 40 milligrams/day of prednisone or other corticosteroid for treatment of lupus-related symptoms; and/or is receiving or had previously received immunosuppressant therapy for treatment of lupus-related symptoms.

[0049] Multiple disease activity indices are well known to clinicians/physicians in the art and can be used to measure the extent of rheumatic disease activity, such as SLE disease activity (see, for example, Strand, et al., J Rheumatol., 26:490-7, (1999)). In one embodiment, the SELENA SLEDAI is used as a disease activity index (DAI) (see, for example, Bombardier, et al., Arthritis Rheum. 35:630-40, (1992)). In another embodiment, the SLE Flare Index is used as a DAI (see, for example, Petri, et al., Lupus, 8:685-91, (1999)). In a further embodiment, the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR) is used as a DAI (see, for example, Gladman, et al., Arthritis Rheum., 39:363-9, (1996)). In another embodiment the Physician's Global assessment (PGA) is used as a DAI, The PGA is a visual analogue scale with a range from 0 to 3 where 0 is no disease activity, 1 is mild disease activity, 2 is moderate disease activity and 3 is severe disease activity, is used as a DAI. In yet another embodiment, The Medical Outcomes Survey Short Form 36 (SF-36) is used as a DAI. The SF-36 is a generic health-related quality of life (HRQOL) instrument that has been shown to reflect the impact of SLE on all domains of HRQOL in observational cohort studies, as well as randomized trials (Cook, et al., J. Rheumatol., 27:1892-1895, (2000); Thumboo, et al., J Rheumatol., 26:97-102, (1999); Thumboo, et al., J Rheumatol., 27:1414-1420, (2000); Ware JE, et al., Med Care, 30:473-483, (1992); Smolen JS, et al., J Rheumatol., 26:504-507, (1999); Gladman, et al., Lupus, 5:190-195, (1996); Alonso J, et al., Qual Life Res., 13:283-298, 2004; and Gladman et al, J Rheumatol., 27:377-9, (1995), each of which is incorporated by reference herein in its entirety).

[0050] In a further embodiment, the EQ-5D (also known as the EuroQol instrument) is used as a DAI. The EQ-5D is a generic health-related quality of life measure. It is intended to be a simple, self-administered questionnaire that not only contains a descriptive health state classification system but also is capable of generating a composite score or index reflecting the preference value associated with a given health state. The EQ-5D descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has three levels, reflecting "no health problems," "moderate health problems," and "extreme health problems" (see, for example, Health Policy. 1990 Dec;16(3):199-208, which is incorporated herein by reference).

[0051] In a further embodiment, the Functional Assessment for Chronic Illness Therapy-Fatigue (FACIT-F) subscale of the Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System is used as a DAI. The FACIT-F subscale is a 27-item compilation of general questions divided into four primary QOL domains: Physical Well-Being, Social/Family Well-Being, Emotional Well-Being, and Functional Well-Being. This measurement tool gathers patient feedback about energy level, listlessness and the ability to start or finish activities (see, for example, Yellen, S.B., et al., Journal of Pain and Symptom Management, 13:63-74, (1997); Cella, D., et al., 94(2):528-538, (2002); and Cella, D., et al., Journal of Pain & Symptom Management, 24 (6):547-561, (2002) , each of which is incorporated by reference herein in its entirety).

[0052] In a further embodiment, the Disease Activity Score (DAS28) is used as a DAI. DAS28 is a standard tool used by rheumatologists for assessment of rheumatic disease activity. This measurement tool calculates an index score based upon assessment of: the number of joints tender to the touch (TEN), the number of swollen joints (SW), the erythrocyte sedimentation rate (ESR), and the patient assessment of disease activity (VAS; mm) (see, for example, Van der Heijde D.M.F.M., et al., J. Rheumatol, 20:579-8, (1993); Prevoo M.L.L., et al., Arthritis Rheum, 38:44-8, (1995), each of which is incorporated by reference herein in its entirety).

[0053] In a further embodiment, the British Isles Lupus Assessment Group (BILAG) is used as a DAI (see, for example, Isenberg, et al., Rheumatology, 44:902-6, (2005); Gordon, et al., Rheumatology, 42(11):1372-9, (2003); Isenberg, et al., Lupus, 9(9):651-4, (2000); Hay et al., Q J Med., 86:447-58, (1993); and the BLIPS™ Version 3.0 Software program users guide, released April 4, 2004, ADS-Limathon Ltd, each of which is incorporated by reference herein in its entirety). The BILAG index comprises 8 body organs/systems known to be affected in lupus and scores each depending on whether the clinical features are new, worse, the same or improving compared to the previous measurement. For each of the 8 body organs/systems, the severity of the SLE disease manifestation is an A, B, C, D or E score with A being the most severe (see, for example, Hay, ibid). The BILAG index provides a composite score to assess disease severity and effectiveness of treatment. This composite score adds contributions across each of the 8 body organs/systems affected in lupus. Using BILAG or other measures known in the art, treatment may be targeted to lupus patients with disease manifestation in specific subsets of organs/systems.

[0054] Accordingly, in a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has achieved a reduction in their SELENA SLEDAI score. In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has achieved a reduction in their SELENA SLEDAI score compared to the same patient's baseline SELENA SLEDAI score, the SELENA SLEDAI score determined prior to that patient's commencing of treatment with the immunomodulatory agent. In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has achieved at least a four point reduction in their

SELENA SLEDAI score. In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has achieved at least a four point reduction in their SELENA SLEDAI score compared to the same patient's baseline SELENA SLEDAI score, the SELENA SLEDAI score determined just prior to that patient's commencing of treatment with the immunomodulatory agent.

[0055] In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has not experienced a worsening of disease activity as determined by the Physician's Global Assessment (PGA). In a specific embodiment, the patient has not experienced a worsening of disease activity if the PGA score has decreased, remained stable or increased by less than 0.3 points. In a specific embodiment, the patient has not experienced a worsening of disease activity if the PGA score has decreased, remained stable or increased by less than 0.3 points from same patient's baseline PGA score, the PGA score determined just prior to that patient's commencing of treatment with the immunomodulatory agent.

[0056] In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has not experienced a worsening of disease activity as determined by the British Isles Lupus Assessment Group (BILAG) disease activity index. In a specific embodiment, the patient has not experienced a worsening of disease activity if the patient has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores. In a specific embodiment, the patient has not experienced a worsening of disease activity if the patient has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores since the patient's baseline BILAG assessment, the BILAG assessment determined just prior to that patient's commencing of treatment with the immunomodulatory agent.

[0057] In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has achieved a reduction in their SELENA SLEDAI score, has not had a substantial worsening of their PGA score, and has not experienced a worsening of disease activity as determined by the British Isles Lupus Assessment Group (BILAG) disease activity index, compared to their baseline SELENA SLEDAI score, PGA score and BILAG assessment, respectively. In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent is considered to be responding/have responded to treatment if the patient has achieved at least a 4 point reduction in their SELENA SLEDAI score, has not had more than a 0.30 point increase in their PGA score and has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores, compared to their baseline SELENA SLEDAI score, PGA score and BILAG assessment, respectively.

[0058] In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced. In another specific embodiment,

a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced compared to the patient's baseline prednisone dose, the prednisone dose the patient was taking just prior to that patient's commencing of treatment with the immunomodulatory agent. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25%. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25% to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25% from the patient's baseline prednisone dose to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50%. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50% to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50% from the patient's baseline prednisone dose to less than or equal to 7.5 mg/day.

[0059] Other measures can be used to measure the quality of a lupus patient's response to treatment. In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved SF-36 Health Survey Score. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved SF-36 Health Survey Score compared to the patient's baseline SF-36 Health Survey Score.

[0060] In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved EQ-5D score. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved EQ-5D score compared to the patient's baseline EQ-5D score.

[0061] In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient demonstrates reduced fatigue as shown by the patient's FACIT-F score.

In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient demonstrates reduced fatigue as shown by the patient's FACIT-F score compared to the patient's baseline FACIT-F score.

[0062] In a specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved DAS28 score. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has an improved DAS28 score compared to the patient's baseline DAS28 score.

[0063] In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or duration of flares. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or duration of flares compared to the frequency and/or duration of flares prior to treatment with the immunomodulatory agent. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased severity of flares. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased severity of flares compared to the severity of flares prior to treatment with the immunomodulatory agent. The SLE flare index assesses the frequency and severity of exacerbations in lupus symptoms (flares). Flares are categorized as "mild or moderate" or "severe". Mild or moderate flares involve one or more of the following: change in SELENA SLEDAI score of 3 points or more; new/worse discoid, photosensitive, profundus, cutaneous vasculitis or bullous lupus; nasopharyngeal ulcers; pleuritis; pericarditis; arthritis; fever (SLE); increase in prednisone, but not to more than 0.5 mg/kg/day; added NSAID or Plaquenil for disease activity; and greater than 1.0 increase in PGA score, but not to more than 2.5. Severe flares involve one or more of the following: change in SELENA SLEDAI score to greater than 12; new/worse CNS-SLE; vasculitis; nephritis; myositis; Plt <60,000; heme anemia (<7% or decrease in Hb >3%); doubling of Prednisone dosage; increase in Prednisone to more than 0.5 mg/kg/day; prescription of Cytoxan; prescription of Azathioprine; prescription of Methotrexate; hospitalization (SLE) and increase in PGA score to more than 2.5. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by the SLE flare index. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as

measured the SLE flare index compared to the patient's previous flare frequency and/or severity. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by a modified version of the SLE flare index. In another specific embodiment, a lupus patient that is being or has been treated with an immunomodulatory agent known in the art and/or described herein is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by a modified version of the SLE flare index compared to the patient's previous flare frequency and/or severity. The modified version of the SLE flare index excludes severe flares triggered by SELENA SLEDAI score change alone.

[0064] Accordingly, in a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has achieved a reduction in his/her SELENA SLEDAI score. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has achieved a reduction in his/her SELENA SLEDAI score compared to the same patient's baseline SELENA SLEDAI score, the SELENA SLEDAI score determined just prior to that patient's commencing of treatment with the antagonist of Neutrokine-alpha. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has achieved at least a four point reduction in his/her SELENA SLEDAI score. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has achieved at least a four point reduction in his/her SELENA SLEDAI score compared to the same patient's baseline SELENA SLEDAI score, the SELENA SLEDAI score determined just prior to that patient's commencing of treatment with the antagonist of Neutrokine-alpha. In a specific embodiment, a Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, a Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, a Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, a Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, a Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0065] In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-

binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has not experienced a worsening of disease activity as determined by the Physician's Global Assessment (PGA). In a specific embodiment, the patient has not experienced a worsening of disease activity if the PGA score has decreased, remained stable or increased by less than 0.3 points. In a specific embodiment, the patient has not experienced a worsening of disease activity if the PGA score has decreased, remained stable or increased by less than 0.3 points from same patient's baseline PGA score, the PGA score determined just prior to that patient's commencing of treatment with the antagonist of Neutrokine-alpha. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0066] In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has not experienced a worsening of disease activity as determined by the British Isles Lupus Assessment Group (BILAG) disease activity index. In a specific embodiment, the patient has not experienced a worsening of disease activity if the patient has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores. In a specific embodiment, the patient has not experienced a worsening of disease activity if the patient has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores since the patient's baseline BILAG assessment, the BILAG score determined just prior to that patient's commencing of treatment with the antagonist of Neutrokine-alpha. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0067] In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment

or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has achieved a reduction in his/her SELENA SLEDAI score, has not had a substantial worsening of his/her PGA score, and has not experienced a worsening of disease activity as determined by the British Isles Lupus Assessment Group (BILAG) disease activity index, compared to his/her baseline SELENA SLEDAI score, PGA score and BILAG score, respectively. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has achieved at least a 4 point reduction in his/her SELENA SLEDAI score, has not had more than a 0.30 point increase in his/her PGA score and has not gained a new BILAG A organ domain score or has not gained 2 new BILAG B organ domain scores, compared to his/her baseline SELENA SLEDAI score, PGA score and BILAG score, respectively. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0068] In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced compared to the patient's baseline prednisone dose, the prednisone dose the patient was taking just prior to that patient's commencing of treatment with the antagonist of Neutrokine-alpha. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25%. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25% to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 25%

from the patient's baseline prednisone dose to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50%. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50% to less than or equal to 7.5 mg/day. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient's prednisone dose has been reduced by at least 50% from the patient's baseline prednisone dose to less than or equal to 7.5 mg/day. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0069] Other measures can be used to measure the quality of a lupus patient's response to treatment. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved SF-36 Health Survey Score. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved SF-36 Health Survey Score compared to the patient's baseline SF-36 Health Survey Score. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0070] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding

fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved EQ-5D score. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved EQ-5D score compared to the patient's baseline EQ-5D score. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0071] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient demonstrates reduced fatigue as shown by the patient's FACIT-F score. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient demonstrates reduced fatigue as shown by the patient's FACIT-F score compared to the patient's baseline FACIT-F score. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha

antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0072] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved DAS28 score. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an improved DAS28 score compared to the patient's baseline DAS28 score. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0073] In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or duration of flares. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or duration of flares compared to the frequency and/or duration of flares prior to treatment with the antagonist of Neutrokine-alpha. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased severity of flares. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has

a decreased severity of flares compared to the severity of flares prior to treatment with the antagonist of Neutrokine-alpha. The SLE flare index assesses the frequency and severity of exacerbations in lupus symptoms (flares). Flares are categorized as "mild or moderate" or "severe". Mild or moderate flares involve one or more of the following: change in SELENA SLEDAI score of 3 points or more; new/worse discoid, photosensitive, profundus, cutaneous vasculitis or bullous lupus; nasopharyngeal ulcers; pleuritis; pericarditis; arthritis; fever (SLE); increase in Prednisone, but not to more than 0.5 mg/kg/day; added NSAID or Plaquenil for disease activity; and greater than 1.0 increase in PGA score, but not to more than 2.5. Severe flares involve one or more of the following: change in SELENA SLEDAI score to greater than 12; new/worse CNS-SLE; vasculitis; nephritis; myositis; Plt <60,000; heme anemia (<7% or decrease in Hb >3%); doubling of Prednisone dosage; increase in Prednisone to more than 0.5 mg/kg/day; prescription of Cytoxan; prescription of Azathioprine; prescription of Methotrexate; hospitalization (SLE) and increase in PGA score to more than 2.5. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by the SLE flare index. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured the SLE flare index compared to the frequency and/or severity of flares prior to treatment with the antagonist of Neutrokine-alpha. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by a modified version of the SLE flare index. In another specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha is considered to be responding/have responded to treatment if the patient has a decreased frequency and/or severity of flares as measured by a modified version of the SLE flare index compared to the frequency and/or severity of flares prior to treatment with the antagonist of Neutrokine-alpha. The modified version of the SLE flare index excludes severe flares triggered by SELENA SLEDAI score change alone. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0074] The above described disease activity indices (e.g., SELENA SLEDAI, PGA, BILAG, SLE flare index, SF-36 Health Survey Score, EQ-5D, FACIT-F, DAS28) may be used to evaluate the status of a lupus patient individually or in combination. Improvements in a patient's health as measured by these disease activity indices may also be assessed at a time following commencement of treatment with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-

Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, in relation to one or more of the patient's previous disease activity index score measurements. Additionally, in a specific embodiment, a lupus patient that is being or has been treated with an an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains an improved disease activity score relative to a previous measurement. In a specific embodiment, one or more disease activity index scores are assessed prior to beginning treatment with an antagonist of Neutrokine-alpha or other immunomodulatory agent at 1, 2, 3, 4, 5, 6 7, 8, 9, 10, 11, or 12 weeks, months and/or years following commencement of treatment with the an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0075] In a specific embodiment, a lupus patient with disease manifestation in one or more organs/systems is treated with an antagonist of Neutrokine-alpha and/or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In specific embodiments, a lupus patient with disease manifestation in one or more internal organ systems, with or without involvement of mucocutaneous and/or musculoskeletal systems, is treated with an antagonist of Neutrokine-alpha and/or other immunomodulatory agent known in the art and/or described herein. In specific embodiments, a lupus patient with disease manifestation in one or more internal organ systems, without involvement of mucocutaneous and/or musculoskeletal systems, is treated with an antagonist of Neutrokine-alpha and/or other immunomodulatory agent known in the art and/or described herein. In lupus, disease manifestations involving the mucocutaneous and/or musculoskeletal systems include, but are not limited to: discoid rash, malar rash, or other skin eruption, mucosal ulceration, panniculitis, cutaneous vasculitis, cutaneous thrombosis, digital infarcts, digital thrombosis, alopecia, peri-ungual erythema, chilblains, splinter hemorrhages, myositis, polyarthritis,

arthritis, tendonitis, arthralgia and myalgia. In lupus, internal organ systems that may be affected by lupus, include, but are not limited to, the nervous system, the circulatory system, the respiratory system, the urinary/excretory system, the digestive system, and the eyes. Lupus disease manifestation in the nervous system include, but are not limited to aseptic meningitis, cerebral vasculitis, demyelinating syndrome, myelopathy, acute confusional state, psychosis, acute inflammatory demyelinating polyradiculoneuropathy, mononeuropathy, cranial neuropathy, plexopathy, polyneuropathy, seizure disorder, status epilepticus, cerebrovascular disease not due to vasculitis, cognitive dysfunction, movement disorder, autonomic disorder, cerebellar ataxia, headache, migraine, mood disorder and anxiety disorder. Lupus disease manifestations in the circulatory system include, but are not limited to myocarditis, cardiac failure, arrhythmia, new valvular dysfunction, serositis, cardiac tamponade, pleural effusion with dyspnoea, pulmonary hemorrhage, pulmonary vasculitis, interstitial alveolitis, interstitial pneumonitis , shrinking lung syndrome, aortitis and coronary vasculitis. Lupus disease manifestations in the digestive system include, but are not limited to peritonitis, abdominal serositis, ascites, lupus enteritris, lupus colitis, malabsorption, protein losing enteropathy, hepatitis, intestinal pseudo-obstruction, acute cholecystitis and acute pancreatitis. Lupus disease manifestations associated with the eye include, but are not limited to orbital inflammation, keratitis, anterior uveitis, posterior uveitis, retinal vasculitis, episcleritis, scleritis, retinal/choroidal vaso-occlusive disease, cutoid bodies, optic neuritis and anterior ischemic optic neuropathy.

[0076] A patient's response to treatment with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein, including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, may also be monitored by assessing biomarkers at one or more intervals after commencing treatment and comparing the patient's biomarker assessment with the patient's baseline and/or a previous measurement for the same biomarker(s). Biomarkers that may be assessed include, but are not limited to, immunoglobulin levels (e.g., total serum immunoglobulin, as well as serum IgM, IgG, IgA, and/or IgE levels), autoantibody levels (e.g., anti-dsDNA antibody, anti-CCP antibody, anti-Ro/SS-A antibody, anti-La/SS-B antibody, anti-RNP antibody, anti-cardiolipin (anti-phospholipid) antibody and anti-Sm antibody levels as well as ANA titer), B cell numbers (e.g., total B cell numbers, activated B cell numbers, naïve B cell numbers, memory B cell numbers, plasma B cell numbers, and plasmacytoid B cell numbers, total CD19+ B cells and/or CD20+ B cells), C4 complement level, C3 complement level. In a specific embodiment, biomarker measurements are assessed prior to beginning treatment with an antagonist of Neutrokine-alpha or other immunomodulatory agent and/or at 1, 2, 3, 4, 5, 6 7, 8, 9, 10, 11, or 12 weeks, months and/or years following commencement of treatment with the antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the

Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0077] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein, including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has a decreased level of immunoglobulin (e.g., total serum immunoglobulin, as well as serum IgM, IgG, IgA, and/or IgE levels) compared to the patient's baseline measurement of immunoglobulin. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient has a decreased level of immunoglobulin (e.g., total serum immunoglobulin, as well as serum IgM, IgG, IgA, and/or IgE levels) compared to one or more of the patient's previous measurements of immunoglobulin. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains a decreased level of immunoglobulin (e.g., total serum immunoglobulin, as well as serum IgM, IgG, IgA, and/or IgE levels) compared to one or more of the patient's previous measurements of immunoglobulin. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient achieves a normal level of immunoglobulin (e.g., total serum immunoglobulin, as well as serum IgM, IgG, IgA, and/or IgE levels).

[0078] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has a decreased level of autoantibody (e.g., anti-dsDNA antibody, anti-CCP

antibody, anti-Ro/SS-A antibody, anti-La/SS-B antibody, anti-RNP antibody, anti-cardiolipin (anti-phospholipid) antibody and anti-Sm antibody levels as well as ANA titer) compared to the patient's baseline measurement of autoantibody. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient has a decreased level of autoantibody (e.g., anti-dsDNA antibody, anti-CCP antibody, anti-Ro/SS-A antibody, anti-La/SS-B antibody, anti-RNP antibody, anti-cardiolipin (anti-phospholipid) antibody and anti-Sm antibody levels as well as ANA titer) compared to one or more of the patient's previous measurements of autoantibody. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains a decreased level of autoantibody (e.g., anti-dsDNA antibody, anti-CCP antibody, anti-Ro/SS-A antibody, anti-La/SS-B antibody, anti-RNP antibody, anti-cardiolipin (anti-phospholipid) antibody and anti-Sm antibody levels as well as ANA titer) compared to one or more of the patient's previous measurements of autoantibody. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient achieves a normal level of autoantibody (e.g., anti-dsDNA antibody, anti-CCP antibody, anti-Ro/SS-A antibody, anti-La/SS-B antibody, anti-RNP antibody, anti-cardiolipin (anti-phospholipid) antibody and anti-Sm antibody levels as well as ANA titer). In a specific embodiment, autoantibodies of the IgG isotype are measured.

[0079] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has a decreased number of B cells (e.g., total B cell numbers, activated B cell numbers, naïve B cell numbers, plasma B cell numbers, and plasmacytoid B cell numbers, total CD19+ B cell numbers and/or CD20+ B cell numbers) compared to the patient's baseline measurement of B cell number. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant

negative. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient has a decreased number of B cells (e.g., total B cell numbers, activated B cell numbers, naïve B cell numbers, plasma B cell numbers, and plasmacytoid B cell numbers, total CD19+ B cell numbers and/or CD20+ B cell numbers) compared to one or more of the patient's previous measurements of B cell number. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains a decreased number of B cells (e.g., total B cell numbers, activated B cell numbers, naïve B cell numbers, plasma B cell numbers, and plasmacytoid B cell numbers, total CD19+ B cell numbers and/or CD20+ B cell numbers) compared to one or more of the patient's previous measurements of B cell number. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient achieves a normal number of B cells (e.g., total B cell numbers, activated B cell numbers, naïve B cell numbers, plasma B cell numbers, and plasmacytoid B cell numbers, total CD19+ B cell numbers and/or CD20+ B cell numbers).

[0080] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an increased serum complement factor C4 level compared to the patient's baseline measurement of C4. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient has an increased level of C4 compared to one or more of the patient's previous measurements of C4. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains an increased level of C4 compared to one or more of the patient's previous measurements of C4. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient achieves a normal level of C4.

[0081] In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has an increased serum complement factor C3 level compared to the patient's baseline measurement of C3. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient has an increased level of C3 compared to one or more of the patient's previous measurements of C3. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient maintains an increased level of C3 compared to one or more of the patient's previous measurements of C3. In a specific embodiment, a lupus patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent is considered to be responding/have responded to treatment if the patient achieves a normal level of C3.

[0082] In specific embodiments, the invention provides a method of treating a patient that has previously been treated with one or more immunosuppressants comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In specific embodiments, the invention provides a method of treating a patient that has previously been diagnosed with systemic lupus erythematosus (lupus) and has previously been treated with one or more immunosuppressants comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha

antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In specific embodiments, the immunosuppressant the patient was previously treated with is azathioprine (e.g., IMURAN™), cyclophosphamide (e.g., Cytoxan®, Neosar®, CTX), a calcineurin inhibitor, for example, FK506, tacrolimus or cyclosporine (e.g., PROGRAF®) and/or CELLCEPT® (mycophenolate motefil, of which the active metabolite is mycophenolic acid).

[0083] Most current therapies for lupus and other autoimmune diseases utilize medications that non-specifically block various inflammatory pathways. Perhaps the most dangerous medications used in this therapy are corticosteroids. While corticosteroids such as prednisone are essential in controlling disease manifestations, they also have numerous adverse effects on patient health such as, global immunosuppression leading to infection, osteoporosis leading to fractures, and atherosclerosis leading to early onset heart attacks and strokes. In a clinical trial, applicants found that treatment with an antibody that neutralizes Neutrokine-alpha protein, given as an IV infusion on days 0, 14, 28 and then every four weeks until week 52, was effective in reducing the dosage of the corticosteroid prednisone which was necessary to ameliorate disease manifestations in lupus patients. Specifically, treatment with the anti-Neutrokine-alpha antibody appeared to be associated with reduced prednisone use during the last three months of the treatment period. In patients that had an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA at baseline, a greater percentage of subjects receiving the anti-Neutrokine-alpha antibody had their prednisone dose reduced, while conversely a greater number of subjects receiving placebo treatment had increases to a prednisone dose greater than 7.5 mg/day.

[0084] Accordingly, in one embodiment, the invention provides a method of reducing the frequency of corticosteroid treatments and/or the quantity of corticosteroid administered to a patient comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In specific embodiments, the corticosteroid is prednisone, prednisolone, hydrocortisone, methylprednisolone or dexamethasone. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a specific embodiment, the patient in whom corticosteroid therapy is reduced is a patient suffering from inflammation. In another specific embodiment, the patient in whom corticosteroid therapy is

reduced is a patient suffering from an autoimmune disease, including but not limited to, rheumatoid arthritis, lupus, Sjogren's syndrome or other autoimmune disease, such as one listed herein.

[0085] Accordingly, in a specific embodiment, the invention provides a method of reducing the frequency of corticosteroid treatments and/or the quantity of corticosteroid administered to a systemic lupus erythematosus (lupus) patient comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In another specific embodiment, the invention provides a method of reducing the frequency of prednisone treatments and/or the quantity of prednisone administered to a systemic lupus erythematosus (lupus) patient comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In this context "therapeutically effective amount" refers to an amount that reduces the corticosteroid necessary to alleviate disease manifestations for which corticosteroids are typically prescribed. These manifestations are well known by clinicians/physicians, as are the methodologies for determining antibody/composition amount effective in reducing the severity of these manifestations. In preferred embodiments, the dosage of the antibody of the invention administered to a patient is 0.1 mg/kg to 100 mg/kg of the patient's body weight. More preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight. In the most preferred embodiments, the dose administered to a patient is 1, 4, 10, or 20 mg/kg.

[0086] In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to $\leq$80 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to $\leq$40 milligrams/day while the same patient is concomitantly on a treatment

regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to less than 20 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤10 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤8 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤6 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤4 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤2 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0087] In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is lowered from a previously higher dose to ≤7.5 milligrams/day while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is ultimately lowered by at least 25% while the patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent compared to the dose of prednisone the patient was taking prior to beginning the treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is ultimately lowered by at least 50% while the patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent compared to the dose of prednisone the patient was taking prior to beginning the treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other

immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is ultimately lowered by at least 25% to ≤ 7.5 milligrams/day while the patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent compared to the dose of prednisone the patient was taking prior to beginning the treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the amount of corticosteroid (e.g., prednisone) administered to a patient is ultimately lowered by at least 50% to ≤ 7.5 milligrams/day while the patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent compared to the dose of prednisone the patient was taking prior to beginning the treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0088] In a specific embodiment, a patient is taken off, either temporarily or permanently, corticosteroid (e.g., prednisone) therapy while the same patient is concomitantly on a treatment regimen comprising administration of an antagonist of Neutrokine-alpha or other immunomodulatory agent. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0089] In specific embodiments, antagonists of Neutrokine-alpha or other immunomodulatory agents known in the art and/or described herein are used to treat patients with clinical diagnosis of rheumatoid arthritis (RA). In specific embodiments, the rheumatoid arthritis patient treated will not have a B cell malignancy. Moreover, the rheumatoid arthritis patient is optionally further treated with any one or more agents employed for treating RA such as salicylate; nonsteroidal anti-inflammatory drugs such as indomethacin, phenylbutazone, phenylacetic acid derivatives (e.g., ibuprofen and fenoprofen), naphthalene acetic acids (naproxen), pyrrolealkanoic acid (tometin), indoleacetic acids (sulindac), halogenated anthranilic acid (meclofenamate sodium), piroxicam, zomepirac and diflunisal; antimalarials such as chloroquine; gold salts; penicillamine; or immunosuppressive agents such as methotrexate or corticosteroids in dosages known for such drugs or reduced dosages. Preferably however, the rheumatoid arthritis patient is only treated with the antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment,

the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. Such immunomodulatory agents are administered to the RA patient according to a dosing schedule as described infra, which may be readily determined by one of ordinary skill in the art. The primary response is determined by the Paulus index (Paulus et al. Arthritis Rheum. 33:477-484 (1990)), i.e., improvement in morning stiffness, number of painful and inflamed joints, erythrocyte sedimentation (ESR), and at least a 2-point improvement on a 5-point scale of disease severity assessed by patient and by physician. Administration of antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein will alleviate one or more of the symptoms of RA in the patient treated as described above.

[0090] In a phase 2 clinical trial (Example 3) in which rheumatoid arthritis patients received treatment an antibody that neutralizes Neutrokine-alpha protein, given as an IV infusion on days 0, 14, 28 and then every four weeks until week 24, treatment was more likely to ameliorate symptoms associated with rheumatoid arthritis in patients that had a DAS28 score greater than 5.1, patients that had not previously received anti-TNF therapy, and/or patients that had rheumatoid factor in his/her blood plasma and/or serum prior to commencing treatment with the antibody that neutralizes Neutrokine-alpha protein. Additional subgroups that appeared to be more likely to respond to treatment with the antibody that neutralizes Neutrokine-alpha protein included male patients, patients that had anti-CCP (cyclic citrullinated peptide) antibodies in his/her blood plasma and/or serum, patients that received methotrexate concomitantly with the antibody that neutralizes Neutrokine-alpha protein, patients that had previously failed treatment with methotrexate, and/or patients that had previously failed methotrexate therapy and at least one other DMARD therapy.

[0091] Accordingly, the invention provides for a method of treating a rheumatoid arthritis patient with antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein, wherein said rheumatoid arthritis patient has any one or more of the following characteristics: the patient has not previously received anti-TNF therapy, e.g., Infliximab (also known as Remicade™ Centocor, Inc.), adalimumab (Humira® from Abbott Laboratories) or etanercept (Enbrel®); the patient has rheumatoid factor in his/her blood plasma and/or serum; the patient has measurable anti-CCP (cyclic citrullinated peptide) antibodies in his/her blood plasma and/or serum; the patient has an elevated CRP (C reactive protein) level in his/her blood plasma and/or serum; the patient previously failed treatment with one or more disease-modifying antirheumatic drugs; that patient has a high modified disease activity score (DAS28); the patient has swollen and tender joints; the patient suffers from morning stiffness; the patient has an increased erythrocyte sedimentation rate (ESR) and/or the patient is male. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In specific embodiments, the rheumatoid arthritis patient has equal to

or greater than 12 IU/ml of rheumatoid factor in his/her blood plasma and/or serum. In specific embodiments, elevated CRP level are defined as at least 1.5 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 5 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 6 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 9 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 10 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 20 milligrams per liter. In specific embodiments, the rheumatoid arthritis patient has equal to or greater than 10 units of anti-CCP antibody in his/her blood plasma and/or serum. In specific embodiments, the rheumatoid arthritis patient has equal to or greater than 20 units of anti-CCP antibody in his/her blood plasma and/or serum. In specific embodiments, the patient previously failed treatment with one or more DMARDs, including but not limited to methotrexate, aminoquinolone, sulfasalazine, and leflunomide. In specific embodiments, the patient previously failed treatment with methotrexate. In specific embodiments, the patient has a DAS28 score greater than 5.1. In specific embodiments, the patient has at least 6 swollen joints and at least 8 tender joints. In specific embodiments, the patient has an ESR greater than 28 mm/hours. In specific embodiments, the patient suffers from morning stiffness for at least 45 minutes. In specific embodiments, the patient suffers from morning stiffness for at least an hour. In specific embodiments, the patient suffers from morning stiffness for at least an hour and a half. In specific embodiments, the patient suffers from morning stiffness for at least 2 hours. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0092] Accordingly, the invention provides for a method of treating a rheumatoid arthritis patient with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, wherein said rheumatoid arthritis patient has any one or more of the following characteristics: the patient has not previously received anti-TNF therapy, e.g., Infliximab (also known as Remicade™ Centocor, Inc.), adalimumab (Humira® from Abbott Laboratories) or etanercept (Enbrel®); the patient has rheumatoid factor in his/her blood plasma and/or serum; the patient has measurable anti-CCP (cyclic citrullinated peptide) antibodies in his/her blood plasma and/or serum; the patient has an elevated CRP (C reactive protein) level in his/her blood plasma and/or serum; the patient previously failed treatment with one or more disease-modifying antirheumatic drugs; that patient has a high modified disease activity score (DAS28); the patient has swollen and tender joints; the patient suffers from morning stiffness; the

patient has an increased erythrocyte sedimentation rate (ESR) and/or the patient is male. In specific embodiments, the rheumatoid arthritis patient has equal to or greater than 12 IU/ml of rheumatoid factor in his/her blood plasma and/or serum. In specific embodiments, elevated CRP level are defined as at least 1.5 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 5 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 6 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 9 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 10 milligrams per liter. In specific embodiments, an elevated CRP level is defined as at least 20 milligrams per liter. In specific embodiments, the rheumatoid arthritis patient has equal to or greater than 10 units of anti-CCP antibody in his/her blood plasma and/or serum. In specific embodiments, the rheumatoid arthritis patient has equal to or greater than 20 units of anti-CCP antibody in his/her blood plasma and/or serum. In specific embodiments, the patient previously failed treatment with one or more DMARDs, including but not limited to methotrexate, aminoquinolone, sulfasalazine, and leflunomide. In specific embodiments, the patient previously failed treatment with methotrexate. In specific embodiments, the patient has a DAS28 score greater than 5.1. In specific embodiments, the patient has at least 6 swollen joints and at least 8 tender joints. In specific embodiments, the patient has an ESR greater than 28 mm/hours. In specific embodiments, the patient suffers from morning stiffness for at least 45 minutes. In specific embodiments, the patient suffers from morning stiffness for at least an hour. In specific embodiments, the patient suffers from morning stiffness for at least an hour and a half. In specific embodiments, the patient suffers from morning stiffness for at least 2 hours.

[0093] In another specific embodiment, a rheumatoid arthritis patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has achieved an ACR20 Response. The ACR20 is an index developed by the American College of Rheumatology (ACR) to assess patient response to treatment for rheumatoid arthritis. An ACR20 response is defined as at least a 20% reduction in tender joint count and swollen joint count, in addition to an improvement of at least 20% on three of five other assessments of symptoms or disease manifestations (i.e., patient pain assessment, patient global assessment, physician global assessment, patient self-assessed disability, acute-phase reactant [ESR or CRP]). In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0094] In another specific embodiment, a rheumatoid arthritis patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein or fragment or variant thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has achieved an ACR50 Response. The ACR50 is an index developed by the American College of Rheumatology (ACR) to assess patient response to treatment for rheumatoid arthritis. An ACR50 response is defined as at least a 50% reduction in tender joint count and swollen joint count, in addition to an improvement of at least 50% on three of five other assessments of symptoms or disease manifestations (i.e., patient pain assessment, patient global assessment, physician global assessment, patient self-assessed disability, acute-phase reactant [ESR or CRP]). In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

[0095] In another specific embodiment, a rheumatoid arthritis patient that is being or has been treated with an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein including but not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein (e.g., TACI, BCMA or BAFF-R) or fragment or variant thereof, an anti-Neutrokine-alpha receptor (e.g., TACI, BCMA or BAFF-R) antibody or antigen-binding fragment thereof, Neutrokine-alpha binding polypeptides, Neutrokine-alpha and/or APRIL polypeptide variants, and antisense or siRNAs that target Neutrokine-alpha, APRIL, TACI, BCMA, BAFF-R or other receptor for Neutrokine-alpha and/or APRIL, is considered to be responding/have responded to treatment if the patient has achieved an ACR70 Response. The ACR70 is an index developed by the American College of Rheumatology (ACR) to assess patient response to treatment for rheumatoid arthritis. An ACR70 response is defined as at least a 70% reduction in tender joint count and swollen joint count, in addition to an improvement of at least 70% on three of five other assessments of symptoms or disease manifestations (i.e., patient pain assessment, patient global assessment, physician global assessment, patient self-assessed disability, acute-phase reactant [ESR or CRP]). In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative.

Immunomodulatory agents

[0096] The present invention provides a method of treating a patient that has an ANA titer of 1:80 or greater and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum with an immunomodulatory agent. The meaning of "immunomodulatory agent" when used herein is discussed supra. In a specific embodiment, the immunomodulatory agent is an antagonist of Neutrokine-alpha. By "antagonist", it is meant agents capable of inhibiting or counteracting the *in vitro* and/or *in vivo* functional and/or biological actions of Neutrokine-alpha (e.g., stimulation of differentiation, proliferation, and/or survival of B cells; stimulation of Ig production by B-cells; and binding to a Neutrokine-alpha receptor. This inhibition may occur with or without direct physical contact between the antagonist and the Neutrokine-alpha polypeptide (e.g., the antagonist may modulate an upstream effector of Neutrokine-alpha activity in order to reduce said activity). Assays for testing the ability of Neutrokine-alpha antagonists to inhibit B cell activity are described herein. Neutrokine-alpha antagonists include, but are not limited to, an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof, a Neutrokine-alpha receptor protein or fragment or variant thereof, an antibody that binds a Neutrokine-alpha receptor or antigen binding fragment thereof, a Neutrokine-alpha binding peptide or polypeptide, a Neutrokine-alpha and/or APRIL polypeptide variant (e.g., a dominant negative form of Neutrokine-alpha and/or APRIL). Additional antagonists of Neutrokine-alpha include small molecule antagonists of Neutrokine-alpha, Neutrokine-alpha peptide mimetics, antisense RNAs and short interfering RNAs (siRNAs) that target Neutrokine-alpha, antisense RNAs and short interfering RNAs (siRNAs) that target APRIL, antisense RNAs and short interfering RNAs (siRNAs) that target receptors for Neutrokine-alpha and/or receptors for APRIL. Each of these are described in more detail below.

Neutrokine-alpha Antagonists

A. Neutrokine-alpha and APRIL polypeptides

[0097] In a specific embodiment, the Neutrokine-alpha antagonist for use in the methods of the present invention is a Neutrokine-alpha or APRIL polypeptide, fragment or variant. Neutrokine-alpha polypeptides, APRIL polypeptides and fragments and variants thereof are described in more detail below. Neutrokine-alpha protein (SEQ ID NO:2) is a member of the TNF family of ligands that shares amino acid sequence identity to APRIL (SEQ ID NO:4; GenBank Accession No. 'AF046888; PCT International Publication Number WO97/33902; Hahne, M., et al., J Exp Med. (1998) 188(6):1185-90), TNFα, and lymphotoxin-α (LTα) (Moore, *et al.*, 1999). The full length Neutrokine-alpha gene encodes a 285 amino acid polypeptide that has an intracellular domain between residues 1 and 46, a transmembrane spanning domain between residues 47 and 73 preceded by a non-hydrophobic sequence characteristic of type II membrane bound proteins, and an extracellular domain between residues 74 and 285. Like other members of the TNF family, Neutrokine-alpha functions as a trimeric protein. Upon expression of Neutrokine-alpha at the surface of the cell, the extracellular domain is cleaved at amino acid 134 to release a biologically active trimer. Structural characterization reveals that while the TNF-family ligands demonstrate sequence diversity, they show high structural homology. The Neutrokine-alpha protein, like other members of the

TNF-family of ligands, is a 2 layered β-sandwich that forms a TNF-like jellyroll form. The Neutrokine-alpha protein is similar to the other TNF-family ligands in overall structure and dimensions. However, the receptor binding region of Neutrokine-alpha is a more pronounced groove than that observed for other cytokines (Oren, *et al.*, (2002) *Nature Structural Biology* 9:288-292). Neutrokine-alpha polypeptides are described in more detail in, for example, International Publication Numbers WO98/18921, WO00/50597, WO02/1820, and WO03/033658 each of which are herein incorporated by reference in ther entireties.

[0098] As described above, Neutrokine-alpha polypeptides function to stimulate B-cell proliferation, differentiation, survival, and Ig secretion. Thus, one would not expect to use the native form of Neutrokine-alpha in the methods of the present invention. However, the Neutrokine-alpha native form may be used as a targeting agent to bring other agents that can inhibit B-cell activity (e.g., cytotoxic moieties or proteins) in proximity with a B-cell (See, for example, Example 12 and 13 of WO00/033658, wherein radiolabelled Neutrokine-alpha is used to target and kill cells expressing Neutrokine-alpha receptors which are predominantly B-cell in origin.) Alternatively, fragments or variants of Neutrokine-alpha which bind to one or more Neutrokine-alpha receptors but do not induce signalling may be used as a Neutrokine-alpha antagonist. Neutrokine-alpha fragments or variants which affect the ability of Neutrokine-alpha to form or maintain stable homotrimers or heterotrimers may also be used as a Neutrokine-alpha antagonist in the methods of the invention. Thus, Neutrokine-alpha polypeptides that may be used in the methods of the present invention include polypeptide fragments or variants of the Neutrokine-alpha protein of SEQ ID NO:2. The polypeptide fragments or variants may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. In specific embodiments, Neutrokine-alpha polypeptides that may be used in the methods of the invention encompass polypeptide fragments comprising, or alternatively consisting of, the predicted extracellular domain of Neutrokine-alpha (amino acid residues 73-285 of SEQ ID NO:2) and the soluble fragment of Neutrokine-alpha (amino acid residues 134-285 of SEQ ID NO:2). In another embodiment, the polypeptide fragments or variants that may be used in the methods of the invention comprise, or alternatively, consist of, a polypeptide fragment or variant at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide fragments of native Neutrokine-alpha described above.

[0099] In another embodiment, Neutrokine-alpha polypeptide variants that may be used in the methods of the present invention include peptide mimetics. Mimetics are peptide-containing molecules that mimic elements of protein secondary structure. See, for example, Johnson et al., "Peptide Turn Mimetics" in BIOTECHNOLOGY AND PHARMACY, Pezzuto et al., Eds., Chapman and Hall, New York (1993), incorporated herein by reference. The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. These principles may be used to engineer second generation molecules having many of the natural properties of the targeting peptides disclosed herein, but with altered and even improved characteristics.

[0100] APRIL (SEQ ID NO:4) is a member of the TNF family of ligands that shares amino acid sequence identity to Neutrokine-alpha (SEQ ID NO:2; GenBank Accession No. NM_006573; Moore, *et al.*, (1999) *Science* 285:260-263; Schneider *et al.*, (1999) *J. Exp. Med.* 189:1747-1756; and Khare *et al.*, (2000) *Proc. Natl. Acad Sci.* 97:3370-3375), TNFα, and lymphotoxin-α (LTα) (Moore, *et al.*, 1999). The full length APRIL gene encodes a 250 amino acid polypeptide that has an intracellular domain between residues 1 and 28, a transmembrane spanning domain between residues 29 and 49, and an extracellular domain between residues 50 and 250. Like other members of the TNF family, APRIL functions as a trimeric protein. Upon expression of APRIL at the surface of the cell, the extracellular domain is cleaved at amino acid 105 to release a biologically active trimer.

[0101] In a specific embodiment, a APRILpolypeptide that may be used in the methods of the present invention includes polypeptide fragments or variants of the APRIL protein of SEQ ID NO:4. The polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. In specific embodiments, APRIL polypeptides that may be used in the methods of the invention encompass polypeptide fragments comprising, or alternatively consisting of, the predicted extracellular domain of APRIL (amino acid residues 50-250 of SEQ ID NO:4) and the soluble fragment of APRIL (amino acid residues 105-250 of SEQ ID NO:4). In another embodiment, the polypeptide fragments or variants that may be used in the methods of the invention comprise, or alternatively, consist of, a polypeptide fragment or variant at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide fragments of native APRIL described above.

[0102] In another embodiment, APRILpolypeptide variants that may be used in the methods of the present invention include peptide mimetics. Mimetics are peptide-containing molecules that mimic elements of protein secondary structure. See, for example, Johnson et al., "Peptide Turn Mimetics" in BIOTECHNOLOGY AND PHARMACY, Pezzuto et al., Eds., Chapman and Hall, New York (1993), incorporated herein by reference. The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. These principles may be used to engineer second generation molecules having many of the natural properties of the targeting peptides disclosed herein, but with altered and even improved characteristics.

[0103] The Neutrokine-alpha and APRIL polypeptides that may be used in the methods of the invention may be expressed or synthesized in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. Such a fusion protein can be made by ligating Neutrokine-alpha or APRIL polynucleotides and the desired nucleic acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for

instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

[0104] A preferred Neutrokine-alpha or APRIL fusion protein that may be used in the methods of the invention comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) and WO00/024782 disclose fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. Neutrokine-alpha immunoglobulin fusion proteins have been described in, for example, Yu, *et al.*, (2000) *Nat Immunol* 1:252-256, herein incorporated by reference in its entirety. APRIL immunoglobulin fusion proteins have been described in, for example, PCT Publication WO01/087977, herein incorporated by reference in its entirety. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995) and K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[0105] As one of skill in the art will appreciate, and as discussed above, the Neutrokine-alpha and APRIL polypeptides can be fused to other polypeptide sequences. For example, the Neutrokine-alpha polypeptides that may be used in the methods of the current invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides.

[0106] Such fusion proteins may facilitate purification, may extend shelf-life and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other

molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0107] Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form (SEQ ID NO:11), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HA for clinical use is produced by extraction from human blood. The production of recombinant HA (rHA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

[0108] The role of albumin as a carrier molecule and its inert nature are desirable properties for use as a carrier and transporter of polypeptides *in vivo*. The use of albumin as a component of an albumin fusion protein as a carrier for various proteins has been suggested in WO 93/15199, WO 93/15200, and EP 413 622. The use of N-terminal fragments of HA for fusions to polypeptides has also been proposed (EP 399 666). Fusion of albumin to a Therapeutic protein may be achieved by genetic manipulation, such that the DNA coding for HA, or a fragment thereof, is joined to the DNA coding for the Therapeutic protein. A suitable host is then transformed or transfected with the fused nucleotide sequences, so arranged on a suitable plasmid as to express a fusion polypeptide. The expression may be effected *in vitro* from, for example, prokaryotic or eukaryotic cells, or *in vivo e.g.*, from a transgenic organism.

[0109] An albumin fusion protein that may be used in methods of the present invention comprises at least a fragment or variant of a Neutrokine-alpha polypeptide and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of Neutrokine-alpha is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The Neutrokine-alpha polypeptide and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "Neutrokine-alpha portion" or an "albumin protein portion").

[0110] In one embodiment, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a Neutrokine-alpha polypeptide and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment of Neutrokine-alpha and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a variant of Neutrokine-alpha and a serum albumin protein In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0111] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a Neutrokine-alpha polypeptide and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a Neutrokine-alpha polypeptide and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Neutrokine-alpha portion of the albumin fusion protein is the full-length Neutrokine-alpha

polypeptide. In a further preferred embodiment, the Neutrokine-alpha protein portion of the albumin fusion protein is the mature, soluble domain of the Neutrokine-alpha polypeptide.

[0112] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of Neutrokine-alpha and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the Neutrokine-alpha polypeptide and the mature portion of serum albumin. (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, Neutrokine-alpha polypeptides (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-x of human serum albumin, where x is an integer from 1 to 585 and the albumin fragment has human serum albumin activity. In another preferred embodiment Neutrokine-alpha polypeptides (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Neutrokine-alpha polypeptides (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

[0113] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins that may be used in the methods of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:11: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins that may be used in the methods of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0114] Preferably, the albumin fusion protein that may be used in the methods of the invention comprises HA as the N-terminal portion, and Neutrokine-alpha polypeptide as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and Neutrokine-alpha polypeptide as the N-terminal portion may also be used.

[0115] In other embodiments, the albumin fusion protein that may be used in methods of the invention has a Neutrokine-alpha polypeptide fused to both the N-terminus and the C-terminus of albumin. In a specific embodiment, the Neutrokine-alpha polypeptides fused at the N- and C- termini are the same. In another embodiment, the Neutrokine-alpha polypeptides fused at the N- and C- termini are different

Neutrokine-alpha polypeptides. In another embodiment, a Neutrokine-alpha polypeptide is fused at either the N- or C- terminus of albumin, and a heterologous polypeptide is fused at the remaining terminus.

[0116] Additionally, the albumin fusion proteins that may be used in the methods of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0117] Generally, the albumin fusion proteins that may be used in the methods of the invention may have one HA-derived region and one Neutrokine-alpha region. Multiple regions of each protein, however, may be used to make an albumin fusion protein that may be used in the methods of the invention. Similarly, more than one protein may be used to make an albumin fusion protein that may be used in the methods of the invention. For instance, a protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the protein portions may be the same or different protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0118] In a specific embodiment, Neutrokine-alpha protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a cytotoxin (e.g., a cytostatic or cytocidal agent). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0119] In another embodiment, a Neutrokine-alpha protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a toxin.

[0120] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium.

[0121] In an additional example, the APRIL polypeptides that may be used in the methods of the current invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides.

[0122] Such fusion proteins may facilitate purification, may extend shelf-life and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0123] An albumin fusion protein that may be used in methods of the present invention comprises at least a fragment or variant of an APRIL polypeptide and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of APRIL is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The APRIL polypeptide and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., an "APRIL portion" or an "albumin protein portion").

[0124] In one embodiment, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, an APRIL polypeptide and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment of APRIL and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a variant of APRIL and a serum albumin protein In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0125] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, an APRIL polypeptide and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, an APRIL polypeptide and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the APRIL portion of the albumin fusion protein is the full-length APRIL polypeptide. In a further preferred embodiment, the APRIL portion of the albumin fusion protein is the mature, soluble domain of the APRIL polypeptide.

[0126] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of APRIL and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the APRIL polypeptide and the mature portion of serum albumin.

[0127] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of APRIL and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the APRIL polypeptide and the mature portion of serum albumin. (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, APRIL polypeptides (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-x of human serum albumin, where x is an integer from 1 to 585 and the albumin fragment has human serum albumin activity. In another preferred embodiment APRIL polypeptides (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. APRIL polypeptides (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

[0128] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins that may be used in the methods of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:11: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins that may be used in the methods of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0129] Preferably, the albumin fusion protein that may be used in the methods of the invention comprises HA as the N-terminal portion, and APRIL polypeptide as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and APRIL polypeptide as the N-terminal portion may also be used.

[0130] In other embodiments, the albumin fusion protein that may be used in methods of the invention has an APRIL polypeptide fused to both the N-terminus and the C-terminus of albumin. In a specific embodiment, the APRIL polypeptides fused at the N- and C- termini are the same. In another embodiment, the APRIL polypeptides fused at the N- and C- termini are different APRIL polypeptides. In another embodiment, a APRIL polypeptide is fused at either the N- or C- terminus of albumin, and a heterologous polypeptide is fused at the remaining terminus.

[0131] Additionally, the albumin fusion proteins that may be used in the methods of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0132] Generally, the albumin fusion proteins that may be used in the methods of the invention may have one HA-derived region and one APRIL region. Multiple regions of each protein, however, may be used to make an albumin fusion protein that may be used in the methods of the invention. Similarly, more than one protein may be used to make an albumin fusion protein that may be used in the methods of the invention. For instance, a protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the protein portions may be the same or different protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0133] In a specific embodiment, an an APRIL protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a cytotoxin (e.g., a cytostatic or cytocidal agent). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof..

[0134] In another embodiment, an APRIL protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a toxin.

[0135] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium.

[0136] Protein engineering may be employed to alter the characteristics of Neutrokine-alpha and APRIL polypeptides to generate polypeptides that may be used in the methods of the invention,. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity, decreased activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. For instance, for many proteins, including the extracellular domain or the mature form(s) of a secreted protein, it is known in the art that one

or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function. For instance, Ron et al., J. Biol. Chem., 268:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

[0137] The Neutrokine-alpha and APRIL polypeptides that may be used in the methods of the present invention may be monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). In specific embodiments, the Neutrokine-alpha and APRIL polypeptides that may be used in the methods of the invention may be homomers or heteromers. A Neutrokine-alpha homomer, refers to a multimer containing only Neutrokine-alpha polypeptides (including Neutrokine-alpha fragments, variants, and fusion proteins, as described herein). These homomers may contain Neutrokine-alpha polypeptides having identical or different amino acid sequences. In specific embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the present invention are Neutrokine-alpha homodimers (e.g., containing two Neutrokine-alpha polypeptides having identical or different amino acid sequences) or are Neutrokine-alpha homotrimers (e.g., containing three Neutrokine-alpha polypeptides having identical or different amino acid sequences). In a preferred embodiment, the Neutrokine-alpha polypeptides that may be used in the methods of the invention are homotrimers of Neutrokine-alpha. In additional embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer. An APRIL homomer, refers to a multimer containing only APRIL polypeptides (including APRIL fragments, variants, and fusion proteins, as described herein). These homomers may contain APRIL polypeptides having identical or different amino acid sequences. In specific embodiments, the APRIL polypeptide that may be used in the methods of the present invention are APRIL homodimers (e.g., containing two APRIL polypeptides having identical or different amino acid sequences) or are APRIL homotrimers (e.g., containing three APRIL polypeptides having identical or different amino acid sequences). In a preferred embodiment, the APRIL polypeptides that may be used in the methods of the invention are homotrimers of APRIL. In additional embodiments, the APRIL polypeptide that may be used in the methods of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[0138] Heteromeric Neutrokine-alpha refers to a multimer containing heterologous polypeptides (i.e., polypeptides of a different protein) in addition to Neutrokine-alpha polypeptides. In a specific embodiment, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is a multimer which is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer. Heteromeric APRIL refers to a multimer containing heterologous polypeptides (i.e., polypeptides of a different protein) in addition to APRIL polypeptides. In a specific embodiment, the APRIL polypeptide that may be used in the methods of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the APRIL polypeptide that may be used in the methods of the invention is a multimer which is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer. In additional embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is a heterotrimer comprising both Neutrokine-alpha polypeptides and APRIL polypeptides or

fragments or variants thereof. In additional embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is a heterotrimer comprising one Neutrokine-alpha polypeptide (including fragments or variants) and two APRIL polypeptides (including fragments or variants). In additional embodiments, the Neutrokine-alpha polypeptide that may be used in the methods of the invention is a heterotrimer comprising two Neutrokine-alpha polypeptides (including fragments or variants) and one APRIL polypeptide (including fragments or variants).

[0139] In additional embodiments, the Neutrokine-alpha polypeptides that may be used in the methods of the invention are homomeric, especially homotrimeric, Neutrokine-alpha polypeptides, wherein the individual protein components of the multimers comprise, or alternatively, consist of the mature form of Neutrokine-alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2) or fragments or variants thereof. In other specific embodiments, the Neutrokine-alpha polypeptides that may be used in the methods of the invention are heteromeric, especially heterotrimeric, Neutrokine-alpha polypeptides such as a heterotrimer containing two Neutrokine-alpha polypeptides and one APRIL polypeptide or a heterotrimer containing one Neutrokine-alpha polypeptide and two APRIL polypeptides, and wherein the individual protein components of the Neutrokine-alpha heteromer comprise, or alternatively, consist of either the mature extracellular soluble portion of Neutrokine-alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2) or fragments or variants thereof, or the mature extracellular soluble portion APRIL (e.g., amino acid residues 105-250 of SEQ ID NO:4) or fragments or variants thereof.

[0140] In additional embodiments, the APRIL polypeptides that may be used in the methods of the invention are homomeric, especially homotrimeric, APRIL polypeptides, wherein the individual protein components of the multimers comprise, or alternatively, consist of the mature form of APRIL (e.g., amino acids residues 105-250 of SEQ ID NO:4) or fragments or variants thereof. In other specific embodiments, the APRIL polypeptides that may be used in the methods of the invention are heteromeric, especially heterotrimeric, APRIL polypeptides such as a heterotrimer containing two APRIL polypeptides and one Neutrokine-alpha polypeptide or a heterotrimer containing one APRIL polypeptide and two Neutrokine-alpha polypeptides, and wherein the individual protein components of the APRIL heteromer comprise, or alternatively, consist of either the mature extracellular soluble portion of APRIL (e.g., amino acid residues 105-250 of SEQ ID NO:4) or fragments or variants thereof, or the mature extracellular soluble portion of Neutrokine-alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2) or fragments or variants thereof.

[0141] Multimers that may be used in the methods of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers, such as, for example, homodimers or homotrimers, are formed when the polypeptides contact one another in solution. In another embodiment, heteromultimers, such as, for example, heterotrimers or heterotetramers, are formed when the polypeptides contact one another in solution. In other embodiments, multimers are formed by covalent associations with and/or between the Neutrokine-alpha polypeptides. In other embodiments, multimers are formed by covalent associations with and/or between the APRIL polypeptides. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:2 for

Neutrokine-alpha or that recited in SEQ ID NO:4 for APRIL). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a Neutrokine-alpha or APRIL fusion protein (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Neutrokine-alpha-Fc fusion protein (as described herein). In a specific example, the covalent associations are between the heterologous sequence contained in an APRIL-Fc fusion protein (as described herein). In another specific example, covalent associations in the fusion proteins that may be used in the methods of the invention are between heterologous polypeptide sequence from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more Neutrokine-alpha polypeptides and/or APRIL polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple Neutrokine-alpha polypeptides and/or APRIL polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0142] In a specific embodiment, a Neutrokine-alpha antagonist that may be used in the methods of the invention is a dominant negative form of Neutrokine-alpha and/or APRIL. In particular, variants of Neutrokine-alpha and/or APRIL, including dominant negative forms, have been described in, for example, International Patent Publication numbers WO06/034106, WO05/113598, WO04/089982, WO04/081043 and WO03/057856 and US Patent Publication numbers US20060014248, US20050221443, US20050130892, US20050048626, US2005003480 and US20030166559. Each of the aforementioned references is herein incorporated by reference in its entirety. Such Neutrokine-alpha and/or APRIL polypeptide variants may antagonize Neutrokine-alpha function, for example, by interfering with Neutrokine-alpha and/or APRIL homo- or hetero-multimerization. Alternatively, Neutrokine-alpha and/or APRIL polypeptide variants may prevent polypeptides comprising them from binding to and/or signaling through Neutrokine alpha–receptors such as TACI, BCMA and BAFF-R.

[0143] In another embodiment, the Neutrokine-alpha antagonist is the Neutorkine-alpha protein mutant dsescribed in Gao *et a.l,* (2006) *Biotechnol. Lett.* 28:1649-54, which is herein incorporated by reference in its entirety.

[0144] In another embodiment, the Neutrokine-alpha antagonist that may be used in the methods of the invention is Δ BAFF (SEQ ID NO:12).

B. Anti-Neutrokine-alpha Antibodies

[0145] In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody or antigen-binding fragment thereof. Anti-Neutrokine-alpha antibodies and fragments thereof have

been described in, for example, PCT Publications WO01/087977, WO03/016468, WO01/60397, WO02/02641 and WO03/55979; US Publication Nos. 2005/0070694 and 2005/0255532; and Cao *et al.*, (2005) *Immunol Lett* 101:87-94; Ch' en *et al.*, (2005) *Cell Immunol* 236:78-85; Liu *et al.*, (2005) *Acta Biochim Biophys Sin (Shanghai)* 37:415-420; Schneider *et al.*, (1999) *J Exp Med* 189:1747-1756; Sun *et al.*, (2006) *Hybridoma* 25:80-85; Sun *et al.*, (2006) *Hybridoma* 25:238-242; and are described in more detail below. Each of the aforementioned references is herein incorporated by reference in its entirety.

[0146] The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. Examples of molecules which are described by the term "antibody" in this application include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, $F(ab')_2$, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term "single chain Fv" or "scFv" as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody. Antibodies that immunospecifically bind to a particular antigen (e.g. Neutrokine-alpha) may have cross-reactivity with other antigens. Preferably, antibodies that immunospecifically bind to a particular antigen do not cross-react with other antigens. Antibodies that immunospecifically bind to a particular antigen can be identified, for example, by immunoassays or other techniques known to those of skill in the art, *e.g.*, the immunoassays described in U.S. Patent Application No. 60/834,152, filed July 31, 2006, which is hereby incorporated by reference in its entirety.

[0147] Antibodies that may be used in the methods of the present invention include, but are not limited to, monoclonal, multispecific, human or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, antiidiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule.

[0148] Antibodies that may be used in the methods of the present invention may also include multimeric forms of antibodies. For example, antibodies that may be used in the methods of the present invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole immunoglobulin molecules or of $F(ab')_2$ fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers within an antibody multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities. Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC [succinimidyl 4-

(maleimidomethyl)cyclohexane-1-carboxylate] and SATA [*N*-succinimidyl *S*-acethylthio-acetate] (available, for example, from Pierce Biotechnology, Inc. (Rockford, IL)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al., *Proceedings of the National Academy of Sciences USA* (1997) 94:7509-7514, which is hereby incorporated by reference in its entirety. Antibody homodimers can be converted to Fab'2 homodimers through digestion with pepsin. Another way to form antibody homodimers is through the use of the autophilic T15 peptide described in Zhao and Kohler, *The Journal of Immunology* (2002) 25:396-404, which is hereby incorporated by reference in its entirety.

[0149] Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the J chain polypeptide. Non-IgA or non-IgM molecules, such as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules. (see, for example, Chintalacharuvu et al., (2001) *Clinical Immunology* 101:21-31. and Frigerio et al., (2000) *Plant Physiology* 123:1483-94., both of which are hereby incorporated by reference in their entireties.) ScFv dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al., (2000) *Cancer Research* 60:6964-6971 which is hereby incorporated by reference in its entirety. Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

[0150] Unless otherwise defined in the specification, specific binding or immunospecific binding by an antibody means that the antibody binds the target antigen but does not significantly bind to (i.e., cross react with) proteins other than the target antigen, such as other proteins in the same family of proteins (e.g., other TNF family ligands). An antibody that binds a target antigen and does not cross-react with other proteins is not necessarily an antibody that does not bind said other proteins in all conditions; rather, the target antigen-specific antibody preferentially binds the target antigen compared to its ability to bind said other proteins such that it will be suitable for use in at least one type of assay or treatment, i.e., give low background levels or result in no unreasonable adverse effects in treatment. It is well known that the portion of a protein bound by an antibody is known as the epitope. An epitope may either be linear (i.e., comprised of sequential amino acids residues in a protein sequences) or conformational (i.e., comprised of one or more amino acid residues that are not contiguous in the primary structure of the protein but that are brought together by the secondary, tertiary or quaternary structure of a protein). Given that target antigen-specific antibodies bind to epitopes of the target antigen, an antibody that specifically binds the target antigen may or may not bind fragments of the target antigen and/or variants of the target antigen (e.g., proteins that are at least 90% identical to the target antigen) depending on the presence or absence of the epitope bound by a given target antigen-specific antibody in the target antigen fragment or variant. Likewise, target antigen-specific antibodies may bind species orthologues of the target antigen (including fragments thereof) depending on the presence or absence of the epitope recognized by the antibody in the orthologue. Additionally, target antigen-specific antibodies may bind modified forms of the target antigen, for example, target antigen fusion proteins. In such a case when antibodies bind target antigen fusion proteins, the

antibody must make binding contact with the target antigen moiety of the fusion protein in order for the binding to be specific.

[0151] Antibodies that specifically bind to any particular target antigen can be identified, for example, by immunoassays or other techniques known to those of skill in the art, *e.g.*, the immunoassays described in U.S. Patent Application No. 60/834,152, filed July 31, 2006, which is hereby incorporated by reference in its entirety. Antibodies that may be used in the methods of the present invention may be "specific" for Neutrokine-alpha, but it is not a requirement. Anti-Neutrokine-alpha antibodies that may be used in the methods of the invention may be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a Neutrokine-alpha polypeptide may be used in the methods of the invention. In a specific embodiment, antibodies that may be used in the methods of the invention cross react with APRIL. In specific embodiments, antibodies that may be used in the methods of the invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof.

[0152] In a specific embodiment, antibodies that bind to a Neutrokine-alpha polypeptide, polypeptide fragment, or variant of SEQ ID NO:2, and/or an Neutrokine-alpha epitope (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding) may be used in the methods of the invention.. In a specific embodiment, antibodies that may be used in the methods of the invention may bind Neutrokine-alpha polypeptides fused to other polypeptide sequences. For example, Neutrokine-alpha polypeptides may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0153] In another embodiment, antibodies that may be used in the methods of the invention bind mutant Neutrokine-alpha polypeptides that have been generated by random mutagenesis of a polynucleotide encoding the Neutrokine-alpha polypeptide, by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, antibodies that may be used in the methods of the invention bind one or more components, motifs, sections, parts, domains, fragments, etc., of Neutrokine-alpha recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or

more heterologous molecules. In preferred embodiments, the heterologous molecules are, for example, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), OPG, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4 IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202),312C2 (International Publication No. WO 98/06842), TR12, CAD, and v-FLIP. In further embodiments, the heterologous molecules are any member of the TNF family.

[0154] In specific embodiments, antibodies that may be used in the methods of the invention bind homomeric, especially homotrimeric, Neutrokine-alpha polypeptides. In other specific embodiments, antibodies that may be used in the methods of the invention bind heteromeric, especially heterotrimeric, Neutrokine-alpha polypeptides such as a heterotrimer containing two Neutrokine-alpha polypeptides and one APRIL polypeptide or a heterotrimer containing one Neutrokine-alpha polypeptide and two APRIL polypeptides. In a specific embodiment, the antibodies that may be used in the methods of the invention bind homomeric, especially homotrimeric, Neutrokine-alpha polypeptides, wherein the individual protein components of the multimers consist of the mature form of Neutrokine-alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2). In other specific embodiments, antibodies that may be used in the methods of the invention bind heteromeric, especially heterotrimeric, Neutrokine-alpha polypeptides such as a heterotrimer containing two Neutrokine-alpha polypeptides and one APRIL polypeptide or a heterotrimer containing one Neutrokine-alpha polypeptide and two APRIL polypeptides, and wherein the individual protein components of the Neutrokine-alpha heteromer consist of either the mature extracellular soluble portion of Neutrokine-alpha (e.g., amino acids residues 134-285 of SEQ ID NO:2) or the mature extracellular soluble portion APRIL (e.g., amino acid residues 105-250 of SEQ ID NO:4).

[0155] In specific embodiments, the antibodies that may be used in the methods of the invention bind conformational epitopes of a Neutrokine-alpha monomeric protein. In specific embodiments, the antibodies that may be used in the methods of the invention bind conformational epitopes of a Neutrokine-alpha multimeric, especially trimeric, protein. In other embodiments, antibodies that may be used in the methods of the invention bind conformational epitopes that arise from the juxtaposition of Neutrokine-alpha with a heterologous polypeptide, such as might be present when Neutrokine-alpha forms heterotrimers (e.g., with APRIL polypeptides), or in fusion proteins between Neutrokine-alpha and a heterologous polypeptide.

[0156] Antibodies that may be used in the methods of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id)

antibodies (including, e.g., anti-id antibodies to anti-Neutrokine-alpha antibodies), and epitope-binding fragments of any of the above. In preferred embodiments, the immunoglobulin is an IgG1 or an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

[0157] In a specific embodiment, the antibodies that may be used in the methods of the invention are Neutrokine-alpha-binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Neutrokine-alpha-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. In a specific embodiment, Neutrokine-alpha-binding fragments that may be used in the methods of the invention comprise any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies that may be used in the methods of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati et al, the contents of which are herein incorporated by reference in its entirety.

[0158] The antibodies that may be used in the methods of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a Neutrokine-alpha polypeptide or may be specific for both a Neutrokine-alpha polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0159] Antibodies that may be used in the methods of the invention may be described or specified in terms of their binding affinity to a Neutrokine-alpha polypeptide. In specific embodiments, antibodies that may be used in the methods of the invention bind Neutrokine-alpha polypeptides, or fragments or variants thereof, with a dissociation constant or K_D of less than or equal to 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, or 10^{-12} M. In a specific embodiment, antibodies that may be used in the methods of the invention bind Neutrokine-alpha polypeptides with a dissociation constant or K_D that is within any one of the ranges that are between each of the individual recited values.

[0160] Neutrokine-alpha antibodies which disrupt the receptor/ligand interactions with Neutrokine-alpha polypeptides either partially or fully may be used in the methods of the invention. Also included are Neutrokine-alpha-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting activation of the transcription

factors NF-AT, AP-1, MAPK8/JNK and/or NF-kappaB (including the noncanonical NF-kappaB signaling pathway) using techniques known in the art, and/or the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis.

[0161] The above Neutrokine-alpha antibodies can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[0162] In a specific embodiment, Neutrokine-alpha antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that may be used in the methods of the invention specifically bind to Neutrokine-alpha or a fragment or variant of Neutrokine-alpha. In particular, antibodies such as, for example, single chain Fvs (scFvs) having an amino acid sequence of any one of SEQ ID NOS:13 - 18, as referred to in Table 1 may be used in the methods of the invention.

Table 1: scFvs that Immunospecifically Bind to Neutrokine-alpha

Clone ID	scFv SEQ ID NO	AAs of VL	AAs of VL CDR1	AAs of VL CDR2	AAs of VL CDR3	AAs of VH	AAs of VH CDR1	AAs of VH CDR2	AAs of VH CDR3
I006D08	13	141 - 249	163 - 173	189 - 195	228 - 238	1 - 123	26 - 35	50 - 66	99 - 112
I050B11	14	143 - 251	166 - 177	193 - 199	232 - 240	1 - 125	26 - 35	50 - 66	99 - 114
I050A12	15	142 - 250	164 - 174	190 - 196	229 - 239	1 - 124	26 - 35	50 - 66	99 - 113
I050B11-15	16	143 - 251	166 - 177	193 - 199	232 - 240	1 - 125	26 - 35	50 - 66	99 - 114
I116A01	17	141 - 249	163 - 173	189 - 195	228 - 238	1 - 123	26 - 35	50 - 66	99 - 112
I026C04-K	18	142 - 250	164 - 176	192 - 198	231 - 239	1 - 125	26 - 35	50 - 66	99 - 114

[0163] In one embodiment of the present invention, antibodies that may be used in the methods of the invention bind to Neutrokine-alpha and comprise a polypeptide having the amino acid sequence of any one of the VH domains referred to in Table 1 and/or any one of the VL domains referred to in Table 1. In preferred embodiments, antibodies that may be used in the methods of the invention comprise the amino acid sequence of a VH domain and VL domain from the same scFv referred to in Table 1. In alternative embodiments, antibodies that may be used in the methods of the invention comprise the amino acid sequence of a VH domain and VL domain from different scFvs referred to in Table 1. In another embodiment, antibodies that may be used in the methods of the invention specifically bind to Neutrokine-alpha and comprise a polypeptide having the amino acid sequence of any one, two, three, or more of the VH CDRs referred to in Table 1 and/or any one, two, three, or more of the VL CDRs referred to in Table 1. In preferred embodiments, antibodies that may be used in the methods of the invention comprise the amino acid sequence of a VH CDR and VL CDR from the same scFv referred to in Table 1. In alternative embodiments, antibodies that may be used in the methods of the invention comprise the amino acid sequence of a VH CDR and VL CDR from different scFvs referred to in Table 1. Molecules comprising, or

alternatively consisting of, antibody fragments or variants of the scFvs referred to in Table 1 that immunospecifically bind to Neutrokine-alpha may also be used in the methods of the invention.

[0164] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:13, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:13, as described in Table 1.

[0165] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:14, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:13, as described in Table 1.

[0166] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:13, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:14, as described in Table 1.

[0167] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:15, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:15, as described in Table 1.

[0168] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:16, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:16, as described in Table 1.

[0169] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:17, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:17, as described in Table 1.

[0170] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of SEQ ID NO:18, as described in Table 1. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of SEQ ID NO:18, as described in Table 1.

[0171] In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of 15C10, a neutralizing anti-Neutrokine-alpha antibody which is described in, for example, US patent publication No. 20050186637. The amino acid sequence of the VH domain of 15C10 is given in SEQ ID NO:19. The amino acid sequence of the VL domain is given in VH domain of 15C10 is given in SEQ ID NO:20. In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention is an antigen binding fragment or variant of 15C10. In a specific embodiment, an antibody that may be used in the methods of the present invention is an humanized version of 15C10. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of 15C10.

[0172] In another specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention comprises the VH and VL domains of the 4A5-3.1.1-B4 anti-Neutrokine-alpha antibody described in International Patent Publication Number WO03/0164468, which is herein incorporated by reference in its entirety. Neutrokine-alpha is referred to as hTNFSF13b in WO03/0164468. The amino acid sequence of the VH domain of 4A5-3.1.1-B4 is given in SEQ ID NO:21. The amino acid sequence of the VL domain is given in VH domain of 4A5-3.1.1-B4 is given in SEQ ID NO:22. In a specific embodiment, an anti-Neutrokine-alpha antibody that may be used in the methods of the invention is an antigen binding fragment or variant of 4A5-3.1.1-B4. In another specific embodiment, an antibody that may be used in the methods of the present invention comprises the VHCDR1, VHCDR2, VHCDR3 and VL CDR1, VLCDR2 and VLCDR3 regions of 4A5-3.1.1-B4.

[0173] In a specific embodiment, Neutrokine-alpha antibodies that may be used in the methods of the invention specifically bind to native Neutrokine-alpha polypeptide expressed from a cell.

C. Neutrokine-alpha Binding Polypeptides

[0174] In a specific embodiment, the Neutrokine-alpha antagonist is a Neutrokine-alpha binding peptide or polypeptide. Neutrokine-alpha binding peptides or polypeptides have been described in, for example International Patent Publication numbers WO05/005462, WO05/000351, WO02/092620, WO02/16412, WO02/02641 and WO02/16411 and US Patent Publication numbers US2006135430, US2006084608, US2003194743, US20030195156 and US2003091565, each of which is herein incorporated by reference in its entirety. Neutrokine-alpha binding peptides or polypeptides have been described in, for example Sun *et al.*, (2006) *Biochem. Biophys. Res. Commun.* 346:1158-1162 which is herein incorporated by reference in its entirety. Neutrokine-alpha binding peptides that may be used in the methods of the present invention include short polypeptides identified from random peptide sequences displayed by fusion with coat proteins of filamentous phage. For discussion of phage display peptide library technology see, for example, Scott et al. (1990), Science 249: 386; Devlin et al. (1990), Science 249: 404; U.S. Pat. No. 5,223,409, issued Jun. 29, 1993; U.S. Pat. No. 5,733,731, issued Mar. 31, 1998; U.S. Pat. No. 5,498,530, issued Mar. 12, 1996; U.S. Pat. No. 5,432,018, issued Jul. 11, 1995; U.S. Pat. No. 5,338,665, issued Aug. 16, 1994; U.S. Pat. No. 5,922,545, issued Jul. 13, 1999; WO 96/40987, published Dec. 19, 1996; and WO

98/15833, published Apr. 16, 1998 (each of which is incorporated by reference in its entirety). Phage expressing the peptides are isolated by successive rounds of affinity purification against an immobilized Neutrokine-alpha target peptide followed by repropagation. The candidates with the highest binding to Neutrokine-alpha can be sequenced to determine the identity of each binding peptide. Each identified Neutrokine-alpha binding peptide may then be attached to a "vehicle" to generate a further Neutrokine-alpha binding peptide for use in the methods of the present experiment. The term "vehicle" refers to a molecule that prevents degradation and/or increases half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of a Neutrokine-alpha binding peptide. Exemplary vehicles include an Fc domain and variants thereof (a "Peptibody" which is preferred); a linear polymer (e.g., polyethylene glycol (PEG), including 5 kD, 20 kD, and 30 kD PEG, polylysine, dextran, etc.); a branched-chain polymer (see, for example, U.S. Pat. No. 4,289,872 to Denkenwalter et al., issued Sep. 15, 1981; 5,229,490 to Tam, issued Jul. 20, 1993; WO 93/21259 by Frechet et al., published Oct. 28, 1993); a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide (e.g., dextran); any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor; albumin, including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety); and a leucine_zipper domain, and other such proteins and protein fragments. Neutrokine-alpha binding polypeptides that may be used in the methods of the invention require the presence of at least one vehicle attached to the peptide through the N-terminus, C-terminus or a sidechain of one of the amino acid residues. Multiple vehicles may also be used; e.g., Fc's at each terminus or an Fc at a terminus and a PEG group at the other terminus or a sidechain. For Neutrokine-alpha binding peptides an Fc domain is the preferred vehicle. The Fc domain may be fused to the N or C termini of the peptides or at both the N and C termini. Fusion to the N terminus is preferred.

[0175] As noted above, Fc variants are suitable vehicles for Neutrokine-alpha binding peptides that may be used in the methods of the invention. A native Fc may be extensively modified to form an Fc variant, provided binding to the salvage receptor is maintained; see, for example WO 97/34631 and WO 96/32478. In such Fc variants, one may remove one or more sites of a native Fc that provide structural features or functional activity not required by the Neutrokine-alpha binding peptides that may be used in the methods of the invention. One may remove these sites by, for example, substituting or deleting residues, inserting residues into the site, or truncating portions containing the site. The inserted or substituted residues may also be altered amino acids, such as peptidomimetics or D-amino acids. Fc variants may be desirable for a number of reasons, several of which are described below. Exemplary Fc variants include molecules and sequences in which:

[0176] 1. Sites involved in disulfide bond formation are removed. Such removal may avoid reaction with other cysteine-containing proteins present in the host cell used to produce the molecules of the invention. For this purpose, the cysteine-containing segment at the N-terminus may be truncated or cysteine residues may be deleted or substituted with other amino acids (e.g., alanyl, seryl). Even when cysteine

residues are removed, the single chain Fc domains can still form a dimeric Fc domain that is held together non-covalently.

[0177] 2. A native Fc is modified to make it more compatible with a selected host cell. For example, one may remove the PA sequence near the N-terminus of a typical native Fc, which may be recognized by a digestive enzyme in E. coli such as proline iminopeptidase. One may also add an N-terminal methionine residue, especially when the molecule is expressed recombinantly in a bacterial cell such as E. coli.

[0178] 3. A portion of the N-terminus of a native Fc is removed to prevent N-terminal heterogeneity when expressed in a selected host cell. For this purpose, one may delete any of the first 20 amino acid residues at the N-terminus.

[0179] 4. One or more glycosylation sites are removed. Residues that are typically glycosylated (e.g., asparagine) may confer cytolytic response. Such residues may be deleted or substituted with unglycosylated residues (e.g., alanine).

[0180] 5. Sites involved in interaction with complement, such as the C1q binding site, are removed. For example, one may delete or substitute the EKK sequence of human IgG1. Complement recruitment may not be advantageous for the molecules that may be used in the methods of the invention and so may be avoided with such an Fc variant.

[0181] 6. Sites are removed that affect binding to Fc receptors other than a salvage receptor. A native Fc may have sites for interaction with certain white blood cells that are not required for the Neutrokine-alpha binding peptide fusion molecules that may be used in the methods of the invention and so may be removed.

[0182] 7. The ADCC site is removed. ADCC sites are known in the art; see, for example, Molec. Immunol. 29 (5): 633-9 (1992) with regard to ADCC sites in IgG1. These sites, as well, are not required for the fusion molecules that may be used in the methods of the invention and so may be removed.

[0183] 8. When the native Fc is derived from a non-human antibody, the native Fc may be humanized. Typically, to humanize a native Fc, one will substitute selected residues in the non-human native Fc with residues that are normally found in human native Fc. Techniques for antibody humanization are well known in the art.

[0184] An alternative vehicle for Neutrokine-alpha binding peptides that may be used in the methods of the invention would be a protein, polypeptide, peptide, antibody, antibody fragment, or small molecule (e.g., a peptidomimetic compound) capable of binding to a salvage receptor. For example, one could use as a vehicle a polypeptide as described in U.S. Pat. No. 5,739,277. Peptides could also be selected by phage display or RNA-peptide screening for binding to the salvage receptor. Such salvage receptor-binding compounds are also included within the meaning of "vehicle" and may be used in the for Neutrokine-alpha binding peptides that may be used in the methods of the invention. Such vehicles should be selected for increased half-life (e.g., by avoiding sequences recognized by proteases) and decreased immunogenicity (e.g., by favoring non-immunogenic sequences, as discovered in antibody humanization).

[0185] As noted above, polymer vehicles may also be used in Neutrokine-alpha binding peptides that may be used in the methods of the invention. Various means for attaching chemical moieties useful as vehicles are currently available, see, e.g., Patent Cooperation Treaty ("PCT") International Publication No.

WO 96/11953, herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water soluble polymers to the N-terminus of proteins.

[0186] In a specific embodiment, a preferred polymer vehicle is polyethylene glycol (PEG). The PEG group may be of any convenient molecular weight and may be linear or branched. The average molecular weight of the PEG will preferably range from about 2 kiloDalton ("kD") to about 100 kD, more preferably from about 5 kD to about 50 kD, most preferably from about 5 kD to about 10 kD. The PEG groups will generally be attached to the for Neutrokine-alpha binding peptides that may be used in the methods of the invention via acylation or reductive alkylation through a reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

[0187] A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are "preactivated" with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

[0188] Polysaccharide polymers are another type of water soluble polymer which may be used for Neutrokine-alpha binding peptides that may be used in the methods of the invention. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by α1-6 linkages. The dextran itself is available in many molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water soluble polymer for use in Neutrokine-alpha binding peptides that may be used in the methods of the invention as a vehicle by itself or in combination with another vehicle (e.g., Fc). See, for example, WO 96/11953 and WO 96/05309. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported; see, for example, European Patent Publication No. 0 315 456, which is hereby incorporated by reference in its entirety. Dextran of about 1 kD to about 20 kD is preferred when dextran is used as a vehicle in accordance with the present invention.

[0189] In a specific embodiment, Neutrokine-alpha binding peptides that may be used in the methods of the invention optionally include a "linker". When present, its chemical structure is not critical, since it serves primarily as a spacer. The linker is preferably made up of amino acids linked together by peptide bonds. Thus, in preferred embodiments, the linker is made up of from 1 to 30 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. Some of these amino acids may be glycosylated, as is well understood by those in the art. In a more preferred embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Even more preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Thus, preferred linkers are polyglycines (particularly $(Gly)_4$, $(Gly)_5$), poly(Gly-Ala),

and polyalanines. Preferred linkers are amino acid linkers comprising greater than 5 amino acids, with suitable linkers having up to about 500 amino acids selected from glycine, alanine, proline, asparagine, glutamine, lysine, threonine, serine or aspartate. Linkers of about 20 to 50 amino acids are most preferred.

[0190] Non-peptide linkers are also useful for Neutrokine-alpha binding peptides that may be used in the methods of the invention. For example, alkyl linkers such as --NH--$(CH_2)_n$--C(O)--, wherein n=2-20 could be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1--C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2, phenyl, etc.

[0191] In preferred embodiments, the Neutrokine-alpha binding peptides that may be used in the methods of the invention include the amino acid sequence of SEQ ID NO:23, the amino acid sequence of SEQ ID NO:24 or the amino acid sequence of SEQ ID NO:25. In a particularly preferred embodiment, the Neutrokine-alpha binding peptide that may be used in the methods of the invention is the amino acid sequence of SEQ ID NO:23 (AMG 623; AGP3 peptibody).

Neutrokine-alpha Receptors

[0192] In a specific embodiment, the Neutrokine-alpha antagonist is a Neutrokine-alpha receptor protein or fragment or variant thereof. Neutrokine-alpha receptors include, e.g., transmembrane activator and CAML interactor (TACI, GenBank accession number AAC51790, SEQ ID NO:6), B cell activating factor receptor (BAFF-R, GenBank Accession Number NP_443177 SEQ ID NO:10), and B-cell maturation antigen (BCMA, GenBank accession number NP_001183 SEQ ID NO:8).. Neutrokine-alpha receptor proteins, fragments and variants thereof, as well as antibodies there to have been described in, for example, PCT Publications WO03/014294, WO02/066516, WO02/024909 WO03/014294, WO03/024991, WO02/094852 and WO04/011611 and U.S. Patent Publication Nos. US20030148445, US20030099990, US2005070689, US2005043516 and US2003012783, and are described in more detail below. Each of the aforementioned references is herein incorporated by reference in its entirety.

D. Neutrokine-alpha Receptors, TACI

[0193] TACI polypeptides, such as those described below, act as Neutrokine-alpha and/or APRIL antagonists and may also be used in the methods of the present invention. TACI, also known as TR17, is a protein of 293 amino acid residues (SEQ ID NO:6), with a deduced molecular weight of about 31.8 kDa. A nucleotide sequence of a cDNA that encodes TACI is given in SEQ ID NO:5. Predicted amino acids from about 1 to about 165 constitute the extracellular domain (SEQ ID NO:6); amino acids from about 166 to about 186 constitute the transmembrane domain (SEQ ID NO:6); and amino acids from about 187 to about 293 constitute the intracellular domain (SEQ ID NO:6).

[0194] Accordingly, in one embodiment, a TACI protein that may be used in the methods of the present invention is an isolated polypeptide comprising, or alternatively, consisting of the amino acid sequence of SEQ ID NO:6, or a polypeptide comprising, or alternatively, consisting of a portion of SEQ ID NO:6, such as for example, the TACI extracellular domain (comprising amino acids 1 to 165 of SEQ ID NO:6) and/or the TACI cysteine rich domain (comprising amino acids 33 to 104 of SEQ ID NO:6); as well

as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above.

[0195] In another embodiment, a TACI protein that may be used in the methods of the present invention is an isolated polypeptide comprising amino acids 1 to 154 of SEQ ID NO:6 as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above.

[0196] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a TACI polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the TACI receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0197] Polypeptide fragments of TACI include polypeptides comprising or alternatively, consisting of: an amino acid sequence contained in SEQ ID NO:6. Polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. In additional embodiments, the polypeptide fragments comprise, or alternatively consist of, one or more TACI domains. Preferred polypeptide fragments include a member selected from the group: (a) a polypeptide comprising or alternatively, consisting of, the TACI extracellular domain (predicted to constitute amino acid residues from about 1 to about 165 of SEQ ID NO:6); (b) a polypeptide comprising or alternatively, consisting of, a TACI cysteine rich domain (predicted to constitute amino acid residues from about 33 to about 104 of SEQ ID NO:6); (c) a polypeptide comprising or alternatively, consisting of, the TACI transmembrane domain (predicted to constitute amino acid residues from about 166 to about 186 of SEQ ID NO:6); (d) a polypeptide comprising or alternatively, consisting of, the TACI intracellular domain (predicted to constitute amino acid residues from about 187 to about 293 of SEQ ID NO:6); or (e) any combination of polypeptides (a)-(d).

[0198] It is believed that the extracellular cysteine rich motifs of TACI are important for interactions between TACI and its ligands, Neutrokine-alpha and APRIL. Accordingly, in preferred embodiments, TACI polypeptide fragments that may be used in the methods of the present invention comprise, or alternatively consist of amino acid residues 33 to 66 and/or 70 to 104 of SEQ ID NO:6. In a specific embodiment the TACI polypeptides that may be used in the methods of the present invention comprise, or alternatively consist of one or both of the extracellular cysteine rich motifs (residues 33 to 66 and residues 70 to 104 of SEQ ID NO:6). Proteins comprising or alternatively consisting of a polypeptide sequence which

is at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide sequences of one or both of these cysteine rich motifs are also preferred.

[0199] Other fragments of the TACI protein that may be used in the methods of the invention are fragments characterized by structural or functional attributes of TACI. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) TACI (SEQ ID NO:6) as is discussed in U.S. Patent No. 6,969,519. Certain preferred regions include, but are not limited to, Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic; Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

[0200] The TACI polypeptide for use in the methods of the invention may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

[0201] A preferred TACI fusion protein that may be used in the methods of the invention comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) and WO00/024782 disclose fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. TACI immunoglobulin fusion proteins have been described in, for example, PCT Publications WO01/60397, WO01/81417, WO01/087977, and WO02/94852; U.S. Publication Nos. US2003103986 and US2006034852 and Gross, *et al.*, (2000) *Nature* 404:995-999 and Yu, *et al.*, (2000) *Nat Immunol* 1:252-256, herein incorporated by reference in their entirety. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner

described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., Journal of Molecular Recognition 8:52-58 (1995) and K. Johanson et al., The Journal of Biological Chemistry 270:16:9459-9471 (1995). In a specific embodiment, the TACI-Fc fusion protein that may be used in the methods of the invention is Atacicept (TACI-Ig).

[0202] As one of skill in the art will appreciate, and as discussed above, the TACI polypeptides can be fused to other polypeptide sequences. For example, the TACI polypeptides may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)) resulting in chimeric polypeptides.

[0203] Such fusion proteins may facilitate purification, may extend shelf-life and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0204] An albumin fusion protein that may be used in methods of the present invention comprises at least a fragment or variant of a TACI polypeptide and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of TACI is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The TACI polypeptide and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "TACI portion" or an "albumin protein portion").

[0205] In one embodiment, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a TACI polypeptide and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment of TACI and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists

of, a variant of TACI and a serum albumin protein In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0206] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a TACI polypeptide and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a TACI polypeptide and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the TACI portion of the albumin fusion protein is the full-length TACI polypeptide. In a further preferred embodiment, the TACI portion of the albumin fusion protein is the mature, soluble domain of the TACI polypeptide.

[0207] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of TACI and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the TACI polypeptide and the mature portion of serum albumin.

[0208] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of TACI and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the TACI polypeptide and the mature portion of serum albumin. (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, TACI polypeptides (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-x of human serum albumin, where x is an integer from 1 to 585 and the albumin fragment has human serum albumin activity. In another preferred embodiment TACI polypeptides (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. TACI polypeptides (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

[0209] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins that may be used in the methods of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:11: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No.

WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins that may be used in the methods of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0210] Preferably, the albumin fusion protein that may be used in the methods of the invention comprises HA as the N-terminal portion, and TACI polypeptide as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and TACI polypeptide as the N-terminal portion may also be used.

[0211] In other embodiments, the albumin fusion protein that may be used in methods of the invention has a TACI polypeptide fused to both the N-terminus and the C-terminus of albumin. In a specific embodiment, the TACI polypeptides fused at the N- and C- termini are the same. In another embodiment, the TACI polypeptides fused at the N- and C- termini are different TACI polypeptides. In another embodiment, a TACI polypeptide is fused at either the N- or C- terminus of albumin, and a heterologous polypeptide is fused at the remaining terminus.

[0212] Additionally, the albumin fusion proteins that may be used in the methods of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0213] Generally, the albumin fusion proteins that may be used in the methods of the invention may have one HA-derived region and one TACI region. Multiple regions of each protein, however, may be used to make an albumin fusion protein that may be used in the methods of the invention. Similarly, more than one protein may be used to make an albumin fusion protein that may be used in the methods of the invention. For instance, a protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the protein portions may be the same or different protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0214] In a specific embodiment a TACI protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a cytotoxin (e.g., a cytostatic or cytocidal agent). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0215] In another embodiment, a TACI protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a toxin.

[0216] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or

induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium.

[0217] To improve or alter the characteristics of TACI polypeptides that may be used in the methods of the invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins". Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions.

[0218] The TACI proteins that may be used in the methods of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers. Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffel, Science 264:667, 1994; Banner et al., Cell 73:431, 1993). Thus, trimeric TACI may offer the advantage of enhanced biological activity.

[0219] In specific embodiments, the multimers may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only TACI proteins (including TACI fragments, variants, and fusion proteins, as described herein). These homomers may contain TACI proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer is a multimer containing only TACI proteins having an identical polypeptide sequence. In another specific embodiment, a homomer is a multimer containing TACI proteins having different polypeptide sequences.

[0220] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to a polypeptide sequences encoded by the TACI gene) in addition to the TACI proteins. Multimers may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers, such as, for example, homodimers, homotrimers, heterotrimers or heterotetramers, are formed when the proteins contact one another in solution. In other embodiments, multimers are formed by covalent associations with and/or between the TACI proteins. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein. In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation.

[0221] Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a TACI fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925, the contents of which are herein incorporated by reference in its entirety). In a specific example, the covalent associations are between the heterologous sequence contained in a TACI-Fc fusion protein (as described herein). In another specific example, covalent associations of fusion proteins are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more TACI polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple TACI polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0222] In a specific embodiment, antibodies that bind to a TACI polypeptide, a polypeptide fragment, a variant of SEQ ID NO:6, and/or an TACI polypeptide epitope (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding) may be used in the methods of the invention. Anti-TACI antibodies and fragments thereof have been described in, for example, PCT Publications WO04/011611, WO01/087977, WO01/60397, and WO02/66516; U.S. Patent Publication US2005043516 and US2003012783; and Ch' en, *et al.*, (2005) *Cell Immunol* 236:78-85 and Liu, *et al.*, (2003) *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 19:168-169. Each of the aforementioned references is herein incorporated by reference in its entirety. Antibodies include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to anti-TACI antibodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In specific embodiments, the immunoglobulin molecules are IgG1. In other specific embodiments, the immunoglobulin molecules are IgG4.

[0223] TACI binding antibody fragments that may be used in the methods of the invention include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat,

guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati et al, the contents of which are herein incorporated by reference in its entirety.

[0224] Anti-TACI antibodies that may be used in the methods of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a TACI polypeptide or may be specific for both a TACI polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992), the contents of which are herein incorporated by reference in their entirety.

[0225] TACI Antibodies may be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a TACI polypeptide may be used in the methods of the invention. In specific embodiments, TACI antibodies cross-react with murine, rat and/or rabbit homologs of human TACI proteins and the corresponding epitopes thereof. In a specific embodiment, an anti-TACI antibody that may be used in the methods of the invention binds not only to TACI, but also binds to BCMA and BAFF-R.

[0226] TACI antibodies that may be used in the methods of the invention may also be described or specified in terms of their binding affinity to a TACI polypeptide. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, or 10^{-12} M.

[0227] TACI antibodies that may be used in the methods of the invention may act as agonists or antagonists of the TACI polypeptides. For example, TACI antibodies which disrupt the receptor/ligand interactions with the TACI polypeptides either partially or fully are included. Also included are receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting activation of the transcription factors NF-AT, AP-1, and/or NF-kappaB using techniques known in the art, and/or the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis.

[0228] In a specific embodiment, receptor-specific TACI antibodies which both prevent ligand binding and receptor activation as well as TACI antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand may be used in the methods of the invention. The above TACI antibodies can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997);

Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

E. Neutrokine-alpha Receptor, BCMA

[0229] BCMA polypeptides, such as those described below, act as Neutrokine-alpha and/or APRIL antagonists and may also be used in the methods of the present invention. BCMA, also known as TR18, is a protein of 184 amino acid residues (SEQ ID NO:8), with a deduced molecular weight of about 20.1 kDa A nucleotide sequence of a cDNA that encodes BCMA is given in SEQ ID NO:7. Predicted amino acids from about 1 to about 54 constitute the extracellular domain (SEQ ID NO:8); amino acids from about 55 to about 80 constitute the transmembrane domain (SEQ ID NO:8); and amino acids from about 81 to about 184 constitute the intracellular domain (SEQ ID NO:8).

[0230] Accordingly, in one embodiment, a BCMA protein that may be used in the methods of the present invention is an isolated polypeptide comprising, or alternatively, consisting of the amino acid sequence of SEQ ID NO:8, or a polypeptide comprising, or alternatively, consisting of a portion of SEQ ID NO:8, such as for example, the BCMA extracellular domain (comprising amino acids 1 to 54 of SEQ ID NO:8) and/or the BCMA cysteine rich domain (comprising amino acids 8 to 41 of SEQ ID NO:8); as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above.

[0231] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a BCMA polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the BCMA receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0232] Polypeptide fragments of BCMA include polypeptides comprising or alternatively, consisting of: an amino acid sequence contained in SEQ ID NO:8. Polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. In additional embodiments, the polypeptide fragments comprise, or alternatively consist of, one or more BCMA domains. Preferred polypeptide fragments include a member selected from the group: (a) a polypeptide comprising or alternatively, consisting of, the BCMA extracellular domain (predicted to constitute amino acid residues from about 1 to about 54 of SEQ ID NO:8); (b) a polypeptide comprising or alternatively, consisting of, a BCMA cysteine rich domain (predicted to constitute amino acid

residues from about 8 to about 41 of SEQ ID NO:8); (c) a polypeptide comprising or alternatively, consisting of, the BCMA transmembrane domain (predicted to constitute amino acid residues from about 55 to about 80 of SEQ ID NO:8); (d) a polypeptide comprising or alternatively, consisting of, the BCMA intracellular domain (predicted to constitute amino acid residues from about 81 to about 184 of SEQ ID NO:8); or (e) any combination of polypeptides (a)-(d).

[0233] It is believed that the extracellular cysteine rich motif of BCMA is important for interactions between BCMA and its ligands, Neutrokine-alpha and APRIL. Accordingly, in preferred embodiments, BCMA polypeptide fragments that may be used in the methods of the present invention comprise, or alternatively consist of, the amino acid sequence of amino acid residues 8 to 41 of SEQ ID NO:8. Proteins comprising or alternatively consisting of a polypeptide sequence which is at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide sequences of the cysteine rich motif are also preferred.

[0234] Other fragments of the BCMA protein that may be used in the methods of the invention are fragments characterized by structural or functional attributes of BCMA. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) BCMA (SEQ ID NO:8) as is discussed in U.S. Patent Application No. 10/786,176. Certain preferred regions include, but are not limited to, Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic; Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

[0235] The BCMA polypeptide for use in the methods of the invention may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

[0236] A preferred BCMA fusion protein that may be used in the methods of the invention comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464

533 (Canadian counterpart 2045869) and WO00/024782 disclose fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. BCMA immunoglobulin fusion proteins have been described in, for example, PCT Publications WO01/087977, WO01/60397, and WO01/24811 and Gross, *et al.*, (2000) *Nature* 404:995-999, Thompson, *et al.*, (2000) *J Exp Med* 192:129-135, and Yu, *et al.*, (2000) *Nat Immunol* 1:252-256, herein incorporated by reference in their entirety. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., Journal of Molecular Recognition 8:52-58 (1995) and K. Johanson et al., The Journal of Biological Chemistry 270:16:9459-9471 (1995).

[0237] As one of skill in the art will appreciate, and as discussed above, the BCMA polypeptides can be fused to other polypeptide sequences. For example, the BCMA polypeptides may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)) resulting in chimeric polypeptides.

[0238] Such fusion proteins may facilitate purification, may extend shelf-life and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0239] An albumin fusion protein that may be used in methods of the present invention comprises at least a fragment or variant of a BCMA polypeptide and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of BCMA is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The BCMA polypeptide and albumin protein, once part of the albumin fusion protein, may be

referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "BCMA portion" or an "albumin protein portion").

[0240] In one embodiment, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BCMA polypeptide and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment of BCMA and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a variant of BCMA and a serum albumin protein In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0241] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BCMA polypeptide and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BCMA polypeptide and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the BCMA portion of the albumin fusion protein is the full-length BCMA polypeptide. In a further preferred embodiment, the BCMA portion of the albumin fusion protein is the mature, soluble domain of the BCMA polypeptide.

[0242] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of BCMA and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the BCMA polypeptide and the mature portion of serum albumin.

[0243] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of BCMA and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the BCMA polypeptide and the mature portion of serum albumin. (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, BCMA polypeptides (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-x of human serum albumin, where x is an integer from 1 to 585 and the albumin fragment has human serum albumin activity. In another preferred embodiment BCMA polypeptides (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as

described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. BCMA polypeptides (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

[0244] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins that may be used in the methods of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:11: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins that may be used in the methods of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0245] Preferably, the albumin fusion protein that may be used in the methods of the invention comprises HA as the N-terminal portion, and BCMA polypeptide as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and BCMA polypeptide as the N-terminal portion may also be used.

[0246] In other embodiments, the albumin fusion protein that may be used in methods of the invention has a BCMA polypeptide fused to both the N-terminus and the C-terminus of albumin. In a specific embodiment, the BCMA polypeptides fused at the N- and C- termini are the same. In another embodiment, the BCMA polypeptides fused at the N- and C- termini are different BCMA polypeptides. In another embodiment, a BCMA polypeptide is fused at either the N- or C- terminus of albumin, and a heterologous polypeptide is fused at the remaining terminus.

[0247] Additionally, the albumin fusion proteins that may be used in the methods of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0248] Generally, the albumin fusion proteins that may be used in the methods of the invention may have one HA-derived region and one BCMA region. Multiple regions of each protein, however, may be used to make an albumin fusion protein that may be used in the methods of the invention. Similarly, more than one protein may be used to make an albumin fusion protein that may be used in the methods of the invention. For instance, a protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the protein portions may be the same or different protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0249] In a specific embodiment, a BCMA protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a cytotoxin (e.g., a cytostatic or cytocidal agent). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0250] In another embodiment, a BCMA protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a toxin.

[0251] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium.

[0252] To improve or alter the characteristics of BCMA polypeptides that may be used in the methods of the invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins". Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. In yet another embodiment of the invention, the BCMA polypeptide mutant can be a "dominant negative." To this end, defective BCMA polypeptides, such as, for example, mutants lacking all or a portion of the TNF-conserved domain, can be used to diminish the activity of BCMA. The non-functional BCMA polypeptides will assemble to form a receptor (e.g., multimer) that may be capable of binding, but which is incapable of inducing signal transduction.

[0253] The BCMA proteins that may be used in the methods of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers. Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffel, Science 264:667, 1994; Banner et al., Cell 73:431, 1993). Thus, trimeric BCMA may offer the advantage of enhanced biological activity.

[0254] In specific embodiments, the multimers may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only BCMA proteins (including BCMA fragments, variants, and fusion proteins, as described herein). These homomers may contain BCMA proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer is a multimer containing only BCMA proteins having an identical polypeptide sequence. In another specific embodiment, a homomer is a multimer containing BCMA proteins having different polypeptide sequences.

[0255] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to a polypeptide sequences encoded by the BCMA gene) in addition to the BCMA proteins. Multimers may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers, such as, for example, homodimers, homotrimers, heterotrimers or heterotetramers, are formed when the proteins contact one another in solution. In other embodiments, multimers are formed by covalent associations with and/or between the BCMA proteins. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein. In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation.

[0256] Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a BCMA fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925, the contents of which are herein incorporated by reference in its entirety). In a specific example, the covalent associations are between the heterologous sequence contained in a BCMA-Fc fusion protein (as described herein). In another specific example, covalent associations of fusion proteins are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more BCMA polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple BCMA polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0257] In a specific embodiment, antibodies that bind to a BCMA polypeptide, a polypeptide fragment, a variant of SEQ ID NO:8, and/or an BCMA polypeptide epitope (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding) may be used in the methods of the invention. Anti-BCMA antibodies and fragments thereof have been described in, for example, PCT Publications WO01/087977, WO01/60397, and WO02/66516 and Ch' en, *et al.*, (2005) *Cell Immunol* 236:78-85. Each of the aforementioned references is herein incorporated by reference in its entirety. Antibodies include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to anti-BCMA antibodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen.

The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In specific embodiments, the immunoglobulin molecules are IgG1. In other specific embodiments, the immunoglobulin molecules are IgG4.

[0258] BCMA binding antibody fragments that may be used in the methods of the invention include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati et al, the contents of which are herein incorporated by reference in its entirety.

[0259] Anti-BCMA antibodies that may be used in the methods of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a BCMA polypeptide or may be specific for both a BCMA polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992), the contents of which are herein incorporated by reference in their entirety.

[0260] BCMA Antibodies may be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a BCMA polypeptide may be used in the methods of the invention. In specific embodiments, BCMA antibodies cross-react with murine, rat and/or rabbit homologs of human BCMA proteins and the corresponding epitopes thereof. In a specific embodiment, an anti-BCMA antibody that may be used in the methods of the invention binds not only to BCMA, but also binds to TACI and BAFF-R.

[0261] BCMA antibodies that may be used in the methods of the invention may also be described or specified in terms of their binding affinity to a BCMA polypeptide. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, or 10^{-12} M.

[0262] BCMA antibodies that may be used in the methods of the invention may act as agonists or antagonists of the BCMA polypeptides. For example, BCMA antibodies which disrupt the receptor/ligand interactions with the BCMA polypeptides either partially or fully are included. Also included are receptor-

specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting activation of the transcription factors NF-AT, AP-1, and/or NF-kappaB using techniques known in the art, and/or the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis.

[0263] In a specific embodiment, receptor-specific BCMA antibodies which both prevent ligand binding and receptor activation as well as BCMA antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand may be used in the methods of the invention. The above BCMA antibodies can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

F. Neutrokine-alpha Receptor, BAFF-R

[0264] BAFF-R polypeptides, such as those described below, act as Neutrokine-alpha and/or APRIL antagonists and may also be used in the methods of the present invention. BAFF-R, also known as TR21, is a protein of 184 amino acid residues (SEQ ID NO:10), with a deduced molecular weight of about 18.9 kDa A nucleotide sequence of a cDNA that encodes BAFF-R is given in SEQ ID NO:9. Predicted amino acids from about 1 to about 81 constitute the extracellular domain (SEQ ID NO:10); amino acids from about 82 to about 101 constitute the transmembrane domain (SEQ ID NO:10); and amino acids from about 102 to about 184 constitute the intracellular domain (SEQ ID NO:10).

[0265] Accordingly, in one embodiment, a BAFF-R protein that may be used in the methods of the present invention is an isolated polypeptide comprising, or alternatively, consisting of the amino acid sequence of SEQ ID NO:10, or a polypeptide comprising, or alternatively, consisting of a portion of SEQ ID NO:10, such as for example, the BAFF-R extracellular domain (comprising amino acids 1 to 81 of SEQ ID NO:10) and/or the BAFF-R cysteine rich domain (comprising amino acids 19 to 35 of SEQ ID NO:10); as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above.

[0266] In another embodiment, a BAFF-R protein that may be used in the methods of the present invention is an isolated polypeptide comprising amino acids 1 to 70 of SEQ ID NO:10 and/or the amino acid sequence of SEQ ID NO:26. SEQ ID NO:26 shows amino acids 1-70 of BAFF-R wherein amino acid 20 (valine) in BAFF-R is substituted with asparagine and amino acid 27 (leucine) in BAFF-R is substituted with proline. In another embodiment, a BAFF-R protein that may be used in the methods of the present invention is an isolated polypeptide comprising amino acids 2 to 70 of SEQ ID NO:26. Polypeptides which

are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98%, 99% or 100% identical to the polypeptides described above may also be used in the methods of the present invention.

[0267] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a BAFF-R polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the BAFF-R receptor. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0268] Polypeptide fragments of BAFF-R include polypeptides comprising or alternatively, consisting of: an amino acid sequence contained in SEQ ID NO:10. Polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. In additional embodiments, the polypeptide fragments comprise, or alternatively consist of, one or more BAFF-R domains. Preferred polypeptide fragments include a member selected from the group: (a) a polypeptide comprising or alternatively, consisting of, the BAFF-R extracellular domain (predicted to constitute amino acid residues from about 1 to about 81 of SEQ ID NO:10); (b) a polypeptide comprising or alternatively, consisting of, a BAFF-R cysteine rich domain (predicted to constitute amino acid residues from about 19 to about 35 of SEQ ID NO:10); (c) a polypeptide comprising or alternatively, consisting of, the BAFF-R transmembrane domain (predicted to constitute amino acid residues from about 82 to about 101 of SEQ ID NO:10); (d) a polypeptide comprising or alternatively, consisting of, the BAFF-R intracellular domain (predicted to constitute amino acid residues from about 102 to about 184 of SEQ ID NO:10); or (e) any combination of polypeptides (a)-(d).

[0269] It is believed that the extracellular cysteine rich motif of BAFF-R is important for interactions between BAFF-R and its ligands, Neutrokine-alpha and APRIL. Accordingly, in preferred embodiments, BAFF-R polypeptide fragments that may be used in the methods of the present invention comprise, or alternatively consist of, the amino acid sequence of amino acid residues 19 to 35 of SEQ ID NO:10. Proteins comprising or alternatively consisting of a polypeptide sequence which is at least 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide sequences of the cysteine rich motif are also preferred.

[0270] Other fragments of the BAFF-R protein that may be used in the methods of the invention are fragments characterized by structural or functional attributes of BAFF-R. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta

amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) BAFF-R (SEQ ID NO:10) as is discussed in U.S. Patent No. 7,112,410. Certain preferred regions include, but are not limited to, Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic; Hopp-Woods predicted hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

[0271] The BAFF-R polypeptide for use in the methods of the invention may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals but also additional heterologous functional regions. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

[0272] A preferred BAFF-R fusion protein that may be used in the methods of the invention comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) and WO0/0024782 disclose fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. BAFF-R immunoglobulin fusion proteins have been described in, for example, Pelletier, *et al.*, (2003) *J Biol Chem* 278:33127-33133 and Carter, *et al.*, (2005) *Arthritis Rheum* 52:3943-3954, herein incorporated by reference in their entirety. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses, it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fc portion proves to be a hindrance to use in therapy and diagnosis, for example, when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., Journal of Molecular Recognition 8:52-58 (1995) and K. Johanson et al., The Journal of Biological Chemistry 270:16:9459-9471 (1995). In a specific embodiment, the BAFF-R-Fc fusion protein that may be used in the methods of the invention is BR3-Fc.

[0273] As one of skill in the art will appreciate, and as discussed above, the BAFF-R polypeptides can be fused to other polypeptide sequences. For example, the BAFF-R polypeptides may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)) resulting in chimeric polypeptides.

[0274] Such fusion proteins may facilitate purification, may extend shelf-life and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0275] One example of a BAFF-R-Fc protein is amino acids 1-70 of SEQ ID NO:10 fused to the Fc region of an IgG1 immunoglobulin molecule. Optionally, amino acid 20 (valine) in BAFF-R is substituted with aspargine and amino acid 27 (leucine) in BAFF-R is substituted with proline. SEQ ID NO:26 shows amino acids 1-70 of BAFF-R with these two amino acid changes.

[0276] An albumin fusion protein that may be used in methods of the present invention comprises at least a fragment or variant of a BAFF-R polypeptide and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of BAFF-R is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The BAFF-R polypeptide and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "BAFF-R portion" or an "albumin protein portion").

[0277] In one embodiment, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BAFF-R polypeptide and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment of BAFF-R and a serum albumin protein. In other embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a variant of BAFF-R and a serum albumin protein In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

[0278] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BAFF-R polypeptide and a biologically active and/or

therapeutically active fragment of serum albumin. In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a BAFF-R polypeptide and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the BAFF-R portion of the albumin fusion protein is the full-length BAFF-R polypeptide. In a further preferred embodiment, the BAFF-R portion of the albumin fusion protein is the mature, soluble domain of the BAFF-R polypeptide.

[0279] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of BAFF-R and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the BAFF-R polypeptide and the mature portion of serum albumin.

[0280] In further embodiments, an albumin fusion protein that may be used in the methods of the invention comprises, or alternatively consists of, a fragment or variant of BAFF-R and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of the BAFF-R polypeptide and the mature portion of serum albumin. (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, BAFF-R polypeptides (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-x of human serum albumin, where x is an integer from 1 to 585 and the albumin fragment has human serum albumin activity. In another preferred embodiment BAFF-R polypeptides (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. BAFF-R polypeptides (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

[0281] In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins that may be used in the methods of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:11: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g., International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins that may be used in the methods of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

[0282] Preferably, the albumin fusion protein that may be used in the methods of the invention comprises HA as the N-terminal portion, and BAFF-R polypeptide as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and BAFF-R polypeptide as the N-terminal portion may also be used.

[0283] In other embodiments, the albumin fusion protein that may be used in methods of the invention has a BAFF-R polypeptide fused to both the N-terminus and the C-terminus of albumin. In a specific embodiment, the BAFF-R polypeptides fused at the N- and C- termini are the same. In another embodiment, the BAFF-R polypeptides fused at the N- and C- termini are different BAFF-R polypeptides. In another embodiment, a BAFF-R polypeptide is fused at either the N- or C- terminus of albumin, and a heterologous polypeptide is fused at the remaining terminus.

[0284] Additionally, the albumin fusion proteins that may be used in the methods of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties. The linker peptide may consist of amino acids such that it is flexible or more rigid.

[0285] Generally, the albumin fusion proteins that may be used in the methods of the invention may have one HA-derived region and one BAFF-R region. Multiple regions of each protein, however, may be used to make an albumin fusion protein that may be used in the methods of the invention. Similarly, more than one protein may be used to make an albumin fusion protein that may be used in the methods of the invention. For instance, a protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the protein portions may be the same or different protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

[0286] In a specific embodiment, a BAFF-R protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a cytotoxin (e.g., a cytostatic or cytocidal agent). A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0287] In another embodiment, a BAFF-R protein or fragment or variant thereof that may be used in the methods of the invention may be conjugated to a toxin.

[0288] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for

example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium.

[0289] To improve or alter the characteristics of BAFF-R polypeptides that may be used in the methods of the invention, protein engineering may be employed. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins". Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. In yet another embodiment of the invention, the BAFF-R polypeptide mutant can be a "dominant negative." To this end, defective BAFF-R polypeptides, such as, for example, mutants lacking all or a portion of the TNF-conserved domain, can be used to diminish the activity of BAFF-R. The non-functional BAFF-R polypeptides will assemble to form a receptor (e.g., multimer) that may be capable of binding, but which is incapable of inducing signal transduction.

[0290] The BAFF-R proteins that may be used in the methods of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers. Certain members of the TNF family of proteins are believed to exist in trimeric form (Beutler and Huffel, Science 264:667, 1994; Banner et al., Cell 73:431, 1993). Thus, trimeric BAFF-R may offer the advantage of enhanced biological activity.

[0291] In specific embodiments, the multimers may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only BAFF-R proteins (including BAFF-R fragments, variants, and fusion proteins, as described herein). These homomers may contain BAFF-R proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer is a multimer containing only BAFF-R proteins having an identical polypeptide sequence. In another specific embodiment, a homomer is a multimer containing BAFF-R proteins having different polypeptide sequences.

[0292] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing only polypeptide sequences that do not correspond to a polypeptide sequences encoded by the BAFF-R gene) in addition to the BAFF-R proteins. Multimers may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers, such as, for example, homodimers, homotrimers, heterotrimers or heterotetramers, are formed when the proteins contact one another in solution. In other embodiments, multimers are formed by covalent associations with and/or between the BAFF-R proteins. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein. In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation.

[0293] Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a BAFF-R fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925, the contents of which are herein incorporated by reference in its entirety). In a specific example, the covalent associations are between the heterologous sequence contained in a BAFF-R-Fc fusion protein (as described herein). In another specific example, covalent associations of fusion proteins are between heterologous polypeptide sequences from another TNF family ligand/receptor member that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication No. WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more BAFF-R polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple BAFF-R polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology.

[0294] In a specific embodiment, antibodies that bind to a BAFF-R polypeptide, a polypeptide fragment, a variant of SEQ ID NO:10, and/or an BAFF-R polypeptide epitope (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding) may be used in the methods of the invention. Anti-BAFF-R antibodies and fragments thereof have been described in, for example, Lee, *et al.*, (2006) Synthetic anti-BR3 antibodies that mimic BAFF binding and target both human and murine B cells *Blood* (Blood First Edition Paper, prepublished online July 13, 2006) Vol. 0, No. 2006, pp. 200603011, Ch' en, *et al.*, (2005) *Cell Immunol* 236:78-85, Nakamura, *et al.*, (2005) *Virchows Arch* 447:53-60, and Carter, *et al.*, (2005) *Arthritis Rheum* 52:3943-3954. Each of the aforementioned references is herein incorporated by reference in its entirety. Antibodies include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to anti-BAFF-R antibodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In specific embodiments, the immunoglobulin molecules are IgG1. In other specific embodiments, the immunoglobulin molecules are IgG4.

[0295] BAFF-R binding antibody fragments that may be used in the methods of the invention include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region,

CH1, CH2, and CH3 domains. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati et al, the contents of which are herein incorporated by reference in its entirety.

[0296] Anti-BAFF-R antibodies that may be used in the methods of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a BAFF-R polypeptide or may be specific for both a BAFF-R polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992), the contents of which are herein incorporated by reference in their entirety.

[0297] BAFF-R Antibodies may be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a BAFF-R polypeptide may be used in the methods of the invention. In specific embodiments, BAFF-R antibodies cross-react with murine, rat and/or rabbit homologs of human BAFF-R proteins and the corresponding epitopes thereof. In a specific embodiment, an anti-BAFF-R antibody that may be used in the methods of the invention binds not only to BAFF-R, but also binds to TACI and BCMA.

[0298] BAFF-R antibodies that may be used in the methods of the invention may also be described or specified in terms of their binding affinity to a BAFF-R polypeptide. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, or 10^{-12} M.

[0299] BAFF-R antibodies that may be used in the methods of the invention may act as agonists or antagonists of the BAFF-R polypeptides. For example, BAFF-R antibodies which disrupt the receptor/ligand interactions with the BAFF-R polypeptides either partially or fully are included. Also included are receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting activation of the transcription factors NF-AT, AP-1, and/or NF-kappaB using techniques known in the art, and/or the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis.

[0300] In a specific embodiment, receptor-specific BAFF-R antibodies which both prevent ligand binding and receptor activation as well as BAFF-R antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand may be used in the methods of the invention. The above BAFF-R antibodies can be made using methods known in the art. See,

e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

G. Anti-APRIL Antibodies

[0301] In a specific embodiment, the Neutrokine-alpha antagonist is an anti-APRIL antibody or antigen-binding fragment thereof. Anti-APRIL antibodies and fragments thereof have been described in, for example, PCT Publications WO01/087977, WO99/12965, WO01/60397, and WO02/094192; U.S. Patent No. 6,506,882; U.S. Patent Publication No. 2003/0166864, filed October 11, 2002; and Ch' en, *et al.*, (2005) *Cell Immunol* 236:78-85; and are described in more detail below. Each of the aforementioned references is herein incorporated by reference in its entirety.

[0302] In a specific embodiment, antibodies that bind to a APRIL polypeptide, polypeptide fragment, or variant of SEQ ID NO:4, and/or an APRIL epitope (as determined by immunoassays well-known in the art for assaying specific antibody-antigen binding) may be used in the methods of the invention. In a specific embodiment, antibodies that may be used in the methods of the invention may bind APRIL polypeptides fused to other polypeptide sequences. For example, APRIL polypeptides may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life *in vivo*. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

[0303] In specific embodiments, antibodies that may be used in the methods of the invention bind homomeric, especially homotrimeric, APRIL polypeptides. In other specific embodiments, antibodies that may be used in the methods of the invention bind heteromeric, especially heterotrimeric, APRIL polypeptides such as a heterotrimer containing two APRIL polypeptides and one Neutrokine-alpha

polypeptide or a heterotrimer containing one APRIL polypeptide and two Neutrokine-alpha polypeptides. In a specific embodiment, the antibodies that may be used in the methods of the invention bind homomeric, especially homotrimeric, APRIL polypeptides, wherein the individual protein components of the multimers consist of the mature form of APRIL (e.g., amino acids residues 105-250 of SEQ ID NO:4). In other specific embodiments, antibodies that may be used in the methods of the invention bind heteromeric, especially heterotrimeric, APRIL polypeptides such as a heterotrimer containing two APRIL polypeptides and one Neutrokine-alpha polypeptide or a heterotrimer containing one APRIL polypeptide and two Neutrokine-alpha polypeptides, and wherein the individual protein components of the APRIL heteromer consist of either the mature extracellular soluble portion of APRIL (e.g., amino acids residues 105-250 of SEQ ID NO:4) or the mature extracellular soluble portion Neutrokine-alpha (e.g., amino acid residues 134-285 of SEQ ID NO:2).

[0304] In specific embodiments, the antibodies that may be used in the methods of the invention bind conformational epitopes of a APRIL monomeric protein. In specific embodiments, the antibodies that may be used in the methods of the invention bind conformational epitopes of a APRIL multimeric, especially trimeric, protein. In other embodiments, antibodies that may be used in the methods of the invention bind conformational epitopes that arise from the juxtaposition of APRIL with a heterologous polypeptide, such as might be present when APRIL forms heterotrimers (e.g., with Neutrokine-alpha polypeptides), or in fusion proteins between APRIL and a heterologous polypeptide.

[0305] Antibodies that may be used in the methods of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-id antibodies to anti-APRIL antibodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. . In preferred embodiments, the immunoglobulin is an IgG1 or an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

[0306] In a specific embodiment, the antibodies that may be used in the methods of the invention are APRIL-binding antibody fragments and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. APRIL-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. In a specific embodiment, APRIL-binding fragments that may be used in the methods of the invention comprise any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies that may be used in the methods of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat),

donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati et al, the contents of which are herein incorporated by reference in its entirety.

[0307] The antibodies that may be used in the methods of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a APRIL polypeptide or may be specific for both a APRIL polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[0308] Anti-APRIL antibodies that may be used in the methods of the invention may be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a APRIL polypeptide may be used in the methods of the invention. In a specific embodiment, antibodies that may be used in the methods of the invention cross react with Neutrokine-alpha. In specific embodiments, antibodies that may be used in the methods of the invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof.

[0309] Antibodies that may be used in the methods of the invention may also be described or specified in terms of their binding affinity to a APRIL polypeptide. In specific embodiments, antibodies that may be used in the methods of the invention bind APRIL polypeptides, or fragments or variants thereof, with a dissociation constant or K_D of less than or equal to 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, or 10^{-12} M. In a specific embodiment, antibodies that may be used in the methods of the invention bind APRIL polypeptides with a dissociation constant or K_D that is within any one of the ranges that are between each of the individual recited values.

[0310] For example, APRIL antibodies which disrupt the receptor/ligand interactions with APRIL polypeptides either partially or fully are included. Also included are APRIL-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting activation of the transcription factors NF-AT, AP-1, and/or NF-kappaB using techniques known in the art, and/or the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis.

[0311] In a specific embodiment, APRIL-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand may be used in the methods of the invention. In a specific embodiment, neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor may be used in the methods of the

invention. The above APRIL antibodies can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

H. APRIL Binding Polypeptides

[0312] In a specific embodiment, the Neutrokine-alpha antagonist is an APRIL binding peptide or polypeptide. APRIL binding peptides or polypeptides have been described in, for example International Patent Publication numbers WO01/87977, WO01/87979 and US Patent Publication numbers US2002081296 and US2002086018, each of which is herein incorporated by reference in its entirety. APRIL binding peptides that may be used in the methods of the present invention include short polypeptides identified from random peptide sequences displayed by fusion with coat proteins of filamentous phage. For discussion of phage display peptide library technology see, for example, Scott et al. (1990), Science 249: 386; Devlin et al. (1990), Science 249: 404; U.S. Pat. No. 5,223,409, issued Jun. 29, 1993; U.S. Pat. No. 5,733,731, issued Mar. 31, 1998; U.S. Pat. No. 5,498,530, issued Mar. 12, 1996; U.S. Pat. No. 5,432,018, issued Jul. 11, 1995; U.S. Pat. No. 5,338,665, issued Aug. 16, 1994; U.S. Pat. No. 5,922,545, issued Jul. 13, 1999; WO 96/40987, published Dec. 19, 1996; and WO 98/15833, published Apr. 16, 1998 (each of which is incorporated by reference in its entirety). Phage expressing the peptides are isolated by successive rounds of affinity purification against an immobilized APRIL target peptide followed by repropagation. The candidates with the highest binding to APRIL can be sequenced to determine the identity of each binding peptide. Each identified APRIL binding peptide may then be attached to a "vehicle" to generate a further APRIL binding peptide for use in the methods of the present experiment. The term "vehicle" refers to a molecule that prevents degradation and/or increases half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of an APRIL binding peptide. Exemplary vehicles include an Fc domain and variants thereof (a "Peptibody" which is preferred); a linear polymer (e.g., polyethylene glycol (PEG), including 5 kD, 20 kD, and 30 kD PEG, polylysine, dextran, etc.); a branched-chain polymer (see, for example, U.S. Pat. No. 4,289,872 to Denkenwalter et al., issued Sep. 15, 1981; 5,229,490 to Tam, issued Jul. 20, 1993; WO 93/21259 by Frechet et al., published Oct. 28, 1993); a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide (e.g., dextran); any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor; albumin, including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety); and a leucine zipper domain, and other such proteins and protein fragments. APRIL binding polypeptides that may be used in the methods of the invention require the presence of at least one vehicle attached to the

peptide through the N-terminus, C-terminus or a sidechain of one of the amino acid residues. Multiple vehicles may also be used; e.g., Fc's at each terminus or an Fc at a terminus and a PEG group at the other terminus or a sidechain. For APRIL binding peptides an Fc domain is the preferred vehicle. The Fc domain may be fused to the N or C termini of the peptides or at both the N and C termini. Fusion to the N terminus is preferred.

[0313] As noted above, Fc variants are suitable vehicles for APRIL binding peptides that may be used in the methods of the invention. A native Fc may be extensively modified to form an Fc variant, provided binding to the salvage receptor is maintained; see, for example WO 97/34631 and WO 96/32478. In such Fc variants, one may remove one or more sites of a native Fc that provide structural features or functional activity not required by the APRIL binding peptides that may be used in the methods of the invention. One may remove these sites by, for example, substituting or deleting residues, inserting residues into the site, or truncating portions containing the site. The inserted or substituted residues may also be altered amino acids, such as peptidomimetics or D-amino acids. Fc variants may be desirable for a number of reasons, several of which are described below. Exemplary Fc variants include molecules and sequences in which:

[0314] 1. Sites involved in disulfide bond formation are removed. Such removal may avoid reaction with other cysteine-containing proteins present in the host cell used to produce the molecules of the invention. For this purpose, the cysteine-containing segment at the N-terminus may be truncated or cysteine residues may be deleted or substituted with other amino acids (e.g., alanyl, seryl). Even when cysteine residues are removed, the single chain Fc domains can still form a dimeric Fc domain that is held together non-covalently.

[0315] 2. A native Fc is modified to make it more compatible with a selected host cell. For example, one may remove the PA sequence near the N-terminus of a typical native Fc, which may be recognized by a digestive enzyme in E. coli such as proline iminopeptidase. One may also add an N-terminal methionine residue, especially when the molecule is expressed recombinantly in a bacterial cell such as E. coli.

[0316] 3. A portion of the N-terminus of a native Fc is removed to prevent N-terminal heterogeneity when expressed in a selected host cell. For this purpose, one may delete any of the first 20 amino acid residues at the N-terminus.

[0317] 4. One or more glycosylation sites are removed. Residues that are typically glycosylated (e.g., asparagine) may confer cytolytic response. Such residues may be deleted or substituted with unglycosylated residues (e.g., alanine).

[0318] 5. Sites involved in interaction with complement, such as the C1q binding site, are removed. For example, one may delete or substitute the EKK sequence of human IgG1. Complement recruitment may not be advantageous for the molecules that may be used in the methods of the invention and so may be avoided with such an Fc variant.

[0319] 6. Sites are removed that affect binding to Fc receptors other than a salvage receptor. A native Fc may have sites for interaction with certain white blood cells that are not required for the Neutrokine-alpha binding peptide fusion molecules that may be used in the methods of the invention and so may be removed.

[0320] 7. The ADCC site is removed. ADCC sites are known in the art; see, for example, Molec. Immunol. 29 (5): 633-9 (1992) with regard to ADCC sites in IgG1. These sites, as well, are not required for the fusion molecules that may be used in the methods of the invention and so may be removed.

[0321] 8. When the native Fc is derived from a non-human antibody, the native Fc may be humanized. Typically, to humanize a native Fc, one will substitute selected residues in the non-human native Fc with residues that are normally found in human native Fc. Techniques for antibody humanization are well known in the art.

[0322] An alternative vehicle for APRIL binding peptides that may be used in the methods of the invention would be a protein, polypeptide, peptide, antibody, antibody fragment, or small molecule (e.g., a peptidomimetic compound) capable of binding to a salvage receptor. For example, one could use as a vehicle a polypeptide as described in U.S. Pat. No. 5,739,277. Peptides could also be selected by phage display or RNA-peptide screening for binding to the salvage receptor. Such salvage receptor-binding compounds are also included within the meaning of "vehicle" and may be used in the for APRIL binding peptides that may be used in the methods of the invention. Such vehicles should be selected for increased half-life (e.g., by avoiding sequences recognized by proteases) and decreased immunogenicity (e.g., by favoring non-immunogenic sequences, as discovered in antibody humanization).

[0323] As noted above, polymer vehicles may also be used in APRIL binding peptides that may be used in the methods of the invention. Various means for attaching chemical moieties useful as vehicles are currently available, see, e.g., Patent Cooperation Treaty ("PCT") International Publication No. WO 96/11953, herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water soluble polymers to the N-terminus of proteins.

[0324] In a specific embodiment, a preferred polymer vehicle is polyethylene glycol (PEG). The PEG group may be of any convenient molecular weight and may be linear or branched. The average molecular weight of the PEG will preferably range from about 2 kiloDalton ("kD") to about 100 kD, more preferably from about 5 kD to about 50 kD, most preferably from about 5 kD to about 10 kD. The PEG groups will generally be attached to the APRIL binding peptides that may be used in the methods of the invention via acylation or reductive alkylation through a reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

[0325] A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are "preactivated" with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

[0326] Polysaccharide polymers are another type of water soluble polymer which may be used for APRIL binding peptides that may be used in the methods of the invention. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by α1-6 linkages. The dextran itself is available in many molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water soluble polymer for use in APRIL binding peptides that may be used in the methods of the invention as a vehicle by itself or in combination with another vehicle (e.g., Fc). See, for example, WO 96/11953 and WO 96/05309. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported; see, for example, European Patent Publication No. 0 315 456, which is hereby incorporated by reference in its entirety. Dextran of about 1 kD to about 20 kD is preferred when dextran is used as a vehicle in accordance with the present invention.

[0327] In a specific embodiment, APRIL binding peptides that may be used in the methods of the invention optionally include a "linker". When present, its chemical structure is not critical, since it serves primarily as a spacer. The linker is preferably made up of amino acids linked together by peptide bonds. Thus, in preferred embodiments, the linker is made up of from 1 to 30 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. Some of these amino acids may be glycosylated, as is well understood by those in the art. In a more preferred embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Even more preferably, a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Thus, preferred linkers are polyglycines (particularly $(Gly)_4$, $(Gly)_5$), poly(Gly-Ala), and polyalanines. Preferred linkers are amino acid linkers comprising greater than 5 amino acids, with suitable linkers having up to about 500 amino acids selected from glycine, alanine, proline, asparagine, glutamine, lysine, threonine, serine or aspartate. Linkers of about 20 to 50 amino acids are most preferred.

[0328] Non-peptide linkers are also useful for APRIL binding peptides that may be used in the methods of the invention. For example, alkyl linkers such as $--NH--(CH_2)_n--C(O)--$, wherein n=2-20 could be used. These alkyl linkers may further be substituted by any non-sterically hindering group such as lower alkyl (e.g., C_1--C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2, phenyl, etc.

I. Antisense and siRNAs

[0329] In a specific embodiment, the Neutrokine-alpha antagonist is an antisense RNA, catalytic RNA (ribozyme) or short interfering RNA (siRNA) that targets Neutrokine-alpha, APRIL or receptors for Neutrokine-alpha (e.g., TACI, BCMA and BAFF-R). In a specific embodiment, antisense molecules directed against Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R may be used in the methods of the invention. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5'

coding portion of a polynucleotide that encodes the extracellular domain of the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R.

[0330] In one embodiment, the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R antisense nucleic acid that may be used in the methods of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) that may be used in the methods of the invention. Such a vector would contain a sequence encoding the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others know in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

[0331] The antisense nucleic acids that may be used in the methods of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0332] Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been

shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of Neutrokine-alpha (SEQ ID NO:1), APRIL (SEQ ID NO:3), TACI (SEQ ID NO:5), BCMA (SEQ ID NO:7) or BAFF-R (SEQ ID NO:9), could be used in an antisense approach to inhibit translation of endogenous Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with methods of the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0333] The polynucleotides that may be used in the methods of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., Proc. Natl. Acad. Sci. 84:648-652 (1987); PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res. 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0334] The antisense oligonucleotide that may be used in the methods of the invention may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0335] The antisense oligonucleotide that may be used in the methods of the invention may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0336] In yet another embodiment, the antisense oligonucleotide that may be used in the methods of the invention comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0337] In yet another embodiment, the antisense oligonucleotide that may be used in the methods of the invention is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res. 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res. 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1997)).

[0338] Polynucleotides that may be used in the methods of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451 (1988)), etc.

[0339] While antisense nucleotides complementary to the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred for use in the methods of the invention.

[0340] In a specific embodiment, Neutrokine-alpha antagonists that may be used in the methods of the invention also include a catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990) directed against Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R. While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R mRNAs, the use of hammerhead ribozymes is preferred for use in the methods of the invention. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0341] As in the antisense approach, the ribozymes that may be used in the methods of the invention can be composed of modified oligonucleotides (e.g., for improved stability, targeting, etc.) and should be

delivered to cells which express Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0342] In a specific embodiment, short interfering RNA directed against Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R may be used in the methods of the invention. siRNA technology can be used to control gene expression through induction of the cells RNA-induced silencing complex (RISC). siRNA techniques are discussed, for example, in Hamilton AJ and Baulcombe DC. Science. 1999 Oct 29;286(5441):950-2; Elbashir SM, et al. Nature. 2001 May 24;411(6836):494-8 and Hanon, Gregory J. and Rossi, John J. Nature 431, 371-378 (2004). The methods are based on the introduction of short double stranded RNA (generally 20-25 nucleotides) into a cell. The doublestranded RNA is unwound and each strand separated. A single strand of the RNA is then incorporated into the RISC. The RISC then directs sequence specific mRNA cleavage, resulting in translational repression. For example, the coding portion of a polynucleotide that encodes a Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R polypeptide may be used to design a siRNA oligonucleotide of about 20 to 25 nucleotides in length. A DNA oligonucleotide is designed with the appropriate 20-25 nucleotide fragment, a spacer of approximately 9 nucleotides, and the reverse complement of the chosen nucleotide fragment. The RNA transcript produced from this construct would be expected to fold on itself to form a hairpin loop. Delivery of the hairpin loop RNA to the cell results in processing by the Rnase, Dicer to yield the short double stranded siRNA. Incorporation of a strand of this siRNA into the RISC effector complex results in cleavage of mRNA targeted by the siRNA in order to inhibit production of Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R.

[0343] In one embodiment, the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R siRNA nucleic acid that may be used in the methods of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an siRNA that may be used in the methods of the invention. Such a vector would contain a sequence encoding the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R siRNA nucleic acid. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Transcription of the sequence encoding the Neutrokine-alpha, APRIL, TACI, BCMA or BAFF-R siRNA is typically carried out using a RNA polymerase III promoter (e.g., U6 or H1), which usually direct the transcription of small nuclear RNAs (snRNAs).

B-Cell Modulators

[0344] In addition to receptors for Neutrokine-alpha and APRIL, B lymphocytes express a variety of cell surface molecules that function to inform B-cells about the extracellular microenvironment and act as transmembrane signals to positively and negatively regulate B-cell function and survival. Among these receptors, CD19, CD20 and CD22 have been identified as promising targets for therapeutic intervention.

[0345] CD20 is an integral membrane protein that acts in a complex as a calcium channel. Inhibitors of the CD20 calcium channel disrupt Ca^{2+} homeostasis and cell cycle progression. In a specific embodiment, an anti-CD20 antibody may be used in the methods of the present invention. In a preferred embodiment, the anti-CD20 antibody that may be used in the methods of the invention is Rituxan® (rituximab). In another preferred embodiment, the anti-CD20 antibody that may be used in the methods of the invention is TRU-015. In another preferred embodiment, the anti-CD20 antibody that may be used in the methods of the invention is ocrelizumab (PRO70769). In another preferred embodiment, the anti-CD20 antibody that may be used in the methods of the invention is IMMU-106. In another preferred embodiment, the anti-CD20 antibody that may be used in the methods of the invention is HuMax-CD20.

[0346] CD22 is a member of the siglec family of sialic acid binding proteins found in a variety of cells, including B lymphocytes. The interaction of CD22 with a variety of cis and trans carbohydrate ligands results in regulation of various aspects of B-cell development, proliferation and activation. In a specific embodiment, an anti-CD22 antibody may be used in the methods of the present invention. In a preferred embodiment, the anti-CD22 antibody that may be used in the methods of the invention is epratuzumab.

Other Immunomodulatory Agents

[0347] In a specific embodiment, the methods of the present invention may be practiced with one or more of the following drugs: CellCept (mycophenolate mofetil; MMF), Orencia® (abatacept; CTLA4-Ig), Riquent™ (abetimus sodium; LJP 394), Prestara™ (praserone), Edratide (TV-4710), Actemra® (tocilizumab; atlizumab), VX-702, TRX 1, IPP-201101, ABR-215757, sphingosine-1-phosphate-1 (S1P1) agonist, HuMax-Inflam™ (MDX 018), MEDI-545 (MDX-1103/1333), RhuDex®, Deoxyspergualin, ENBREL™ (Etanercept), anti-TNF antibody, anti-interferon-alpha antibody.

Patient Populations

[0348] As described herein, data from a clinical trial in which lupus patients were treated with an antibody that neutralizes Neutrokine-alpha protein, significantly ameliorated symptoms associated with lupus in patients having an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA (Example 1). Surprisingly, statistically significant improvements in clinical endpoints measuring disease activity (such as reduction in SELENA SLEDAI score, explained in more detail below) were only obtained in a subset of the patients, as opposed to the entire patient population enrolled in the clinical trial. Thus, the present invention relates to the identification of subgroups of patients that are more likely to respond to treatment with an immunomodulatory agent such as an antagonist of Neutrokine-alpha.

[0349] Additionally, as described herein, systemic lupus erythematosus (SLE) is an extremely heterogeneous disease that is difficult to correctly diagnose due to the broad nature of symptoms that a patient may have and the fact that many of the symptoms associated with lupus are also seen in a number of other autoimmune diseases. Thus, one embodiment of the present invention provides a method of treating a patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein irrespective of the disease diagnosis. Another embodiment of the present invention provides a method of treating a patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum comprising administering a therapeutically effective amount an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein irrespective of the disease diagnosis, and further comprising making a determination that the patient has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum, prior to administering the immunomodulatory agent.

[0350] In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has an autoimmune disease that is not SLE. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has rheumatoid arthritis. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has Sjögren's syndrome. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has scleroderma. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has polymyositis-dermatomyositis. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has Felty's syndrome. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has mixed connective tissue disease. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has Raynaud's syndrome. In further embodiments, the patient that has an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA antibodies in their blood plasma or serum has juvenile chronic arthritis.

[0351] Moreover, it is noted that in both the phase 2 clinical trials using an antibody that neutralizes Neutrokine-alpha protein to treat patients with systemic lupus erythematosus and rheumatoid arthritis (See, Examples 1 and 3) it was observed that patients with autoantibody positive disease at baseline were more likely to respond to treatment. As described herein, SLE patients that had an ANA titer of 1:80 or greater, and/or greater than or equal to 30 IU/mL of anti-dsDNA at baseline showed a stronger response as a group than SLE patients whose ANA titer was less than 1:80 and whose level of anti-dsDNA antibody was less

than 30 IU/mL. Similarly, in rheumatoid arthritis, it was observed that patients that were positive for rheumatoid factor [RF] or anti-cyclic citrullinated peptide [CCP] antibodies at baseline showed a stronger response as a group than rheumatoid arthritis patients that were not positive for rheumatoid factor [RF] or anti-cyclic citrullinated peptide [CCP] antibodies at baseline.

[0352] Thus, in another aspect of the present invention, there is provided a method of treating a patient that is positive for rheumatoid factor [RF] and/or anti-cyclic citrullinated peptide [CCP] antibodies at baseline, comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art an/or described herein irrespective of the disease diagnosis. Another embodiment of the present invention provides a method of treating a patient that that is positive for rheumatoid factor [RF] and/or anti-cyclic citrullinated peptide [CCP] antibodies at baseline comprising administering a therapeutically effective amount an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art an/or described herein irrespective of the disease diagnosis, and further comprising making a determination that the patient that is positive for rheumatoid factor [RF] and/or anti-cyclic citrullinated peptide [CCP] antibodies at baseline, prior to administering the immunomodulatory agent. In specific embodiments, a patient is considered to be positive for rheumatoid factor if they have $\geq$ 12 IU/ml of rheumatoid factor in his/her blood plasma and/or serum. In specific embodiments, a patient is considered to be positive for anti-CCP antibody if the patient has $\geq$ 10 units of anti-CCP antibody in his/her blood plasma and/or serum.

[0353] In a further aspect of the present invention, there is provided a method of treating a patient that is autoantibody positive at baseline, comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art an/or described herein irrespective of the disease diagnosis. Another embodiment of the present invention provides a method of treating a patient is autoantibody positive at baseline comprising administering a therapeutically effective amount an antagonist of Neutrokine-alpha or other immunomodulatory agent known in the art an/or described herein irrespective of the disease diagnosis, and further comprising making a determination that the patient that is autoantibody positive at baseline, prior to administering the immunomodulatory agent.

Making Immunomodulatory Agents

[0354] Methods of making and/or isolating immunomodulatory agents that can be used in the present invention are known to those of skill in the relevant arts. Below, methods available for making immunomodulatory agents that are proteinacious in nature (e.g., anti-Neutrokine-alpha antibodies, Neutrokine-alpha binding peptides and polypeptides, Neutrokine-alpha receptor proteins as well as fragments and variants of the aforementioned polypeptides) are briefly reviewed.

[0355] In one embodiment, a polynucleotide encoding an immunomodulatory protein is inserted in a vector (e.g., a cloning or expression vector). The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells. The polynucleotides encoding an immunomodulatory protein may be joined to a vector containing a selectable marker for propagation in a

host. Introduction of the vector construct into the host cell can be effected by techniques known in the art which include, but are not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986).

[0356] Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, for example, stabilization or simplified purification of expressed recombinant product.

[0357] In one embodiment, the polynucleotide encoding an immunomodulatory protein is operatively associated with an appropriate heterologous regulatory element (e.g., promoter or enhancer), such as, the phage lambda PL promoter, the E. coli lac, trp, phoA, and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan.

[0358] As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293 and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

[0359] The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed. Selection of appropriate vectors and promoters for expression in a host cell is a well-known procedure and the requisite techniques for expression

vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

[0360] Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium, and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice. As a representative, but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Among vectors preferred for use in bacteria include pHE4-5 (ATCC Accession No. 209311; and variations thereof), pQE70, pQE60 and pQE-9, available from QIAGEN, Inc., supra; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL (available from Pharmacia). Other suitable vectors will be readily apparent to the skilled artisan.

[0361] Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

[0362] Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

[0363] In one embodiment, the yeast Pichia pastoris is used to express Neutrokine-alpha protein in a eukaryotic system. Pichia pastoris is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, Pichia pastoris must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (AOX1) is

highly active. In the presence of methanol, alcohol oxidase produced from the AOX1 gene comprises up to approximately 30% of the total soluble protein in Pichia pastoris. See, Ellis, S.B., et al., Mol. Cell. Biol. 5:1111-21 (1985); Koutz, P.J, et al., Yeast 5:167-77 (1989); Tschopp, J.F., et al., Nucl. Acids Res. 15:3859-76 (1987). Thus, a heterologous coding sequence under the transcriptional regulation of all or part of the AOX1 regulatory sequence is expressed at exceptionally high levels in Pichia yeast grown in the presence of methanol.

[0364] In one example, the plasmid vector pPIC9K is used to express DNA encoding a immunomodulatory protein or portion thereof as set forth herein, in a Pichea yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of an immunomodulatory protein by virtue of the strong AOX1 promoter linked to the Pichia pastoris alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

[0365] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

[0366] In one embodiment, high-level expression of a heterologous coding sequence may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

[0367] Transcription of the DNA encoding immunomodulatory proteins by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0368] Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman (Cell 23:175 (1981)), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

[0369] In a specific embodiment, constructs designed to express a portion of an immunomodulatory protein, such as the extracellular domains of the Neutrokine-alpha receptors, (e.g., TACI, BCMA and BAFF-R) are used. One of skill in the art would be able to use the polynucleotide and polypeptide sequences provided as SEQ ID NO:5 and SEQ ID NO:6, respectively, SEQ ID NO:7 and SEQ ID NO:8, respectively,

or SEQ ID NO:9 and SEQ ID NO:10, respectively, to design polynucleotide primers to generate such an expression construct.

[0370] Host cell are used to express the polynucleotides encoding an immunomodulatory protein. Such host cells include primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin. In some cases, the host cells will have been engineered to delete or replace endogenous genetic material (e.g., Neutrokine-alpha coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences). In some instance, the host cell is modified so as to promote and/or alter expression of the endogenous polynucleotide encoding the immunomodulatory protein. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[0371] The host cells described herein can be used in a conventional manner to produce the immunomodulatory protein. Alternatively, cell-free translation systems can also be employed to produce an immunomodulatory polypeptide using RNAs derived from the DNA constructs of the present invention.

[0372] The frame AUG as required may be expressed or synthesized in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. Such a fusion protein can be made by ligating polynucleotides encoding the immunomodulatory protein and the desired nucleic acid sequence encoding the desired amino acid sequence to each other, by methods known in the art, in the proper reading frame, and expressing the fusion protein product by methods known in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

[0373] In one embodiment, a polynucleotide encoding an immunomodulatory protein may be fused to signal sequences which will direct the localization of an immunomodulatory protein to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of the immunomodulatory protein from a prokaryotic or eukaryotic cell. For example, in E. coli, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the polypeptides of the invention may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the pelB signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the ompA signal sequence, the signal sequence of the

periplasmic E. coli heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, polynucleotides encoding an immunomodulatory protein may be fused to the pelB pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. See, U.S. Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

[0374] Examples of signal peptides that may be fused to an immunomodulatory protein in order to direct its secretion in mammalian cells include, but are not limited to, the MPIF-1 signal sequence (amino acids 1-21 of GenBank Accession number AAB51134), the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:27), and a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:28). A suitable signal sequence that may be used in conjunction with baculoviral expression systems is the gp67 signal sequence, (amino acids 1-19 of GenBank Accession Number AAA72759).

[0375] A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to stabilize and purify proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5 has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995) and K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[0376] Immunomodulatory proteins that may be used in the methods of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the immunomodulatory proteins may be glycosylated or may be non-glycosylated. In addition, immunomodulatory proteins may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0377] Immunomodulatory proteins that may be used in the methods of the present invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, Nature 310:105-111). For example, a peptide corresponding to a fragment of an immunomodulatory protein can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical

amino acid analogs can be introduced as a substitution or addition into the polynucleotide sequence encoding the immunomodulatory protein. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0378] Immunomodulatory proteins that may be used in the methods of the present invention may be differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

[0379] Additional post-translational modifications include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the protein.

[0380] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, glucose oxidase or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Tin.

[0381] In specific embodiments, immunomodulatory proteins that may be used in the methods of the present invention may be labeled with Europium. For example, immunomodulatory proteins (*e.g.*, antagonists of Neutrokine-alpha) may be labelled with Europium using the DELFIA Eu-labeling kit (catalog# 1244-302, Perkin Elmer Life Sciences, Boston, MA) following manufacturer's instructions.

[0382] In specific embodiments, immunomodulatory proteins (*e.g.*, are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, ^{111}In, ^{177}Lu, ^{90}Y, ^{166}Ho, and ^{153}Sm, to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to an immunomodulatory protein is ^{111}In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to an immunomodulatory protein is ^{90}Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N",N'"-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to an immunomodulatory protein via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the method disclosed therein in order to conjugate chelating agents to other polypeptides.

[0383] In one embodiment, an immunomodulatory protein that may be used in the methods of the present invention may be labeled with biotin.

[0384] Chemically modified derivatives of immunomodulatory proteins which may provide additional advantages such as increased solubility, stability and in vivo or in vitro circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337) may also be used in the methods of the present invention. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0385] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0386] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo et al., Appl. Biochem. Biotechnol. 56:59-72 (1996); Vorobjev et al., Nucleosides Nucleotides 18:2745-2750 (1999); and Caliceti et al., Bioconjug. Chem. 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0387] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include, for example, lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0388] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0389] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0390] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in

Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0391] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

[0392] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0393] The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

[0394] Immunomodulatory proteins that may be used in the methods of the present invention can be recovered and purified by known methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Formulations and Administration

[0395] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a pharmaceutical composition comprising an immunomodulatory agent, such as an antagonist of Neutrokine-alpha. In a specific embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha antibody. In a specific embodiment, the Neutrokine-alpha antagonist is a TACI-Fc protein. In a specific embodiment, the Neutrokine-alpha antagonist is a BAFF-R-Fc protein. In a specific

embodiment, the Neutrokine-alpha antagonist is an anti-Neutrokine-alpha peptibody. In a specific embodiment, the Neutrokine-alpha antagonist is Neutrokine-alpha protein fragment or variant that functions as a dominant negative. In a preferred embodiment, the immunomodulatory agent is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[0396] An immunomodulatory agent will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the immunomodulatory agent alone), the site of delivery of the composition containing the immunomodulatory agent, the method of administration, the scheduling of administration, and other factors known to practitioners. The "therapeutically effective amount" of an immunomodulatory agent for purposes herein is thus determined by such considerations.

[0397] Various delivery systems are known and can be used to administer a pharmaceutical composition comprising an immunomodulatory agent, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. A pharmaceutical composition comprising an immunomodulatory agent may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical composition comprising an immunomodulatory agent into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0398] In a specific embodiment, the present invention is directed to pharmaceutical formulations of therapeutic agents (e.g., immunomodulatory agents known in the art and/or described herein). In particular, the present invention is directed to pharmaceutical formulations of therapeutic agents that are proteinacious in nature (e.g., proteins and antibodies). A pharmaceutical formulation of the invention contains pharmaceutically acceptable excipients. In general, a pharmaceutical formulation of the invention is formulated such that a therapeutic agent retains its physical, chemical and biological activity. A pharmaceutical formulation of the invention may be stored at suitable temperatures. For example, a pharmaceutical formulation of the invention may be stored at 2-8° C, at -40° C, or at -80° C. In a specific embodiment, a stable formulation is one in which less than about 1% aggregation of the therapeutic agent is observed over 2 years, less than about 1% oxidation of the therapeutic agent is observed over 2 years, and/or less than about 4% deamidation of the therapeutic agent is observed over 2 years. The amount of therapeutic

agent present in a pharmaceutical formulation of the invention is determined, for example, by taking into account the desired dose volumes and mode(s) of administration. In one embodiment of the invention, the concentration of a therapeutic agent in a pharmaceutical formulation of the invention is about 1-160 mg/ml, about 10-155 mg/ml, about 20-150 mg/ml, about 30-145 mg/ml, about 40-140 mg/ml, about 50-135 mg/ml, about 60-130 mg/ml, about 70-125 mg/ml, about 80-120 mg/ml, about 90-115 mg/ml, about 95-110 mg/ml, about 100-105 mg/ml, or about 100 mg/ml. Ranges intermediate to the above recited concentrations, e.g., about 11-154 mg/ml, are also intended to be part of the invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) mg/ml, at the upper and/or lower limits of the range.

[0399] Aqueous pharmaceutical formulations of the invention comprise a pH-buffered solution. In one embodiment of the invention, the buffer used in pharmaceutical formulations of the invention has a pH ranging from about 5 to about 7. In a preferred embodiment, the buffer used in pharmaceutical formulations of the invention has a pH ranging from about 5.5 to about 6.5. In another preferred embodiment, the buffer used in pharmaceutical formulations of the invention has a pH ranging from about 5.8 to about 6.2. In another preferred embodiment, the buffer used in pharmaceutical formulations of the invention has a pH of about 6.0. Ranges intermediate to the above recited pH's are also intended to be part of the invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by 0.5, 0.4, 0.3, 0.2, or 0.1 pH units, at the upper and/or lower limits of the range. Examples of buffers that will control the pH within preferred ranges include acetate (e.g., sodium acetate), succinate (such as sodium succinate), gluconate, histidine, citrate, Tris, phosphate, glycylglycine and other organic acid buffers. Additional exemplary buffers are those which are pharmaceutically acceptable and may be created from suitable acids, bases and salts thereof, such as those which are defined below.

[0400] Pharmaceutically acceptable acids include inorganic and organic acids which are non toxic at the concentration and manner in which they are formulated. For example, suitable inorganic acids include hydrochloric, perchloric, hydrobromic, hydroiodic, nitric, sulfuric, sulfonic, sulfinic, sulfanilic, phosphoric, carbonic, etc. Suitable organic acids include straight and branched-chain alkyl, aromatic, cyclic, cycloaliphatic, arylaliphatic, heterocyclic, saturated, unsaturated, mono-, di- and tri-carboxylic, including for example, formic, acetic, 2-hydroxyacetic, trifluoroacetic, phenylacetic, trimethylacetic, t-butyl acetic, anthranilic, propanoic, 2-hydroxypropanoic, 2-oxopropanoic, propandioic, cyclopentanepropionic, cyclopentane propionic, 3-phenylpropionic, butanoic, butandioic, benzoic, 3-(4-hydroxybenzoyl)benzoic, 2-acetoxy-benzoic, ascorbic, cinnamic, lauryl sulfuric, stearic, muconic, mandelic, succinic, embonic, fumaric, malic, maleic, hydroxymaleic, malonic, lactic, citric, tartaric, glycolic, glyconic, gluconic, pyruvic, glyoxalic, oxalic, mesylic, succinic, salicylic, phthalic, palmoic, palmeic, thiocyanic, methanesulphonic, ethanesulphonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulphonic, 4-chorobenzenesulfonic, napthalene-2-sulphonic, p-toluenesulphonic, camphorsulphonic, 4-

methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 4,4'-methylenebis-3-(hydroxy-2-ene-1-carboxylic acid), hydroxynapthoic, etc.

[0401] Pharmaceutically-acceptable bases include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated. For example, suitable bases include those formed from inorganic base forming metals such as lithium, sodium, potassium, magnesium, calcium, ammonium, iron, zinc, copper, manganese, aluminum, N-methylglucamine, morpholine, piperidine and organic nontoxic bases including, primary, secondary and tertiary amine, substituted amines, cyclic amines and basic ion exchange resins, [e.g., $N(R')_4^+$ (where R' is independently H or C_{1-4} alkyl, e.g., ammonium, Tris)], for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

[0402] Additional pharmaceutically acceptable acids and bases useable with the present invention include those which are derived from the amino acids, for example, histidine, glycine, phenylalanine, aspartic acid, glutamic acid, lysine and asparagine..

[0403] Pharmaceutically acceptable buffers include those derived from both acid and base addition salts of the above indicated acids and bases. In one embodiment of the invention, the buffer of a pharmaceutical formulation of the invention is succinate, histidine, citrate and/or phosphate. In a preferred embodiment, the buffer of a pharmaceutical formulation of the invention is histidine. In another preferred embodiment, the buffer of a pharmaceutical formulation of the invention is citrate.

[0404] In another embodiment of the invention, the buffer concentration in a pharmaceutical formulation of the invention is about 5-50 mM, about 5-20 mM, about 5-15 mM, or about 10 mM. Ranges intermediate to the above recited concentrations, e.g., about 6-48 mM, are also intended to be part of the invention. For example, ranges of values using a combination of any of the above recited values as upper and/or lower limits are intended to be included. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) mM, at the upper and/or lower limits of the range.

[0405] A surfactant may also be added to a pharmaceutical formulation of the invention. Exemplary surfactants include nonionic surfactants such as polysorbates (e.g., polysorbates 20 or 80) or poloxamers (e.g., poloxamer 188). Other pharmaceutically acceptable surfactants are well known in the art and are also contemplated. In a specific embodiment, an amount of surfactant is added in an amount sufficient to reduce aggregation of a therapeutic agent (such as that which occurs upon shaking or shipping), to minimize the formation of particulates in a pharmaceutical formulation of the invention, and/or to reduce non-specific adsorption of a therapeutic agent. In a preferred embodiment, a pharmaceutical formulation of the invention includes a surfactant which is a polysorbate.

[0406] In one embodiment, a pharmaceutical formulation of the invention contains the surfactant polysorbate 20. In one preferred embodiment, a pharmaceutical formulation of the invention contains between at least 0.007% and about 0.07% of polysorbate 20. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.01% and about 0.05% of polysorbate 20. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.01% and about 0.03% of polysorbate 20. In another preferred embodiment, about 0.01% polysorbate 20 is found in a pharmaceutical formulation of the invention. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by 0.01%, 0.009%, 0.008%, 0.007%, 0.006% or 0.005%, at the upper and/or lower limits of the range, with the proviso that the percentage of polysorbate 20 is not lower than 0.007%.

[0407] In another preferred embodiment, a pharmaceutical formulation of the invention contains the surfactant polysorbate 80. In one preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.0015% and about 0.07% of polysorbate 80. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.003% and about 0.05% of polysorbate 80. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.005% and about 0.03% of polysorbate 80. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 0.01% and about 0.03% of polysorbate 80. In another preferred embodiment, about 0.01% polysorbate 80 is found in a pharmaceutical formulation of the invention. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by 0.01%, 0.009%, 0.008%, 0.007%, 0.006% or 0.005%, at the upper and/or lower limits of the range, with the proviso that the percentage of polysorbate 80 is not lower than 0.0015%.

[0408] A tonicity modifier may also be added to a pharmaceutical formulation of the invention. Useful tonicity modifiers include salts and amino acids. Salts that are pharmaceutically acceptable and suitable for pharmaceutical formulations of the invention include sodium chloride, sodium succinate, sodium sulfate, potassuim chloride, magnesium chloride, magnesium sulfate, and calcium chloride. Preferred salts for use in pharmaceutical formulations of the invention are NaCl and $MgCl_2$. NaCl may improve stability of a therapeutic agent by protecting the agent from deamidation and aggregation. In one preferred embodiment, a pharmaceutical formulation of the invention contains NaCl. In another preferred embodiment, a pharmaceutical formulation of the invention contains between about 150 and about 500 mM NaCl. In another preferred embodiment, a pharmaceutical formulation of the invention contains about 150 mM NaCl. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by 1, 2, 3, 4, 5, 10, 25 or 50 mM, at the upper and/or lower limits of the range. In a preferred embodiment, pharmaceutical formulations of the invention are isotonic. By isotonic, it is meant that a pharmaceutical formulation of the invention has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to about 350 mOsm, preferably from about 290 to about 310 mOsm. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) mOsm, at the upper and/or lower limits of the range. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

[0409] In one embodiment, a pharmaceutical formulation of the invention contains the above-identified agents (i.e. therapeutic agent, buffer, surfactant and tonicity modifier) and is essentially free of one or more preservatives, such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium Cl. In another embodiment, a preservative may be included in a pharmaceutical formulation of the invention, particularly where the formulation is a multidose formulation. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in a pharmaceutical formulation of the invention provided that they do not significantly adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g., Zn-protein complexes); biodegradable polymers such as polyesters; preservatives; cryoprotectants; lyoprotectants; bulking agents and the like. Examples of suitable cryoprotectants include polyols, polyethylene glycol (PEG), Bovine Serum Albumin (BSA), glutamic acid, other amino acids and the like. Additional suitable cryoprotectants include sugars and sugar alcohols such as sucrose, mannose, trehalose, glucose, sorbitol, and mannitol and the like. Suitable lyoprotectants can encompass sugars including sucrose, trehalose, lactose, and maltose and the like. Suitable bulking agents include mannitol, glycine, and sorbital and the like.

[0410] In a specific embodiment, a pharmaceutical formulation of the invention does not comprise a cryoprotectant. In a further embodiment, a pharmaceutical formulation of the invention does not comprise sucrose.

[0411] EDTA, which is commonly used to stabilize a protein formulation, may also be included in a pharmaceutical formulation of the invention. EDTA, as a chelating agent, may inhibit the metal-catalyzed oxidation of the sulfhydryl groups, thus reducing the formation of disulfide-linked aggregates. A preferred concentration of EDTA is from about 0.01% to about 0.2%.

[0412] A pharmaceutical formulation of the invention may also be combined with one or more other therapeutic agents as necessary, for the particular indication being treated, preferably those with complementary activities that do not adversely affect a therapeutic agent in a pharmaceutical formulation of the invention. Combinations are contemplated where the additional therapeutic agents are formulated as a mixture with the immunomodulatory agents. In addition, combinations are contemplated where the additional therapeutic agents are formulated independently but are intended for simultaneous or overlapping administration with an immunomodulatory agent. Such additional therapeutic agents are suitably present in combination in amounts that are effective for the purpose intended. Additional therapeutic agents which can be combined with the formulation of the invention are further described herein.

[0413] The present invention provides, in a preferred embodiment, a pharmaceutical formulation comprising, or alternatively consisting of, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 10 mM histidine buffer, 150 mM NaCl, and 0.01%

polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5) for intravenous administration. In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5) for subcutaneous administration. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) mg/ml, at the upper and/or lower limits of the range.

[0414] In a preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5) for intravenous administration. In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 (± 0.5) for subcutaneous administration.

[0415] The present invention provides, in a preferred embodiment, a pharmaceutical formulation comprising, or alternatively consisting of, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5) for intravenous administration. In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, an antibody in an amount from about 1 mg/ml to about 160 mg/ml, preferably from about 80 mg/ml to about 120 mg/ml, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5) for subcutaneous administration. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) mg/ml, at the upper and/or lower limits of the range.

[0416] In a preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5). In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl,

and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5) for intravenous administration. In another preferred embodiment, a pharmaceutical formulation of the invention comprises, or alternatively consists of, 100 mg/ml of an antibody, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl, and 0.1 mg/ml polysorbate 80, pH 6.0 (± 0.5) for subcutaneous administration.

[0417] In a preferred embodiment, the antibody in a pharmaceutical formulation of the invention is a monoclonal antibody. In another preferred embodiment, the antibody in a pharmaceutical formulation of the invention is an IgG antibody. In another preferred embodiment, the antibody in a pharmaceutical formulation of the invention is an IgG1 antibody. In another preferred embodiment, the antibody in a pharmaceutical formulation of the invention is an IgG1/λ antibody. In another preferred embodiment, the antibody in a pharmaceutical formulation of the invention is a human or humanized antibody.

[0418] In a preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 6 months at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 9 months at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 1 year at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 1.5 years at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 2 years at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 3 years at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 4 years at 2-8° C. In another preferred embodiment, a pharmaceutical formulation of the invention is stable for at least 5 years at 2-8° C.

[0419] In a preferred embodiment, an antibody in a pharmaceutical formulation of the invention can display significant stability over repeated freeze-thaw cycles, and following such treatment can remain stable after being thawed. In general, a formulation to be frozen is rapidly frozen, for example, frozen in liquid nitrogen. Thawing can be at a range of temperatures, e.g., from about 0° C to about 25° C, which is slow thawing; or from about 26° C to 40° C, which is rapid thawing. In this context "about" includes the particularly recited ranges, and ranges that are larger or smaller by several (5, 4, 3, 2, or 1) degrees Celsius, at the upper and/or lower limits of the range. An example of rapid thawing is thawing a pharmaceutical formulation of the invention in a 37° C water bath. In a preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least one freeze-thaw cycle. In another preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least two freeze-thaw cycles. In another preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least three freeze-thaw cycles. In another preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least four freeze-thaw cycles. In another preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least five freeze-thaw cycles. In another preferred embodiment, an antibody in a pharmaceutical formulation of the invention is stable for at least ten freeze-thaw cycles.

[0420] It may be desirable to determine an optimal regime for freeze-thawing a pharmaceutical formulation of the invention to preserve stability, or it may be desirable to identify a pharmaceutical

formulation of the invention that provides the greatest stability for an antibody that will be subjected to a particular freeze-thaw cycle. Accordingly, in an embodiment of the invention, this parameter is assessed. For example, a pharmaceutical formulation of the invention can be assayed for stability under a variety of freeze-thaw conditions such as rapid freezing, slow freezing, rapid thawing, slow thawing in various combinations to determine the procedure that produces the fewest degradation products (e.g., that has the greatest stability).

[0421] A concentration study has shown that an IgG1/λ antibody may be concentrated up to at least 160 mg/ml in a pharmaceutical formulation of the invention comprising 10 mM histidine buffer, 150 mM NaCl, and 0.01% polysorbate 80, pH 6.0 without detrimental effects on purity (as determined by SEC-HPLC) and aggregation (no particulates were observed)(data not shown). In addition, an increase in viscosity was observed with concentration. As viscosity increases, the possibility of administration difficulties increases. Studies have shown that samples with viscosities less than 7.75 cP can be easily injected through a 30G ½ inch needle in less than 10 seconds. As shown in Table X, even at an IgG1/λ antibody concentration of 160 mg/ml, the viscosity of the pharmaceutical formulation is below 7.75 cP and thus is still easily injected through a syringe.

Table X. Viscosity as a Function of IgG1/λ Antibody Concentration

Concentration (mg/ml)	Viscosity (cP)
0.0	0.93
18.9	0.97
35.5	1.13
65.7	1.48
79.2	1.80
89.4	1.96
104.0	2.38
113.2	2.68
122.2	3.15
128.0	3.43
136.5	3.89
144.1	4.58
161.7	6.74

[0422] The formulations to be used for in vivo administration is most preferably sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to, or following, preparation of the formulation.

[0423] In a preferred embodiment the antibody of the invention is formulated in 10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 6.5 (± 0.3). In another preferred

embodiment, the antibody of the invention is formulated in 10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 6.5 (± 0.3) for intravenous administration.

[0424] In a preferred embodiment the antibody of the invention is formulated in 10 mM sodium citrate, 8% sucrose, 0.04% (w/v) polysorbate 80 (pH 6.5) (± 0.3). In another preferred embodiment, the antibody of the invention is formulated in 10 mM sodium citrate, 8% sucrose, 0.04% (w/v) polysorbate 80 (pH 6.5) for intravenous administration. In another preferred embodiment, the antibody of the invention is formulated in 10 mM sodium citrate, 8% sucrose, 0.04% (w/v) polysorbate 80 (pH 6.5) for subcutaneous administration.

[0425] Generally, the formulations are prepared by contacting the Neutrokine-alpha antagonist or other immunomodulatory agent known in the art and/or described herein uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0426] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, sucrose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; preservatives, such as cresol, phenol, chlorobutanol, benzyl alcohol and parabens, and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0427] Compositions comprising immunomodulatory polypeptides are typically formulated in such vehicles at a concentration of about 0.001 mg/ml to 100 mg/ml, or 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml or 1-10 mg/ml, at a pH of about 3 to 10, or 3 to 8, more preferably 5-8, most preferably 6-7. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

[0428] Compositions to be used for therapeutic administration are most preferably sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0429] Pharmaceutical compositions comprising immunomodulatory agents that may be used the methods of the present invention ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an

example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Neutrokine-alpha polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Neutrokine-alpha polypeptide using bacteriostatic Water-for-Injection.

[0430] Alternatively, pharmaceutical compositions comprising immunomodulatory agents that may be used the methods of the present invention are stored in single dose containers in lyophilized form. The infusion selection is reconstituted using a sterile carrier for injection.

[0431] In specific embodiments, immunomodulatory agents that maybe used in the present invention are radiolabelled polypeptides such as a radiolabelled form of Neutrokine-alpha or anti-CD20 antibody. Such pharmaceutical compositions comprising radiolabelled molecules may also comprise radioprotectants and plasma expanders such as sodium ascorbate, gentran-40, and glycerol. In specific embodiments, compositions that may be used in the methods of the present invention comprise iodinated forms of Neutrokine-alpha polypeptides or fragments or variants thereof which are formulated in 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Genetran-40.

[0432] In specific embodiments, a composition that may be used in the methods of the present invention comprises at least 1 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise at least 2 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise at least 3 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise at least 4 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise about 4.6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40.

[0433] In specific embodiments, a composition that may be used in the methods of the present invention comprise about between 0.1 mg/mL and 20mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise between 1mg/mL and 10mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise between 2mg/mL and 8mg/mL of an

iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition that may be used in the methods of the present invention comprise between 3mg/mL and 6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40.

[0434] In preferred embodiments, a composition that may be used in the methods of the present invention comprises an anti-Neutrokine-alpha antibody. In other embodiments, a composition that may be used in the methods of the present invention comprises an antibody that specifically binds Neutrokine-alpha. In other embodiments, a composition that may be used in the methods of the present invention comprises an antagonistic anti-Neutrokine-alpha antibody. In other embodiments, a composition that may be used in the methods of the present invention comprises antibody that specifically binds Neutrokine-alpha and neutralizes Neutrokine-alpha biological activity. In other embodiments, a composition that may be used in the methods of the present invention comprises an anti-Neutrokine-alpha antibody that binds a recombinant Neutrokine-alpha protein purified from a cell culture wherein said recombinant Neutrokine-alpha protein is encoded by a polynucleotide encoding at least amino acids 134 to 285 of SEQ ID NO:2. In other embodiments, a composition that may be used in the methods of the present invention comprises an antibody that specifically binds Neutrokine-alpha wherein said antibody binds a recombinant Neutrokine-alpha protein purified from a cell culture wherein said recombinant Neutrokine-alpha protein is encoded by a polynucleotide encoding at least amino acids 134 to 285 of SEQ ID NO:2.

[0435] Pharmaceutical compositions containing immunomodulatory agents may be administered orally, rectally, parenterally, subcutaneously, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray (e.g., via inhalation of a vapor or powder).

[0436] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0437] In a preferred embodiment, compositions containing immunomodulatory agents are administered subcutaneously.

[0438] In another preferred embodiment, compositions containing immunomodulatory agents are administered intravenously.

[0439] Compositions containing immunomodulatory agents may also be administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0440] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981),

and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

[0441] In a specific embodiment, compositions containing immunomodulatory agents are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific embodiments compositions containing immunomodulatory agents are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado). In other specific embodiments, compositions containing immunomodulatory agents are formulated using the ProLease® sustained release system available from Alkermes, Inc. (Cambridge, MA).

[0442] Examples of biodegradable polymers which can be used in the pharmaceutical formulations, include but are not limited to, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic. These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of compositions containing immunomodulatory agents are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug release profile (See, e.g., Ravivarapu et al., Journal of Pharmaceutical Sciences 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

[0443] It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alchohols, dils, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alchohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate,

and triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

[0444] Additionally, formulations compositions containing immunomodulatory agents and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebecate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C.sub.6 -C.sub.12 alkanols, 2-ethoxyethanol, and the like. The release rate modification agent may be used singly or in combination with other such agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to, sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

[0445] In specific embodiments compositions containing immunomodulatory agents are formulated using the BEMA™ BioErodible Mucoadhesive System, MCA™ MucoCutaneous Absorption System, SMP™ Solvent MicroParticle System, or BCP™ BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

[0446] Sustained-release compositions also include liposomally entrapped compositions (see generally, Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes may be prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal immunomodulatory therapy.

[0447] In another embodiment sustained release compositions include crystal formulations known in the art.

[0448] In yet an additional embodiment, the compositions comprising an immunomodulatory agent are delivered by way of a pump (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0449] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0450] For parenteral administration, in one embodiment, the immunomodulatory agent is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, when the active ingredient is an immunomodulatory protein, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0451] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0452] In another embodiment, the immunomodulatory agent can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[0453] In yet another embodiment, the immunomodulatory agent can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Press, Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0454] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

[0455] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it

becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0456] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0457] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an

ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0458] Immunomodulatory agents may be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0459] The amount of the immunomodulatory agent which will be effective in the methods of the invention as described herein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0460] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. In preferred embodiments, a dose of 1, 4, 10, or 20 mg/kg is administered intravenously to a patient. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[0461] As a general proposition, the total pharmaceutically effective amount of a polypeptide administered parenterally per dose will be in the range of about 1 microgram/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day.

[0462] In another embodiment, a polypeptide is administered to a human at a dose between 0.0001 and 0.045 mg/kg/day, preferably, at a dose between 0.0045 and 0.045 mg/kg/day, and more preferably, at a dose of about 45 microgram/kg/day in humans; and at a dose of about 3 mg/kg/day in mice.

[0463] If given continuously, the polypeptide is typically administered at a dose rate of about 1 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0464] The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0465] Compositions comprising immunomodulatory agents may be administered as a continuous infusion, multiple discreet injections per day (e.g., three or more times daily, or twice daily), single injection per day, or as discreet injections given intermittently (e.g., twice daily, once daily, every other day, twice weekly, weekly, biweekly, monthly, bimonthly, and quarterly). If given continuously, a polypeptide is typically administered at a dose rate of about 0.001 to 10 microgram/kg/ hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump.

[0466] Effective dosages of the compositions comprising immunomodulatory agents to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Such determination is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0467] Bioexposure of an organism to an immunomodulatory agent may also play an important role in determining a therapeutically and/or pharmacologically effective dosing regime. Variations of dosing such as repeated administrations of a relatively low dose of an immunomodulatory agent for a relatively long period of time may have an effect which is therapeutically and/or pharmacologically distinguishable from that achieved with repeated administrations of a relatively high dose of an immunomodulatory agent for a relatively short period of time.

[0468] Using the equivalent surface area dosage conversion factors supplied by Freireich, E. J., et al. (Cancer Chemotherapy Reports 50(4):219-44 (1966)), one of ordinary skill in the art is able to conveniently convert data obtained from the use of immunomodulatory agent in a given experimental system into an accurate estimation of a pharmaceutically effective amount of immunomodulatory agent to be administered per dose in another experimental system. Experimental data obtained through the administration of the immunomodulatory agent in mice, for example, may converted through the conversion factors supplied by Freireich, et al., to accurate estimates of pharmaceutically effective doses of Neutrokine-alpha in rat, monkey, dog, and human. The following conversion table (Table III) is a summary of the data provided by Freireich, et al. Table III gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent surface area dose expressed as mg/kg in another species tabulated.

Table III. Equivalent Surface Area Dosage Conversion Factors.

			--TO--		
	Mouse	Rat	Monkey	Dog	Human
--FROM--	(20g)	(150g)	(3.5kg)	(8kg)	(60kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	5/3	1	1/2
Human	12	7	3	2	1

[0469] Thus, for example, using the conversion factors provided in Table III, a dose of 50 mg/kg in the mouse converts to an appropriate dose of 12.5 mg/kg in the monkey because (50 mg/kg) x (1/4) = 12.5 mg/kg. As an additional example, doses of 0.02, 0.08, 0.8, 2, and 8 mg/kg in the mouse equate to effect doses of 1.667 micrograms/kg, 6.67 micrograms/kg, 66.7 micrograms/kg, 166.7 micrograms/kg, and 0.667 mg/kg, respectively, in the human.

[0470] In certain embodiments, administration of radiolabeled forms of Neutrokine-alpha or anti-Neutrokine-alpha antibody is contemplated. The radiometric dosage to be applied can vary substantially. The radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.1 to about 100 mCi per 70 kg body weight. In another embodiment, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.1 to about 50 mCi per 70 kg body weight. In another embodiment, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 or 100 mCi per 70 kg body weight.

[0471] The radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.1 to about 10 mCi/kg body weight. In another embodiment, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.25 to about 5 mCi/kg body weight. In specific embodiments, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 0.35, 0.70, 1.35, 1.70, 2.0, 2.5 or 3.0 mCi/kg.

[0472] The radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 1 to about 50 mCi/m^2. In another embodiment, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 10 to about 30 mCi/m^2. In specific embodiments, the radiolabeled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition can be administered at a dose of about 10, 15, 20, 25, or 30 mCi/m2.

[0473] The concentration of total Neutrokine-alpha protein, Neutrokine-alphaSV protein, anti-Neutrokine-alpha antibody and/or anti-Neutrokine-alphaSV antibody in a radiolabelled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition may also vary, for example from about 1 microgram/kg to about 1 mg/kg. In specific embodiments, the total concentration of Neutrokine-alpha protein, Neutrokine-alphaSV protein, anti-Neutrokine-alpha antibody and/or anti-Neutrokine-alphaSV antibody in a radiolabelled Neutrokine-alpha or anti-Neutrokine-alpha antibody composition may be about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 , 55, 60, 65, 70, 75, 80, 85, 90, 95 100 micrograms/kg.

[0474] For example, lymphomas are known to be radiosensitive tumors. For immunodiagnostic imaging, trace-labeling by the complex may be used, typically 1-20 mg of Neutrokine-alpha protein is labeled with about 1 to 60 mCi of radioisotope. The dose may be somewhat dependent upon the isotope used for imaging; amounts in the higher end of the range, preferably 40 to 60 mCi, may be used with ^{99m}Tc; amounts in the lower end of the range, preferably 1-20 mCi, may be used with ^{111}In. For imaging purposes, about 1 to about 30 mg of Neutrokine-alpha complex can be given to the subject. For radioimmunotherapeutic purposes, the Neutrokine-alpha complex is administered to a subject in sufficient

amount so that the whole body dose received is up to about 1100 cGy, but preferably less than or equal to 500 cGy. The total amount of Neutrokine-alpha protein administered to a subject, including Neutrokine-alpha protein, Neutrokine-alpha conjugate and Neutrokine-alpha complex, can range from 1.0 μg/kg to 1.0 mg/kg of patient body weight. In another embodiment, total amount of Neutrokine-alpha protein administered to a subject, can range from 20 μg/kg to 100 μg/kg of patient body weight.

[0475] An amount of radioactivity which would provide approximately 500 cGy to the whole body of a human is estimated to be about 825 mCi of ^{131}I. The amounts of radioactivity to be administered depend, in part, upon the isotope chosen. For ^{90}Y therapy, from about 1 to about 200 mCi amounts of radioactivity are considered appropriate, with preferable amounts being 1 to 150 mCi, and 1 to 100 mCi (e.g., 60 mCi) being most preferred. The preferred means of estimating tissue doses from the amount of administered radioactivity is to perform an imaging or other pharmacokinetic regimen with a tracer dose, so as to obtain estimates of predicted dosimetry. In determining the appropriate dosage of radiopharmaceutical to administer to an individual, it is necessary to consider the amount of radiation that individual organs will receive compared to the maximum tolerance for such organs. Such information is known to those skilled in the art, for example, see Emami et al., International Journal of Radiation Oncology, Biology, Physics 21:109-22 (1991); and Meredith, Cancer Biotherapy & Radiopharmaceuticals 17:83-99 (2002), both of which are hereby incorporated by reference in their entireties.

[0476] A "high-dose" protocol, for example in the range of 200 to 600 cGy (or higher) to the whole body, may require the support of a bone-marrow replacement protocol, as the bone-marrow is the tissue which limits the radiation dosage due to toxicity.

[0477] In specific embodiments, a patient receives multiple administrations of a composition (e.g., antibody that specifically binds Neutrokine-alpha or other immunomodulatory agent known in the art and/or described herein). One set of multiple administrations is referred to as a cycle. A single cycle may comprise, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more administrations. For any one administration, the dose may be fixed or variable to allow for initial drug loading and/or to account for patient-specific differences in mass, body surface area, disease activity, disease responsiveness, drug tolerability, recovery times, PK parameters, and/or pharmacological response(s).

[0478] The time between any two administrations within a given cycle may be may be fixed or variable to accommodate patient-specific differences in disease activity, disease responsiveness, drug tolerability, recovery times, PK parameters, and/or pharmacological response(s). In specific embodiments, patients are given an initial loading dose that is twice the amount given in subsequent administrations. In other embodiments, the time between any two administrations may be 1, 2, 3, 4, 5, 6, or 7 days (1week) or greater. In specific embodiments, the time between any two administrations may be 1, 2, 3, 4, 5, 6, 7, or 8 weeks or greater. Patients may also receive multiple cycles of treatment. If more than one cycle is needed, the time between any two treatment cycles may be fixed or variable to accommodate patient-specific differences in disease activity, disease responsiveness, drug tolerability, recovery times, PK parameters, and/or pharmacological response(s). In specific embodiments, the time between any two cycles may be 1, 2, 3, 4, 5, 6 weeks or greater. In specific embodiments, the time between any two cycles may be 1, 2, 3, 4, 5, 6,

7, 8, 9, 10, 11, 12 months or greater. In specific embodiments, the time between any two cycles may be 1, 2, 3, 4, 5 years or greater. In specific embodiments, a patient receives an initial bolus administration followed by one or multiple cycle treatments.

[0480] In one embodiment, the initial bolus administration comprises a dose of more than or equal to 2 mg/kg of an antibody antagonist of Neutrokine-alpha administered intravenously to a patient. In one embodiment, the initial bolus administration comprises a dose of more than or equal to 5 mg/kg of an antibody antagonist of Neutrokine-alpha administered intravenously to a patient. In preferred embodiments, the initial bolus administration is a dose of more than or equal to 10 mg/kg of an antibody antagonist of Neutrokine-alpha administered intravenously to a patient. In other embodiments, the initial bolus administration is a dose of more than or equal to 15 mg/kg of an antibody antagonist of Neutrokine-alpha administered intravenously to a patient. In one embodiment, the initial bolus administration comprises a dose of more than or equal to 20 mg/kg of the antibody of the invention administered intravenously to a patient.

[0481] In other specific embodiments, the initial bolus comprises an anti-CD20 antibody.

[0482] In other specific embodiments, the initial bolus comprises a B-cell depleting agent.

[0483] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0484] An immunomodulatory agent may be administered alone or in combination with other therapeutic agents, including but not limited to, one or more additional immunomodualory agents, chemotherapeutic agents, antibiotics, antivirals, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0485] In the succeeding paragraphs, it is disclosed that an immunomodulatory agent may be administered in combination with another compound. In certain instances, the additional compound is itself also an immunomodulatory agent. The disclosure in those paragraphs is intended to convey that it is specifically contemplated that two or more distinct immunomodulatory agents may be administered in combination with one another in conjunction with the methods of the present invention. For example, it is specifically contemplated that an anti-Neutrokine-alpha antibody may be used in conjunction with an anti-CD20 antibody in conjunction with the methods of the present invention.

[0486] Conventional nonspecific immunosuppressive agents, that may be administered in combination an immunomodulatory agent include, but are not limited to, steroids, cyclosporine, cyclosporine analogs

cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents, that may be administered in combination with an immunomodulatory agent include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685).

[0487] In specific embodiments, an immunomodulatory agent is administered in combination with an immunosuppressant. Immunosuppressant preparations that may be administered with an immunomodulatory agent include, but are not limited to, ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate motefil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrxate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0488] In another embodiment, an immunomodulatory agent is administered in combination with steroid therapy. Steroids that may be administered in combination with an immunomodulatory agent, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, an immunomodulatory agent is administered in combination with prednisone. In a further specific embodiment, an immunomodulatory agent is administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with an immunomodulatory agent and prednisone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV. In another specific embodiment, an immunomodulatory agent is administered in combination with methylprednisolone. In a further specific embodiment, an immunomodulatory agent is administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with an immunomodulatory agent and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV.

[0489] In a preferred embodiment, an immunomodulatory agent is administered in combination with an antimalarial. Antimalarials that may be administered with an immunomodulatory agent include, but are not limited to, hydroxychloroquine (e.g., PLAQUENIL™), chloroquine, and/or quinacrine.

[0490] In a preferred embodiment, an immunomodulatory agent is administered in combination with an NSAID.

[0491] In a nonexclusive embodiment, an immunomodulatory agent is administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasermin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000

(Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0492] In one embodiment, an immunomodulatory agent is administered in combination with one or more of the following drugs: Infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.), SCIO-469 (p38 kinase inhibitor from Scios, Inc), Humira® (adalimumab from Abbott Laboratories) and/or ASLERA™ (prasterone, dehydroepiandrosterone, GL701) from Genelabs Technologies Inc.

[0493] In another embodiment, an immunomodulatory agent is administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California), and prednisolone.

[0494] In another embodiment, an immunomodulatory agent is administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, an immunomodulatory agent is administered in combination with methotrexate. In another embodiment, an immunomodulatory agent is administered in combination with anti-TNF antibody. In another embodiment, an immunomodulatory agent is administered in combination with methotrexate and anti-TNF antibody. In another embodiment, an immunomodulatory agent is administered in combination with suflasalazine. In another specific embodiment, an immunomodulatory agent is administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, an immunomodulatory agent is administered in combination ENBREL™. In another embodiment, an immunomodulatory agent is administered in combination with ENBREL™ and methotrexate. In another embodiment, an immunomodulatory agent is administered in combination with ENBREL™, methotrexate and suflasalazine. In another embodiment, an immunomodulatory agent is administered in combination with ENBREL™, and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specfic embodiment, an immunomodulatory agent is administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specfic embodiment, an immunomodulatory agent is administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0495] In an additional embodiment, an immunomodulatory agent is administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with an immunomodulatory agent include, but are not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a

specific embodiment, an immunomodulatory agent is administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0496] In an additional embodiment, an immunomodulatory agent is administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with an immunomodulatory agent include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0497] In specific embodiments, an immunomodulatory agent is administered alone or in combination with anti-CD4 antibody. In one embodiment, coadministration of an immunomodulatory agent with anti-CD4 antibody is envisioned for treatment of rheumatoid arthritis. In one embodiment, coadministration of an immunomodulatory agent with anti-CD4 antibody is envisioned for treatment of systemic lupus erythematosus.

[0498] In specific embodiments, an immunomodulatory agent is administered alone or in combination with anti-IL-15 antibody. In one embodiment, coadministration of an immunomodulatory agent with anti-IL-15 antibody is envisioned for treatment of rheumatoid arthritis. In one embodiment, coadministration of an immunomodulatory agent with anti-IL-15 antibody is envisioned for treatment of systemic lupus erythematosus.

[0499] In specific embodiments, an immunomodulatory agent is administered alone or in combination with CTLA4-Ig and LEA29Y. In one embodiment, coadministration of an immunomodulatory agent with CTLA4-Ig and LEA29Y is envisioned for treatment of rheumatoid arthritis. In one embodiment, coadministration of an immunomodulatory agent with CTLA4-Ig and LEA29Y is envisioned for treatment of systemic lupus erythematosus.

[0500] In specific embodiments, an immunomodulatory agent is administered alone or in combination with anti-IL-6 Receptor antibody. In one embodiment, coadministration of an immunomodulatory agent with anti-IL-6 Receptor antibody is envisioned for treatment of rheumatoid arthritis. In one embodiment, coadministration of an immunomodulatory agent with anti-IL-6 Receptor antibody is envisioned for treatment of systemic lupus erythematosus.

[0501] In specific embodiments, an immunomodulatory agent is administered alone or in combination with anti-C5 (complement component) antibody. In one embodiment, coadministration of an immunomodulatory agent with anti-C5 antibody is envisioned for treatment of rheumatoid arthritis. In one embodiment, coadministration of an immunomodulatory agent with anti-C5 antibody is envisioned for treatment of systemic lupus erythematosus.

[0502] In specific embodiments, an immunomodulatory agent is administered alone or in combination with complement cascade inhibitors. Complement cascade inhibitors include, but are not limited to, anti-

properdin antibodies (Gliatech); TP-10, a recombinant soluble type I complement receptor (AVANT Immunotheragenetics Inc.); Pexelizmab, a Complement C5 inhibitor (Alexion Pharmaceuticals Inc.); and 5G1.1, a monoclonal antibody that prevents cleavage of complement component C5 into its pro-inflammatory components. In one embodiment, coadministration of an immunomodulatory agent with complement cascade inhibitors is envisioned for treatment of Inflammation, Rheumatoid arthritis and/or systemic lupus erythematosus.

[0503] In another embodiment, an immunomodulatory agent is administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with an immunomodulatory agent include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0504] In a specific embodiment, an immunomodulatory agent is administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, an immunomodulatory agent is administered in combination with anti-CD20 antibodies, such as human monoclonal anti-CD20 antibodies. In another embodiment, an immunomodulatory agent is administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, an immunomodulatory agent is administered in combination with Rituximab. In a further embodiment, an immunomodulatory agent is administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, an immunomodulatory agent is administered in combination with tositumomab (anti-CD20 antibody from Coulter Pharmaceuticals, San Francisco, CA). In a further embodiment, an immunomodulatory agent is administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Tositumomab may optionally be associated with 131I. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

[0505] In another specific embodiment, an immunomodulatory agent is administered in combination Zevalin™. In a further embodiment, an immunomodulatory agent is administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly

cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radioisotopes. Particularly preferred isotopes are ^{90}Y and ^{111}In.

[0506] In additional embodiments, an immunomodulatory agent is administered in combination with Rituximab (Rituxan™) and/or Ibritumomab Tiuxetan (Zevalin™, e.g., either (In-111) Ibritumomab Tiuxetan or (Y-90) Ibritumomab Tiuxetan). In a specific embodiment, an immunomodulatory agent is administered in combination with Rituximab and/or Ibritumomab Tiuxetan for the treatment of non-Hodgkin's lymphoma

[0507] In specific embodiments, an immunomodulatory agent is administered as a chronic treatment that is supplemented with anti-CD20 administration following disease flare, e.g., after a lupus flare.

[0508] In additional embodiments, an immunomodulatory agent is administered in combination with imatinib mesylate (Gleevec®: 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide methanesulfonate).

[0509] In additional embodiments, an immunomodulatory agent is administered in combination with bortezomib (Velcade™ [(1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino]propyl]amino]butyl] boronic acid).

[0510] In additional embodiments, an immunomodulatory agent is administered in combination with Alemtuzumab (Campath®).

[0511] In additional embodiments, an immunomodulatory agent is administered in combination with fludarabine phosphate (Fludara®: 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-β-D-arabinofuranosyl) (2-fluoro-ara-AMP)).

[0512] An immunomodulatory agent may be administered in combination with one or more therapeutic agents useful in the treatment of multiple myeloma including but not limited to, Alkylating agents, Anthracyclines, Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Prednisone, Thalidomide and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0513] Preferred combinations of therapeutic agents useful in the treatment of multiple myeloma which may be administered in combination with an immunomodulatory agent include, but are not limited to, Cyclophosphamide + Prednisone, Melphalan + Prednisone (MP), Vincristine + Adriamycin® + Dexamethasone (VAD), Vincristine + Carmustine + Melphalan + Cyclophosphamide + Prednisone (VBMCP; the M2 protocol), and Vincristine + Melphalan + Cyclophosphamide + Prednisone alternating with Vincristine + Carmustine + Doxorubicin + Prednisone (VMCP/VBAP).

[0514] An immunomodulatory agent may be administered in combination with one or more therapeutic agents useful in the treatment of non-Hodgkin's lymphoma including but not limited to, 2-chlorodeoxyadenosine, Amifostine (Ethyol®, Ethiofos®, WR-272), Bexarotene (Targretin®, Targretin gel®, Targretin oral®, LGD1069), Bleomycin (Blenoxane®), Busulfan (Busulfex®, Myleran®), Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Chlorambucil (Leukeran®), Cisplatin (Platinol®, CDDP), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside,

DepoCyt®), Dacarbazine (DTIC), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Denileukin diftitox (Ontak®), Dexamethasone (Decadron®), Dolasetron mesylate (Anzemet®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Erythropoietin (EPO®, Epogen®, Procrit®), Etoposide phosphate (Etopophos®), Etoposide (VP-16, Vepesid®), Fludarabine (Fludara®, FAMP), Granisetron (Kytril®), Hydrocortisone, Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Interferon alpha (Alfaferone®, Alpha-IF®), Interferon alpha 2a (Intron A®), Mechlorethamine (Nitrogen Mustard, HN_2, Mustargen®), Melphalan (L-PAM, Alkeran®, Phenylalanine mustard), Methotrexate® (MTX, Mexate®, Folex®), Methylprednisolone (Solumedrol®), Mitoxantrone (Novantrone®, DHAD), Ondansetron (Zofran®), Pentostatin (Nipent®, 2-deoxycoformycin), Perfosfamide (4-hydroperoxycyclophosphamide, 4-HC), Prednisone, Procarbazine (Matulane®), Rituximab® (Rituxan®, anti-CD20 MAb), Thiotepa (triethylenethiophosphaoramide, Thioplex®), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Vinblastine (Velban®, VLB), Vincristine (Oncovin®, Onco TCS®, VCR, Leurocristine®) and Vindesine (Eldisine®, Fildesin®).

[0515] Preferred combinations of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with an immunomodulatory agent include, but are not limited to, Adriamycin® + Blenoxane + Vinblastine + Dacarbazine (ABVD), Anti-idiotype therapy (BsAb) + Interferon alpha, Anti-idiotype therapy (BsAb) + Chlorambucil, Anti-idiotype therapy (BsAb) ± Interleukin-2, BCNU (Carmustine) + Etoposide + Ara-C (Cytarabine) + Melphalen (BEAM), Bleomycin + Etoposide + Adriamycin + Cyclophosphamide + Vincristine + Procarbazine + Prednisone (BEACOPP), Bryostatin + Vincristine, Cyclophosphamide + BCNU (Carmustine) + VP-16 (Etoposide) (CBV), Cyclophosphamide + Vincristine + Prednisone (CVP), Cyclophosphamide + Adriamycin® (Hydroxyldaunomycin) + Vincristine (Oncovorin) + Prednisone (CHOP), Cyclophosphamide + Novantrone® (Mitoxantrone) + Vincristine (Oncovorin) + Prednisone (CNOP), Cyclophosphamide + Doxorubicin + Teniposide + Prednisone, Cyclophosphamide + Adriamycin® (Hydroxyldaunomycin) + Vincristine (Oncovorin) + Prednisone + Rituximab (CHOP + Rituximab), Cyclophosphamide + Doxorubicin + Teniposide + Prednisone + Interferon alpha, Cytarabine + Bleomycin + Vincristine + Methotrexate (CytaBOM), Dexamethasone + Cytarabine + Cisplatin (DHAP), Dexamethasone + Ifosfamide + Cisplatin + Etoposide (DICE), Doxorubicin + Vinblastine + Mechlorethamine + Vincristine + Bleomycin + Etoposide + Prednisone (Stanford V), Etoposide + Vinblastine + Adriamycin (EVA), Etoposide + Methylprednisone + Cytarabine + Cisplatin (ESHAP), Etoposide + Prednisone + Ifosfamide + Cisplatin (EPIC), Fludarabine, Mitoxantrone + Dexamethasone (FMD), Fludarabine, Dexamethasone, Cytarabine (ara-C), + Cisplatin (Platinol®) (FluDAP), Ifosfamide + Cisplatin + Etoposide (ICE), Mechlorethamine + Oncovin® (Vincristine) + Procarbazine + Prednisone (MOPP), Mesna + Ifosfamide + Idarubicin + Etoposide (MIZE), Methotrexate with leucovorin rescue + Bleomycin + Adriamycin + Cyclophosphamide + Oncovorin + Dexamethasone (m-BACOD), Prednisone + Methotrexate + Adriamycin + Cyclophosphamide + Etoposide (ProMACE), Thiotepa + Busulfan + Cyclophosphamide, Thiotepa + Busulfan + Melphalan, Topotecan + Paclitaxel, and Vincristine (Oncovin®) + Adriamycin® + Dexamethasone (VAD).

[0516] Further examples of therapeutic agents useful in the treatment of non-Hodgkin's lymphoma which may be administered in combination with an immunomodulatory agent include, but are not limited to, A007 (4-4'-dihydroxybenzophenone-2, 4-dinitrophenylhydrazone), AG-2034 (AG-2024, AG-2032, GARFT [glycinamide ribonucleoside transformylase] inhibitor), Aldesleukin (IL-2, Proleukin®), Alemtuzumab (Campath®), Alitretinoin (Panretin®, LGN-1057), Altretamine (Hexalen®, hexamethylmelamine, Hexastat®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Anti-CD19/CD3 MAb (anti-CD19/CD3 scFv, anti-NHL MAb), Anti-idiotype therapy (BsAb), Arabinosylguanine (Ara-G, GW506U78), Arsenic trioxide (Trisenox®, ATO), B43-Genistein (anti-CD19 Ab/genistein conjugate), B7 antibody conjugates, Betathine (Beta-LT), BLyS antagonists, Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), CHML (Cytotropic Heterogeneous Molecular Lipids), Clofarabine (chloro-fluoro-araA), Daclizumab (Zenapax®), Depsipeptide (FR901228, FK228), Dolastatin-10 (DOLA-10, NSC-376128), Epirubicin (Ellence®, EPI, 4' epi-doxorubicin), Epratuzumab (Lymphocide®, humanized anti-CD22, HAT), Fly3/flk2 ligand (Mobista®), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Hu1D10 (anti-HLA-DR MAb, SMART 1D10), HumaLYM (anti-CD20 MAb), Ibritumomab tiuxetan (Zevalin®), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), Irinotecan (Camptosar®, CPT-11, Topotecin®, CaptoCPT-1), ISIS-2053, ISIS-3521 (PKC-alpha antisense), Lmb-2 immunotoxin (anti-CD25 recombinant immuno toxin, anti-Tac(Fv)-PE38), Leuvectin® (cytofectin + IL-2 gene, IL-2 gene therapy), Lym-1 (131-I LYM-1), Lymphoma vaccine (Genitope), Nelarabine (Compound 506, U78), Neugene compounds (Oncomyc-NG®, Resten-NG®, myc antisense), NovoMAb-G2 scFv (NovoMAb-G2 IgM), O6-benzylguanine (BG, Procept®), Oxaliplatin (Eloxatine®, Eloxatin®), Paclitaxel (Paxene®, Taxol®), Paclitaxel-DHA (Taxoprexin®), Peldesine (BCX-34, PNP inhibitor), Rebeccamycin and Rebeccamycin analogues, SCH-66336, Sobuzoxane (MST-16, Perazolin®), SU5416 (Semaxanib®, VEGF inhibitor), TER-286, Thalidomide, TNP-470 (AGM-1470), Tositumomab (Bexxar®), Valspodar (PSC 833), Vaxid (B-cell lymphoma DNA vaccine), Vinorelbine (Navelbine®), WF10 (macrophage regulator) and XR-9576 (XR-9351, P-glycoprotein/MDR inhibitor).

[0517] An immunomodulatory agent may be administered in combination with one or more therapeutic agents useful in the treatment of acute lymphocytic leukemia including but not limited to, Amsacrine, Carboplatin (Paraplatin®, CBDCA), Carmustine (DTI-015, BCNU, BiCNU, Gliadel Wafer®), Cholecaliferol, Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®), Daunorubicin (Daunomycin, DaunoXome®, Daunorubicin®, Cerubidine®), Dexamethasone (Decadron®), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Etoposide (VP-16, Vepesid®), Filgrastam® (Neupogen®, G-CSF, Leukine®), Fludarabine (Fludara®, FAMP), Idarubicin (Idamycin®, DMDR, IDA), Ifosfamide (IFEX®), Imatinib mesylate (STI-571, Imatinib®, Glivec®, Gleevec®, Abl tyrosine kinase inhibitor), Interferon gamma (Gamma-interferon, Gamma 100®, Gamma-IF), L-asparaginase (Elspar®, Crastinin®, Asparaginase medac®, Kidrolase®), Mercaptopurine (6-mercaptopurine, 6-MP), Methotrexate® (MTX, Mexate®, Folex®), Mitoxantrone (Novantrone®, DHAD), Pegaspargase® (Oncospar®), Prednisone, Retinoic acid, Teniposide (VM-26, Vumon®), Thioguanine (6-

thioguanine, 6-TG), Topotecan (Hycamtin®, SK&F-104864, NSC-609699, Evotopin®), Tretinoin (Retin-A®, Atragen®, ATRA, Vesanoid®) and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0518] Further examples of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be administered in combination with an immunomodulatory agent include, but are not limited to, Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78, Nelzarabine®), Arsenic trioxide (Trisenox®, ATO, Atrivex®), B43-Genistein (anti-CD19 Ab/genistein conjugate), B43-PAP (anti-CD19 Ab/pokeweed antiviral protein conjugate), Cordycepin, CS-682, Decitabine (5-aza-2'-deoxyytidine), Dolastatin-10 (DOLA-10, NSC-376128), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Quinine, TNP-470 (AGM-1470, Fumagillin), Trimetrexate (Neutrexin®), Troxacitabine (BCH-204, BCH-4556, Troxatyl®), UCN-01 (7-hydroxystaurosporine), WHI-P131 and WT1 Vaccine.

[0519] Preferred combinations of therapeutic agents useful in the treatment of acute lymphocytic leukemia which may be administered in combination with an immunomodulatory agent include, but are not limited to, Carboplatin + Mitoxantrone, Carmustine + Cyclophosphamide + Etoposide, Cytarabine + Daunorubicin, Cytarabine + Doxorubicin, Cytarabine + Idarubicin, Cytarabine + Interferon gamma, Cytarabine + L-asparaginase, Cytarabine + Mitoxantrone, Cytarabine + Fludarabine and Mitoxantrone, Etoposide + Cytarabine, Etoposide + Ifosfamide, Etoposide + Mitoxantrone, Ifosfamide + Etoposide + Mitoxantrone, Ifosfamide + Teniposide, Methotrexate + Mercaptopurine, Methotrexate + Mercaptopurine + Vincristine + Prednisone, Phenylbutyrate + Cytarabine, Phenylbutyrate + Etoposide, Phenylbutyrate + Topotecan, Phenylbutyrate + Tretinoin, Quinine + Doxorubicin, Quinine + Mitoxantrone + Cytarabine, Thioguanine + Cytarabine + Amsacrine, Thioguanine + Etoposide + Idarubicin, Thioguanine + Retinoic acid + Cholecaliferol, Vincristine + Prednisone, Vincristine + Prednisone and L-asparaginase, Vincristine + Dexamethasone/Prednisone + Asparaginase + Daunorubicin/ Doxorubicin, Vincristine + Dexamethasone/Prednisone + Asparaginase + Daunorubicin/ Doxorubicin + Filgrastim, Vincristine + Dexamethasone/Prednisone + Asparaginase + Daunorubicin/Doxorubicin + Cyclophosphamide + Methotrexate, and Vincristine + Dexamethasone/Prednisone + Asparaginase + Daunorubicin/Doxorubicin + Cyclophosphamide + Methotrexate + Filgrastim.

[0520] An immunomodulatory agent may be administered in combination with one or more therapeutic agents useful in the treatment of chronic lymphocytic leukemia including but not limited to, Chlorambucil (Leukeran®), Cladribine (2-CdA, Leustatin®), Cyclophosphamide (Cytoxan®, Neosar®, CTX), Cytarabine (Cytosar-U®, ara-C, cytosine arabinoside, DepoCyt®, cytarabine ocfosfate, ara-CMP), Doxorubicin (Adriamycin®, Doxil®, Rubex®), Fludarabine (Fludara®, FAMP), Pentostatin (Nipent®, 2-deoxycoformycin), Prednisone and Vincristine (Oncovorin®, Onco TCS®, VCR, Leurocristine®).

[0521] Further examples of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with an immunomodulatory agent include, but are not limited to, Alemtuzumab (Campath®), Aminocamptothecin (9-AC, 9-Aminocamptothecin, NSC 603071), Aminopterin, Annamycin (AR-522, annamycin LF, Aronex®), Arabinosylguanine (Ara-G, GW506U78,

Nelzarabine®, Compound 506U78), Arsenic trioxide (Trisenox®, ATO, Atrivex®), Bryostatin-1 (Bryostatin®, BMY-45618, NSC-339555), CS-682, Dolastatin-10 (DOLA-10, NSC-376128), Filgrastim (Neupogen®, G-CSF, Leukine), Flavopiridol (NSC-649890, HMR-1275), G3139 (Genasense®, GentaAnticode®, Bcl-2 antisense), Irofulven (MGI-114, Ivofulvan, Acylfulvene analogue), MS-209, Phenylbutyrate, Rituximab® (Rituxan®, anti-CD20 MAb), Thalidomide, Theophylline, TNP-470 (AGM-1470, Fumagillin), UCN-01 (7-hydroxystaurosporine) and WHI-P131.

[0522] Preferred combinations of therapeutic agents useful in the treatment of chronic lymphocytic leukemia which may be administered in combination with an immunomodulatory agent include, but are not limited to, Fludarabine + Prednisone, and Cyclophosphamide + Doxorubicin + Vincristine + Prednisone (CHOP).

[0523] In an additional embodiment, an immunomodulatory agent is administered in combination with cytokines. Cytokines that may be administered with an immunomodulatory agent include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In another embodiment, an immunomodulatory agent may be administered with any interleukin, including but not limited to, IL-1alpha, IL-1beta, 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, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, an immunomodulatory agent is administered in combination with IL4 and IL10. Both IL4 and IL10 have been observed by the inventors to enhance Neutrokine-alpha mediated B cell proliferation.

[0524] In vitro, IFN gamma and IL-10 have each been observed by the inventors to enhance cell surface expression of Neutrokine-alpha in monocytes and macrophages (macrophages were obtained by culturing primary monocytes with 20ng/mL of M-CSF for 12-15 days), whereas IL-4 treatment decreased cell surface expression of Neutrokine-alpha in monocytes and macrophages. IL-4 administered with IL-10 resulted in a complete inhibition of the IL-10 induced cell surface expression of Neutrokine-alpha. IL-4 administered with IFN-gamma resulted in increased cell-surface expression of Neutrokine-alpha. Treatment of macrophages with IFN-gamma and IL-10 resulted in a 3 fold increase of soluble (active) Neutrokine-alpha released into the culture medium compared to untreated macrophages.

[0525] In an additional embodiment, an immunomodulatory agent is administered with a chemokine. In another embodiment, an immunomodulatory agent is administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, an immunomodulatory agent is administered with chemokine beta-8.

[0526] In an additional embodiment, an immunomodulatory agent is administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with an immunomodulatory agent include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4.

Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0527] In an additional embodiment, an immunomodulatory agent is administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with an immunomodulatory agent include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0528] In an additional embodiment, an immunomodulatory agent is administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with an immunomodulatory agent include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0529] In an additional embodiment, an immunomodulatory agent is administered in combination with an antihypertensive. Antihypertensives that may be administered with an immunomodulatory agent include, but are not limited to, calcium channel blocking agents, such as nifedipine (ADALAT™, PROCARDIA™); peripheral vasodilators, such as hydralazine (APRESOLINE™); Beta-adrenergic blocking agents, such as propranolol (INDERAL™); alpha/beta adrenergic blockers, such as labetolol (NORMODYNE™, TRANDATE™); agents which inhibit the production of angiotensin II, such as captopril (CAPOTEN™); agents which directly inhibit the activity of angiotensin II, such as losartan (COZAAR™); and thiazide diuretics, such as hydrochlorothiazide (HYDRODIURIL™, ESIDREX™).

[0530] Immunomodulatory agents may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with an immunomodulatory agent include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, an immunomodulatory agent is administered in combination with alum. In another specific embodiment, an immunomodulatory agent is administered in combination with QS-21. Further adjuvants that may be administered with an immunomodulatory agent include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with an immunomodulatory agent include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™. In another specific embodiment, an immunomodulatory agent is used in combination with PNEUMOVAX-23™.

[0531] In one embodiment, an immunomodulatory agent is administered in combination with another member of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with an immunomodulatory agent include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL/AIM-I (International Publication No. WO 97/33899), LIGHT/AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International

Publication No. WO 98/07880), FASTR/TR6 (International Publication No. WO 98/30694), Osteoprotegrin (OPG), and Neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), TRAIL-R1/DR4 (International Publication No. WO 98/32856), TRAIL-R3, TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TRAIL-R2/TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TRAIL-R4/TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12.

[0532] In another embodiment, an immunomodulatory agent is administered in combination with one or more Neutrokine-alpha receptors (e.g., TACI, BCMA and BAFF-R). In preferred in embodiments, the Neutrokine-alpha receptor is soluble. In other preferred embodiments, the Neutrokine-alpha receptor is fused to the Fc region of an immunoglobulin molecule such as the Fc region of Ian IgG molecule. For example, amino acid residues 1-154 of TACI (GenBank accession number AAC51790), amino acids 1-48 of BCMA (GenBank accession number NP_001183 or amino acids 1 to 81 of BAFF-R (GenBank Accession Number NP_443177 may be fused to the Fc region of an IgG molecule and used in combination with another immunomodulatory agent known in the art and/or described herein. In another embodiment a BAFF-R-Fc protein that may be administered in combination with an immunomodulatory agent is amino acids 1-70 of SEQ ID NO:10 fused to the Fc region of an IgG1 immunoglobulin molecule. Optionally, amino acid 20 (valine) in BAFF-R is substituted with aspargine and amino acid 27 (leucine) in BAFF-R is substituted with proline.

[0533] In a preferred embodiment, an immunomodulatory agent is administered in combination with anti-CD40L antibodies and/or anti-CD40 antibodies.

[0534] In an additional embodiment, an immunomodulatory agent is administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with an immunomodulatory agent include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0535] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0536] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0537] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0538] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

[0539] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman J Pediatr. Surg. 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., J Clin. Invest. 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0540] Anti-angiogenic agents that may be administered in combination with an immunomodulatory agent may work through a variety of mechanisms including but not limited to, inhibiting proteolysis of the

extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with an immunomodulatory agent include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with an immunomodulatory agent include, but are not limited to, EMD-121974 (Merck KcgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with an immunomodulatory agent include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with an immunomodulatory agent include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

[0541] In particular embodiments, the use of an immunomodulatory agent in combination with an anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

[0542] In a particular embodiment, the use of an immunomodulatory agent in combination with an anti-angiogenic agent is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of an immunomodulatory agent in combination with an anti-angiogenic agent is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

[0543] In another embodiment, an immunomodulatory agent is administered in combination with an anticoagulant. Anticoagulants that may be administered with an immunomodulatory agent include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, an immunomodulatory agent is administered in combination with heparin and/or warfarin. In another specific embodiment, an immunomodulatory agent is administered in combination with warfarin. In another specific embodiment, an immunomodulatory agent is administered in combination with warfarin and aspirin. In another specific embodiment, an immunomodulatory agent is administered in combination with heparin. In another specific embodiment, an immunomodulatory agent is administered in combination with heparin and aspirin.

[0544] In another embodiment, an immunomodulatory agent is administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In specific embodiments, an immunomodulatory agent is administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0545] In certain embodiments, an immunomodulatory agent is administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with an immunomodulatory agent, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with an immunomodulatory agent, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with an immunomodulatory agent, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir).

[0546] In certain embodiments, an immunomodulatory agent is administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with an immunomodulatory agent, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with an immunomodulatory agent is, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with an immunomodulatory agent is, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir).

[0547] Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity in vitro; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β-L-FD4C and β-L-FddC (WO 98/17281).

[0548] Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N

mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

[0549] Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myres Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with in vitro activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Welcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

[0550] Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

[0551] Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ~~ALX~~40-4~~C~~ (a cationic peptide), T22 (an 18 amino-acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES , NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1α, MIP-1β, etc., may also inhibit fusion.

[0552] Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

[0553] Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

[0554] Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

[0555] Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1α, MIP-1β, SDF-1α, IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-8, IL-10, IL-12, and IL-13; interferons such as IFN-α2a; antagonists of TNFs, NFκB, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporine and prednisone; vaccines such as Remune™ (HIV

Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targeted to the ER to block surface expression of newly synthesized CCR5 (Yang et al., PNAS 94:11567-72 (1997); Chen et al., Nat. Med. 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF-α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α-naphthoflavone (WO 98/30213); and antioxidants such as γ-L-glutamyl-L-cysteine ethyl ester (γ-GCE; WO 99/56764).

[0556] In other embodiments, an immunomodulatory agent may be administered in combination with an anti-opportunistic infection agent. Anti-opportunistic agents that may be administered in combination with an immunomodulatory agent, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, an immunomodulatory agent is used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic Pneumocystis carnii pneumonia infection. In another specific embodiment, , an immunomodulatory agent is used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic Mycobacterium avium complex infection. In another specific embodiment, an immunomodulatory agent is used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic Mycobacterium tuberculosis infection. In another specific embodiment, , an immunomodulatory agent is used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, an immunomodulatory agent is used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, an immunomodulatory agent is used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another

specific embodiment, an immunomodulatory agent is used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic Toxoplasma gondii infection. In another specific embodiment, an immunomodulatory agent is used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0557] In a further embodiment, an immunomodulatory agent is administered in combination with an antiviral agent. Antiviral agents that may be administered with an immunomodulatory agent include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0558] In a further embodiment, an immunomodulatory agent is administered in combination with an antibiotic agent. Antibiotic agents that may be administered with an immunomodulatory agent include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0559] Additionally, an immunomodulatory agent may be administered alone or in combination with other therapeutic regimens, including but not limited to, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

Kits

[0560] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions. Optionally, associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds. In a specific embodiment, the kit contains a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration in patients that have an ANA titer greater than or equal to 1:80 and/or greater than or equal to 30 IU of anti-dsDNA antibodies in his/her blood plasma or serum.

Examples

[0561] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1: Summary of Results from a clinical trial testing the use of an antibody (belimumab) that neutralizes Neutrokine-alpha protein to treat systemic lupus erythematosus (SLE).

[0562] A prospective, randomized, double-blind, placebo-controlled trial tested belimumab, an antibody that neutralizes Neutrokine-alpha protein, added to standard of care therapy for SLE. 449 subjects with SLE by ACR criteria (Tan et al., Arthritis Rheum. 25:1271-7, (1982); and Hochberg et al., Arthritis Rheum. 40:1725, (1997)), with a history of measurable autoantibodies and SELENA SLEDAI score ≥4 at screening were dosed.

[0563] Study agent (1, 4, 10 mg/kg belimumab) or placebo was administered intravenously on days 0, 14, 28 then every 28 days over 52 weeks. Subjects who completed the 52-week treatment period were given the option to continue the study for a 24-week extension period. Belimumab was formulated in 10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% (w/v) polysorbate 80, pH 6.5 (± 0.3). Subjects receiving placebo dose received the formulation (10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% (w/v) polysorbate 80, pH 6.5 (± 0.3) without belimumab. Efficacy was assessed every 1-2 months by SELENA SLEDAI (SS), SLE Flare Index, Physician's Global Assessment (PGA). BILAG and SF-36 disease activity scores were also assessed regularly. Predefined primary efficacy endpoints were percent reduction in SS score at week 24 and time to flare over 52 weeks as defined by SLE Flare Index. Biologic markers included ANA, anti-dsDNA antibody (Ab), C3/C4, Ig isotypes, and peripheral B cell FACS. B-cells were analyzed every 1-2 months by 4-color FACS (CD19, CD20, CD27, CD69, CD38, CD138 and CD45). Serum autoantibody, including anti-dsDNA Ab, Ig isotypes, total protein, and albumin levels were obtained at the same visits. The likelihood ratio chi-squared, Wilcoxon test or t-test was used to analyze the changes in the biological markers.

[0564] The mean age of the subjects in this study was 42; the mean duration of SLE in these subjects was 8.8 years. The baseline level of disease activity in these subjects was relatively high, with approximately 67% of subjects having an SS score 8 points or greater (mean SS score: 9.6). Ninety-three percent of the subjects enrolled in this study were female. 70% of subjects were Caucasian; 24% of subjects African American; 3% of subjects Asian; and 18% of subjects Hispanic (categories overlap). Ninety-eight percent of subjects had historically scored positive for ANA and 71.5% of subjects were ANA+ at entry (ANA titer ≥ 1:80 and/or anti-dsDNA Ab ≥ 30 IU/ml at screen/day 0). 50% of subjects had an anti-dsDNA titer ≥ 30 IU/ml at entry. The most common concomitant medications for SLE used at baseline included the following: steroids (approximately 70% of subjects), aminoquinolines (e.g., anti-malarials) (70%), COX-2 inhibitors (28%), COX-1 inhibitors (26%), azathioprine (20%), methotrexate (16%) and mycophenolate mofetil (16%). Thirty-four percent and 42% of active and placebo subjects, respectively, were receiving clinically meaningful doses of systemic corticosteroids (defined as a prednisone or prednisone equivalent dose > 7.5 mg/day) at baseline. There were no significant differences in baseline characteristics or completion rates across treatment arms (81% completed).

[0565] Primary efficacy endpoints did not reach statistical significance, but SS score was significantly reduced by 29% at week 52 in ANA+ subjects (p=0.0435, see Figure 1). SLE flares decreased in belimumab subjects during weeks 24-52 using a 24 week baseline (log rank p= 0.036). Although no significant

differences in composite numerical BILAG scores (BILAG composite calculated by converting organ system grades to numerical scores as follows: A=9, B=3, C=1, D=0, E=0) were observed, in ANA+ subjects, analysis of scores for the 8 individual organ domains revealed fewer increases in score in two organ domains (musculoskeletal, $p < 0.008$; neurological, $p < 0.038$) and a trend toward fewer increases in score in three organ domains (cardiovascular & respiratory, $p = 0.060$; general, $p < 0.15$; renal, $p < 0.15$) in belimumab treated subjects at week 52. The PGA score improved by week 16 ($p=0.016$) through week 52 ($p<0.002$, all active vs. placebo.) Improvements occurred despite increases of prednisone in placebo vs. belimumab-treated (increases to > 7.5mg/day, ~15% vs. ~7%). In ANA+ subjects, a significant reduction in the frequency of increase in prednisone, from low dose of ≤7.5mg/day to high dose of >7.5 mg/day, was observed as early as week 8 ($p<0.05$ over weeks 8-12 and over weeks 32-40). There was no dose response in efficacy, suggesting all doses are equally active. No clinically significant differences were noted in safety, including adverse events (AE), AE severity, infections or lab toxicity in all belimumab arms vs. placebo. Fewer subjects on belimumab had pleurisy (3.3% vs. 8%, $p<0.05$), while more had urticaria (4% vs. 0%, $p<0.05$). Infusion reactions were rare, with only 1 severe event reported. Immunogenicity to belimumab was observed in 1 subject (1 mg/kg).

[0566] As shown in Table IX, analysis of ANA+ subjects at week 52, revealed belimumab treatment resulted in significant stabilization of the disease relative to placebo as measured by the BILAG index (row 3) and by PGA (row 4). Further, response rates among the treatment groups in the trial were also analyzed using a combined response endpoint (row 1), which combined a measure of global disease activity as measured by the SS score, with a measure of a patient's overall condition as assessed by the PGA disease activity index and a measure of disease in specific organ systems as measured by the BILAG scale. A patient was scored as responding to treatment in the combined endpoint if they had a reduction in SELENA SLEDAI score ≥ 4 points, no worsening in their PGA score defined as < 0.3 point increase in PGA score and no worsening in any specific organ system defined as no new BILAG A organ domain score or no 2 new BILAG B organ domain scores. Analysis of ANA+ subjects using the above described composite endpoint revealed a significant response to belimumab ($p=0.0058$).

[0567] Additionally, in ANA+ subjects, significant improvements of PGA and SF-36 Physical Component Scores (SF-36 PCS) were observed early in treatment. The mean percent change from baseline PGA showed significant improvement ($p<0.05$) as early as week 4 in ANA+ subjects treated with belimumab as compared to ANA+ placebo treated subjects. The values for mean percent change in PGA score at 8 weeks, 16 weeks, 48 weeks and 52 weeks also showed significant improvement in belimumab treated ANA+ subjects compared to ANA+ placebo treated subjects ($p<0.05$ at 8, 16 and 48 weeks; $p<0.01$ at week 52). The mean SF-36 PCS also showed significant improvements in quality of life in belimumab treated ANA+ subjects compared to ANA+ placebo treated subjects at weeks 12, 24, 48 and 52 ($p<0.05$ at each time point).

[0568] Significant reductions in B cell counts (expressed as median percent change from baseline) were observed for belimumab-treated subjects over the course of the study, including CD19+ B cells ($p<0.01$ for each measurement taken during weeks 8-52), activated B cells (CD20+/CD69+; $p<0.01$ for each

measurement taken during weeks 8-52), naïve B cells (CD20+/CD27-; p<0.01 for each measurement taken during weeks 8-52), and plasmacytoid B cells (CD20+/CD138+; p<0.01 for each measurement taken during weeks 16-52). Measurements of B cell counts at week 24 demonstrated that belimumab (all treated combined) significantly reduced B-cell by week 24 compared to placebo treated subjects. At week 24, a significant reduction in cell counts (expressed as median percent change from baseline; p<0.0001) was observed for CD19+ B cells, naïve B cells (CD20+/CD27-), activated B cells (CD20+/CD69+), and plasmacytoid B cells (CD20+/CD138+). Belimumab (all treated combined) significantly reduced B-cell counts at week 52 (medians). At week 52, the median percent change in CD20+ B cells was 54%* for all treatment groups combined with significant reduction observed as early as week 8 (p<0.0001). At week 52, the median percent change in plasmacytoid B cells (CD20+/CD138+) was 62%* for all treatment groups combined. And, the median percent change in activated B cells (CD20+/CD69+ B cells) was 70%* at week 52 (* all p<0.002). At week 52, CD19+ B cells and naïve B cells (CD20+/CD27-) were significantly reduced, while memory cell populations were preserved. In contrast, plasma cells (CD20-/CD138+) increased 72.5% over baseline (2.7%) in belimumab treated subjects vs. 30.6% in placebo/standard of care (p=0.02) at week 52. In addition, the belimumab induced reduction in B cell counts continued through week 76. At week 76, the median percent change in CD20+ B cells was 61% for all treatment groups combined. At week 76, the median percent change in plasmacytoid B cells (CD20+/CD138+) was 60% for all treatment groups combined. And the median percent change in activated B cells (CD20+/CD69+ B cells) at week 76 was 84% for all treatment groups combined. Among subjects that had an anti-dsDNA titer ≥ 30 IU/ml at entry, a significant reduction in anti-dsDNA titer (expressed as median percent change from baseline) was observed as early as week 4 in belimumab treated subjects versus placebo treated subjects (p<0.01 for each measurement taken during weeks 4-12; p<0.03 for each measurement taken during weeks 16-24 and p<0.01 for each measurement taken during weeks 32-52). Belimumab reduced anti-dsDNA Ab at week 52 by 30% (p<0.002, baseline positive) vs. 9% in placebo. This effect was sustained as measurement at week 76 showed a 28% reduction in anti-dsDNA Ab. Significant belimumab-induced reductions in serum IgG, IgA, IgE and IgM levels (expressed as median percent change from baseline) were evident as early as week 8 (p<0.0001) in belimumab treated subjects compared to placebo treated controls. At week 52, serum IgG, IgA, IgE and IgM were reduced (10%, 14%, 34% and 29%, respectively). The reductions continued through week 76 (12% 15%, 35% and 34%, respectively). Moreover, for those subjects with elevated Ig isotype levels at baseline, 41% (52/128, p=0.0014)) of the subjects receiving belimumab returned to normal Ig isotype levels while only 16% (7/45) of the control subjects normalized. A significant increase was observed in C4 complement levels (expressed as median percent change from baseline) for each measurement taken during weeks 4-52 among patients with low C4 complement at baseline in belimumab-treated arms (p≤0.01). At week 52, C4 had increased by 33% (p=0.0126, low baseline C4) in belimumab-treated arms. Again, the belimumab effect was sustained, with C4 improving to 46% at week 76, in belimumab-treated arms. At week 52, 14.5% (24/165) of anti-dsDNA+ subjects receiving belimumab converted to negative compared with 3.5% (2/58) on placebo (p=0.012). At week 76, 3 additional anti-dsDNA+ subjects receiving belimumab converted to negative.

[0569] Belimumab was well tolerated and demonstrated significant bioactivity. Belimumab improved PGA scores, reduced B cell counts, increased C4, reduced anti-dsDNA, and reduced/normalized Ig isotype levels. Belimumab delayed flare onset after 6 months. In subjects with ANA positivity at entry, the SS score improved significantly at Week 52. Finally, a combined response endpoint revealed a significant response to belimumab treatment by ANA+ subjects (see TABLE IX).

TABLE IX. Response Rate in ANA+ Subjects at Week 52

		Placebo N=86	1.0 mg/kg N=78	4.0 mg/kg N=79	10.0 mg/kg N=78	All Active N=235	P-value[a]
1	Response rate (% of subjects with reduction in SELENA SLEDAI ≥ 4 and no worsening by BILAG index (no new BILAG A organ domain score or 2 new BILAG B organ domain scores) and no worsening by PGA (< 0.3 point increase)	25 (29.1%)	38 (48.7%)	34 (43.0%)	36 (46.2%)	108 (46.0%)	0.0058
2	% of subjects with reduction in SELENA SLEDAI ≥ 4	34 (39.5%)	41 (52.6%)	38 (48.1%)	37 (47.4%)	116 (49.4%)	0.1169
3	% of subjects with no worsening by BILAG index (no new BILAG A organ domain score or 2 new BILAG B organ domain scores)	70 (81.4%)	69 (88.5%)	75 (94.9%)	71 (91.0%)	215 (91.5%)	0.0152
4	% of subjects with no worsening by PGA (< 0.3 point increase from baseline)	66 (76.7%)	70 (89.7%)	70 (88.6%)	72 (92.3%)	212 (90.2%)	0.0027

[a]P-value from likelihood ratio test for pairwise comparison between combined all active vs. placebo

Example 2: Scoring Proteinuria for SELENA SLEDAI

[0570] Kidney malfunction is often associated with Systemic lupus erythematosus. One of skill in the art would be aware of a variety of standard measures that can be used to assess kidney function, for example, progression to end-stage renal disease, sustained doubling of serum creatinine, creatinine clearance, iothalamate clearance, protein concentration in a single urine sample and protein concentration in a 24-hour urine sample.

[0571] Changes in proteinuria calculated from "24 hour urine samples" is one of the categories scored in the SELENA SLEDAI. Proteinuria measurements may be performed by any method known in the art. In a specific embodiment, a single urine specimen is collected and the amount of protein and/or creatinine clearance is measured, see, for example, Lemann, et al., Clin Chem., 33:297-9, 1987. and Schwab, et al., Arch Intern Med., May;147(5):943-4, 1987. In a specific embodiment, urine is collected over 24 hours and the amount of protein and/or creatinine clearance is determined. In a specific embodiment, a single urine specimen is collected, the ratio of the amount of protein to the amount of creatinine clearance is determined, and this ratio is used to estimate the amount of protein in a 24-hour urine sample, see, for example, Ruggenenti, et al., BMJ. 316(7130):504-9, 1998. Therefore, in this example, a "24 hour urine sample" can refer to either the grams of protein in the urine based on a 24 hour urine sample, or an estimate of the grams of protein in a 24 hour urine sample. An estimate of the grams of protein in a 24 hour urine sample can be

based on, for example, the ratio of the amount of protein in a single urine specimen to the amount of creatinine clearance in a single urine sample.

[0572] In the standard SELENA SLEDAI scoring system, a patient that exhibits a new onset of proteinuria or a recent increase in proteinuria that results in a proteinuria value in the current 24 hour urine sample that is at least 0.5 grams higher that the proteinuria value determined in the immediate prior 24 hour urine sample, will be assigned a score of 4 for proteinuria in the published SELENA SLEDAI scale, see, for example, Bombardier, et al., Arthritis Rheum. Jun;35(6):630-40, 1992. Accordingly, under the standard SELENA SLEDAI scoring system, a subject that is assigned 4 points at baseline for proteinuria will have an improving SELENA SLEDAI at a subsequent visit as long as proteinuria does not continue to rise by > 0.5 g in a 24 hour urine sample (i.e., the patient will have 4 points deducted from their total score even in the face of stable proteinuria or increases ≤ 0.5 g/24).

[0573] A modification to the SELENA SLEDAI proteinuria scoring rules is described below. As in the standard SELENA SLEDAI scoring system, a patient that exhibits a new onset of proteinuria or a recent increase in proteinuria that results in a proteinuria value in the current 24 hour urine sample that is at least 0.5 grams higher that the proteinuria value determined in the immediate prior 24 hour urine sample, will be assigned a score of 4 for proteinuria. Further, if a patient's proteinuria value has not improved (i.e., there has not been a decrease in proteinuria in the current 24 hour urine sample by at least 0.5 grams compared to proteinuria value determined for the immediate prior 24 hour urine sample) a patient will continue to be assigned a score of 4 for proteinuria. If however, a patients proteinuria value has improved (i.e., there has been a decrease in proteinuria in the current 24 hour urine sample of at least 0.5 grams compared to proteinuria value determined for the immediate prior 24 hour urine sample) a patient will be assigned a score of 0 for proteinuria.

[0574] In a specific embodiment, the previous proteinuria measurement was made on a 24 hour urine sample that was obtained ≤ 26 weeks before the current measurement.

Example 3: Summary of Results from a clinical trial testing the use of an antibody (belimumab) that neutralizes Neutrokine-alpha protein to treat rheumatoid arthritis (RA).

[0575] A Phase 2, multi-center, randomized, double-blind, placebo-controlled study was performed in subjects with RA. Subjects were randomized into 4 treatment groups (placebo, 1 mg/kg, 4 mg/kg and 10 mg/kg). Belimumab or placebo was administered at doses of 1, 4 and 10 mg/kg on Days 0, 14 and 28 and every 28 days thereafter for 24 weeks, followed by an optional 24-week extension period. Belimumab was formulated in 10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% (w/v) polysorbate 80, pH 6.5 (± 0.3). Subjects receiving placebo dose received the formulation (10 mM sodium citrate, 1.9% glycine, 0.5% sucrose, 0.01% (w/v) polysorbate 80, pH 6.5 (± 0.3) without belimumab. A total of 283 subjects participated in the study. Belimumab was administered to 214 subjects at doses of 1, 4, or 10 mg/kg during the 24-week treatment phase of the study. Sixty-nine subjects received placebo.

[0576] A statistically superior ACR20 response was achieved in the 1 mg/kg (p=0.0097) treatment group, as well as in all active treatment groups combined (p=0.0213). The ACR20 is an index developed by

the American College of Rheumatology (ACR) to assess patient response to treatment for rheumatoid arthritis. An ACR20 response is defined as at least a 20% reduction in tender joint count and swollen joint count, in addition to an improvement of at least 20% on three of five other assessments of symptoms or disease manifestations (i.e., patient pain assessment, patient global assessment, physician global assessment, patient self-assessed disability, acute-phase reactant [ESR or CRP]). Moreover, the result for the 1 mg/kg treatment group remained statistically significant under adjustment for multiple comparisons using the Bonferroni-closed procedure ($p<0.0166$). As in subjects with SLE, belimumab was associated with improved ACR20 responses in subjects with autoantibody positive disease (rheumatoid factor [RF] or anti-cyclic citrullinated peptide [CCP]), as well as in subjects positive for C-reactive protein (CRP) at baseline. Biological activity was observed including statistically significant reductions in CD20+ B-cells, naïve B-cells, activated B-cells and RF; memory cells increased within first month of treatment and slowly declined with continued treatment. Belimumab was well tolerated at all doses. A dose-response relationship was not apparent in this study for efficacy, safety nor biomarker effects. Continued treatment in the extension period of the study was well tolerated. ACR20 response increased to approximately 40% at Week 48. Effects on biomarkers increased or were sustained with continued treatment, while memory cells continued to decline towards baseline levels. Serum concentrations in this study were within the range expected based on the Phase 1 data and concomitant medications (i.e., methotrexate, leflunomide or hydroxychloroquine) had no significant effect on belimumab exposures.

Example 4: Neutrokine-Alpha Extends B Cell Lifespan Through Two Independent Signaling Pathways

[0577] Neutrokine-alpha, also called BLyS, (B lymphocyte stimulator), BAFF, TALL-1, THANK, TNFSF13B and zTNF4, is essential for the survival of resting peripheral B lymphocytes (Rolink, A. G., and Melchers, F. (2002). *Curr Opin Immunol* 14, 266-275) The importance of Neutrokine-alpha in naïve B cell homeostasis is demonstrated best by the finding that Neutrokine-alpha deficient mice produced by targeted gene deletion or introduction of soluble decoy receptors have striking deficits in marginal zone and follicular B cells, the major mature peripheral B cell populations (Gross, J. A., et al. (2001). *Immunity* 15, 289-302; Schiemann, B., et al. (2001). *Science* 293, 2111-2114) Conversely, ectopic expression of Neutrokine-alpha from a transgene markedly expands follicular and marginal peripheral zone B cells without affecting T cells, B1 cells, early (T1) transitional peripheral B cells, or developing B cells in the marrow (Mackay, F., et al (2003). *Annu Rev Immunol* 21, 231-264). Neutrokine-alpha is also required for the maintenance of numerous B cell tumors and dysregulated Neutrokine-alpha stimulation rescues autoreactive B cells from deletion, thereby promoting the production of autoantibodies (Kalled, S. L. (2005). *Immunol Rev* 204, 43-54). Thus, Neutrokine-alpha has a critical role in the homeostasis of both normal and pathogenic B cells. This Example details the results of experiments performed to understand the mechanism by which Neutrokine-alpha promotes B cell survival

Experimental Procedures

[0578] Mice: Pim-$1^{+/+}2^{+/+}$, Pim-$1^{-/-}2^{+/+}$, Pim-$1^{+/+}2^{-/-}$ and Pim-$1^{-/-}2^{-/-}$ mice were generated from Pim-$1^{+/-}$ $2^{+/-}$ stock of Paul Rothman Columbia University, New York, NY. C57BL/6 (B6) mice were from The Jackson Laboratory, Bar Harbor ME or from the National Cancer Institute Production Program, NCI-Fredrick, Fredrick MD. Animals were bred and maintained at the Univ. of Pennsylvania, Harvard Medical School, or the Univ. of Massachusetts Medical School in accordance with Institutional Animal Care and Use Committee guidelines.

[0579] B cell purification: Splenic B cells were obtained by anti-thy1.2 and complement treatment of splenocytes followed by purification of resting B cells using a step wise percoll gradient and harvesting cells at the 60-70% interface. In some experiments $CD23^+$ B cells were obtained by positive selection and magnetic separation of splenocytes suspensions using biotinylated anti-CD23 antibody (BD Biosciences-Pharmingen, San Diego CA) and streptavidin-coated microbeads (Miltenyi Biotec, Auburn CA). $CD23^+$ B cells were not size selected on Percoll as environmental activation *in vivo* leads to the loss of CD23. B cells prepared by antibody and Percoll were >90% $B220^+$, whereas $CD23^+$ B cells were >95% pure.

[0580] Cell cultures: Purified B cells or $CD23^+$ B cells were cultured in RPMI-1640 supplemented with 2-mercaptoethanol, MEM-non-essential amino acids, glutamine, penicillin and streptomycin (complete media, CM). For B cell survival and other assays recombinant human Neutrokine-alpha made at Human Genome Sciences, Rockville MD was used at 50-100 ng/ml. FLAG-tagged human Neutrokine-alpha was from Dr. Randolph Noelle, Dartmouth Medical School. Murine Neutrokine-alpha was purchased from Alexis Biochemicals, San Diego CA and human interferon alpha (IFNα) from PBL Biomedical, Piscataway, NJ. Rapamycin was used at a final concentration of 50 nM, added to cultures from a stock dissolve in methanol. Control B cells in experiments using rapamycin were treated with methanol as a vehicle control. For kinetic assays B cells were prepared and refrigerated overnight at 4°C. 5-6 x 10^6 purified B cells per sample were spun onto 24-well plates that had been coated with 5 ug/ml monoclonal anti-FLAG M2 antibody (Sigma), washed, blocked with 1% BSA in PBS, followed by the addition of FLAG-tagged human Neutrokine-alpha 2 ug/well an hour prior to washing and cell addition. Unstimulated control B cells were those spun onto wells treated with anti-FLAG antibody alone. B cells were also activated by incubation with anti-murine IgM (5 ug/ml), anti-CD40 (0.5 ug/ml) or 100ng/ml of recombinant human Neutrokine-alpha added to kinetic assay buffer (Hank's balanced salt solution plus 2% BSA).

[0581] Antibodies and Western Blotting: Mouse anti-Pim 2 (1D12), Pim 1 (19F7), goat anti-actin (1-19), anti-mouse Ig, anti-rabbit Ig and anti-goat Ig coupled to HRP were obtained from Santa Cruz Biotechnology, Santa Cruz CA. Rabbit anti-phosphoserine 473 Akt, phosphothreonine 389 p70 S6 kinase, phosphothreonine 24/32 FKHR/FKRHL1, phospho$GSK3_{\alpha/\beta}$, GSK, p70S6K, FKHR and Akt were purchased from Cell Signaling, Beverly MA. Rabbit anti-mouse Mcl-1 was purchased from Rockland, Wilmington MA. Whole cell lysates were prepared by washing B cells in ice cold PBS and lysing in RIPA (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris pH8.0) supplemented with protease inhibitors (minitab, Roche, Indianapolis IN) and phosphatase inhibitor cocktails I and II (Sigma). 10-50 ug of protein was resolved on 4-12% NuPage bis-tris polyacrylamide gels (Invitrogen, Carlsbad CA)

and transferred to nitrocellulose. Blots were blocked with 3% BSA (Sigma, IgG free), 0.2% Tween-20 in PBS and incubated with primary antibody in the same buffer overnight at 4°C. Blots were washed with PBS-0.2% Tween-20, incubated with secondary antibody conjugated with HRP and developed using ECLplus (Amersham Bioscience, Piscataway NJ). Blots were stripped for reprobing by incubation for 20 minutes at 65°C in PBS supplemented with 1% SDS and 100uM β-mercaptoethanol. Blots were then washed and blocked as above.

[0582] Survival assays: B cells at 5 x 10^6/ml were cultured in 24-well tissue culture dishes in CM at 37°C. B cells were supplemented with 50-100ng/ml of rhuNeutrokine-alpha, 50nM rapamycin, 200U of human IFNα, or a combination of these reagents. B cells were pretreated with 50nM rapamycin or vehicle 1 hour before culture with the test supplements, fresh rapamycin was added after 48 hours of culture. Survival was monitored daily by counting viable cells using trypan blue exclusion with each determination done in triplicate.

Results

[0583] Investigation into the mechanism by which Neutrokine-alpha promotes B cell survival revealed that Neutrokine-alpha activates the Akt/mTOR pathway in B cells. Purified B cells were stimulated for the 0, 5, 20, 60 ~~or 120 minutes with~~ 100 ng/ml recombinant human or murine Neutrokine-alpha or ~~anti-Ig~~ (positive control) at 37°C in pregassed medium. Lysates were prepared from iced samples and analyzed by Western Blot. Such stimulation of primary B cells with recombinant Neutrokine-alpha results in activation of the Akt pathway as determined by increased phosphorylation of the serine 473 and threonine 308 residues of Akt. Additional experiments in which purified B cells were stimulated with plate bound FLAG-tagged Neutrokine-alpha, soluble Neutrokine-alpha (100 ng/ml) or 0.5ug/ml anti-CD40 (positive control) showed that Akt itself had been activated as seen by the phosphorylation of the Akt substrates, GSKβ and the forkhead transcription factors FOXO1 and FOXO3a. mTOR is the major downstream effector of Akt. Subsequent to Neutrokine-alpha stimulation, activation of mTOR in primary B cells was also shown by the phosphorylation of the mTOR substrates, p70 S6 kinase and the translation inhibitor 4E-BP1. Phosphorylation patterns were studied by Western blotting.

[0584] Rapamycin is a potent inhibitor of mTOR and a potent suppressor of B cell proliferation and differentiation. Small resting B cells from normal donors were cultured for 4 days with and without 100 ng/ml rhuNeutrokine-alpha, vehicle or 50nM rapamycin which was used to pretreat B cells before culture, added directly to cultures upon initiation and re-added every 2 days. Viable cells were determined at day 4. Coculture of total B or CD23+ B cells with Neutrokine-alpha and rapamycin did not prevent Neutrokine-alpha-mediated enhancement of survival as measured by the number of viable cells present after 4 days in culture. This result suggested that another survival pathway may be active in Neutrokine-alpha treated B cells.

[0585] Pims are a family of three serine/threonine kinases that can provide rapamycin resistant apoptosis protection, induced in hematopoietic cells by a variety of activators (Fox, C. J., et al., (2003). *Genes Dev* 17, 1841-1854. and Fox, C. J., et al., (2005). *J Exp Med* 201, 259-266). It was shown by

western blot, that subsequent to 2 days of treatment with 100 ng/ml rhuNeutrokine-alpha, primary B cells upregulate Pim1 and Pim 2 expression.

[0586] To test the involvement of Pim1 and 2 in Neutrokine-alpha mediated B cell survival, $CD23^+$ B cells from wild type or Pim $1^{-/+}2^{-/+}$ heterozygotes, Pim $1^{-/-}$ $2^{-/-}$ double deficient or Pim 2 deficient (Pim $1^{+/-}$ $2^{-/-}$) donors were cultured in CM for 4 days with vehicle, 100 ng/ml rhuNeutrokine-alpha and with or without 50 nM rapamycin. Viability was determined daily by trypan blue exclusion. Interestingly, B cells from mice doubly deficient in Pim 1 and Pim 2 (Pim-$1^{-/-}2^{-/-}$ B cells) did exhibit enhanced survival when exposed to Neutrokine-alpha. This result could be explained if mTOR and Pims 1 and 2 operate in distinct signaling pathways that each mediate Neutrokine-alpha promotion of cell survival. To test the theory that two separate pathways were involved, the effect of rapamycin on Neutrokine-alpha mediated survival in Pim-$1^{-/-}$ $2^{-/-}$ B cells was tested. The addition of rapamycin abrogated Neutrokine-alpha's ability to enhance B cell survival in Pim-$1^{-/-}2^{-/-}$ B cells. Further investigation showed that Pim-$1^{+/-}2^{-/-}$ B cells, deficient only in Pim 2 function, were as sensitive to rapamycin as Pim-$1^{-/-}2^{-/-}$ B cells, indicating that Pim1 is not necessary for Neutrokine-alpha's effects on B cell survival. All together, these data show that there are two independent pathways which work to mediate Neutrokine-alpha mediated survival, and that either pathway alone is sufficient for this activity of Neutrokine-alpha.

[0587] Further experiments indicated that expression of the Mcl-1, a Bcl-2 family member that plays a role in promoting peripheral B and T cell homeostasis is required for effective enhancement of B cell survival (protection against apoptosis induction) (data not shown).

[0588] Accordingly, a composition comprising an inhibitor of the akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to mimic the effects induced by a Neutrokine-alpha antagonist. Thus, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used as an antagonist of Neutrokine-alpha to inhibit B cell survival or to treat one or more of the diseases or disorders disclosed herein. For instance, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to decrease B cell lifespan. Additionally, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to treat an autoimmune disease. In specific embodiments, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to treat B cell mediated autoimmune diseases. In other specific embodiments, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to treat autoimmune diseases in which autoantibodies are prevalent. In specific embodiments, a composition comprising an inhibitor of the Akt/mTOR pathway (e.g., rapamycin) and an inhibitor of the Pim 2 pathway may be used to treat rheumatoid arthritis, systemic lupus erythematosus multiple sclerosis, myasthenia gravis, Sjogren's syndrome, type 1 diabetes, idiopathic thrombocytopenia purpura, Gullian-Barre syndrome, Hashimoto's thyroiditis, or Graves' disease.

[0589] Additionally, a composition comprising an inhibitor Mcl-1 may be used to mimic the effects induced by a Neutrokine-alpha antagonist Thus, a composition comprising an inhibitor of Mcl-1 may be

used as an antagonist of Neutrokine-alpha to inhibit B cell survival or to treat one or more of the diseases or disorders disclosed herein. For instance, a composition comprising an inhibitor of Mcl-1 may be used to decrease B cell lifespan. Additionally, a composition comprising an inhibitor of Mcl-1 may be used to treat an autoimmune disease. In specific embodiments, a composition comprising an inhibitor of Mcl-1 may be used to treat B cell mediated autoimmune diseases. In other specific embodiments, a composition comprising an inhibitor of Mcl-1 may be used to treat autoimmune diseases in which autoantibodies are prevalent. In specific embodiments, a composition comprising an inhibitor of Mcl-1 may be used to treat rheumatoid arthritis, systemic lupus erythematosus multiple sclerosis, myasthenia gravis, Sjogren's syndrome, type 1 diabetes, idiopathic thrombocytopenia purpura, Gullian-Barre syndrome, Hashimoto's thyroiditis, or Graves' disease.

Example 5: Characterization of antibody formulations.

[0590] Analysis of 1 mg/ml IgG1/λ antibody formulated in 10 mM histidine and 10 mM citrate buffers by differential scanning calorimetry was used to assess the thermal stability of the antibody in each formulation. The particular antibody used in this study was an IgG1/λ antibody that is specific for Neutrokine-alpha and is capable of neutralizing Neutrokine-alpha activity. The analysis revealed that the melting temperature was highest for both buffers in the pH range of 6.0-7.5, and higher melting temperature generally indicates higher thermal stability. The melting temperature of the citrate buffer was ~2° C higher than the histidine buffer in this pH range, suggesting that the citrate buffer may yield a more stable antibody formulation. However, the thermal reversibility of the antibody was higher in the histidine buffer than the citrate buffer. This suggests that the antibody has greater biophysical stability in histidine than in citrate despite its lower melting temperature. This was confirmed by stability studies of antibody formulations which found that 10 mM histidine resulted in less aggregation than 10 mM citrate when stored at 2-8° C over 18 months. During the stability study, the buffering capacity of the two buffers was assessed by repeated pH measurements. In addition to providing greater biophysical stability for the antibody, histidine appears to provide greater buffering capacity at pH 6.0-6.5 than citrate in a pH range of 6.5-7.0. In the 18 month stability study, the histidine formulations remained at a stable pH over time at all temperatures tested (2-8° C, 25° C, and 40° C). In contrast, the citrate formulations had wider variances at the higher temperatures (data not shown).

Example 6: Long-term stability study of an antibody formulation.

[0591] To determine the shelf-life of an antibody formulation, a long-term stability study of 100 mg/ml antibody in 10 mM histidine, 150 mM NaCl, 0.01% (w/v) polysorbate 80, pH 6.0 was performed. The particular antibody used in this study was an IgG1/λ antibody that is specific for Neutrokine-alpha and is capable of neutralizing Neutrokine-alpha activity. Two ml aliquots in 5 ml glass vials were stored upright for 24 months at -80° C, 2-8° C, 25° C and 40° C. Samples were stored at -80° C as a control, at 2-8° C to determine shelf-life, and at accelerated conditions (25° C and 40° C) to monitor any possible degradation pathways that could occur. Periodically over 24 months, samples were analyzed by multiple assays,

including: visual inspection, pH, concentration, SDS-PAGE, SEC-HPLC, ion-exchange-HPLC (IE-HPLC), bioassay, capillary isoelectric focusing (cIEF), peptide mapping, RP-HPLC and ISOQUANT®.

[0592] Analysis of samples stored for 24 months at 2-8° C and -80° C by SEC-HPLC, IE-HPLC and RP-HPLC were visually comparable by all three methods, with only minor differences observed. The 2-8° C sample decreased in SEC-HPLC purity at an approximate rate of 0.03% per month, and increased in early-eluting IE-HPLC peaks (mostly due to deamidation) at an approximate rate of 0.14% per month (data not shown). The 2-8° C sample showed only small changes in aggregation (<1%), deamidation (~4%), and oxidation (1%) of the antibody after 24 months of storage. However, significant degradation was observed by all assays for samples stored under accelerated conditions. Degradation observed by SEC-HPLC under accelerated conditions included both aggregation and fragmentation. IE-HPLC assays showed that storage at accelerated conditions results in an increase in early eluting peaks. Deamidation and fragmentation were observed by peptide mapping at 25° C; deamidation, oxidation, fragmentation and rearrangement of aspartate to isoaspartate were observed at 40° C. Thus, 100 mg/ml of an IgG1/λ antibody in a pharmaceutical formulation of the invention is stable at 2-8° C for at least 24 months of storage.

Example 7: In vitro assay to test for Inhibition of Neutrokine-alpha –Neutrokine-alpha Receptor Interaction

[0593] The following describes an assay that can be used to test if a compound works as an antagonist of Neutrokine-alpha. Specifically, this assay measures the ability of compound to inhibit soluble Neutrokine-alpha binding to its cognate receptor on IM9 cells.

Preparation of biotinylated Neutrokine-alpha

[0594] One hundred μg of either human or mouse Neutrokine-alpha is dialysed overnight at 4° C against 50 mM sodium bicarbonate (sodium hydrogen carbonate) pH8.5 using a slide-a-lyzer cassette (Pierce). The next day, NHS-biotin (Pierce) is dissolved in DMSO to 13.3 mg/ml. This is then added to the Neutrokine-alpha at a molar ratio of 20:1 biotin:Neutrokine-alpha, mixed and incubated on ice for 2 hours. The biotinylated Neutrokine-alpha is then dialysed back into sterile PBS (Sigma) using a slide-a-lyzer cassette overnight at 4°C. The biological activity of the biotinylated Neutrokine-alpha is confirmed using the receptor binding inhibition assay (see below).

Maintenance of IM9 cells

[0595] IM9 cells are a human B lymphocyte cell line that express Neutrokine-alpha receptors. IM9 cells can be maintained in RPMI-1640 supplemented with 4 mM L-glutamine, 10% FCS, 10 U penicillin, 100 g/ml streptomycin (all reagents from Sigma). The cells are thawed from frozen stock and can be used in assays after 5 days in culture when they reach a density of 4 - 8 x 10^5 /ml.

Receptor binding inhibition assay

Flat-bottomed 96-well plates (Costar) are coated with 100 µl per well of a 1:10 dilution of poly-L-lysine (Sigma) in PBS for 1 hour at room temperature. The plates are then washed twice with water, allowed to air-dry and placed at 4° C overnight. One hundred µl of IM9 cells (at 10^6/ml in RPMI-1640 culture medium) are then added to each well. Plates are then centrifuged at 3200 rpm for 5 mins to pellet the cells. The media is carefully aspirated and 200 µl of MPBS (PBS containing 3% Marvel blocking solution) added to each well. The plates are then allowed to block for 1 hour at room temperature.

[0596] In a separate 96-well plate, 10 µl of biotinylated Neutrokine-alpha (at 162.5 ng/ml) in MPBS is added to each well to give a final concentration of 25 ng/ml. Fifty-five µl of each test compound is added to each well. The final volume in each well is 65 µl. Preferably the test compound is also diluted in MPBS. Plates are then incubated at room temperature for 30 minutes.

[0597] The IM9 coated plates are washed twice in PBS, tapped dry and immediately 50 µl of the phage/biotinylated-Neutrokine-alpha mix is added and incubated at room temperature for 1 hour. Plates are washed three times in PBST and three times in PBS, tapped dry and 50 µl of streptavidin-Delfia (Wallac) is added to each well at 1:1000 dilution in the Manufacturer's assay buffer. The plates are then incubated at room temperature for 1 hour and washed six times in Delfia wash solution (Wallac). After tapping the plates dry, 100 µl per well of Delfia enhancement solution (Wallac) is added. The plates are gently tapped to encourage micelle formation, incubated at room temperature for 10 minutes, and fluorescence read on a Wallac 1420 workstation at 6520 nM.

[0598] Appropriate controls to include this assay include a bio-Neutrokine-alpha only sample to demonstrate what the maximal binding of biotinylated Neutrokine-alpha to its receptor is in this assay and sample that does not contain bio-Neutrokine-alpha to demonstrate the background signal in this assay. An additional useful control is non-Neutrokine-alpha specific, or "irrelevant", compound - a compound that is structurally similar to the test compound but that is not believed to interact with either Neutrokine-alpha or one of Neutrokine-alpha's receptors. If the test compound was an anti-Neutrokine-alpha antibody of the IgG1 isotype, a suitable "irrelevant control would be another IgG1 antibody that is not specific for Neutrokine-alpha or one of its receptors.

Example 8: Human B cell proliferation assay for in vitro screening of Neutrokine-alpha antagonist molecules

[0599] One bioassay for assessing the effects of a putative Neutrokine-alpha antagonist is performed in triplicate in 96 well format by mixing equal volumes of Neutrokine-alpha, responder cells, and putative antagonist each of which is prepared as a 3X stock reagent.

[0600] B-lymphocytes are purified from human tonsil by MACS (anti-CD3 depletion), washed, and resuspended in complete medium (CM) (RPMI 1640 with 10% FBS containing 100U/ml penicillin, 100µg/ml streptomycin, 4mM glutamine, 5x10E-5 M beta-mercaptoethanol) at a concentration of 3 x 10^6 cells/mL. *Staphylococcus aureus,* Cowan I (SAC, CalBiochem) is added to cells at 3X concentration (3X = 1:33,333 dilution of stock).

[0601] Meanwhile, eight serial dilutions (3-fold) of potential antagonist are prepared in CM such that the diluted antagonists are at 3X the final concentrations to be tested in the assay. For example, antibodies are routinely tested starting at a final concentration of 10ug/mL and going down to about 1.5 ng/mL.

[0602] Human rNeutrokine-alpha is prepared in CM to 3X concentration (3X = 300 ng/mL, 30 ng/mL, and 3 ng/mL) in CM. Potential antagonists are routinely tested at several concentrations of Neutrokine-alpha to avoid false negatives due to unexpectedly low affinity or antagonist concentration.

[0603] Fifty microliters of diluted antagonist and 50uL of diluted Neutrokine-alpha are than added to wells containing 50uL of the cells mixture.

[0604] Cells are then incubated for 72 hours (37°C, 5% CO_2) in a fully humidified chamber. After 72 hrs, the cells are supplemented with 0.5 μCi/well ^{3}H-thymidine (6.7 Ci/mmol) and incubated for an additional 24 hours. Plates are harvested using a Tomtec Cell Harvester and filters counted in a TopCount Scintillation counter (Packard).

[0605] Appropriate controls to include this assay include a sample in which no antagonist was included to demonstrate what the maximal ^{3}H-thymidine incorporation is in this assay and a sample that does not contain Neutrokine-alpha to demonstrate the background signal in this assay. An additional useful control is a non-Neutrokine-alpha specific, or "irrelevant", test compound - a compound that is structurally similar to the test compound but that is not believed to interact with either Neutrokine-alpha or one of Neutrokine-alpha's receptors. For instance, if the test compound was an anti-Neutrokine-alpha antibody of the IgG1 isotype, a suitable "irrelevant" control would be another IgG1 antibody that is not specific for Neutrokine-alpha or one of its receptors.

[0606] One of skill in the art will be aware of modifications that may be made to this assay, for example, in the order of steps or the reagents used. As a specific example, the B cells may be primed with anti-IgM instead of SAC. One of skill in the art is also aware of other assays that may be used to test the ability of a compound to act as an antagonist of Neutrokine-alpha.

Example 9: Murine B cell proliferation assay for in vitro screening of Neutrokine-alpha antagonist molecules

[0607] To determine if a potential Neutrokine-alpha antagonist inhibits Neutrokine-alpha mediated B cell proliferation, a murine splenocyte proliferation assay may be performed Briefly, murine splenocytes are isolated by flushing a spleen using a 25g needle and 10 ml of complete medium (RPMI 1640 with 10% FBS containing 100U/ml penicillin, 100μg/ml streptomycin, 4mM glutamine, $5x10^{-5}$M β-mercaptoethanol). The cells are passed through a 100 micron nylon filter to remove cell clumps. The cell suspension is then ficolled at 400 x g for 25 minutes at room temperature (one 15 ml conical tube/spleen; 3 ml ficol, 10 ml cell suspension/spleen; Ficol 1083 from Sigma). The recovered cells are washed 3 times in complete medium and counted. Recovered cells are then diluted to a concentration of $3x10^6$/ml in complete medium containing a 3X concentration of SAC (3X = 1:33,333 dilution of stock; stock is a 10% suspension of Staph. aureus (Cowan I strain) available from Calbiochem).

[0608] For each antibody, 50 microliters of antibody dilutions at 30μg/ml, 3.0μg/ml, and 0.3μg/ml concentrations are aliquotted into individual wells of a 96 well plate in triplicate. Medium containing no

antibody (and human isotype controls (purchased commercially) when necessary) are used as negative controls.

[0609] Neutrokine-alpha protein is diluted in complete medium to concentrations of 300ng/ml, 90ng/ml and 30ng/ml. 50 microliters of each of the Neutrokine-alpha dilutions are then added to the antibody dilution series in the plates. The plate containing the antibody and Neutrokine-alpha dilutions are then incubated for 30 minutes at 37°C, 5% CO_2, after which 50 microliters of the splenocyte cell suspension containing SAC is added to all wells. The plates are then incubated for 72 hours (37°C, 5% CO_2).

[0610] After 72 hours, each well is supplemented with 50µl of complete medium containing 0.5µCi of 3H-thymidine (6.7 Ci/mM; Amersham) and cells are incubated for an additional 20-24 hours at (37°C, 5% CO_2). Following incubation cells are harvested using a Tomtec Cell Harvester and filters counted in a TopCount Scintillation counter (Packard).

[0611] One of skill in the art will be aware of modifications that may be made to this assay, for example, in the order of steps or the reagents used. As a specific example, the B cells may be primed with anti-IgM instead of SAC. One of skill in the art is also aware of other assays that may be used to test the ability of a compound to act as an antagonist of Neutrokine-alpha.

[0612] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[0613] The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

[0614] Further, the Sequence Listing submitted herewith in both computer and paper forms are hereby incorporated by reference in their entireties. Additionally, the entire disclosure (including the specification, sequence listing, and drawings) of each of the following U.S. Provisional and Non-Provisional Patent Applications and International Patent Applications are herein incorporated by reference in their entireties: U.S. Provisional Application Nos. 60/725,625, filed October 13, 2005; 60/735,967, filed November 14, 2005; 60/776,664, filed February 27, 2006; 60/781,387, filed March 13, 2006; 60/787,557, filed March 31, 2006; 60/797,360, filed May 4, 2006; 60/814,870, filed June 20, 2006; 60/815,558, filed June 22, 2006; 60/815,827, filed June 23, 2006; 60/834,150, filed July 31, 2006; 60/725,626, filed October 13, 2005; 60/735,988, filed November 14, 2005; 60/776,665, filed February 27, 2006; 60/797,351, filed May 4, 2006; 60/814,869, filed June 20, 2006; 60/815,559, filed June 22, 2006; 60/834,152, filed July 31, 2006; 60/725,627, filed October 13, 2005; 60/735,964, filed November 14, 2005; 60/776,658, filed February 27, 2006; 60/725,629, filed October 13, 2005; 60/735,963, filed November 14, 2005; 60/776,660, filed February 27, 2006; 60/725,628, filed October 13, 2005; 60/735,987, filed November 14, 2005; 60/776,659, filed February 27, 2006; 60/543,261 filed February 11, 2004, 60/580,387 filed Jun 18, 2004, 60/617,191 filed October 12, 2004, 60/368,548 filed April 1, 2002, 60/336,726 filed December 7, 2001, 60/331,478 filed November 16, 2001, 60/330,835 filed Oct 31, 2001, 60/329,747 filed October 18, 2001, and 60/329,508 filed October 17, 2001, 60/225,628 filed August 15, 2000, 60/227,008 filed August 23, 2000, 60/234,338 filed September 22, 2000, 60/240,806 filed October 17, 2000, 60/250,020 filed November 30, 2000,

60/276,248 filed March 6, 2001, 60/293,499 filed May 25, 2001, 60/296,122 filed June 7, 2001, 60/304,809 filed July, 13 2001,60/122,388 filed March 2, 1999, 60/124,097 filed March 12, 1999, 60/126,599 filed March 26, 2000, 60/127,598 filed April 2,1999, 60/130,412 filed April 16, 1999, 60/130,696 filed April 23, 1999, 60/131,278 filed April 27, 1999, 60/131,673 filed April 29, 1999, 60/136,784 filed May 28, 1999, 60/142,659 filed July 6, 1999, 60/145,824 filed July 27, 1999, 60/167,239 filed November 24, 1999, 60/168,624 filed December 3, 1999, 60/171,108 filed December 16, 1999, 60/171,626 filed December 23, 1999, 60/176,015 filed January 14, 2000, and 60/036,100 filed January 14, 1997; U.S. Nonprovisional Application Serial Nos.: 11/054,539 filed February 10, 2005, 10/739, 042 filed December 19, 2003, 10/735,865 filed December 16, 2003, 10/270,487 filed October 16, 2002, 09/929,493, filed August 14, 2001, 09/588,947 filed June 8, 2000, 09/589,285 filed June 8, 2000, 09/589,286 filed June 8, 2000, 09/589,287 filed June 8, 2000, 09/589,288 filed June 8, 2000, 09/507,968 filed February 22, 2000, 09/255,794 filed February 23, 1999, and 09/005,874 filed January 12, 1998; and International Patent Application Serial Nos. PCT/US01/25549 filed August 15, 2001, PCT/US00/04336, filed February 22, 2000, and PCT/US96/17957, filed October 25, 1996.

What is claimed is:

1. A method of treating a patient that has an ANA titer ≥ 1:80 or ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha.

2. The method of claim 1 wherein the patient has an ANA titer ≥ 1:80 and ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

3. The method of claim 1 wherein the antagonist of Neutrokine-alpha is administered in combination with an anti-CD20 antibody

4. The method of claim 1 which comprises making a determination, prior to administering the antagonist of Neutrokine-alpha, that the patient has an ANA titer ≥ 1:80 or ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

5. The method of claim 4 wherein the determination is made on the basis of the patient's medical record.

6. The method of claim 4 wherein the determination is made on the basis of laboratory tests.

7. The method of claim 1 wherein the antagonist of Neutrokine-alpha is an anti-Neutrokine-alpha antibody.

8. The method of claim 1 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of TACI (SEQ ID NO:6).

9. The method of claim 1 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BCMA (SEQ ID NO:8).

10. The method of claim 1 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BAFF-R (SEQ ID NO:10) or a variant of the BAFF-R Neutrokine-alpha binding domain having the amino acid sequence of amino acid residues 2-70 of SEQ ID NO:26.

11. The method of claim 1 wherein the antagonist of Neutrokine-alpha is a Neutrokine-alpha-binding peptide, a peptibody, a Neutrokine-alpha protein variant or an anti-Neutrokine-alpha receptor antibody.

12. The method of claim 11 wherein the Neutrokine-alpha protein variant acts as a dominant negative.

13. The method of claim 1 wherein the patient has an autoimmune disease.

14. The method of claim 13 wherein the autoimmune disease is systemic lupus erythematosus (SLE).

15. The method of claim 14 wherein the antagonist of Neutrokine-alpha is administered in combination with an anti-CD20 antibody.

16. The method of claim 13 wherein the autoimmune disease is rheumatoid arthritis, Sjögren's syndrome, scleroderma, polymyositis, dermatomyositis, Felty's syndrome, mixed connective tissue disease, Raynaud's syndrome, or juvenile chronic arthritis.

17. The method of claim 7 wherein the antibody comprises the amino acid sequences of a set of VH and VL domains selected from the group consisting of:

(a) the VH domain and the VL domain of SEQ ID NO:13;

(b) the VH domain and the VL domain of SEQ ID NO:14;

(c) the VH domain and the VL domain of SEQ ID NO:15;

(d) the VH domain and the VL domain of SEQ ID NO:16;

(e) the VH domain and the VL domain of SEQ ID NO:17;

(f) the VH domain and the VL domain of SEQ ID NO:18;

(g) the VH domain of SEQ ID NO:19 and the VL domain of SEQ ID NO:20; and

(h) the VH domain of SEQ ID NO:21 and the VL domain of SEQ ID NO:22.

18. A method of treating a patient with systemic lupus erythematosus comprising:

(a) making a determination that the patient has an ANA titer $\geq$ 1:80 or $\geq$ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum; and

(b) administering a therapeutically effective amount of an antagonist of Neutrokine-alpha to said patient after making said determination.

19. The method of claim 18 wherein the patient has ANA titer $\geq$ 1:80 and $\geq$ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

20. The method of claim 18 wherein the antagonist of Neutrokine-alpha is administered in combination with an anti-CD20 antibody.

21. The method of claim 18 which also comprises making a determination, prior to administering the antagonist of Neutrokine-alpha, that the patient has at least one characteristic selected from the group consisting of:

(a) a SELENA SLEDAI score $\geq$ 6.

(b) a depressed level of C3 complement factor in his/her blood plasma or serum;

(c) a depressed level of C4 complement factor in his/her blood plasma or serum;

(d) the patient is receiving $\geq$7.5 milligrams/day of prednisone; and

(e) the patient is receiving or had previously received immunosuppressant therapy for the treatment of lupus-related symptoms.

22. The method of claim 21 which comprises making a determination that the patient has a SELENA SLEDAI score greater $\geq$ 6 prior to administering the antagonist of Neutrokine-alpha.

23. The method of claim 21 which comprises making a determination that the patient has less than 90 milligrams/deciliter of C3 complement factor in his/her blood plasma or serum prior to administering the antagonist of Neutrokine-alpha.

24. The method of claim 21 which comprises making a determination that the patient has less than 16 milligrams/deciliter of C4 complement factor in his/her blood plasma or serum prior to administering the antagonist of Neutrokine-alpha.

25. The method of claim 21 which comprises making a determination that the patient is receiving ≥7.5 milligrams/day of prednisone prior to administering the antagonist of Neutrokine-alpha.

26. The method of claim 21 which comprises making a determination that the patient is receiving or had previously received immunosuppressant therapy for the treatment of lupus-related symptoms prior to administering the antagonist of Neutrokine-alpha.

27. The method of claim 18 wherein the determination is made on the basis of the patient's medical record.

28. The method of claim 18 wherein the determination is made on the basis of laboratory tests.

29. The method of claim 18 wherein the antagonist of Neutrokine-alpha is an anti-Neutrokine-alpha antibody.

30. The method of claim 18 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of TACI (SEQ ID NO:6).

31. The method of claim 18 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BCMA (SEQ ID NO:8).

32. The method of claim 18 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BAFF-R (SEQ ID NO:10) or a variant of the BAFF-R Neutrokine-alpha binding domain having the amino acid sequence of amino acid residues 2-70 of SEQ ID NO:26

33. The method of claim 18 wherein the antagonist of Neutrokine-alpha is a Neutrokine-alpha-binding peptide, a peptibody, a Neutrokine-alpha protein variant or an anti-Neutrokine-alpha receptor antibody.

34. The method of claim 33 wherein the Neutrokine-alpha protein variant acts as a dominant negative.

35. A method of reducing the frequency or quantity of corticosteroid administered to a patient with systemic lupus erythematosus comprising administering a therapeutically effective amount of an antagonist of Neutrokine-alpha to said patient.

36. The method of claim 35 which also comprises making a determination, prior to administering the antagonist of Neutrokine-alpha, that the patient has at least one characteristic selected from the group consisting of:

(a) an ANA titer ≥ 1:80;

(b) ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum;

(c) a SELENA SLEDAI score ≥ 6;

(d) a depressed level of C3 complement factor in his/her blood plasma or serum;

(e) a depressed level of C4 complement factor in his/her blood plasma or serum;

(f) the patient is receiving ≥7.5 milligrams/day of prednisone; and

(g) the patient is receiving or had previously received immunosuppressant therapy for the treatment of lupus-related symptoms.

37. The method of claim 36 which comprises making a determination that the patient has an ANA titer ≥ 1:80.

38. The method of claim 36 which comprises making a determination that the patient has ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

39. The method of claim 36 which comprises making a determination that the patient has an ANA titer ≥ 1:80 and ≥ 30 IU/mL of anti-dsDNA antibodies in his/her blood plasma or serum.

40. The method of claim 36 which comprises making a determination that the patient has a SELENA SLEDAI score greater ≥ 6 prior to administering the antagonist of Neutrokine-alpha.

41. The method of claim 36 which comprises making a determination that the patient has less than 90 milligrams/deciliter of C3 complement factor in his/her blood plasma or serum prior to administering the antagonist of Neutrokine-alpha.

42. The method of claim 36 which comprises making a determination that the patient has less than 16 milligrams/deciliter of C4 complement factor in his/her blood plasma or serum prior to administering the antagonist of Neutrokine-alpha.

43. The method of claim 36 which comprises making a determination that the patient is receiving ≥7.5 milligrams/day of prednisone prior to administering the antagonist of Neutrokine-alpha.

44. The method of claim 36 which comprises making a determination that the patient is receiving or had previously received immunosuppressant therapy for the treatment of lupus-related symptoms prior to administering the antagonist of Neutrokine-alpha.

45. The method of claim 36 wherein the determination is made on the basis of the patient's medical record.

46. The method of claim 36 wherein the determination is made on the basis of laboratory tests.

47. The method of claim 35 wherein the antagonist of Neutrokine-alpha is an anti-Neutrokine-alpha antibody.

48. The method of claim 35 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of TACI (SEQ ID NO:6).

49. The method of claim 35 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BCMA (SEQ ID NO:8).

50. The method of claim 35 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BAFF-R (SEQ ID NO:10) or a variant of the BAFF-R Neutrokine-alpha binding domain having the amino acid sequence of amino acid residues 2-70 of SEQ ID NO:26.

51. The method of claim 35 wherein the antagonist of Neutrokine-alpha is a Neutrokine-alpha-binding peptide, a peptibody, a Neutrokine-alpha protein variant or an anti-Neutrokine-alpha receptor antibody.

52. The method of claim 51 wherein the Neutrokine-alpha protein variant acts as a dominant negative.

53. The method of claim 35 wherein the corticosteroid is selected from the group consisting of prednisone, prednisolone, hydrocortisone, methylprednisolone and dexamethasone.

54. The method of claim 35 wherein the corticosteroid is prednisone.

55. The method of claim 54 wherein the quantity of prednisone administered to a patient is reduced by at least 25% to $\leq$ 7.5 milligrams/day.

56. A method of determining if a lupus patient is responding to medical treatment comprising:

(a) determining the patient's SELENA SLEDAI, BILAG and PGA score prior to administration of medical treatment;

(b) administering the medical treatment; and

(c) determining the patient's SELENA SLEDAI, BILAG and PGA score following the administration of the medical treatment;

wherein the patient is considered to have responded to medical treatment if the SELENA SLEDAI score determined in step (c) is 4 or more points less than the SELENA SLEDAI score determined in step (a), the BILAG index score determined in step (c) does not include a new BILAG A organ domain score or 2 new BILAG B organ domain scores compared to the BILAG score determined in step (a), and the PGA score determined in step (c) is < 0.3 point higher than the PGA score determined in step (a).

57. The method of claim 56 wherein the medical treatment is a pharmaceutical composition comprising an antagonist of Neutrokine-alpha.

58. The method of claim 56 wherein the antagonist of Neutrokine-alpha is an anti-Neutrokine-alpha antibody.

59. The method of claim 56wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of TACI (SEQ ID NO:6).

60. The method of claim 56 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BCMA (SEQ ID NO:8).

61. The method of claim 56 wherein the antagonist of Neutrokine-alpha is a protein comprising the Neutrokine-alpha binding domain of BAFF-R (SEQ ID NO:10) or a variant of the BAFF-R

Neutrokine-alpha binding domain having the amino acid sequence of amino acid residues 2-70 of SEQ ID NO:26.

62. The method of claim 56 wherein the antagonist of Neutrokine-alpha is a Neutrokine-alpha-binding peptide, a peptibody, a Neutrokine-alpha protein variant or an anti-Neutrokine-alpha receptor antibody.

63. The method of claim 62 wherein the Neutrokine-alpha protein variant acts as a dominant negative.

64. An aqueous pharmaceutical formulation comprising a therapeutically effective amount of an antibody, a buffer in an amount from about 5 mM to about 50 mM, NaCl in an amount from about 150 mM to about 500 mM, a surfactant in an amount from about 0.003% to about 0.05%, with a pH from about 5.5 to about 6.5.

65. The formulation of claim 64 wherein the antibody is a human IgG1/λ antibody, the buffer is 10 mM histidine, the surfactant is polysorbate 80 in an amount of 0.01% w/v, the NaCl is 150mM and wherein the formulation has a pH of 6.0.

66. The formulation of claim 65 which is stable at a temperature of about 2-8° C for at least one year.

67. The formulation of claim 65 which is stable at a temperature of about 2-8° C for at least two years.

68. The formulation of claim 65 wherein the antibody is present in an amount of 100 mg/ml.

69. The aqueous pharmaceutical formulation of claim 64 comprising 100 mg/ml IgG1/λ antibody, 0.74 mg/ml L-histidine, 1.1 mg/ml L-histidine monohydrochloride, 8.8 mg/ml NaCl and 0.1 mg/ml polysorbate 80 and wherein the formulation has a pH of 6.0.