The present invention is directed to therapeutic methods using IL-6 antagonists such as anti-IL-6 antibodies and fragments thereof having binding specificity for IL-6 to prevent or treat anemia (e.g., anemia associated with chemotherapy) including persons on a treatment regimen with a drug or chemotherapy and/or radiation for cancer (e.g., head and neck cancer) that is associated with increased risk of anemia.
with sequence listing part of description (Rule 5.2(a))
ANTI-IL-6 ANTIBODIES FOR THE TREATMENT OF ANEMIA

CROSS-REFERENCE TO RELATED APPLICATIONS


FIELD OF THE INVENTION

[0002] IL-6 antagonists, including anti-IL-6 antibodies and antigen-binding fragments thereof, may be used to reduce C-reactive protein ("CRP levels") and inflammation and in methods and compositions for the treatment and prevention of anemia, including anemia associated with chemotherapy or radiography.

BACKGROUND OF THE INVENTION

Interleukin-6 (IL-6)

[0003] Interleukin-6 ("IL-6") is a multifunctional cytokine involved in numerous biological processes such as the regulation of the acute inflammatory response, the modulation of specific immune responses including B- and T-cell differentiation, bone metabolism, thrombopoiesis, epidermal proliferation, menses, neuronal cell differentiation, neuroprotection, aging, cancer, and the inflammatory reaction occurring in Alzheimer's disease. See Papassotiropoulos, et al. (2001) Neurobiology of Aging 22: 863-871.

[0004] IL-6 is a member of a family of cytokines that promote cellular responses through a receptor complex consisting of at least one subunit of the signal-transducing glycoprotein gp130 and the IL-6 receptor ("IL-6R") (also known as gp80). The IL-6R may also be present in a soluble form ("sIL-6R"). IL-6 binds to IL-6R, which then dimerizes the signal-transducing receptor gp130. See Jones (2005) Immunology 175: 3463-3468.

[0005] IL-6 is a pleiotropic pro-inflammatory cytokine, which regulates the acute phase response and the transition from the innate to the adaptive immune response. IL-6 increases hepatic synthesis of proteins that are involved in the 'acute phase response' leading to symptoms such as fever, chills, and fatigue. It stimulates B cell differentiation and secretion of antibodies and prevents apoptosis of activated B cells. IL-6 activates and induces proliferation of T cells and in the presence of IL-2, induces differentiation of mature and immature CD8 T cells into

[0006] In humans, the gene encoding IL-6 is organized in five exons and four introns, and maps to the short arm of chromosome 7 at 7p21. Translation of IL-6 RNA and post-translational processing result in the formation of a 21 to 28 kDa protein with 184 amino acids in its mature form. See Papassotiropoulos, et al. (2001) Neurobiology of Aging 22:863-871.


[0009] As noted above, IL-6 stimulates the hepatic acute phase response, resulting in increased production of CRP and elevated serum CRP levels. For this reason, C-reactive protein (CRP) has been reported to comprise a surrogate marker of IL-6 activity. Thus, elevated IL-6 activity can be
detected through measurement of serum CRP. Conversely, effective suppression of IL-6 activity, e.g., through administration of a neutralizing anti-IL-6 antibody, can be detected by the resulting decrease in serum CRP levels.

IL-6 is believed to play a role in the development of a multitude of diseases and disorders, including but not limited to fatigue, cachexia, autoimmune diseases, diseases of the skeletal system, cancer, heart disease, obesity, diabetes, asthma, Alzheimer’s disease and multiple sclerosis. See, e.g., WO 201 1/066374, WO 201 1/066371, WO 201 1/066378, and WO 201 1/066369.

A recent clinical trial demonstrated that administration of rosvastatin to apparently healthy individuals having elevated CRP (greater than 2.0 mg/l) reduced their CRP levels by 37% and greatly decreased the incidence of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular causes. Ridker et al., N Engl J Med. 2008 Nov 9 [Epub ahead of print].


Anemia

Anemia is a condition where a decrease in the number of red blood cells (RBCs) or hemoglobin results in a diminished ability of the blood to carry oxygen. A cardinal sign of anemia is a serum hemoglobin level less than about 14.0 g/dL for men and less than 12.0 g/dL for women (or less than about 11.0 g/L hemoglobin for both men and women). See Auerbach, et al. (2004) Journal of Clinical Oncology, 22(7): 1301-1307. Symptoms of anemia generally include fatigue, lack of energy, lightheadedness or dizziness, especially when sitting up rapidly, or standing, shortness of breath, headaches, a pale appearance, rapid heart rate or palpitations, and chest pain. Anemia may be experienced in patients with cancer (e.g., cancer-related anemia), as well as patients undergoing chemotherapy (e.g., chemotherapy-related anemia), radiotherapy (e.g., intensity-modulated radiotherapy (IMRT)), or drug therapy (e.g., drug-induced immune

[0014] Anemia is common in cancer where about 30% of newly-diagnosed untreated cancer patients exhibit anemia and 75% of cancer patients suffering from anemia at some time during the illness. Over 62% of cancer patients experience anemia during treatment and 38% suffer from anemia during follow-up. Cancer-related anemia has been linked to IL-6 expression in mouse models inoculated with IL-6 producing tumor cells. This cancer-related anemia was successfully prevented by blocking the IL-6 receptor by administration of an anti-IL-6 receptor antibody. Mori, et al. (2009) Biomedical Research 30(1): 47-51; Groopman & Itri (1999) Journal of National Cancer Institute 91(19): 1616-1634; and Prabhush, et al. (2011) Indian J Cancer 48: 1-10.

[0015] Anemia a major side effects of chemotherapy. Common symptoms of anemia include fatigue, lack of energy, dizziness, headaches, diminished sex drive, rapid heartbeat, inability to concentrate, paleness, and shortness of breath. Seventy-eight percent of chemotherapy patients experience fatigue. "Chemotherapy-Related Anemia Guide." Patient Advocate Foundation Website (2011). In response to chemotherapy the patient experiences an inflammatory response including the production of IL-6 which acts on the liver to produce hepcidin which, in turn, inhibits ferroportin, macrophage iron release, and intestinal iron absorption. Thus, IL-6 production, via hepcidin, causes a drop in iron level and leads to anemia. Inflammatory cytokines also appear to affect other important elements of iron metabolism, including decreasing ferroportin expression, and probably directly blunting erythropoiesis by decreasing the ability of the bone marrow to respond to erythropoietin. Nemeth, et al. (2004) J Clinical Invest 113(9): 1251-3 and Andrews (2004) The Journal of Clinical Investigation 113(9): 1251-1253; See also Atkins, et al. (1995) Blood 86(4): 1288-1291.

[0016] Treatment of anemia includes blood transfusion, iron supplements (e.g., oral or intravenous), and medications that stimulate the formation of red blood cells (e.g., Epoetin alfa (Epogen®), Procrit® and Darbepoetin alfa (Aranesp®). See Groopman & Itri (1999) Journal of the National Cancer Institute 91(19): 1616-1634. However, many patients with anemia, including chemotherapy-associated anemia do not response well to blood transfusion, iron supplements, or erythropoietin therapy. See, e.g., Auerbach, et al. (2004) Journal of Clinical Oncology 22(7): 1301-1307 and Smith, et al. (2008) Journal of Clinical Oncology 26(7): 1040-1050. Therefore, a need exists for an improved therapeutics for anemia including chemotherapy-associated anemia. The invention described herein provides compositions IL-6 antagonists, including anti-IL-6 antibodies and antibody fragments thereof, and methods of use which may be
used for the prevention and treatment of anemia, including anemia associated with chemotherapy, anemia associated with radiotherapy, and drug-induced immune hemolytic anemia (DIIHA).

SUMMARY OF THE INVENTION

[0017] The present invention provides compositions comprising IL-6 antagonists and methods of use thereof for treating anemia. In one embodiment, the anemia may be associated with cancer, chemotherapy, radiotherapy, the combination of chemotherapy and radiotherapy, or drug-induced immune hemolytic anemia (DIIHA). In one embodiment of the invention, the IL-6 antagonist may target IL-6, IL-6 receptor alpha, gp130, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, SYK, or any combination thereof. In one embodiment of the invention, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. In one embodiment of the invention, the IL-6 antagonist may be an anti-IL-6R, anti-gp130, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, anti-STAT3, or anti-SYK antibody or antibody fragment. In one embodiment of the invention, the IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. In one embodiment of the invention, the IL-6 antagonist may be an anti-IL-6 antibody or antibody fragment.

[0018] The present invention provides compositions comprising humanized monoclonal antibodies that selectively bind IL-6 and methods of treating anemia. In one embodiment, anti-IL-6 antibodies (e.g., ALD518 antibodies, also known as Ab\) may be used in methods for the treatment of anemia. In this embodiment of the invention anti-IL-6 antibody or antibody fragment may be administered prophylactically to patients at significant risk of developing anemia. The invention also provides for humanized monoclonal anti-IL-6 antibodies may be used in the treatment of anemia. The present invention further includes the prevention or treatment of inflammatory conditions by administration of anti-IL-6 antibodies according to the invention.

[0019] In one embodiment, the invention provides for a method of treating or preventing anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist. In another embodiment, a method of treating or preventing drug-induced immune hemolytic anemia (DIIHA) may comprise administration of a composition comprising an effective amount of an IL-6 antagonist. In another embodiment, a method of treating or preventing anemia associated with chemotherapy may comprise administration of a composition comprising an effective amount of an IL-6 antagonist. In another embodiment, a method of treating or preventing anemia associated with radiotherapy may comprise administration of a composition comprising an effective amount of an IL-6 antagonist. In another embodiment, a
method of treating or preventing anemia associated with cancer may comprise administration of a composition comprising an effective amount of an IL-6 antagonist.

[0020] In one embodiment, the invention provides for the use of an IL-6 antagonist in the manufacture of a medicament for the treatment or prevention of anemia. In further embodiment, the invention provides for the use of an IL-6 antagonist in the manufacture of a medicament for the treatment or prevention of drug-induced immune hemolytic anemia (DIHA). In further embodiment, the invention provides for the use of an IL-6 antagonist in the manufacture of a medicament for the treatment or prevention of anemia associated with chemotherapy. In further embodiment, the invention provides for the use of an IL-6 antagonist in the manufacture of a medicament for the treatment or prevention of anemia associated with radiotherapy. In further embodiment, the invention provides for the use of an IL-6 antagonist in the manufacture of a medicament for the treatment or prevention of anemia associated with cancer.

[0021] The invention provides a method of treating or preventing anemia comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Abl 1, Abl 2, Abl 3, Abl4, Abl 5, Abl 6, Abl 7, Abl 8, Abl 9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0022] The invention also provides a method of treating anemia comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Abl0, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0023] The invention further provides a method of preventing anemia comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Abl0, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl 6, Abl 7, Abl 8, Abl 9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0024] The invention provides a composition for the treatment or prevention of anemia comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Abl0, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or
an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0025] The invention also provides a composition for the treatment of anemia comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0026] The invention further provides a composition for the prevention of anemia comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0027] The invention provides a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0028] The invention also provides for a pharmaceutical composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0029] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment or prevention of anemia. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0030] The invention also provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4,
Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment of anemia. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0031] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the prevention of anemia. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0032] The invention provides a method of treating or preventing drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0033] The invention also provides a method of treating drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0034] The invention further provides a method of preventing drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.
The invention provides a composition for the treatment or prevention of drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention also provides a composition for the treatment of drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention further provides a composition for the prevention of drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment or prevention of drug-induced immune hemolytic anemia (DIIHA). In a further embodiment, said composition may be formulated for subcutaneous administration.

The invention also provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment of drug-induced immune hemolytic anemia (DIIHA). In a further embodiment, said composition may be formulated for subcutaneous administration.
[0040] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the prevention of drug-induced immune hemolytic anemia (DIIHA). In a further embodiment, said composition may be formulated for subcutaneous administration.

[0041] The invention provides a method of treating or preventing anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0042] The invention also provides a method of treating anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0043] The invention further provides a method of preventing anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0044] The invention provides a composition for the treatment or prevention of anemia associated with chemotherapy comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Abl, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0045] The invention also provides a composition for the treatment of anemia associated with chemotherapy comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8,
Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0046] The invention further provides a composition for the prevention of anemia associated with chemotherapy comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Ab 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0047] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Ab 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment or prevention of anemia associated with chemotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0048] The invention also provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Ab 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment of anemia associated with chemotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0049] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Ab 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the prevention of anemia associated with chemotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.
The invention provides a method of treating or preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention also provides a method of treating anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention further provides a method of preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention provides a composition for the treatment or prevention of anemia associated with radiotherapy comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention also provides a composition for the treatment of anemia associated with radiotherapy comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

The invention further provides a composition for the prevention of anemia associated with radiotherapy comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbI0, AbI 1, AbI2, AbI3, AbI4, AbI5, AbI6, AbI7, AbI8, AbI9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or
Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0056] The invention provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment or prevention of anemia associated with radiotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0057] The invention also provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment of anemia associated with radiotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0058] The invention provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the prevention of anemia associated with radiotherapy. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0059] The invention provides a method of treating or preventing anemia associated with cancer comprising administration of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0060] The invention also provides a method of treating anemia associated with cancer comprising administration of a composition comprising an effective amount of an Ab1, Ab2,
Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl 9, Ab20, Abl21, Abl22, Abl23, Abl24, Abl25, Abl26, Abl27, Abl28, Abl29, Abl30, Abl31, Abl32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0061] The invention further provides a method of preventing anemia associated with cancer comprising administration of a composition comprising an effective amount of an Ab 1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl 8, Abl 9, Ab20, Abl21, Abl22, Abl23, Abl24, Abl25, Abl26, Abl27, Abl28, Abl29, Abl30, Abl31, Abl32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0062] The invention provides a composition for the treatment or prevention of anemia associated with cancer comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Abl9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0063] The invention also provides a composition for the treatment of anemia associated with cancer comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Abl7, Ab8, Abl9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Abl32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0064] The invention further provides a composition for the prevention of anemia associated with cancer comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Abl9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Abl32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6.

[0065] The invention provides for the use of a composition comprising an effective amount of an Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment or prevention of anemia associated
with cancer. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0066] The invention also provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab1O, Ab1 1, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the treatment of anemia associated with cancer. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0067] The invention provides for the use of a composition comprising an effective amount of an Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab1O, Ab1 1, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody, or antibody fragment thereof, specifically binds to IL-6, for the manufacture of a medicament for the prevention of anemia associated with cancer. In a further embodiment, said composition may be formulated for subcutaneous administration.

[0068] In one embodiment, the antibody may comprise at least one light chain selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 2, 20, 21, 37, 53, 69, 85, 101, 119, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 647, 648, 649, 650, 651, 655, 660, 666, 667, 671, 675, 679, 683, 687, 693, 699, 702, 706, or 709. In a further embodiment, the antibody may comprise at least one light chain selected from the group consisting of an amino acid sequence of SEQ ID NO: 2, 20, 21, 37, 53, 69, 85, 101, 119, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 647, 648, 649, 650, 651, 655, 660, 666, 667, 671, 675, 679, 683, 687, 693, 699, 702, 706, or 709. In another embodiment, the antibody may comprise at least one light chain selected from the group consisting of nucleic acid sequences with at least 90% sequence identity to a nucleic acid sequence of SEQ ID NO: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723, wherein said nucleic acid sequence encodes said light chain. In further embodiment, the antibody may comprise at least one light chain selected from the group consisting of nucleic acid sequences of SEQ ID NO: 10, 29, 45, 61, 77, 93, 109,
In one embodiment, the antibody may comprise at least one heavy chain selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86, 102, 117, 118, 123, 139, 155, 171, 187, 203, 219, 225, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 507, 523, 539, 555, 571, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 668, 672, 676, 680, 684, 688, 691, 692, 704, or 708. In further embodiment, the antibody may comprise at least one heavy chain selected from the group consisting of an amino acid sequence of SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86, 102, 117, 118, 123, 139, 155, 171, 187, 203, 219, 225, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 668, 672, 676, 680, 684, 688, 691, 692, 704, or 708. In another embodiment, the antibody may comprise at least one heavy chain selected from the group consisting of nucleic acid sequences with at least 90% sequence identity to a nucleic acid sequence of SEQ ID NO: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725, wherein said nucleic acid sequence encodes said heavy chain. In further embodiment, the antibody may comprise at least one heavy chain selected from the group consisting of SEQ ID NO: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725, wherein said nucleic acid sequence encodes said heavy chain.


In a further embodiment, the antibody may comprise at least one CDR selected from the group consisting of nucleic acid sequences of SEQ ID NO: 12, 15, 31, 34, 47, 50, 63, 66, 79, 82, 95, 98, 111, 114, 132, 135, 148, 151, 164, 167, 180, 183, 196, 199, 212, 215, 228, 231, 244, 247, 260, 263, 276, 279, 292, 295, 308, 311, 324, 327, 340, 343, 356, 359, 372, 375, 388, 391, 404, 407,
In another embodiment, the antibody or antibody fragment thereof may comprise at least one light chain CDR polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 4, 23, 39, 55, 71, 74, 87, 103, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 710, 711, 712, 5, 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 713, 714, 715, 718, 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574. In another embodiment, the antibody or antibody fragment thereof may comprise at least one light chain CDR1 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 4, 23, 39, 55, 71, 74, 87, 103, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 710, 711, 712, 5, 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 713, 714, 715, 718, 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574. In another embodiment, the antibody or antibody fragment thereof may comprise at least one light chain CDR2 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 5, 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 713, 714, 715, or 718. In another embodiment, the antibody or antibody fragment thereof may comprise at least one light chain CDR3 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 6, 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574. In another embodiment, the antibody or antibody fragment thereof may
comprise at least two light chain CDR polypeptides. In another embodiment, the antibody or antibody fragment thereof may comprise three light chain CDR polypeptides.

[0073] In another embodiment, the antibody or antibody fragment thereof may comprise at least one heavy chain CDR polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 7, 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, 575, 716, 8, 27, 43, 59, 75, 91, 107, 120, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, 659, 717, 718, 9, 28, 44, 60, 76, 92, 108, 129, 145, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, or 577. In a further embodiment, the antibody or antibody fragment thereof may comprise at least one heavy chain CDR1 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 7, 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, 575, or 716. In a further embodiment, the antibody or antibody fragment thereof may comprise at least one heavy chain CDR2 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 8, 27, 43, 59, 75, 91, 107, 120, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, 659, 717, or 718. In a further embodiment, the antibody or antibody fragment thereof may comprise at least one heavy chain CDR3 polypeptide selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 9, 28, 44, 60, 76, 92, 108, 129, 145, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, or 577. In a further embodiment, the antibody or antibody fragment thereof may comprise at least two heavy chain CDR polypeptides. In a further embodiment, the antibody or antibody fragment thereof may comprise three heavy chain CDR polypeptides.

[0074] In one embodiment, the light chain of said antibody may be selected from the amino acid sequences of light chains listed in TABLE 4. In one embodiment, the light chain of said antibody may be selected from the amino acid sequences of heavy chains listed in TABLE 4. In one embodiment, at least one CDR of said antibody may be selected from the amino acid sequences of CDRs listed in TABLE 4. In another embodiment, the light chain may have at least 90% sequence identity to an amino acid sequence listed in TABLE 4. In another embodiment,
the light chain may have at least 95% sequence identity to an amino acid sequence listed in TABLE 4. In another embodiment, the light chain may comprise an amino acid sequence listed in TABLE 4. In further embodiment, the heavy chain may have at least 90% sequence identity to an amino acid sequence listed in TABLE 4. In further embodiment, the heavy chain may have at least 95% sequence identity to an amino acid sequence listed in TABLE 4. In further embodiment, the heavy chain may comprise an amino acid sequence listed in TABLE 4. In a still further embodiment, the CDR sequence of the antibody may have at least 90% sequence identity to an amino acid sequence listed in TABLE 4. In a still further embodiment, the CDR sequence of the antibody may have at least 95% sequence identity to an amino acid sequence listed in TABLE 4. In a still further embodiment, the CDR sequence of the antibody may comprise an amino acid sequence listed in TABLE 4.


[0076] In one embodiment, the antibody may be an Ab1 antibody. In one embodiment, the antibody may comprise a light chain comprising the amino acid sequence of SEQ ID NO: 2, 20, 647, 648, 649, 650, 651, 660, 666, 699, 702, 706, or 709. In one embodiment, the antibody may comprise a humanized light chain comprising the amino acid sequence of SEQ ID NO: 648, 649, and 650. In one embodiment, the antibody may comprise at least one light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 4, 5, 6, 710, 711, 712, 713, 714, and 715. In one embodiment, the antibody may comprise at least one humanized light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 710, 711, 712, 713, 714, and 715. In another embodiment, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO: 3, 18, 19, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 704, 708. In another embodiment, the antibody may comprise a humanized heavy chain comprising the amino acid sequence of SEQ ID NO: 653, 654, and 655. In another embodiment, the antibody may comprise at least one heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID
In another embodiment, the antibody may comprise at least one humanized heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 74, 716, 717, and 718. In a further embodiment, the Ab1 antibody may comprise a light chain comprising the amino acid sequence of SEQ ID NO: 709 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 657. In a further embodiment, the Ab1 antibody may comprise a light chain comprising the amino acid sequence of SEQ ID NO: 20 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 19.

In one embodiment, the antibody or antibody fragment thereof may be administered to the subject in the form of at least one nucleic acids that encode the antibody. In one embodiment, the light chain of said antibody or antibody fragment thereof may be encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723. In another embodiment, the heavy chain of said antibody or antibody fragment thereof may be encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725. In another embodiment, at least one of the CDRs of said antibody or antibody fragment thereof may be encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 12, 15, 31, 34, 47, 50, 63, 66, 79, 82, 95, 98, 111, 114, 132, 135, 148, 151, 164, 167, 180, 183, 196, 199, 212, 215, 228, 231, 244, 247, 260, 263, 276, 279, 292, 295, 308, 311, 324, 327, 340, 343, 356, 359, 372, 375, 388, 391, 404, 407, 420, 423, 436, 439, 452, 455, 468, 471, 484, 487, 500, 503, 516, 519, 532, 535, 548, 551, 564, 567, 580, 583, 694, 13, 16, 32, 35, 48, 51, 64, 67, 80, 83, 96, 99, 112, 115, 133, 136, 149, 152, 165, 168, 181, 184, 197, 200, 213, 216, 229, 232, 245, 248, 261, 264, 277, 280, 293, 296, 309, 312, 325, 328, 341, 344, 357, 360, 373, 376, 389, 392, 405, 408, 421, 424, 437, 440, 453, 456, 469, 472, 485, 488, 501, 504, 517, 520, 533, 536, 549, 552, 565, 568, 581, 584, 696, 14, 17, 33, 36, 49, 52, 65, 68, 81, 84, 97, 100, 113, 116, 134, 137, 150, 153, 166, 169, 182, 185, 198, 201, 214, 217, 230, 233, 246, 249, 262, 265, 278, 281, 294, 297, 310, 313, 326, 329, 342, 345, 358, 361, 374, 377, 390, 393, 406, 409, 422, 425, 438, 441, 454, 457, 470, 473, 486, 489, 502, 505, 518, 521, 534, 537, 550, 553, 566, 569, 582, 585, 695, or 697. In another embodiment, at least one nucleic acids may comprise the heavy and light chain polynucleotide sequences of SEQ ID NO: 723 and SEQ ID NO: 700; SEQ ID NO: 701 and SEQ ID NO: 703; SEQ ID NO: 705 and SEQ ID NO: 707; SEQ ID NO: 700 and SEQ ID NO: 704; and SEQ ID NO: 10 and SEQ ID NO: 11.
In one embodiment, the antibody or antibody fragment thereof may be asialated. In one embodiment, the antibody or antibody fragment thereof may have a half-life of at least about 30 days. In one embodiment, the antibody or antibody fragment thereof may comprise the humanized variable light sequence of amino acid sequence of SEQ ID NO: 709. In one embodiment, the antibody or antibody fragment thereof may comprise humanized variable heavy sequence of amino acid sequence of SEQ ID NO: 657. In another embodiment, the antibody or antibody fragment thereof may comprise at least one light chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 4, 5, or 6. In another embodiment, the antibody or antibody fragment thereof may comprise at least one heavy chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 7, 120, or 9. In further embodiment, the antibody or antibody fragment thereof may be an asialated, humanized anti-IL-6 monoclonal antibody with a half-life of -30 days comprising the humanized variable light and heavy sequences as set forth in SEQ ID NO: 20 and 19. In further embodiment, the antibody or antibody fragment thereof may be an asialated, humanized anti-IL-6 monoclonal antibody with a half-life of -30 days comprising the humanized variable light and heavy sequences as set forth in SEQ ID NO: 709 and 657.

In a preferred embodiment this is effected by the administration of the antibodies described herein, comprising the sequences of the $V_H$, $V_L$, and CDR polypeptides described in Table 4, or humanized or chimeric or single chain versions thereof containing at least one of the CDRs of the exemplified anti-IL-6 antibody sequences and the polynucleotides encoding them. Preferably these antibodies will be aglycosylated. In more specific embodiments of the invention these antibodies will block gp130 activation and/or possess binding affinities (Kds) less than 50 picomolar and/or $K_{off}$ values less than or equal to $10^4$ S$^{-1}$.

The invention also contemplates methods of making said humanized anti-IL-6 or anti-IL-6/IL-6R complex antibodies and binding fragments and variants thereof. In one embodiment, binding fragments include, but are not limited to, Fab, Fab', F(ab')2, Fv and scFv fragments.

In one embodiment, the anti-IL-6 antibodies block the effects of IL-6. In another embodiment, the anti-IL-6 antibody is a humanized monoclonal antibody that binds to free human IL-6 and soluble IL-6R/IL-6 complex with an affinity of at least about 4 pM. In another embodiment, the anti-IL-6 antibody, has a serum half-life about at least 30 days. In another embodiment, the anti-IL-6 antibody is based on a consensus human IgGl kappa framework that had asparagines modified to alanine to eliminate N-glycosylation sites.

In another embodiment, the antibodies and humanized versions may be derived from rabbit immune cells (B lymphocytes) and may be selected based on their homology (sequence
identity) to human germ line sequences. These antibodies may require minimal or no sequence modifications, thereby facilitating retention of functional properties after humanization. In exemplary embodiments, the humanized antibodies may comprise human frameworks which are highly homologous (possess high level of sequence identity) to that of a parent (e.g., rabbit) antibody.

[0083] In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may specifically bind to the same linear or conformational epitopes on an intact IL-6 polypeptide or fragment thereof which may include at least fragments selected from those encompassing amino acid residues 37-51, amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45 and/or amino acid residues 58-72.

[0084] In a preferred exemplary embodiment, the anti-IL-6 antibody will comprise at least one of the CDRs in listed in Table 4. In a more preferred embodiment the anti-IL-6 antibody will comprise the variable heavy and light chain sequences in SEQ ID NO: 657 and SEQ ID NO: 709, or variants thereof.

[0085] In a preferred embodiment the humanized anti-IL-6 antibody will comprise the variable heavy and variable light chain sequences respectively set forth in SEQ ID NO: 657 and SEQ ID NO: 709, and preferably further comprising the heavy chain and light chain constant regions respectively set forth in SEQ ID NO: 588 and SEQ ID NO: 586, and variants thereof comprising at least one amino acid substitutions or deletions that do not substantially affect IL-6 binding and/or desired effector function. This embodiment also contemplates polynucleotides comprising, or alternatively consisting of, at least one of the nucleic acids encoding the variable heavy chain (SEQ ID NO: 700) and variable light chain (SEQ ID NO: 723) sequences and the constant region heavy chain (SEQ ID NO: 589) and constant region light chain (SEQ ID NO: 587) sequences. This embodiment further contemplates nucleic acids encoding variants comprising at least one amino acid substitutions or deletions to the variable heavy and variable light chain sequences respectively set forth in SEQ ID NO: 657 and SEQ ID NO: 709 and the heavy chain and light chain constant regions respectively set forth in SEQ ID NO: 588 and SEQ ID NO: 586, that do not substantially affect IL-6 binding and/or desired effector function.

[0086] In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may be aglycosylated or substantially aglycosylated, e.g., as a result of one or more modifications in the Fc region of the antibody.

[0087] In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may contain an Fc region that has been modified to alter effector function, half-
life, proteolysis, and/or glycosylation. Preferably the Fc region is modified to eliminate glycosylation.

[0088] In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may be a human, humanized, single chain or chimeric antibody.

[0089] In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may be a humanized antibody derived from a rabbit (parent) anti-IL-6 antibody.

[0090] In an embodiment of the invention, the framework regions (FRs) in the variable light region and the variable heavy regions of said anti-IL-6 antibody or antibody fragment or variant thereof respectively may be human FRs which are unmodified or which have been modified by the substitution of at most 2 or 3 human FR residues in the variable light or heavy chain region with the corresponding FR residues of the parent rabbit antibody, and the human FRs may have been derived from human variable heavy and light chain antibody sequences which have been selected from a library of human germline antibody sequences based on their high level of homology to the corresponding rabbit variable heavy or light chain regions relative to other human germline antibody sequences contained in the library. As disclosed in detail infra in a preferred embodiment the antibody will comprise human FRs which are selected based on their high level of homology (degree of sequence identity) to that of the parent antibody that is humanized.

[0091] In one embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may comprise a heavy chain polypeptide sequence comprising: SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, or 708; and may further comprise a VL polypeptide sequence comprising: SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, or 709 or a variant thereof wherein at least one of the framework residues (FR residues) in said VH or VL polypeptide may have been substituted with another amino acid residue resulting in an anti-IL-6 antibody or antibody fragment or variant thereof that specifically binds human IL-6, or may comprise a polypeptide wherein the CDRs therein are incorporated into a human framework homologous to said sequence. Preferably the variable heavy and light sequences comprise those in SEQ ID NO: 657 and 709.

[0092] In an embodiment of the invention, at least one of said FR residues may be substituted with an amino acid present at the corresponding site in a parent rabbit anti-IL-6 antibody from which the complementarity determining regions (CDRs) contained in said VH or VL polypeptides have been derived or by a conservative amino acid substitution.
In an embodiment of the invention, said anti-IL-6 antibody, or antibody fragment or variant thereof, may be humanized. In an embodiment of the invention, said anti-IL-6 antibody, or antibody fragment or variant thereof, may be chimeric.

In an embodiment of the invention, said anti-IL-6 antibody, or antibody fragment or variant thereof, further may comprise a human Fc, e.g., an Fc region comprised of the variable heavy and light chain constant regions set forth in SEQ ID NO: 704 and 702.

In an embodiment of the invention, said human Fc may be derived from IgGl, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgGlO, IgGl 1, IgG12, IgG13, IgG14, IgG15, IgGl 6, IgGl 7, IgGl 8 or IgGl 9.

In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may comprise a polypeptide having at least about 90% sequence homology to at least one of the polypeptide sequences of SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, 708, 2, 20, 647, 651, 660, 666, 699, 702, 706, and 709.

In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may have an elimination half-life of at least about 30 days.

In one embodiment, the antibody, or antibody fragment thereof, may inhibit with at least one activity associated with IL-6. In another embodiment, the at least one activity associated with IL-6 may be an in vitro activity comprising stimulation of proliferation of T1 165 cells; binding of IL-6 to IL-6R; activation (dimerization) of the gpl30 signal-transducing glycoprotein; formation of IL-6/IL-6R/gpl30 multimers; stimulation of haptoglobin production by HepG2 cells modified to express human IL-6 receptor; or any combination thereof. In one embodiment, prior to administration of the antibody, or antibody fragment thereof, the subject may have exhibited or may be at risk for developing at least one of the following symptoms: elevated serum C-reactive protein ("CRP"); elevated erythrocyte sedimentation rate; or a combination thereof.

In one embodiment, the antibody or antibody fragment thereof may comprise a Fab, Fab', F(ab')2, Fv, scFv, IgNAR, SMIP, camelbody, or nanobody. In one embodiment, the antibody or antibody fragment thereof may have an in vivo half-life of at least about 30 days in a healthy human subject. In one embodiment, the antibody or antibody fragment thereof may have a binding affinity (Kd) for IL-6 of less than about 50 picomolar, or a rate of dissociation (Kdoff) from IL-6 of less than or equal to 10^-4 S^-1. In one embodiment, the antibody or antibody fragment thereof may specifically binds to the same linear or conformational epitope(s) and/or competes for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof as an anti-IL-6 antibody comprising the polypeptides of SEQ ID NO: 702 and
SEQ ID NO: 704 or the polypeptides of SEQ ID NO: 2 and SEQ ID NO: 3. In one embodiment, the binding to the same linear or conformational epitope(s) and/or competition for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof is ascertained by epitopic mapping using overlapping linear peptide fragments which span the full length of the native human IL-6 polypeptide and includes at least one residues comprised in IL-6 fragments selected from those respectively encompassing amino acid residues 37-51, amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45 and/or amino acid residues 58-72 of SEQ ID NO: 1.

[0100] In one embodiment, the antibody or antibody fragment thereof, may be aglycosylated. In one embodiment, the antibody, or antibody fragment thereof, may contain an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation. In one embodiment, the antibody, or antibody fragment thereof, may be a human, humanized, single chain, or chimeric antibody. In one embodiment, the antibody, or antibody fragment thereof, may comprise a Fab, Fab', F(ab')2, Fv, or scFv. In one embodiment, the antibody, or antibody fragment thereof, may further comprise a human Fc. In another embodiment, the Fc may be derived from IgGl, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgGl, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18, or IgG19.

[0101] In one embodiment, the composition may comprise at least about 25, 80, 100, 160, 200, or 320 mg. In one embodiment, the effective amount may be between about 0.1 and 100 mg/kg of body weight of the subject. In one embodiment, the subject may be administered at least 1, 2, 3, 4, or 5 doses. In one embodiment, composition may be administered every 4 weeks. In one embodiment, the subject may be administered 25 mg every 4 weeks. In one embodiment, the subject may be administered 80 mg every 4 weeks. In one embodiment, the subject may be administered 100 mg every 4 weeks. In one embodiment, the subject may be administered 160 mg every 4 weeks. In one embodiment, the subject may be administered 200 mg every 4 weeks. In one embodiment, the subject may be administered 320 mg every 4 weeks. In another embodiment, the composition may be administered every 4 weeks for at least 16 weeks. In another embodiment, the composition may be administered every 4 weeks for at least 24 weeks.

[0102] In this embodiment, anti-IL-6 antibodies, or antibody fragments thereof may be administered at effective doses to less inflammation, pain, and loss of mobility experienced from anemia, optionally dosages ranging from about 25-500 mg, more preferably at least about 25, 80, 100, 120, 160, 200, 240, or 320 mg dosages.

[0103] In one embodiment, the antibody may comprise a light chain polypeptide that comprises at least one Abl light chain CDR polypeptide comprising a light chain CDR1 having at
least 72.7% sequence identity to SEQ ID NO: 4; a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5; a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6; a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4; a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6; and wherein the heavy chain polypeptide comprises at least one Abl heavy chain CDR polypeptide comprising a heavy chain CDR1 having at least 80% sequence identity to SEQ ID NO: 7; a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120; a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9; a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7; a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9.

[0104] In a further embodiment, the antibody or antibody fragment may comprise a light chain polypeptide comprises at least one Abl light chain CDR polypeptide comprising a light chain CDR1 having at least 81.8% sequence identity to SEQ ID NO: 4; a light chain CDR2 having at least 71.4% sequence identity to SEQ ID NO: 5; or a light chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 6; and wherein the heavy chain polypeptide comprises at least one Abl heavy chain CDR polypeptide comprising a heavy chain CDR1 having at least 60% sequence identity to SEQ ID NO: 7; a heavy chain CDR2 having at least 87.5% sequence identity to SEQ ID NO: 120; or a heavy chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 9. In a further embodiment, the antibody or antibody fragment may comprise antibody or antibody fragment comprises at least two of said light chain CDR polypeptides and at least two of said heavy chain CDR polypeptides.

[0105] In a further embodiment, the antibody or antibody fragment may comprise two or more Abl light chain CDR polypeptides comprising a light chain CDR1 having at least 72.7% sequence identity to SEQ ID NO: 4; a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5; or a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6; and two or more Abl heavy chain CDR polypeptide comprising a heavy chain CDR1 having at least 80% sequence identity (identical to at least 4 out of 5 residues) to SEQ ID NO: 7; a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120; or a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9; wherein the Abl antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.
In a further embodiment, the antibody or antibody fragment may comprise two or more Abl light chain CDR polypeptides comprising a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4; a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6; and two or more Abl heavy chain CDR polypeptide comprising a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7; a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9; wherein the Abl antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.

In a further embodiment, the Abl antibody or antibody fragment comprises said light chain CDR1, said light chain CDR3, said heavy chain CDR2, and said heavy chain CDR3.

In one embodiment, the antibody or antibody fragment may comprise antibody or antibody fragment thereof is administered to the subject in the form of at least one nucleic acids that encode the antibody or antibody fragment thereof.

In one embodiment, the antibody or antibody fragment may comprise a light chain of encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723.

In one embodiment, the antibody or antibody fragment may comprise a heavy chain of said antibody or antibody fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725.

In one embodiment, the antibody or antibody fragment may comprise at least one of the CDRs of said antibody or antibody fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 12, 15, 31, 34, 47, 50, 63, 66, 79, 82, 95, 98, 111, 114, 132, 135, 148, 151, 164, 167, 180, 183, 196, 199, 212, 215, 228, 231, 244, 247, 260, 263, 276, 279, 292, 295, 308, 311, 324, 327, 340, 343, 356, 359, 372, 375, 388, 391, 404, 407, 420, 423, 436, 439, 452, 455, 468, 471, 484, 487, 500, 503, 516, 519, 532, 535, 548, 551, 564, 567, 580, 583, 694, 13, 16, 32, 35, 48, 51, 64, 67, 80, 83, 96, 99, 112, 115, 133, 136, 149, 152, 165, 168, 181, 184, 197, 200, 213, 216, 229, 232, 245, 248, 261, 264, 277, 280, 293, 296, 309, 312, 325, 328, 341, 344, 357, 360, 373, 376, 389, 392, 405, 408, 421, 424, 437, 440, 453, 456, 469, 472, 485, 488, 501, 504, 517, 520, 533, 536, 549, 552, 565, 568, 581, 584, 696, 14, 17, 33, 36, 49, 52,

[0112] In one embodiment, the antibody or antibody fragment may comprise at least one of the nucleic acids comprise the heavy and light chain polynucleotide sequences of SEQ ID NO: 723 and SEQ ID NO: 700; SEQ ID NO: 701 and SEQ ID NO: 703; SEQ ID NO: 705 and SEQ ID NO: 707; SEQ ID NO: 720 and SEQ ID NO: 724; and SEQ ID NO: 10 and SEQ ID NO: 11.

[0113] In one embodiment, the antibody or antibody fragment may comprise a humanized variable light sequence of amino acid sequence of SEQ ID NO: 709.

[0114] In one embodiment, the antibody or antibody fragment may comprise a humanized variable heavy sequence of amino acid sequence of SEQ ID NO: 657.

[0115] In one embodiment, the antibody or antibody fragment may comprise at least one light chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 4, 5, or 6.

[0116] In one embodiment, the antibody or antibody fragment may comprise at least one heavy chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 7, 120, or 9.

[0117] In one embodiment, the antibody or antibody fragment may be an asialated, humanized anti-IL-6 monoclonal antibody with a half-life of -30 days comprising the humanized variable light and heavy sequences as set forth in SEQ ID NO: 20 and 19 or SEQ ID NO: 709 or 657.

[0118] In one embodiment, the antibody or antibody fragment may be expressed from a recombinant cell. In another embodiment, the cell may be a mammalian, yeast, bacterial, and insect cell. In another embodiment, the cell may be a yeast cell. In another embodiment, the cell may be a diploidal yeast cell. In another embodiment, the yeast cell may be a Pichia yeast. In one embodiment, the antibody may be asialated. In one embodiment, the antibody may be humanized.

[0119] In one embodiment, the antibody or antibody fragment thereof may comprise a Fab, Fab', F(ab')2, Fv, scFv, IgNAR, SMIP, camelbody, ornanobody.

[0120] In one embodiment, the antibody or antibody fragment thereof may have an in vivo half-life of at least about 30 days.

[0121] In one embodiment, the antibody or antibody fragment thereof may have a binding affinity (Kd) for IL-6 of less than about 50 picomolar, or a rate of dissociation (Kd) from IL-6 of less than or equal to 10^-4 S^-1.

[0122] In one embodiment, the antibody or antibody fragment thereof may specifically binds to the same linear or conformational epitope(s) and/or competes for binding to the same linear or
conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof as an anti-IL-6 antibody comprising the polypeptides of SEQ ID NO: 702 and SEQ ID NO: 704 or the polypeptides of SEQ ID NO: 2 and SEQ ID NO: 3.

[0123] In one embodiment, the antibody or antibody fragment thereof may have binding to the same linear or conformational epitope(s) and/or competition for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof is ascertained by epitopic mapping using overlapping linear peptide fragments which span the full length of the native human IL-6 polypeptide and includes at least one residues comprised in IL-6 fragments selected from those respectively encompassing amino acid residues 37-51, amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45 and/or amino acid residues 58-72 of SEQ ID NO: 1.

[0124] In one embodiment, the antibody or antibody fragment thereof may be aglycosylated. In one embodiment, the antibody or antibody fragment thereof may comprise an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation. In one embodiment, the antibody or antibody fragment thereof may be a human, humanized, single chain, or chimeric antibody. In one embodiment, the antibody or antibody fragment thereof may further comprise a human Fc. The method or use of claim 126, wherein said human Fc is derived from IgGl, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgGlO, IgGl 1, IgGl2, IgGl3, IgGl4, IgGl 5, IgGl 6, IgGl 7, IgGl 8, or IgGl 9.

[0125] In one embodiment, the chemotherapy may comprise administration of a chemotherapy agent selected from the group consisting of Alemtuzumab (Campath®), Asparaginase (Elspar®), Bleomycin (Blenoxane®), Busulfan (Myleran®, Busulfex®), Capecitabine (Xeloda®), Carboplatin (Paraplatin®), Cisplatin (PLATINOL®), Cyclophosphamide (Cytoxan®), Cytarabine (Cytosar-U®), Daunorubicin (Cerubidine®), Docetaxel (Taxotere®), Doxorubicin (Adriamycin®), Epirubicin (Ellence®), Etoposide (VePesid®), Fluorouracil (5-FU®), Gemcitabine (Gemzar®), Gemtuzumab ozogamicin (Mylotarg®), Hydroxyurea (Hydrea®), Idarubicin (Idamycin®), Interleukin 2 (Proleukin®), Irinotecan (Camptosar®), Lomustine (CeeNU®), Mechlorethamine (Mustargen®), Melphalan (Alkeran®), Methotrexate (Rheumatrex®), Mitomycin (Mutamycin®), Mitoxantrone (Novantrone®), Oxaliplatin (Eloxatin®), Paclitaxel (Taxol®), Pemetrexed (Alimta®), Pentostatin (Nipent®), Procarbazine (Matulane®), Thiopeta (Thioplex®), Topotecan (Hycamtin®), Trastuzumab (Herceptin®), Tretinoin (Vesanoid®), Vinblastine (Velban®), or Vincristine (Oncovin®).
In one embodiment, the patient may have elevated C-reactive protein ("CRP"). In one embodiment, the patient may have elevated IL-6 serum level. In one embodiment, the patient may have elevated IL-6 level in the joints.

In one embodiment, the IL-antagonist may inhibit at least one activity associated with IL-6. In another embodiment, the at least one activity associated with IL-6 is an in vitro activity comprising stimulation of proliferation of T1 165 cells; binding of IL-6 to IL-6R; activation (dimerization) of the gpl30 signal-transducing glycoprotein; formation of IL-6/IL-6R/gpl30 multimers; stimulation of haptoglobin production by HepG2 cells modified to express human IL-6 receptor; or any combination thereof.

In another embodiment, prior to administration of the IL-6 antagonist, optionally an antibody or antibody fragment, the subject has exhibited or is at risk for developing at least one of the following symptoms: decreased serum albumin; elevated serum C-reactive protein ("CRP"); elevated erythrocyte sedimentation rate; fatigue; fever; anorexia (loss of appetite); weight loss; cachexia; weakness; decreased Glasgow Prognostic Score ("GPS"); elevated serum D-dimer; abnormal coagulation profile; and any combination thereof.

In another embodiment, the symptom may be a side-effect of another therapeutic agent administered to the subject prior to, concurrent with, or subsequent to administration of the antibody or antibody fragment. In another embodiment, the method may further comprise monitoring the subject to assess said symptom subsequent to administration of the antibody. In another embodiment, the symptom may be exhibited prior to administration of said IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment. In another embodiment, the symptom may be improved or restored to a normal condition within about 1-5 weeks of administration of said IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment. In another embodiment, the symptom may thereafter remains improved for an entire period intervening two consecutive administrations of said IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment. In another embodiment, the patient treated may have at least one symptom of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer.

In another embodiment, the patient treated may have cancer or is being treated for cancer. In one embodiment, the cancer is selected from the group consisting of Acanthoma, Acinic cell carcinoma, Acoustic neuroma, Acral lentiginous melanoma, Acrospiroma, Acute eosinophilic leukemia, Acute lymphoblastic leukemia, Acute megakaryoblastic leukemia, Acute monocytic leukemia, Acute myeloblastic leukemia with maturation, Acute myeloid dendritic cell leukemia, Acute myeloid leukemia, Acute promyelocytic leukemia, Adamantinoma,

In one embodiment, the patient suffers from a disease or disorder selected from the group consisting of general fatigue, exercise-induced fatigue, cancer-related fatigue, inflammatory disease-related fatigue, chronic fatigue syndrome, cancer-related cachexia, cardiac-related cachexia, respiratory-related cachexia, renal-related cachexia, age-related cachexia, rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic juvenile idiopathic arthritis, psoriasis, psoriatic arthropathy, ankylosing spondylitis, inflammatory bowel disease (IBD), polymyalgia rheumatica, giant cell arteritis, autoimmune vasculitis, graft versus host disease (GVHD), Sjogren's syndrome, adult onset Still's disease, rheumatoid arthritis, systemic juvenile idiopathic arthritis, osteoarthritis, osteoporosis, Paget's disease of bone, osteoarthritis, multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, prostate cancer, leukemia, renal cell cancer, multicentric Castleman's disease, ovarian cancer, drug resistance in cancer chemotherapy, cancer chemotherapy toxicity, ischemic heart disease, atherosclerosis, obesity, diabetes, asthma, multiple sclerosis, Alzheimer's disease, cerebrovascular disease, fever, acute phase response, allergies, anemia, anemia of inflammation (anemia of chronic disease), hypertension, depression, depression associated with a chronic illness, thrombosis, thrombocytosis, acute heart failure,
metabolic syndrome, miscarriage, obesity, chronic prostatitis, glomerulonephritis, pelvic inflammatory disease, reperfusion injury, transplant rejection, graft versus host disease (GVHD), avian influenza, smallpox, pandemic influenza, adult respiratory distress syndrome (ARDS), severe acute respiratory syndrome (SARS), sepsis, and systemic inflammatory response syndrome (SIRS).

In one embodiment, the patient has or is to receive autologous stem cell or bone marrow transplant.

In one embodiment, the IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment, may be administered prior, concurrent or after chemotherapy or radiotherapy. In one embodiment, the chemotherapeutic is an EGFR inhibitor. In one embodiment, the EGFR inhibitor is selected from the group consisting of Cetuximab (Erbitux), Erlotinib (Tarceva), Gefitinib (Iressa), Lapatinib (Tykerb), Panitumimab (Vectibix), Sunitinib or Sutent (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-lH-indol-3-ylidene)methyl]-2,4-dimethyl-lH-pyrrole-3-carboxamide), Gefitinib or N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine, and Zalutumumab. In one embodiment, the patient may have a cancer that has exhibited resistance to said chemotherapeutic or radiation after at least one round of chemotherapy or radiation. In one embodiment, the chemotherapeutic or radiation reduces or prevents the treated cancer from invading or metastasizing to other sites in the body. In one embodiment, the chemotherapeutic or radiation results in increased apoptosis of the treated cancer cells.

In one embodiment, the treated cancer is selected from advanced and non-advanced cancers including metastasized cancers such as metastatic and non-metastatic lung cancer, breast cancer, head and neck cancer, (HNSCC), pharyngeal cancer, pancreatic cancer, colorectal cancer, anal cancer, glioblastoma multiforme, epithelial cancers, renal cell carcinomas, acute or chronic myelogenous leukemia and other leukemias.

In one embodiment, the results are used to facilitate design of an appropriate therapeutic regimen for anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer or a disease associated with anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer.

In one embodiment, the IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment, is co-administered with another therapeutic agent selected from the group consisting of analgesics, antibiotics, anti-cachexia agents, anti-coagulants, anti-cytokine agents, antiemetic...
agents, anti-fatigue agent, anti-fever agent, anti-inflammatory agents, anti-nausea agents, antipyretics, antiviral agents, anti-weakening agent, chemotherapy agents, cytokine antagonist, cytokines, cytotoxic agents, gene therapy agents, growth factors, IL-6 antagonists, immunosuppressive agents, local anesthetic, statins, other therapeutic agents, or any combination thereof.

[0137] In another embodiment, the analgesic is acetaminophen, amitriptyline, benzocaine, carbamazepine, codeine, dyclonine hydrochloride (HQ), dihydromorphine, fentanyl patch, Flupirtine, flurbiprofen, gabapentin, hydrocodone APAP, hydromorphone, ibuprofen, ketoprofen, lidocaine, morphine, an opiate and derivatives thereof, oxycodone, pentazocine, pethidine, phenacetin, pregabalin, propoxyphene, propoxy APAP, salicylamide, tramadol, tramadol APAP, Ulcerease® (0.6% Phenol), or voltaren.

[0138] In another embodiment, the local anesthetic is amethocaine, articaine, benzocaine, bupivacaine, mepivacaine, cocaine, cinchocaine, chloroprocaine, cyclomethycaine, dibucaine, dimethocaine, EMLA® (eutectic mixture of lidocaine and prilocaine), etidocaine, larocaine, levobupivacaine, lidocaine, lignocaine, procaine, piperocaine, prilocaine, propoxycaine, ropivacaine, saxitoxin, tetracaine, tetrodotoxin, or trimecaine.

[0139] In another embodiment, the anti-cachexia agent is cannabis, dronabinol (Marinol®), nabilone (Cesamet), cannabidiol, cannabichromene, tetrahydrocannabinol, Sativex, megestrol acetate, or any combination thereof.

[0140] In another embodiment, the anti-coagulant is abciximab (ReoPro®), acenocoumarol, antithrombin III, argatroban, aspirin, bivalirudin (Angiomax®), clopidogrel, dabigatran, dabigatran etexilate (Pradaxa®/Pradax®), desirudin (Revasc®/Iprivask®), dipyridamole, eptifibatide (Integrilin®), fondaparinux, heparin, hirudin, idraparinux, lepirudin (Refludan®), low molecular weight heparin, melagatran, phenindione, phenprocoumon, ticlopidine, tirofiban (Aggrastat®), warfarin, ximelagatran, ximelagatran (Exanta®/Exarta®), or any combination thereof.

[0141] In another embodiment, the anti-inflammatory agent is acetaminophen, azapropazone, diclofenac, diflunisal, etodolac, fenbufen, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, mefenamic, meloxicam, nabumetone, naproxen, phenylbutazone, piroxicam, a salicylate, sulindac, tenoxicam, tiaprofenic acid, or tolfenamic acid. In still further embodiment, the salicylate is acetylsalicylic acid, amoxiprin, benorylate, choline magnesium salicylate, ethenzamide, faislamine, methyl salicylate, magnesium salicylate, salicyl salicylate, or salicylamide.
[0142] In another embodiment, the anti-nausea agent or antiemetic agent is comprising 5-HT3 receptor antagonists, ajwain, alizapride, anticholinergics, antihistamines, apreptian, benzodiazepines, cannabichromene, cannabidiol, cannabinoids, cannabis, casopitant, chlorpromazine, cyclizine, dexamethasone, dexamethasone, dimenhydrinate (Gravol®), diphenhydramine, dolasetron, domeridon, dopamine antagonists, doxylamine, dronabinol (Marinol®), droperidol, emetrol, ginger, granisetron, haloperidol, hydroxyzine, hyoscine, lorazepam, meclizine, metoclopramide, midazolam, muscimol, nabilone (Cesanet), nkl receptor antagonists, ondansetron, palonosetron, peppermint, Phenergan, prochlorperazine, Promacot, promethazine, Pentazine, propofol, sativex, tetrahydrocannabinol, trimethobenzamide, tropisetron, nandrolone, stilbestrol, thalidomide, lenalidomide, ghrelin agonists, myostatin antagonists, anti-myostatin antibodies, selective androgen receptor modulators, selective estrogen receptor modulators, angiotensin All antagonists, beta two adenergic receptor agonists, beta three adenergic receptor agonists, or any combination thereof.

[0143] In another embodiment, the antiviral agent is selected from the group consisting of abacavir, aciclovir, acyclovir, adeovir, amantadine, amprenavir, an antiretroviral fixed dose combination, an antiretroviral synergistic enhancer, arbidol, atazanavir, atripla, brivudine, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, famciclovir, fomivirsen, fosaprenavir, foscarinet, fosfonet, fusion inhibitor, ganciclovir, gardsasil, ibacitabine, idoxuridine, imiquimod, imunovir, indinavir, inosine, integrase inhibitor, interferon, interferon type I, interferon type II, interferon type III, lamivudine, lopinavir, loviride, maraviroc, MK-0518, moroxydine, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine, or any combination thereof.

[0144] In another embodiment, the cytotoxic agent, chemotherapeutic agent, or immunosuppressive agent is comprising 1-dehydrotestosterone, 1-methylnitrosourea, 5-fluorouracil, 6-mercaptopurine, 6-mercaptopurine, 6-thioguanine, Abatacept, abraxane, acitretin, aclarubicin, Actinium-225 (225Ac), actinomycin, Adalimumab, adenosine deaminase inhibitors, Afelimomab, Aflibercept, Afutuzumab, Alefacect, altretinoin, alkyl sulfonates, alkylating agents, altretamine, alvocidib, aminolevulinic acid/methyl aminolevulinate, aminopterin, aminopterin, amrubicin, amsacrine, amsacrine, anagrelide, Anakinra, anthracenenediones, anthracyclines, anthracyclines, anthracyclines, antithromycin (AMC); antimyotatic agents, antibiotics, anti-CD20
antibodies, antifolates, Anti-lymphocyte globulin, Antimetabolites, Anti-thymocyte globulin, arsenic trioxide, Aselizumab, asparaginase, asparagine depleters, Astatine-211 (\(^{211}\text{At}\)), Atlizumab, Atorolimumab, atrasentan, Avastin®, azacitidine, Azathioprine, azelastine, aziridines, Basiliximab, BAYX antibodies, Belatacept, Belimumab, belotecan, bendamustine, Bertilimumab, bexarotene, bisantrene, Bismuth-213 (\(^{213}\text{Bi}\)), Bismuth-212 (\(^{212}\text{Bi}\)), bleomycin, bleomycin, bleomycin, BLyS antibodies, bortezomib, busulfan, busulfan, Calcineurin inhibitors, calicheamicin, camptothecin, camptothecins, capecitabine, carboplatin (paraplatin), carboquone, carminomycin, carmofur, Carmustine, Carmustine (BSNU), CAT antibodies, CD147/Basigin antibodies, CD154 antibodies, CD18 antibodies, CD20 antibodies, CD23 antibodies, CD3 antibodies, CD4 antibodies, CD40 antibodies, CD62L/L-selectin antibodies, CD80 antibodies, CDK inhibitors, Cedelizumab, celecoxib, Certolizumab pegol, chlorambucil, chlorambucils, Ciclosporin, cis-dichlorodiamine platinum (II) (DDP) cisplatin, cladribine, Clenoliximab, clofarabine, colchicin, Complement component 5 antibodies, Copper-67 (\(^{65}\text{Cu}\)), corticosteroids, CTLA-4 antibodies, CTLA-4 fusion proteins, Cyclophilin inhibitors, cyclophosphamides, cyclophosphamide, cytarabine, cytarabine, cytochalasin B, cytotoxic ribonucleases, dacarbazine, Daclizumab, dactinomycin, dactinomycin (actinomycin D), daunorubicin, daunorubicin, daunorubicin (formerly daunomycin), decitabine, Deforolimus, demecolcine, detorubicin, dibromomannitol, dihydrofolate reductase inhibitors, dihydroxy anthracybin dione, diphereria toxin, DNA polymerase inhibitors, docetaxel, Dorlimomab aritox, Dorlixizumab, doxorubicin (adriamycin), DXL625, Eculizumab, Efalizumab, efaproxiral, EGFR antagonists, elesclomol, elasmirucin, Elsilimomab, emetine, endothelin receptor antagonists, epipodophyllotoxins, epirubicin, epothilones, Erbitux®, Erlizumab, estramustine, Etaanercept, ethidium bromide, etoglucid, etoposide, etoposide phosphate, Everolimus, Faralimomab, farnesyltransferase inhibitors, FKBP inhibitors, floxuridine, fludarabine, fluorouracil, Fontolizumab, fotemustine, Galiximab, Gallium-67 (\(^{68}\text{Ga}\)), Gantenerumab, Gavilimomab, gemcitabine, glucocorticoids, Golimumab, Gomiliximab, gramicidin D, Gusperimus, Herceptin®, hydrazines, hydroxyurea, hypomethylating agents, idarubicin, Idarubicine, ifosfamide, IL-1 antagonists, IL-1 receptor antagonists, IL-12, IL-12 antibodies, IL-12R antagonists, IL-13 antibodies, IL-2, IL-2 inhibitors, IL-2 receptor/CD25 antibodies, IL-6 antibodies, imatinib mesylate, Immunoglobulin E antibodies, IMP dehydrogenase inhibitors, Infliximab, Inolimomab, Integrin antibodies, Interferon antibodies, interferons, Interleukin 5 antibodies, Interleukin-6 receptor antibodies, interleukins, Iodine-125 (\(^{125}\text{I}\)), Iodine-131 (\(^{131}\text{I}\)), Ipilimumab, irinotecan, ixabepilone, Keliximab, larotaxel, Lead-212 (\(^{212}\text{Pb}\)), Lebrilizumab, Leflunomide, Lenalidomide, Lerdelimumab, leucovorine, LFA-1
antibodies, lidocaine, lipoxygenase inhibitors, lomustine (CCNU), lonidamine, lucanthone, Lumiliximab, Lutetium-177 (177Lu), Macrolides, mannosulfan, Maslimomab, masoprolcol, mechlorethamine, melphalan, Mepolizumab, mercaptopurine, Metelimumab, Methotrexate, microtubule assembly inhibitors, microtubule stability enhancers, mithramycin, mitobronitol, mitoguazone, mitomycin, mitomycin C, mitotane, mitoxantrone, Morolimumab, mTOR inhibitors, Muromonab-CD3, mustines, Mycophenolic acid, mytotane (0,P’-(DDD)), Natalizumab, nedaplatin, Nerelimomab, nimustine, nitrogen mustards, nitrosoureas, nordihydroguaiaretic acid, oblimersen, ocrelizumab, Ocrelizumab, Odulimomab, ofatumumab, olaparib, Omalizumab, ortataxel, Otelixizumab, oxaliplatin, oxaliplatin, paclitaxel (taxol), Pascolizumab, PDGF antagonists, pegasparagase, pemetrexed, Pentostatin, Pertuzumab, Pexelizumab, phosphodiesterase inhibitors, Phosphorus-32 (32P), Pimecolomim Abetimus, pirarubicin, pixantrone, platins, plicamycin, poly ADP ribose polymerase inhibitors, porfimer sodium, porphyrin derivatives, prednimustine, procaine, procarbazine, procarbazine, propranolol, proteasome inhibitors, pseudomonas exotoxin, Pseudomonas toxin, purine synthesis inhibitors, puromycin, pyrimidine synthesis inhibitors, radionuclides, radiotherapy, raltitrexed, ranimustine, Reslizumab, retinoid X receptor agonists, retinoids, Rhenium-186 (186Re), Rhenium-188 (188Re), ribonucleotide reductase inhibitors, ricin, Rilonacept, Rituxan®, Rovelizumab, rubitecan, Ruplizumab, Samarium-153 (153Sm), satraplatin, Scandium-47 (47Sc), selective androgen receptor modulators, selective estrogen receptor modulators, seliciclib, semustine, sex hormone antagonists, siplizumab, sirolimus, steroid aromatase inhibitors, steroids, streptozocin, streptozotocin, Tacrolimus, talaporfin, Talizumab, taxanes, taxols, tegafur, Telimomab aritox, temoporfin, temozolomide, temsirolimus, Tensirolimus, Teneliximab, teniposide, Teplizumab, Teriflunomide, tesetaxel, testolactone, tetracaine, Thalidomide, thioepa chlorambucil, thiopurines thioguanine, ThioTEPA, thymidylate synthase inhibitors, tiazofurin, tipifarnib, T-lymphocyte antibodies, TNF antagonists, TNF antibodies, TNF fusion proteins, TNF receptor fusion proteins, TNF-alpha inhibitors, Tocilizumab, topoisomerase inhibitors, topotecan, Toralizumab, trabectedin, Tremelimumab, treosulfan, tretinoin, triazenes, triaziquone, triethyleneemelamine, triplatin tetraniitrate, trofosfamide, tumor antigen specific monoclonal antibodies, tyrosine kinase inhibitors, uramustine, Ustekinumab, valrubicin, Valrubicine, Valapliximab, VEGF antagonists, Vepalimomab, verteporfin, vinblastine, vinca alkaloids, vincristine, vindesine, vinflunine, vinorelbine, Visilizumab, vorinostat, Yttrium-88 (88Y), Yttrium-90 (90Y), Zanolimumab, zileuton, Ziralimumab, Zolimomab aritox, zorubicin, Zotarolimus, or any combination thereof.

[0145] In another embodiment, the chemotherapy agent is selected from the group consisting of VEGF antagonists, EGFR antagonists, platins including cisplatin and carboplatin, taxols,
irinotecan, 5-fluorouracil, gemcytabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids, vinblastine, vincristine, vindesine, vinorelbine, mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins, IL-12, IL-2, IL-12R antagonists, Toxin conjugated monoclonal antibodies, tumor antigen specific monoclonal antibodies, Erbitux®, Avastin®, Pertuzumab, anti-CD20 antibodies, Rituxan®, ocrelizumab, ofatumumab, DXL625, Herceptin®, or any combination thereof.

In another embodiment, the cytokine antagonist is an antagonist of tumor necrosis factor-alpha, interferon gamma, interleukin 1 alpha, interleukin 1 beta, interleukin 6, TNF-a, IL-la. IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-a, IFN-γ, BAFF, CXCL13, IP-10, leukemia-inhibitory factor, or a combination thereof.

In another embodiment, the growth factor is VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Heparin, or any combination thereof.

In another embodiment, the statin is comprising atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or any combination thereof.

In another embodiment, the other therapeutic agent is an antagonist of a factor comprising tumor necrosis factor-alpha, Interferon gamma, Interleukin 1 alpha, Interleukin 1 beta, Interleukin 6, proteolysis inducing factor, leukemia-inhibitory factor, tamoxifen, BCL-2 antagonists, estrogen, bisphosphonates, teriparatide, strontium ranelate, sodium alendronate (Fosamax), risedronate (Actonel), raloxifene, ibandronate (Boniva), Obatoclax, ABT-263, gossypol, gefitinib, epidermal growth factor receptor tyrosine kinase inhibitors, erlotinib, epidermal growth factor receptor inhibitors, psoralens, trioxysalen, methoxsalen, bergapten, retinoids, etretinate, acitretin, infliximab (Remicade®), adalimumab, infliximab, etanercept, Zenapax®, Cyclosporine, Methotrexate, granulocyte-colony stimulating factor, filgrastim, lenograstim, Neupogen, Neulasta, 2-Arylpropionic acids, Aceclofenac, Acemetacin, Acetylsalicylic acid (Aspirin), Aclofenac, Alminopronfen, Amoxiprin, Amaryne, Aroyalkanoic acids, Azapropazone, Benorylate/Benorilate, Benoxaprofen, Bromfenac, Carprofen, Celecoxib, Choline magnesium salicylate, Clofazine, COX-2 inhibitors, Dexibuprofen, Dextropropafen, Diclofenac, Diflunisal, Droxicam, Ethenzamide, Etodolac, Etoricoxib, Faislamine, fenamic acids, Fenbufen, Fenoprofen, Flufenamic acid, Flunoxaprofen, Flurbiprofen, Ibuprofen, Ibuprofen, Indometacin, Indoprofen, Kebuzone, Ketoprofen, Ketorolac, Lornoxicam, Loxoprofen, Lumiracoxib, Magnesium salicylate, Meflofenamic acid, Mefenamic acid, Meloxicam, Metamizole, Methyl salicylate, Mofebutazone, Nabumetone, Naproxen, N-Arylanthranilic acids,
Oxametacin, Oxaprozin, Oxicams, Oxyphenbutazone, Parecoxib, Phenazone, Phenylbutazone, Phenylbutazone, Piroxicam, Pirprofen, profens, Proglumetacin, Pyrazolidine derivatives, Rofecoxib, Salicyl salicylate, Salicylamide, Salicylates, Sulfapyrazone, Sulindac, Suprofen, Tenoxicam, Tiaprofenic acid, Toltenamic acid, Tolmetin, and Valdecoxib. Antibiotics include Amikacin, Aminoglycosides, Amoxicillin, Ampicillin, Ansamycins, Arsenicals, Azithromycin, Azlocillin, Aztreonam, Bacitracin, Carbacephem, Carbenicillin, Ceftazidine, Ceftobiprole, Ceftriaxone, Cefuroxime, Cephalosporins, Chloramphenicol, Cilastatin, Ciprofloxacin, Clarithromycin, Clindamycin, Cloxacillin, Colistin, Co-trimoxazole, Dalfopristin, Demeclocycline, Dicloxacillin, Dirithromycin, Doripenem, Doxycycline, Enoxacin, Ertapenem, Erythromycin, Ethambutol, Flucloxacillin, Fosfomycin, Furazolidone, Fusidic acid, Gatifloxacin, Geldanamycin, Gentamicin, Glycopeptides, Herbstimycin, Imipenem, Isoniazid, Kanamycin, Levofloxacin, Lincomycin, Linezolid, Lomefloxacin, Loracarbef, Macrolides, Mafenide, Meropenem, Metcillin, Metronidazole, Mezlocillin, Minocycline, Monobactams, Moxifloxacin, Mupirocin, Nafcillin, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacinill, Oxytetracycline, Paromomycin, Penicillin, Penicillins, Piperacillin, Platensimycin, Polymyxin B, Polypeptides, Prontosil, Pyrazinamide, Quinolones, Quinupristin, Rifampicin, Rifampin, Roxithromycin, Spectinomycin, Streptomycin, Sulfaetamide, Sulfamethizole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Sulfamides, Teicoplanin, Telithromycin, Tetracycline, Tetracyclines, Ticarcillin, Tinidazole, Tobramycin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Troleandomycin, Trovafloxacin, and Vancomycin. Active agents also include Aldosterone, Beclometasone, Betamethasone, Corticosteroids, Cortisol, Cortisone acetate, Deoxycorticosterone acetate, Dexamethasone, Fludrocortisone acetate, Glucocorticoids, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone, Steroids, and Triamcinolone, an agonist, antagonist, or modulator of a factor comprising TNF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, WN-α, WN-γ, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof. [0150] In one embodiment, the IL-6 antagonist comprises anti-IL-6 antibodies or antibody fragments thereof, antisense nucleic acids, polypeptides, small molecules, or any combination thereof. In another embodiment, the antisense nucleic acid comprises at least approximately 10 nucleotides of a sequence encoding IL-6, IL-6 receptor alpha, gp130, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, or SYK. In another embodiment, the antisense nucleic acid comprises
DNA, RNA, peptide nucleic acid, locked nucleic acid, morpholino (phosphorodiamidate morpholino oligo), glycerol nucleic acid, threose nucleic acid, or any combination thereof. In another embodiment, the IL-6 antagonist polypeptide comprises a fragment of a polypeptide having a sequence selected from the group consisting soluble IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, and SYK.

[0151] In one embodiment, the antibody or antibody fragment may be directly or indirectly coupled to a detectable label, half-life increasing moiety, cytotoxic agent, therapeutic agent, or an immunosuppressive agent. In another embodiment, the detectable label is comprising fluorescent dyes, bioluminescent materials, radioactive materials, chemiluminescent moieties, streptavidin, avidin, biotin, radioactive materials, enzymes, substrates, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, β-galactosidase, luciferase, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin, dansyl chloride, luminol, luciferin, aequorin, Iodine 125 (125I), Carbon 14 (14C), Sulfur 35 (35S), Tritium (3H), Phosphorus 32 (32P), or any combination thereof.

[0152] In one embodiment, the subject may recieve concomitant chemotherapy. In another embodiment, the subject may recieve receiving concomitant radiotherapy.

[0153] In another embodiment, the antibody may be the Abl antibody.

[0154] In another embodiment, the composition may be administered intravenously for at least about 1 hour. In another embodiment, the effective amount is or medicament comprises between about 0.1 and 20 mg/kg of body weight of recipient subject of said IL-6 antagonist. In another embodiment, the effective amount is or medicament comprises at least about 25, 80, 100, 160, 200, or 320 mg. In another embodiment, the effective amount is or medicament comprises between about 0.1 and 100 mg/kg of body weight of the subject.

[0155] In another embodiment, the subject may be administered at least 1, 2, 3, 4, or 5 doses. In another embodiment, the composition may be administered every 4 weeks. In another embodiment, the composition may be administered administered 160 mg every 4 weeks for a total of 2 doses. In another embodiment, the composition may be administered administered 160 mg every 4 weeks for a total of 2 doses. In another embodiment, the composition may be administered administered 320 mg every 4 weeks for a total of 2 doses.

[0156] In another embodiment, the anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer may be induced by chemoradiation (CRT) regimens or HSCT used for the treatment of cancers of the head and neck.
In another embodiment, the method may further comprise assessment of the status of the anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer.

In another embodiment, the assessment may comprise imaging modality selected from the group consisting of CAT, PET, and MRI exams.

In another embodiment, the subject may be administered 5-fluoracil (5-FU) or Irinotecan.

The invention also provides a method of identifying cancers that are potentially resistant to the effects of a chemotherapeutic or radiation by assaying for IL-6 using an antibody according to the invention in order to detect whether elevated IL-6 levels are present at the site of the treated cancer.

In another embodiment, a method for the reduction of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy comprises administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6.

In another embodiment, a method for the treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in a subject with head and neck cancer receiving concomitant chemotherapy comprises administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Abl.

In another embodiment, a method for the treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in a subject with head and neck cancer receiving concomitant chemotherapy comprises administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Ab 1.

In another embodiment, the invention provides for the use of an antibody according to the invention for preparing a diagnostic composition for identifying cancers that are potentially
resistant to the effects of a chemotherapeutic or radiation by assaying for IL-6 in order to detect whether elevated IL-6 levels are present at the site of the treated cancer.

[0166] In another embodiment, the invention provides for the use of an antibody according to the invention for preparing a composition for the reduction of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy comprising administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6.

[0167] In another embodiment, the invention provides for the use of an antibody according to the invention for preparing a composition for the treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in a subject with head and neck cancer receiving concomitant chemotherapy comprising administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Ab 1.

[0168] In another embodiment, the invention provides for the use of an antibody according to the invention for preparing a composition for the treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in a subject with head and neck cancer receiving concomitant chemotherapy comprising administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Ab 1.

[0169] In one embodiment, the composition may be administered subcutaneously. In another embodiment, the composition may be a pharmaceutical composition. In a further embodiment, the composition may be formulated for subcutaneous administration.

[0170] In one embodiment, the patient may have an elevated C-reactive protein ("CRP"). In one embodiment, the patient may have an elevated IL-6 serum level. In one embodiment, the patient may have an elevated IL-6 level in the joints. In one embodiment, the patient may have had an inadequate response to non-steroidal anti-inflammatory drugs (NSAIDs). In one embodiment, the patient may have had an inadequate response to non-biologic Disease Modifying Anti-Rheumatic Drugs (DMARDs).

[0171] In one embodiment, the antibody or antibody fragment may be directly or indirectly coupled to a detectable label, cytotoxic agent, therapeutic agent, or an immunosuppressive agent. In one embodiment, the detectable label may comprise a fluorescent dye, bioluminescent material, radioactive material, chemiluminescent moiety, streptavidin, avidin, biotin, radioactive material, enzyme, substrate, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase,
β-galactosidase, luciferase, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin, dansyl chloride, luminol, luciferin, aequorin, Iodine 125 (125I), Carbon 14 (14C), Sulfur 35 (35S), Tritium (3H), Phosphorus 32 (32P), or any combination thereof. In another embodiment, the IL-6 antagonist may be coupled to a half-life increasing moiety.

[0172] In one embodiment, the antibody or antibody fragment may be co-administered with another therapeutic agent selected from the group consisting of analgesics, antibiotics, anti-cachexia agents, anti-coagulants, anti-cytokine agents, antiemetic agents, anti-fatigue agent, anti-fever agent, anti-inflammatory agents, anti-nausea agents, antipyretics, antiviral agents, anti-weakened agent, chemotherapy agents, cytokine antagonist, cytokines, cytotoxic agents, gene therapy agents, growth factor, IL-6 antagonists, immunosuppressive agents, statins, or any combination thereof. In one embodiment, the cytokine antagonist may be an antagonist of a factor comprising tumor necrosis factor-alpha, interferon gamma, interleukin 1 alpha, interleukin 1 beta, interleukin 6, or any combination thereof. In one embodiment, the cytokine antagonist may be an antagonist of TNF-a, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-a, IFN-γ, BAFF, CXCL13, IP-10, leukemia-inhibitory factor, or a combination thereof. In one embodiment, the growth factor may be VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof. In one embodiment, the IL-6 antagonist may comprise an anti-IL-6 antibodies or antibody fragments thereof, antisense nucleic acids, polypeptides, small molecules, or any combination thereof.

[0173] In another embodiment, the antisense nucleic acid may comprise at least approximately 10 nucleotides of a sequence encoding IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, or SYK. In another embodiment, the antisense nucleic acid may comprise DNA, RNA, peptide nucleic acid, locked nucleic acid, morpholinol (phosphorodiamidate morpholino oligo), glycerol nucleic acid, theose nucleic acid, or any combination thereof. In another embodiment, the IL-6 antagonist polypeptide may comprise a fragment of a polypeptide having a sequence selected from the group consisting IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, STAT3, or any combination thereof. In a further embodiment, the IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, anti-STAT3, or anti-SYK antibody or antibody fragment.

[0174] One embodiment encompasses specific humanized antibodies and fragments and variants thereof for treatment or prevention of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer capable of binding to IL-6 and/or the IL-6/IL-6R complex. These
antibodies may bind soluble IL-6 or cell surface expressed IL-6. Also, these antibodies may
inhibit the formation or the biological effects of at least one of IL-6, IL-6/IL-6R complexes, IL-
6/IL-6R/gpl30 complexes and/or multimers of IL-6/IL-6R/gpl30. The present invention relates
to novel therapies and therapeutic protocols using anti-IL-6 antibodies, preferably those described
herein.

The invention also contemplates the administration of conjugates of anti-IL-6
antibodies and humanized, chimeric or single chain versions thereof and other binding fragments
and variants thereof conjugated to at least one functional or detectable moieties.

In an embodiment of the invention, the anti-IL-6 antibody or antibody fragment or
variant thereof may be directly or indirectly attached to a detectable label or therapeutic agent.

In one embodiment, the IL-6 antagonist may be an antisense nucleic acid. In another
embodiment of the invention, the IL-6 antagonist may be an antisense nucleic acid, for example
comprising at least approximately 10 nucleotides of a sequence encoding IL-6, IL-6 receptor
alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, or SYK. In a further embodiment
of the invention, the antisense nucleic acid may comprise DNA, RNA, peptide nucleic acid,
locked nucleic acid, morpholino (phosphorodiamidate morpholino oligo), glycerol nucleic acid,
threose nucleic acid, or any combination thereof.

In one embodiment, the IL-6 antagonist may comprise Actemra® (Tocilizumab),
Remicade®, Zenapax® (daclizumab), or any combination thereof.

In one embodiment, the IL-6 antagonist may comprise a polypeptide having a sequence
comprising a fragment of IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2,
JAK3, SYK, or any combination thereof, such as a fragment or full-length polypeptide that is at
least 40 amino acids in length. In another embodiment of the invention, the IL-6 antagonist may
comprise a soluble IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK,
STAT3, or any combination thereof.

In another aspect the invention provides pharmaceutical compositions and their use in
novel combination therapies and comprising administration of an anti-IL-6 antibody, such as any
one of Abl-Ab36 antibodies described in Table 4 or a fragment or variant thereof, and at least
one other therapeutic compound such as an anti-cytokine agent.

In an embodiment of the invention, the IL-6 antagonist may target IL-6, IL-6 receptor
alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. In one
embodiment, the IL-6 antagonist may comprise an antibody, an antibody fragment, a peptide, a
glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an averm, a small molecule, or any
combination thereof. In one embodiment, the IL-6 antagonist may comprise an anti-IL-6R, anti-
gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody, anti-STAT3, or antibody fragment. In an embodiment of the invention, the antagonist may comprise an anti-IL-6 antibody (e.g., any one of Abl-Ab36 antibodies described in Table 4) or antibody fragment or variant thereof.

[0182] The present invention also pertains to methods of improving survivability or quality of life of a patient having or at risk of developing anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer comprising administering to the patient an anti-IL-6 antibody (e.g., ALD5 18 antibody) or antibody fragment or variant thereof, whereby the patient's C-reactive protein ("CRP") level is lowered.

[0183] In one embodiment of the invention, the anti-IL-6 antibody or antibody fragment or variant thereof may be administered to the patient with a frequency at most once per period of approximately 4, 8, 12, 16, 20, or 24 weeks.

[0184] In an embodiment of the invention, the patient's quality of life may be improved.

[0185] This invention relates to novel anti-IL-6 antibodies, novel therapies and therapeutic protocols utilizing anti-IL-6 antibodies, and pharmaceutical formulations containing anti-IL-6 antibodies. In preferred embodiments, an anti-IL-6 antibody is any one of Abl-Ab36 antibodies described in Table 4, which includes rabbit or humanized forms thereof, as well as heavy chains, light chains, fragments, variants, and CDRs thereof, or an antibody or antibody fragment that specifically binds to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide fragment thereof as Abl. The subject application pertains in particular to preferred formulations and therapeutic uses of an exemplary humanized antibody referred to herein as any one of Abl-Ab36 antibodies described in Table 4 and variants thereof. In preferred embodiments, the anti-IL-6 antibody has an in vivo half-life of at least about 30 days, has an in vivo effect of lowering C-reactive protein, possesses a binding affinity (Kd) for IL-6 of less than about 50 picomolar, and/or has a rate of dissociation (Koff) from IL-6 of less than or equal to 10^9 s^-1.

[0186] In one aspect, this invention pertains to methods of improving survivability or quality of life of a patient in need thereof, comprising administering to a patient with or at risk of developing anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer as a result of disease or a therapeutic regimen comprising the administration of an anti-IL-6 antibody, such as any one of Abl-Ab36 antibodies described in Table 4 antibody or a fragment or variant thereof (e.g., Abl).
Another embodiment relates to methods of improving survivability or quality of life of a patient diagnosed with anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer, comprising administering to the patient an anti-IL-6 antibody or antibody fragment or variant thereof, whereby the patient's serum C-reactive protein ("CRP") level is stabilized and preferably reduced, and monitoring the patient to assess the reduction in the patient's serum CRP level. In an embodiment, the patient may have an elevated C-reactive protein (CRP) level prior to treatment. In an embodiment, the patient may have an elevated serum CRP level prior to treatment.

In an embodiment of the invention, the patient's serum CRP level may remain decreased for an entire period intervening two consecutive anti-IL-6 antibody administrations.

In one embodiment, the patient may have been diagnosed anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer.

In one embodiment, the antibody, or antibody fragment thereof, may be expressed from a recombinant cell. In another embodiment, the cell may be selected from a mammalian, yeast, bacterial, and insect cell. In another embodiment, the cell may be a yeast cell. In another embodiment, the cell may be a diploid yeast cell. In another embodiment, the yeast cell may be a Pichia yeast. In another embodiment, the anti-IL-6 antibody may be produced in a yeast based (Pichia pastoris) expression system using conventional fermentation processes and downstream purification. In one embodiment, the antibodies and antibody fragments described herein may be expressed in yeast cells. In one embodiment, the mating competent yeast may a member of the Saccharomycetaceae family, which includes the genera Arxiozyma; Ascobotryozyma; Citeromyces; Debaryomyces; Dekkera; Eremothecium; Issatchenkia; Kazachstaniza; Kluyveromyces; Kodamaea; Lodderomyces; Pachysolen; Pichia; Saccharomyces; Saturnispora; Tetrapisispora; Torulaspora; Williopsis; and Zygosaccharomyces. Other types of yeast potentially useful in the invention include Yarrowia, Rhodospiridium, Candida, Hansenula, Filobasium, Filobasidella, Sporidiobolus, Ballera, Leuconosporidium, and Filobasidella. In a preferred embodiment, the mating competent yeast may a member of the genus Pichia. In a further preferred embodiment, the mating competent yeast of the genus Pichia is one of the following species: Pichia pastoris, Pichia methanolica, and Hansenula polymorpha (Pichia angusta). In a particularly preferred embodiment, the mating competent yeast of the genus Pichia may the species Pichia pastoris.
In one embodiment, a composition for the reduction of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy may comprise an effective amount of a humanized monoclonal antibody that selectively binds IL-6.

In one embodiment, a composition for the treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer in a subject with head and neck cancer receiving concomitant chemotherapy may comprise an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Ab 1.

In one embodiment, a composition comprising a humanized monoclonal antibody or fragment thereof that selectively binds IL-6 for treating anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer induced by chemoradiation (CRT) regimens used for the treatment of cancers of the head and neck.

In one embodiment, a composition for treatment or prevention of anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer may comprise a humanized monoclonal antibody that selectively binds IL-6 and saline solution.

In one embodiment, the anemia, drug-induced immune hemolytic anemia (DIIHA), anemia associated with chemotherapy, anemia associated with radiotherapy, or anemia associated with cancer may be induced by chemoradiation (CRT) regimens or HSCT regimens used for the treatment of cancers of the head and neck.

In one embodiment, a method of treating rheumatoid arthritis by subcutaneously administering a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab 1 or an antibody that competes with Ab 1 for binding to IL-6 to a patient in need thereof.

In one embodiment, the invention provides for the use of anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab 1 or an antibody that competes with Ab 1 for binding to IL-6 for the preparation of a subcutaneously administrable composition for treating rheumatoid arthritis in a patient in need thereof.

In a further embodiment, a composition for treating rheumatoid arthritis may comprise a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same
epitopic specificity as Abl or an antibody that competes with Abl for binding to IL-6 to a patient in need thereof that is formulated for subcutaneous administration.

[0199] In one embodiment, the composition may comprise an anti-IL-6 antibody or antibody fragment contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HC1 to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

[0200] In one embodiment, the composition may comprise an anti-IL-6 antibody or antibody fragment contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HC1 to make final pH 6, 250 to 280 mM sorbitol or sorbitol in combination with sucrose, and 0.015% (w/w) Polysorbate 80, said formulation having a nitrogen headspace in the shipping vials.

[0201] The invention also provides a composition for treating rheumatoid arthritis comprising a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Abl or an antibody that competes with Abl for binding to IL-6 to a patient in need thereof that is formulated for intravenous administration.

[0202] In one embodiment, the composition may comprise an anti-IL-6 antibody or antibody fragment contained in a composition comprising, or alternatively consisting of, anti-IL-6 antibody or antibody fragment, 25 mM Histidine base, Phosphoric acid q.s. to pH 6, and 250 mM sorbitol.

[0203] In one embodiment, the composition may comprise an anti-IL-6 antibody or antibody fragment contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, 12.5 mM Histidine base, 12.5 mM Histidine HC1 (or 25 mM Histidine base and Hydrochloric acid q.s. to pH 6), 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

[0204] In one embodiment, the composition may comprise an anti-IL-6 antibody or antibody fragment contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HC1 to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

[0205] In one embodiment, the composition may comprise a concentration of an anti-IL-6 antibody or antibody fragment is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/mL or at least about 10-100 mg/mL.

[0206] In one embodiment, the composition may comprise at least about 50 or 100 mg of an anti-IL-6 antibody or antibody fragment.

[0207] In one embodiment, the composition may comprise at least about 80 mg, about 160 mg, or about 320 mg of an anti-IL-6 antibody or antibody fragment.
In one embodiment, the effective amount is between about 0.1 and 20 mg/kg of body weight of recipient subject.

In one embodiment, the effective amount is between about 0.1 and 100 mg/kg of body weight of the subject.

In one embodiment, the composition may comprise at least about 25, 80, 100, 160, 200, or 320 mg.

In one embodiment, the composition may be formulated for intravenous administration.

In one embodiment, the composition may comprise an excipient selected from the group consisting of histidine, sorbitol, and polysorbate 80.

In one embodiment, the composition may be administered every 4 weeks. In one embodiment, the composition may be administered 80 mg every 4 weeks for a total of 2 doses. In one embodiment, the composition may be administered 160 mg every 4 weeks for a total of 2 doses. In one embodiment, the composition may be administered 320 mg every 4 weeks for a total of 2 doses.

In one embodiment, the anti-IL-6 antibody may comprise a light chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 709.

In one embodiment, the anti-IL-6 antibody may comprise a light chain polypeptide comprising a polypeptide encoded by a polynucleotide that has at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 723.

In one embodiment, the anti-IL-6 antibody may comprise a heavy chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 657.

In one embodiment, the anti-IL-6 antibody may comprise a heavy chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 700.

In one embodiment, the anti-IL-6 antibody may comprise a light chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 709, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 723, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency
conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723; and a heavy chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 657, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 700, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 700, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 700; wherein the Abl antibody or antibody fragment specifically binds to IL-6 and antagonizes one or more activity associated with IL-6.

[0219] In one embodiment, the anti-IL-6 antibody may comprise anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 90% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

[0220] In one embodiment, the anti-IL-6 antibody may comprise anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 95% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

[0221] In one embodiment, the anti-IL-6 antibody may comprise anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 98% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

[0222] In one embodiment, the anti-IL-6 antibody may comprise anti-IL-6 antibody comprises the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

[0223] In one embodiment, the anti-IL-6 antibody may comprise anti-IL-6 antibody further comprises the constant light chain sequence contained in SEQ ID NO: 586.

[0224] In one embodiment, the anti-IL-6 antibody may comprise the constant heavy chain sequence contained in SEQ ID NO: 588.

[0225] In one embodiment, the composition may further comprise methotrexate.

[0226] In one embodiment, the composition may further comprise at least one anti-inflammatory agent, analgesic agent, or disease-modifying antirheumatic drug (DMARD).

[0227] In one embodiment, the anti-inflammatory agent is selected from the group consisting of steroids, Cortisone, Glucocorticoids, prednisone, prednisolone, Hydrocortisone (Cortisol), Cortisone acetate, Methylprednisolone, Dexamethasone, Betamethasone, Triamcinolone, Beclometasone, and Fludrocortisone acetate, non-steroidal anti-inflammatory drug (NSAIDs), ibuprofen, naproxen, meloxicam, etodolac, nabumetone, sulindac, tolementin, choline magnesium
salicylate, diclofenac, diflusinal, indomethicin, Ketoprofen, Oxaprozin, piroxicam, and nimesulide, Salicylates, Aspirin (acetylsalicylic acid), Diflunisal, Salsalate, p-amino phenol derivatives, Paracetamol, phenacetin, Propionic acid derivatives, Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Acetic acid derivatives, Indomethacin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone, Enolic acid (Oxicam) derivatives, Piroxicam, Meloxicam, Tenoxicam, Droxicalm, Lornoxicam, ISOxicam, Fenamic acid derivatives (Fenamates), Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Selective COX-2 inhibitors (Coxibs), Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, Sulphonanilides, Nimesulide, and Licofelone.

[0228] In one embodiment, the analgesic agent is selected from the group consisting of NSAIDs, COX-2 inhibitors, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, acetaminophen, opiates, Dextropropoxyphene, Codeine, Tramadol, Anileridine, Pethidine, Hydrocodone, Morphine, Oxycodone, Methadone, Dicetyl morphine, Hydromorphone, Oxymorphone, Levorphanol, Buprenorphine, Fentanyl, Sufentanyl, Etorphine, Carfentanil, dihydromorphine, dihydrocodeine, Thebaine, Papaverine, Diproqualone, Flupirtine, Tricyclic antidepressants, and lidocaine.

[0229] In one embodiment, the DMARD may be selected from the group consisting of mycophenolate mofetil (CellCept), calcineurin inhibitors, cyclosporine, sirolimus, everolimus, oral retinoids, azathioprine, fumaric acid esters, D-penicillamine, cyclophosphamide, immunoadsorption column, Pro sorba(r) column, a gold salt, auranofin, sodium aurothiomalate (Myocrisin), hydroxychloroquine, chloroquine, leflunomide, methotrexate (MTX), minocycline, sulfasalazine (SSZ), tumor necrosis factor alpha (TNFa) blockers, etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi)), Interleukin 1 (IL-1) blockers, e.g., anakinra (Kineret), monoclonal antibodies against B cells, rituximab (Rituxan)), T cell costimulation blockers, abatacept (Orencia), Interleukin 6 (IL-6) blockers, tocilizumab, RoActemra, and Actemra.

[0230] In one embodiment, the DMARD is not an antibody.

[0231] In one embodiment, the administration of a composition described herein to a patient in need thereof results in an improvement in at least one of the following: (i) improved DAS-28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy is measured relative to said patient's baseline prior to administration of said antibody or antibody fragment, relative
untreated patients, relative to patients receiving a placebo or control formulation, or relative to age/gender norms.

[0232] In one embodiment, the administration of a composition described herein to a patient in need thereof results in a prolonged improvement in disease (observed at least 4, 6, 8, 10, 12, 14 or 16 weeks after antibody administration) as manifested by at least one of the following: (i) improved DAS-28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy is measured relative to said patient's baseline prior to administration of said antibody or antibody fragment, relative untreated patients, relative to patients receiving a placebo or control formulation, or relative to age/gender norms.

[0233] In a further embodiment, the improvement in SF-6D score is at least equal to the Minimum Important Difference (MID) relative to the patient's SF-6D prior to said administration.

[0234] In a further embodiment, the improvement in SF-6D score is at least twice the MID relative to the patient's SF-6D prior to said administration. In a further embodiment, the improvement in SF-6D score is at least three times the MID relative to the patient's SF-6D prior to said administration. In another embodiment, the improvement in SF-36 may comprise an improvement in the physical functioning domain score, said improvement being at least equal to the minimum clinically important difference (MCID), at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score. In another embodiment, the improvement in SF-36 may comprise an improvement in the role physical domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

[0235] In another embodiment, the improvement in SF-36 may comprise an improvement in the bodily pain domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score. In another embodiment, the improvement in SF-36 may comprise an improvement in the general health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score. In another embodiment, the improvement in SF-36 may comprise an improvement in the role emotional domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at
least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

[0236] In another embodiment, the improvement in SF-36 may comprise an improvement in the vitality domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

[0237] In another embodiment, the improvement in SF-36 may comprise an improvement in the social functioning domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

[0238] In another embodiment, the improvement in SF-36 may comprise an improvement in the mental health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

[0239] In one embodiment, a method for treating rheumatoid arthritis may comprise administering a composition comprising at least about 10 mg/mL of an anti-IL-6 antibody having the epitopic specificity of Ab 1 to a patient in need thereof.

[0240] The invention also provides for the use of an anti-IL-6 antibody having the epitopic specificity of Ab 1 or any of the other anti-IL-6 antibodies disclosed herein for preparing a pharmaceutical composition for treating rheumatoid arthritis comprising at least about 10 mg/mL of an anti-IL-6 antibody having the epitopic specificity of Ab 1 to a patient in need thereof.

[0241] The invention also provides for a composition for treating rheumatoid arthritis comprising at least about 10 mg/mL of an anti-IL-6 antibody to a patient in need thereof. In one embodiment, the composition may comprise at least about 20, 30, 40, 50, 60, 70, 80, or 100 mg/mL of an anti-IL-6 antibody. In one embodiment, the composition may comprise at least about 10-100 mg/mL of an anti-IL-6 antibody. In one embodiment, the composition may be formulated for subcutaneous administration and comprises at least about 100 mg/mL of an anti-IL-6 antibody. In one embodiment, the composition may be formulated for intravenous administration and comprises at least about 10, 20, 30, or 40 mg/mL, or 10-40 mg/mL of an anti-IL-6 antibody.

[0242] In one embodiment, the anemia is severe anemia. In another embodiment, the patient treated has at least one symptom of anemia, optionally wherein the patient exhibits: hematocrit levels below about 42-52% for men or about 36-48% for women; serum ferritin levels below about 30-400 ng/mL for men or about 13-150 ng/mL for women; serum iron levels below about
60-170 µg/dL; reticulocyte count below about 0.5%—1.5%; white blood cell (WBC) count of below about 5,000-10,000/mL; red blood cell (RBC) count of below about 4.5-5.5x10^6/mL for men and below about 4.0-5.0x10^6/mL for women; platelet count below about 1.4-4.0 x10^5/mL; or total iron binding capacity (TIBC) below about 250-370 µg/dL. In another embodiment, the patient treated has at least one symptom of anemia, optionally wherein the patient exhibits fatigue, lack of energy, dizziness, headaches, diminished sex drive, rapid heartbeat, inability to concentrate, paleness, or shortness of breath. In another embodiment, the patient patient has or is to receive autologous stem cell or bone marrow transplant.

**BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS**

[0243] FIGURE 1 depicts alignments of variable light and variable heavy sequences between a rabbit antibody variable light and variable heavy sequences and homologous human sequences and the humanized sequences. Framework regions are identified FR1-FR4. Complementarity determining regions are identified as CDR1-CDR3. Amino acid residues are numbered as shown. The initial rabbit sequences are called RbtVL and RbtVH for the variable light and variable heavy sequences respectively. Three of the most similar human germline antibody sequences, spanning from Framework 1 through to the end of Framework 3, are aligned below the rabbit sequences. The human sequence that is considered the most similar to the rabbit sequence is shown first. In this example those most similar sequences are L12A for the light chain and 3-64-04 for the heavy chain. Human CDR3 sequences are not shown. The closest human Framework 4 sequence is aligned below the rabbit Framework 4 sequence. The vertical dashes indicate a residue where the rabbit residue is identical with at least one of the human residues at the same position. The bold residues indicate that the human residue at that position is identical to the rabbit residue at the same position. The final humanized sequences are called VLh and VHh for the variable light and variable heavy sequences respectively. The underlined residues indicate that the residue is the same as the rabbit residue at that position but different than the human residues at that position in the three aligned human sequences.

[0244] FIGURES 2 and 3 depicts alignments between a rabbit antibody light and variable heavy sequences and homologous human sequences and the humanized sequences. Framework regions are identified as FR1-FR4. Complementarity determining regions are identified as CDR1-CDR3.

[0245] FIGURES 4A-B and 5A-B depicts alignments between light and variable heavy sequences, respectively, of different forms of Abl. Framework regions are identified as FR1-FR4. Complementarity determining regions are identified as CDR1-CDR3. Sequence differences within the CDR regions highlighted.
FIGURE 6 provides the α-2-macroglobulin (A2M) dose response curve for antibody Abl administered intravenously at different doses one hour after a 100 μg/kg s.c. dose of human IL-6. See also WO 2011/066371.

FIGURE 7 provides survival data for the antibody Ab1 progression groups versus control groups. See also WO 201 1/066371.

FIGURE 8 provides additional survival data for the antibody Abl regression groups versus control groups. See also WO 201 1/066371.

FIGURE 9 provides survival data for polyclonal human IgG at 10 mg/kg i.v. every three days (270-320 mg tumor size) versus antibody Abl at 10 mg/kg i.v. every three days (270-320 mg tumor size). See also WO 201 1/066371.

FIGURE 10 provides survival data for polyclonal human IgG at 10 mg/kg i.v. every three days (400-527 mg tumor size) versus antibody Ab1 at 10 mg/kg i.v. every three days (400-527 mg tumor size). See also WO 201 1/066371.

FIGURE 11 shows increased hemoglobin concentration following administration of Abl to patients with advanced cancer. See also WO 201 1/066371.

FIGURE 12 depicts mean plasma lipid concentrations following administration of Abl to patients with advanced cancer. See also WO 201 1/066371.

FIGURE 13 depicts mean neutrophil counts following administration of Abl to patients with advanced cancer. See also WO 201 1/066371.

FIGURE 14A demonstrates suppression of serum CRP levels in healthy individuals.

FIGURE 14B demonstrates suppression of serum CRP levels in advanced cancer patients.

FIGURE 15A depicts the mean CRP values for each dosage concentrations (placebo, 80 mg, 160 mg, and 320 mg) of the Abl monoclonal antibody in NSCLC patients.

FIGURE 15B depicts the change in median values of CRP from each dosage concentration group corresponding to FIGURE 15A in NSCLC patients.

FIGURE 16 depicts the mean plasma CRP concentration in patients with advanced cancer after a single I.V. infusion of 80, 160, or 320 mg of Abl (ALD518) (n=8).

FIGURE 17 depicts the mean serum CRP levels in patients with rheumatoid arthritis patients with an inadequate response to methotrexate after dosing at 80, 160, or 320 mg of Abl (ALD518).

FIGURE 18A depicts that Abl increases mean hemoglobin concentration (g/dL) at 80, 160 and 320 mg after 12 weeks of dosing in NSCLC patients versus placebo. See also WO 201 1/066371.
[0261] FIGURE 18B depicts the mean change from baseline in hemoglobin concentration (g/dL) for NSCLC patients versus placebo. See also WO 201 1/066371.

[0262] FIGURE 18C depicts the mean hemoglobin concentration (g/dL) in NSCLC patients with a baseline hemoglobin below 11 g/L at baseline versus time with Abl compared to placebo.

[0263] FIGURE 19 depicts the mean change from baseline in hemoglobin concentration (g/dL) for rheumatoid arthritis patients with an inadequate response to methotrexate versus placebo. The normal range of hemoglobin concentration is approximately 11.5-15.5 g/dL. See also WO 201 1/066371.

[0264] FIGURE 20A depicts that Abl increases mean albumin concentration at 80, 160 and 320 mg in NSCLC patients. See also WO 201 1/066371.

[0265] FIGURE 20B depicts the change from baseline for mean albumin concentration from each dosage concentration group corresponding to Figure 20A in NSCLC patients. See also WO 201 1/066371.

[0266] FIGURE 20C depicts the mean albumin concentration in NSCLC patients with a baseline albumin ≤ 35 g/l at baseline versus time for Abl versus placebo. See also WO 201 1/066371.

[0267] FIGURE 21A depicts the mean plasma CRP levels concentration after subcutaneous or intravenous dosing of humanized Ab l.

[0268] FIGURE 21B depicts the mean plasma CRP levels concentration after subcutaneous or intravenous dosing of humanized Ab l at dosing of 50 mg or 100 mg through 12 weeks.

[0269] FIGURE 22 depicts percentage of mice ulcerated at any timepoint after single dose radiation.

[0270] FIGURE 23 depicts median tumor volume over time.

[0271] FIGURE 24 depicts the percentage of mice with no ulcerations versus ulcerations on Day 10.

[0272] FIGURE 25 depicts median number of days ulcerated after single dose of radiation.

[0273] FIGURE 26 depicts patient disposition in a Phase II clinical trial for administration of ALD518 to patients with active rheumatoid arthritis (RA). An asterisk indicates that one patient did not receive treatment as randomized (the patient was randomized to receive 160 mg ALD518, but received 320 mg on Day 1 and 160 mg ALD518 at Week 8; AE=adverse event.

[0274] FIGURE 27 graphically illustrates the mean changes in SF-36 composite scores at Week 12 in a Phase II clinical trial for administration of ALD518 to patients with active RA. Data are mean and error bars represent 95% confidence intervals (for each group, the left bar shows the PCS score and the right bar shows the MCS score). Mean changes in PCS and MCS
scores at Week 12 exceeded the MCID in all ALD-5 18 treatment groups. Greater improvements in MCS score in favor of all ALD-5 18 treatment groups were demonstrated at Week 12 (p<0.05). MCS scores changes also exceeded the PCS scores in all ALD-5 18 treatment groups. SF-36=Short Form Health Survey-36; PCS=physical component score; MCS=mental component score; MCID=minimum clinically important difference.

[0275] FIGURE 28A-D presents spydergrams summarizing the changes from baseline to week 12 in SF-36 domain scores compared with age/gender matched norms for a Phase II clinical trial for administration of ALD5 18 to patients with active RA. The spydergrams summarize age/gender norms, average baseline scores prior to treatment, and average scores after treatment in each of eight tested domains for patients receiving 80 mg (panel A), 160 mg (panel B), or 320 mg (panel C) ALD-5 18, or placebo (panel D). PF=physical function; RP=role physical; BP=bodily pain; GH=general health; VT=vitality; SF=social functioning; RE=role emotional; MH=mental health; SF-36=Short Form-36.

[0276] FIGURE 29A-B presents spydergrams summarizing the changes from baseline to weeks 12 (A) and 16 (B) in SF-36 domain scores compared with age/gender matched norms for a Phase II clinical trial for administration of ALD5 18 to patients with active RA. The spydergrams summarize scores in eight tested domains for age/gender norms, combined average baseline scores prior to treatment, and average scores after treatment for each treatment group (ALD-5 18 dosages of 80 mg, 160 mg, or 320 mg), and the placebo group. Abbreviations are as in FIG. 28.

[0277] FIGURE 30 depicts WHO oral mucositis grade versus cumulative IMRT (Gy): ALD5 18 160 mg intravenous at week 0 and week 4 for three patients.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

[0278] It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0279] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.
Amplification as used herein, refers broadly to the amplification of polynucleotide sequences is the in vitro production of multiple copies of a particular nucleic acid sequence. The amplified sequence is usually in the form of DNA. A variety of techniques for carrying out such amplification are known in the art. See, e.g., Van Brunt (1990) Bio/Technol. 8(4): 291-294. Polymerase chain reaction or PCR is a prototype of nucleic acid amplification, and use of PCR herein should be considered exemplary of other suitable amplification techniques.

Antibody, as used herein, refers broadly to any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where at least one non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, from all sources, e.g., human, rodent, rabbit, cow, sheep, pig, dog, chicken, are considered to be "antibodies." Antibodies include but are not limited to chimeric antibodies, human antibodies and other non-human mammalian antibodies, humanized antibodies, single chain antibodies (scFvs), camelbodies, nanobodies, IgNAR (single-chain antibodies derived from sharks), small-modular immunopharmaceuticals (SMIPs), and antibody fragments (e.g., Fabs, Fab', F(ab')₂). Numerous antibody coding sequences have been described; and others may be raised by methods well-known in the art. See Streltsov, et al. (2005) Protein Sci. 14(11): 2901-9; Greenberg, et al. (1995) Nature 374(6518): 168-173; Nuttall, et al. (2001) Mol Immunol. 38(4): 313-26; Hamers-Casterman, et al. (1993) Nature 363(6428): 446-8; Gill, et al. (2006) Curr Opin Biotechnol. 17(6): 653-8.

Antigen-binding fragment, as used herein, refers broadly to a fragment of an antibody which recognizes an antigen (e.g., paratopes, antigen-binding fragment.) The antigen-binding fragment may comprise a paratope that may be a small region (e.g., 15-22 amino acids) of the antibody's Fv region and may contain parts of the antibody's heavy and light chains. See Goldsby, et al. Antigens (Chapter 3) Immunology (5th Ed.) New York: W.H. Freeman and Company, pages 57-75.

C-Reactive Protein (CRP), as used herein, refers broadly to a 224 amino acid protein found in the blood that rise in response to inflammation [e.g., GenBank Protein Accession No. NP_000558 (SEQ ID NO: 726)]. CRP also encompasses any pre-pro, pro- and mature forms of this CRP amino acid sequence, as well as mutants and variants including allelic variants of this sequence. CRP levels, e.g. in the serum, liver, or elsewhere in the body, can be readily measured using routine methods and commercially available reagents, e.g. ELISA, antibody test strip, immunoturbidimetry, rapid immunodiffusion, visual agglutination, Western blot, Northern blot
As mentioned above CRP levels may in addition be measured in patients having or at risk of developing thrombosis according to the invention.

[0284] Coding sequence, as used herein refers broadly to an in-frame sequence of codons that (in view of the genetic code) correspond to or encode a protein or peptide sequence. Two coding sequences correspond to each other if the sequences or their complementary sequences encode the same amino acid sequences. A coding sequence in association with appropriate regulatory sequences may be transcribed and translated into a polypeptide. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. Promoter sequences typically contain additional sites for binding of regulatory molecules (e.g., transcription factors) which affect the transcription of the coding sequence. A coding sequence is "under the control" of the promoter sequence or "operatively linked" to the promoter when RNA polymerase binds the promoter sequence in a cell and transcribes the coding sequence into mRNA, which is then in turn translated into the protein encoded by the coding sequence. A polynucleotide sequence "corresponds" to a polypeptide sequence if translation of the polynucleotide sequence in accordance with the genetic code yields the polypeptide sequence (i.e., the polynucleotide sequence "encodes" the polypeptide sequence), one polynucleotide sequence "corresponds" to another polynucleotide sequence if the two sequences encode the same polypeptide sequence.

[0285] Complementarity determining region, hypervariable region, or CDR, as used herein refer broadly to at least one of the hyper-variable or complementarity determining regions (CDRs) found in the variable regions of light or heavy chains of an antibody (See Kabat, E. A. et al. (1987) Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md.). These expressions include the hypervariable regions as defined by Kabat et al. ("Sequences of Proteins of Immunological Interest," Kabat E., et al. (1983) US Dept. of Health and Human Services) or the hypervariable loops in 3-dimensional structures of antibodies. Chothia and Lesk (1987) J Mol. Biol. 196: 901-917. The CDRs in each chain are held in close proximity by framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site. Within the CDRs there are select amino acids that have been described as the selectivity determining regions (SDRs) which represent the critical contact residues used by the CDR in the antibody-antigen interaction (Kashmiri (2005) Methods 36:25-34). CDRs for exemplary anti-IL-6 antibodies are provided herein.

[0286] Disease or condition, as used herein, refers broadly to a disease or condition that a patient has been diagnosed with or is suspected of having, particularly a disease or condition
associated with elevated IL-6. A disease or condition encompasses, without limitation thereto, anemia, as well as idiopathic conditions characterized by symptoms that include elevated IL-6.

[0287] Effective amount, as used herein, refers broadly to an amount of an active ingredient that is effective to relieve or reduce to some extent at least one of the symptoms of the disease in need of treatment, or to retard initiation of clinical markers or symptoms of a disease in need of prevention, when the compound is administered. Thus, an effective amount refers to an amount of the active ingredient which exhibit effects such as (i) reversing the rate of progress of a disease; (ii) inhibiting to some extent further progress of the disease; and/or, (iii) relieving to some extent (or, preferably, eliminating) at least one symptoms associated with the disease. The effective amount may be empirically determined by experimenting with the compounds concerned in known in vivo and in vitro model systems for a disease in need of treatment. The context in which the phrase "effective amount" is used may indicate a particular desired effect. For example, "an amount of an anti-IL-6 antibody effective to prevent or treat a hypercoagulable state" and similar phrases refer to an amount of anti-IL-6 antibody that, when administered to a subject, will cause a measurable improvement in the subject's coagulation profile, or prevent, slow, delay, or arrest, a worsening of the coagulation profile for which the subject is at risk. Similarly, "an amount of an anti-IL-6 antibody effective to reduce serum CRP levels" and similar phrases refer to an amount of anti-IL-6 antibody that, when administered to a subject, will cause a measurable decrease in serum CRP levels, or prevent, slow, delay, or arrest, an increase in serum CRP levels for which the subject is at risk. Similarly, "an amount of an anti-IL-6 antibody effective to increase serum albumin levels" and similar phrases refer to an amount of anti-IL-6 antibody that, when administered to a subject, will cause a measurable increase in serum albumin levels, or prevent, slow, delay, or arrest, a decrease in serum albumin levels for which the subject is at risk. Similarly, "an amount of an anti-IL-6 antibody effective to reduce weakness" and similar phrases refer to an amount of anti-IL-6 antibody that, when administered to a subject, will cause a measurable decrease in weakness as determined by the hand grip strength test. Similarly, "an amount of an anti-IL-6 antibody effective to increase weight" and similar phrases refer to an amount of anti-IL-6 antibody that, when administered to a subject, will cause a measurable increase in a patient's weight. An effective amount will vary according to the weight, sex, age and medical history of the individual, as well as the severity of the patient's condition(s), the type of disease(s), mode of administration, and the like. An effective amount may be readily determined using routine experimentation, e.g., by titration (administration of increasing dosages until an effective dosage is found) and/or by reference to amounts that were effective for prior
patients. Generally, the anti-IL-6 antibodies of the present invention will be administered in dosages ranging between about 0.1 mg/kg and about 20 mg/kg of the patient's body-weight.

**Expression Vector**, as used herein, refers broadly to a DNA vectors contain elements that facilitate manipulation for the expression of a foreign protein within the target host cell. Conveniently, manipulation of sequences and production of DNA for transformation is first performed in a bacterial host (e.g., *E. coli*) and usually vectors will include sequences to facilitate such manipulations, including a bacterial origin of replication and appropriate bacterial selection marker. Selection markers encode proteins necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media. Exemplary vectors and methods for transformation of yeast are described, for example, in Burke, Dawson, & Stearns (2000) *Methods in Yeast Genetics: a Cold Spring Harbor Laboratory course manual*, Cold Spring Harbor Laboratory Press.

**Folding**, as used herein, refers broadly to the three-dimensional structure of polypeptides and proteins, where interactions between amino acid residues act to stabilize the structure. While non-covalent interactions are important in determining structure, usually the proteins of interest will have intra- and/or intermolecular covalent disulfide bonds formed by two cysteine residues. For naturally occurring proteins and polypeptides or derivatives and variants thereof, the proper folding is typically the arrangement that results in optimal biological activity, and can conveniently be monitored by assays for activity, e.g. ligand binding, enzymatic activity.

**Framework region or FR**, as used herein refers broadly to at least one of the framework regions within the variable regions of the light and heavy chains of an antibody. See Kabat, *et al.* (1987) *Sequences of Proteins of Immunological Interest*, National Institutes of Health, Bethesda, MD. These expressions include those amino acid sequence regions interposed between the CDRs within the variable regions of the light and heavy chains of an antibody. As mentioned in the preferred embodiments, the FRs may comprise human FRs highly homologous to the parent antibody (e.g., rabbit antibody).

**Glasgow Prognostic Score (GPS)**, as used herein, refers broadly to an inflammation-based prognostic score that awards one point for a serum albumin level less than < 35 mg/L and one point for a CRP level above 10 mg/L. Thus, a GPS of 0 indicates normal albumin and CRP, a GPS of 1 indicates reduced albumin or elevated CRP, and a GPS of 2 indicates both reduced albumin and elevated CRP.
[0292]  *gpl30* (also called Interleukin-6 receptor subunit beta), as used herein, refers broadly to a transmembrane protein that forms one subunit of type I cytokine receptors in the IL-6 receptor family [(e.g., 918 precursor amino acid sequence available as Swiss-Prot Protein Accession No. P40189 (SEQ ID NO: 728)].  *gpl30* also encompasses any pre-pro, pro- and mature forms of this amino acid sequence, such as the mature form encoded by amino acids 23 through 918 of the sequence shown, as well as mutants and variants including allelic variants of this sequence.

[0293]  *Heterologous* region or domain of a DNA construct, as used herein, refers broadly to an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous region is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[0294]  *Homology*, as used herein, refers broadly to a degree of similarity between a nucleic acid sequence and a reference nucleic acid sequence or between a polypeptide sequence and a reference polypeptide sequence. Homology may be partial or complete. Complete homology indicates that the nucleic acid or amino acid sequences are identical. A partially homologous nucleic acid or amino acid sequence is one that is not identical to the reference nucleic acid or amino acid sequence. The degree of homology can be determined by sequence comparison. The term "sequence identity" may be used interchangeably with "homology."

[0295]  *Host cell*, as used herein, refers broadly to a cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, insect (e.g., SF9), amphibian, or mammalian cells such as CHO, HeLa, HEK-293 (e.g., cultured cells, explants, and cells *in vivo*)

[0296]  *Isolated*, as used herein, refers broadly to material removed from its original environment in which it naturally occurs, and thus is altered by the hand of man from its natural environment. Isolated material may be, for example, exogenous nucleic acid included in a vector system, exogenous nucleic acid contained within a host cell, or any material which has been removed from its original environment and thus altered by the hand of man (e.g., "isolated antibody").

[0297]  *Improved*, as used herein, refers broadly to any beneficial change resulting from a treatment. A beneficial change is any way in which a patient's condition is better than it would
have been in the absence of the treatment. "Improved" includes prevention of an undesired condition, slowing the rate at which a condition worsens, delaying the development of an undesired condition, and restoration to an essentially normal condition. For example, improvement in anemia encompasses any increase in hemocrit, hemoglobin, or reduction in fatigue.

[0298] **IL-6 antagonist**, as used herein, refers broadly to any composition that prevents, inhibits, or lessens the effect(s) of IL-6 signaling. Generally, such antagonists may reduce the levels or activity of IL-6, IL-6 receptor alpha, gp130, or a molecule involved in IL-6 signal transduction, or may reduce the levels or activity complexes between the foregoing (e.g., reducing the activity of an IL-6 / IL-6 receptor complex). Antagonists include antisense nucleic acids, including DNA, RNA, or a nucleic acid analogue such as a peptide nucleic acid, locked nucleic acid, morpholino (phosphorodiamidate morpholino oligo), glycerol nucleic acid, or threose nucleic acid. See Heasman (2002) Dev Biol. 243(2): 209-14; Hannon and Rossi (2004) Nature 431(7006):371-8; Paul, et al. (2002) Nat Biotechnol. 20(5):505-8; Zhang, et al. (2005) J Am Chem Soc. 127(12):4174-5; Wahlestedt, et al. (2000) Proc Natl Acad Sci USA. 97(10):5633-8; Hanvey, et al. (1992) Science 258 (5087): 1481-5; Braasch, et al. (2002) Biochemistry 41(14): 4503-10; Schoning, et al. (2000) Science 290(5495): 1347-51. In addition IL-6 antagonists specifically include peptides that block IL-6 signaling such as those described in any of U.S. Patent Nos. 5,210,075; 6,172,042; 6,599,875; 6,841,533; and 6,838,433. Also, IL-6 antagonists according to the invention may include p38 MAP kinase inhibitors such as those reported in U.S. Patent Application No. 2007/0010529 given this kinase's role in cytokine production and more particularly IL-6 production. Further, IL-6 antagonists according to the invention include the glycoalkaloid compounds reported in U.S. Patent Application Publication No. 2005/0090453 as well as other IL-6 antagonist compounds isolatable using the IL-6 antagonist screening assays reported therein. Other IL-6 antagonists include antibodies, such as anti-IL-6 antibodies, anti-IL-6 receptor alpha antibodies, anti-gp130 antibodies, and anti-p38 MAP kinase antibodies including (but not limited to) the anti-IL-6 antibodies disclosed herein, Actemra® (Tocilizumab), Remicade®, Zenapax® (daclizumab), or any combination thereof.

Other IL-6 antagonists include portions or fragments of molecules involved in IL-6 signaling, such as IL-6, IL-6 receptor alpha, and gp130, which may be native, mutant, or variant sequence, and may optionally be coupled to other moieties (such as half-life-increasing moieties, e.g. an Fc domain). For example, an IL-6 antagonist may be a soluble IL-6 receptor or fragment, a soluble IL-6 receptor:Fc fusion protein, a small molecule inhibitor of IL-6, an anti-IL-6 receptor antibody or antibody fragment or variant thereof, antisense nucleic acid. Other IL-6 antagonists include
avemirs, such as C326 (Silverman, et al. (2005) Nat Biotechnol. 23(12): 1556-61) and small molecules, such as synthetic retinoid AM80 (tamibarotene) (Takeda, et al. (2006) Arterioscler Thromb Vase Biol. 26(5): 1177-83). Such IL-6 antagonists may be administered by any means known in the art, including contacting a subject with nucleic acids which encode or cause to be expressed any of the foregoing polypeptides or antisense sequences.

[0299] **Interleukin-6 (IL-6),** as used herein, refers broadly to interleukin-6 (IL-6) encompasses not only the following 212 amino acid sequence available as GenBank Protein Accession No. NP_000591 (*e.g.*, SEQ ID NO: 1), but also any pre-pro, pro- and mature forms of this IL-6 amino acid sequence, as well as mutants and variants including allelic variants of this sequence.

[0300] **Interleukin-6 receptor (IL-6R) (IL-6 receptor alpha (IL-6RA) [CD126],** as used herein, refers broadly to 468 amino acid protein that binds IL-6, a potent pleiotropic cytokine that regulates cell growth and differentiation and also plays an important role in immune response (*e.g.*, Swiss-Prot Protein Accession No. P08887 and SEQ ID NO: 727). IL-6R also includes any pre-pro, pro- and mature forms of this amino acid sequence, as well as mutants and variants including allelic variants of this sequence.

[0301] **Mammal,** as used herein, refers broadly to any and all warm-blooded vertebrate animals of the class Mammalia, including humans, characterized by a covering of hair on the skin and, in the female, milk-producing mammary glands for nourishing the young. Examples of mammals include but are not limited to alpacas, armadillos, capybaras, cats, camels, chimpanzees, chinchillas, cattle, dogs, goats, gorillas, hamsters, horses, humans, lemurs, llamas, mice, non-human primates, pigs, rats, sheep, shrews, squirrels, and tapirs. Mammals include but are not limited to bovine, canine, equine, feline, murine, ovine, porcine, primate, and rodent species. Mammal also includes any and all those listed on the Mammal Species of the World maintained by the National Museum of Natural History, Smithsonian Institution in Washington DC.

[0302] **Meiosis,** as used herein, refers broadly to a process by which a diploid yeast cell undergoes reductive division to form four haploid spore products. Each spore may then germinate and form a haploid vegetatively growing cell line.

[0303] **Nucleic acid or nucleic acid sequence,** as used herein, refers broadly to a deoxyribonucleotide or ribonucleotide oligonucleotide in either single- or double-stranded form. The term encompasses nucleic acids, *i.e.*, oligonucleotides, containing known analogs of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and
complementary sequences, as well as the sequence explicitly indicated. The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

[0304] Operatively linked, as used herein, refers broadly to when two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0305] Paratope, as used herein, refers broadly to the part of an antibody which recognizes an antigen (e.g., the antigen-binding site of an antibody.) Paratopes may be a small region (e.g., 15-22 amino acids) of the antibody's Fv region and may contain parts of the antibody's heavy and light chains. See Goldsby, et al. Antigens (Chapter 3) Immunology (5th Ed.) New York: W.H. Freeman and Company, pages 57-75.

[0306] Patient, as used herein, refers broadly to any animal who is in need of treatment either to alleviate a disease state or to prevent the occurrence or reoccurrence of a disease state. Also, "Patient" as used herein, refers broadly to any animal who has risk factors, a history of disease, susceptibility, symptoms, signs, was previously diagnosed, is at risk for, or is a member of a patient population for a disease. The patient may be a clinical patient such as a human or a veterinary patient such as a companion, domesticated, livestock, exotic, or zoo animal. The term "subject" may be used interchangeably with the term "patient".

[0307] Polyploid yeast that stably expresses or expresses a desired secreted heterologous polypeptide for prolonged time, as used herein, refers broadly to a yeast culture that secretes said polypeptide for at least several days to a week, more preferably at least a month, still more preferably at least about 1-6 months, and even more preferably for more than a year at threshold expression levels, typically at least about 10-25 mg/liter and preferably substantially greater.

[0308] Polyploidal yeast culture that secretes desired amounts of recombinant polypeptide, as used herein, refers broadly to cultures that stably or for prolonged periods secrete at least about 10-25 mg/liter of heterologous polypeptide, more preferably at least about 50-500 mg/liter, and most preferably at least about 500-1000 mg/liter or more.

[0309] Prolonged reduction in serum CRP, and similar phrases, as used herein refer broadly to a measurable decrease in serum CRP level relative to the initial serum CRP level (i.e. the serum CRP level at a time before treatment begins) that is detectable within about a week from when a treatment begins (e.g. administration of an anti-IL-6 antibody) and remains below the initial serum CRP level for an prolonged duration, e.g. at least about 14 days, at least about 21 days, at least about 28 days, at least about 35 days, at least about 40 days, at least about 50 days, at least about 60 days, at least about 70 days, at least about 11 weeks, or at least about 12 weeks from when the treatment begins.
[0310] **Promoter**, as used herein, refers broadly to an array of nucleic acid sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. An "inducible" promoter is a promoter that is active under environmental or developmental regulation.

[0311] **Prophylactically effective amount**, as used herein, refers broadly to the amount of a compound that, when administered to a patient for prophylaxis of a disease or prevention of the reoccurrence of a disease, is sufficient to effect such prophylaxis for the disease or reoccurrence. The prophylactically effective amount may be an amount effective to prevent the incidence of signs and/or symptoms. The "prophylactically effective amount" may vary depending on the disease and its severity and the age, weight, medical history, predisposition to conditions, preexisting conditions, of the patient to be treated.

[0312] **Prophylaxis**, as used herein, refers broadly to a course of therapy where signs and/or symptoms are not present in the patient, are in remission, or were previously present in a patient. Prophylaxis includes preventing disease occurring subsequent to treatment of a disease in a patient. Further, prevention includes treating patients who may potentially develop the disease, especially patients who are susceptible to the disease (e.g., members of a patent population, those with risk factors, or at risk for developing the disease).

[0313] **Recombinant** as used herein, refers broadly with reference to a product, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0314] **Selectable Marker**, as used herein, refers broadly to a selectable marker is a gene or gene fragment that confers a growth phenotype (physical growth characteristic) on a cell receiving that gene as, for example through a transformation event. The selectable marker allows that cell to survive and grow in a selective growth medium under conditions in which cells that do not receive that selectable marker gene cannot grow. Selectable marker genes generally fall into several types, including positive selectable marker genes such as a gene that confers on a cell
resistance to an antibiotic or other drug, temperature when two ts mutants are crossed or a ts mutant is transformed; negative selectable marker genes such as a biosynthetic gene that confers on a cell the ability to grow in a medium without a specific nutrient needed by all cells that do not have that biosynthetic gene, or a mutagenized biosynthetic gene that confers on a cell inability to grow by cells that do not have the wild type gene; and the like. Suitable markers include but are not limited to ZEOMYCIN® (zeocin), neomycin, G418, LYS3, MET1, MET3a, ADE1, ADE3, and URA3.

Specifically (or selectively) binds to an antibody or "specifically (or selectively) immunoreactive with," or "specifically interacts or binds," as used herein, refers broadly to a protein or peptide (or other epitope), refers, in some embodiments, to a binding reaction that is deterministic of the presence of the protein in a heterogeneous population of proteins and other biologies. For example, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times greater than the background (non-specific signal) and do not substantially bind in a significant amount to other proteins present in the sample. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than about 10 to 100 times background.

Signs of disease, as used herein, refers broadly to any abnormality indicative of disease, discoverable on examination of the patient; an objective indication of disease, in contrast to a symptom, which is a subjective indication of disease.

Solid support, support, and substrate, as used herein, refers broadly to any material that provides a solid or semi-solid structure with which another material can be attached including but not limited to smooth supports (e.g., metal, glass, plastic, silicon, and ceramic surfaces) as well as textured and porous materials.

Subjects as used herein, refers broadly to anyone suitable to be treated according to the present invention include, but are not limited to, avian and mammalian subjects, and are preferably mammalian. Mammals of the present invention include, but are not limited to, canines, felines, bovines, caprines, equines, ovines, porcines, rodents (e.g., rats and mice), lagomorphs, primates, humans. Any mammalian subject in need of being treated according to the present invention is suitable. Human subjects of both genders and at any stage of development (i.e., neonate, infant, juvenile, adolescent, adult) can be treated according to the present invention. The present invention may also be carried out on animal subjects, particularly mammalian subjects such as mice, rats, dogs, cats, cattle, goats, sheep, and horses for veterinary purposes, and for drug screening and drug development purposes. "Subjects" is used interchangeably with "patients."
Mating competent yeast species, as used herein refers broadly encompass any diploid or tetraploid yeast which can be grown in culture. Such species of yeast may exist in a haploid, diploid, or tetraploid form. The cells of a given ploidy may, under appropriate conditions, proliferate for indefinite number of generations in that form. Diploid cells can also sporulate to form haploid cells. Sequential mating can result in tetraploid strains through further mating or fusion of diploid strains. In the present invention the diploid or polyploidal yeast cells are preferably produced by mating or spheroplast fusion.

Haploid Yeast Cell, as used herein, refers broadly to a cell having a single copy of each gene of its normal genomic (chromosomal) complement.

Polyploid Yeast Cell, as used herein, refers broadly to a cell having more than one copy of its normal genomic (chromosomal) complement.

Diploid Yeast Cell, as used herein, refers broadly to a cell having two copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two haploid cells.

Tetraploid Yeast Cell, as used herein, refers broadly to a cell having four copies (alleles) of essentially every gene of its normal genomic complement, typically formed by the process of fusion (mating) of two haploid cells. Tetraploids may carry two, three, four, or more different expression cassettes. Such tetraploids might be obtained in S. cerevisiae by selective mating homozygotic heterothallic a/a and alpha/alpha diploids and mPichia by sequential mating of haploids to obtain auxotrophic diploids. For example, a [met his] haploid can be mated with [ade his] haploid to obtain diploid [his]; and a [met arg] haploid can be mated with [ade arg] haploid to obtain diploid [arg]; then the diploid [his] x diploid [arg] to obtain a tetraploid prototroph. It will be understood by those of skill in the art that reference to the benefits and uses of diploid cells may also apply to tetraploid cells.

Yeast Mating, as used herein, refers broadly to a process by which two haploid yeast cells naturally fuse to form one diploid yeast cell.

Variable region or VR as used herein refers broadly to the domains within each pair of light and heavy chains in an antibody that are involved directly in binding the antibody to the antigen. Each heavy chain has at one end a variable domain (\(V_H\)) followed by a number of constant domains. Each light chain has a variable domain (\(V_L\)) at one end and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.
Variants, as used herein refers broadly to single-chain antibodies, dimers, multimers, sequence variants, and domain substitution variants. Single-chain antibodies such as SMIPs, shark antibodies, nanobodies (e.g., Camelidiae antibodies). Sequence variants can be specified by percentage identity (similarity, sequence homology) e.g., 99%, 95%, 90%, 85%, 80%, 70%, 60%, or by numbers of permitted conservative or non-conservative substitutions. Domain substitution variants include replacement of a domain of one protein with a similar domain of a related protein. A similar domain may be identified by similarity of sequence, structure (actual or predicted), or function. For example, domain substitution variants include the substitution of at least one CDRs and/or framework regions.

The techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook, et al. (2001) Molec. Cloning: Lab. Manual [3rd Ed] Cold Spring Harbor Laboratory Press. Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture, and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

ANEMIA

The IL-6 antagonists described herein, include but are not limited to anti-IL-6 antibodies and antibody fragments, and may be used in methods and compositions for the treatment of anemia (e.g., anemia associated with chemotherapy).

Anemia

Normal hemoglobin ranges for humans are about 14-18 g/dl for men and 12-16 for women g/dl with the average hemoglobin value for men at about 16 g/dL and for women at about 14 g/dL. Anemia may be considered a drop of hemoglobin levels below about 11 g/dL and severe anemia may be considered a drop in hemoglobin below about 8 g/dL. See Table 1; See also Groopman & Itri (1999) Journal of National Cancer Institute 91(19): 1616-1634. Anemia may be caused by cancer (e.g., cancer-related anemia), chemotherapy (e.g., chemotherapy-related
Anemia may be assessed by assays well-known in the art such as a Complete Blood Count (CBC) test that measures the red blood cell (RBC) count, hematocrit, hemoglobin levels, white blood cell count (CBC), differential blood count, and platelet count. The first three parameters, the RBC, hematocrit, and hemoglobin levels are the most commonly used in determining whether or not the patient is suffering from anemia. Other anemia marker include the measurement of the levels of serum ferritin and serum iron.

**TABLE 1: WHO and NCI Grading Systems for Anemia**

<table>
<thead>
<tr>
<th>Severity</th>
<th>World Health Organization</th>
<th>National Cancer Institute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>≥11.0 g/dL</td>
<td>12.0–16.0 g/dL for women</td>
</tr>
<tr>
<td>Grade 1 (mild)</td>
<td>9.5–10.9 g/dL</td>
<td>10.0–12.0 g/dL for women</td>
</tr>
<tr>
<td>Grade 2 (moderate)</td>
<td>8.0–9.4 g/dL</td>
<td>10.0–14.0 g/dL for men</td>
</tr>
<tr>
<td>Grade 3 (severe)</td>
<td>6.5–7.9 g/dL</td>
<td>6.5–7.9 g/dL</td>
</tr>
<tr>
<td>Grade 4 (life-threating)</td>
<td>&lt;6.5 g/dL</td>
<td>&lt;6.5 g/dL</td>
</tr>
</tbody>
</table>

**TABLE 2: Common Parameters Measured in Diagnosing Anemia**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range (men)</th>
<th>Normal Range (women)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>42–52%</td>
<td>36–48%</td>
</tr>
<tr>
<td>Ferritin (serum)</td>
<td>30–400 ng/mL</td>
<td>13–150 ng/mL</td>
</tr>
<tr>
<td>Iron (serum)</td>
<td>60–170 µg/dL</td>
<td>60–170 µg/dL</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>0.5–1.5%</td>
<td>0.5–1.5%</td>
</tr>
<tr>
<td>White Blood Cell (WBC)</td>
<td>5,000–10,000/mL</td>
<td>5,000–10,000/mL</td>
</tr>
<tr>
<td>Red Blood Cell (RBC)</td>
<td>4.5–5.5x10^12/mL</td>
<td>4.0–5.0x10^12/mL</td>
</tr>
<tr>
<td>Platelet</td>
<td>1.4–4.0x10^11/mL</td>
<td>1.4–4.0x10^11/mL</td>
</tr>
<tr>
<td>TIBC</td>
<td>250–370 µg/dL</td>
<td>250–370 µg/dL</td>
</tr>
</tbody>
</table>

Lower values of hematocrit, serum ferritin, serum iron, white blood cells, red blood cells, and platelets below those levels presented in Table 2 are signs of anemia. The upper normal limit of reticulocytes (immature red blood cells) is about 1.5%, a low count suggests problems with the bone marrow and a high count suggests hemolytic anemia (e.g., the patient's body is attempting to make up for a loss of RBCs). MD Medical Center (2011) "Anemia-Diagnosis". Additionally, total iron binding capacity (TIBC) measures the level for transferring in the blood. Transferrin is a protein that carries iron in the blood and a higher than normal TIBC value is a sign of iron-deficiency anemia and a lower than normal level indicates chronic anemia,
pernicious anemia, or hemolytic anemia. Additionally, tests for anemia include direct or indirect Coombs’ test, indirect bilirubin levels, serum haptoglobin, vitamin B12 levels, folate levels, and urine hemoglobin. MedlinePlus website "Drug-induced immune hemolytic anemia." (2011).

**[0332]** Anemia is also common in cancer where about 30% of newly-diagnosed untreated cancer patients exhibit anemia. Mori, *et al.* (2009) *Biomedical Research* 30(1): 47-51. Over 70% of patients who receive chemotherapy will develop some degree of anemia during the course of their treatment. Further, patients receiving radiation to the head, neck, or chest areas, and patients who undergo bone marrow or stem cell transplant, often develop anemia. Patient Advocate Foundation (2011) "Chemotherapy-Related Anemia." Certain chemotherapy agents known to cause anemia are listed in Table 3.

**TABLE 3 Common Chemotherapy Agents.**

<table>
<thead>
<tr>
<th>Agent (Brand Name)</th>
<th>Chemotherapy Drug</th>
<th>Agent (Brand Name)</th>
<th>Chemotherapy Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemtuzumab (Campath®)</td>
<td>Bleomycin (Blenoxane®)</td>
<td>Asparaginase (Elspar®)</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide (Cytoxan®)</td>
<td>Cytarabine (Cytosar-U®)</td>
<td>Busulfan (Myleran®, Busulfex®)</td>
<td></td>
</tr>
<tr>
<td>Docetaxel (Taxotere®)</td>
<td>Doxorubicin (Adriamycin®)</td>
<td>Capecitabine (Xeloda®)</td>
<td></td>
</tr>
<tr>
<td>Fluorouracil (5-FU®)</td>
<td>Gemcitabine (Gemzar®)</td>
<td>Carboplatin (Paraplatin®)</td>
<td></td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin (Mylotarg®)</td>
<td>Hydroxyurea (Hydrea®)</td>
<td>Daunorubicin (Cerubidine®)</td>
<td></td>
</tr>
<tr>
<td>Idarubicin (Idamycin®)</td>
<td>Interleukin 2 (Proleukin®)</td>
<td>Epirubicin (Ellence®)</td>
<td></td>
</tr>
<tr>
<td>Lomustine (CeeNU®)</td>
<td>Melphalan (Alkeran®)</td>
<td>Etoposide (VePesid®)</td>
<td></td>
</tr>
<tr>
<td>Mitomycin (Mutamycin®)</td>
<td>Mitoxantrone (Novantrone®)</td>
<td>Irinotecan (Camptosar®)</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin (Eloxatin®)</td>
<td>Paclitaxel (Taxol®)</td>
<td>Methotrexate (Rheumatrex®)</td>
<td></td>
</tr>
<tr>
<td>Pentostatin (Nipent®)</td>
<td>Procarbazine (Matulane®)</td>
<td>Mechlorethamine (Mustargen®)</td>
<td></td>
</tr>
<tr>
<td>Topotecan (Hycamtin®)</td>
<td>Trastuzumab (Herceptin®)</td>
<td>Pemetrexed (Alimta®)</td>
<td></td>
</tr>
<tr>
<td>Vinblastine (Velban®)</td>
<td>Vincristine (Oncovin®)</td>
<td>Thiotepa (Thioplex®)</td>
<td></td>
</tr>
<tr>
<td>Tretinoin (Vesanoid®)</td>
<td>Cisplatin (PLATINOL®)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**[0333]** Anemia is also a common side-effect of radiation therapy (radiotherapy). In one study, 41% of all patients were anemic (hemoglobin < 12 g/dL); by the end of radiation therapy, this percentage increased to 54%. The most common tumor types were prostate (16%), breast (14%), head and neck (12%), colorectal (11%), lung/bronchus (11%), and uterine-cervix (9%). Anemia was most prevalent in patients with uterine-cervical tumors (75%), increasing to 79% by the end of radiation therapy. The prevalence of lung/bronchus and colorectal cancer was 55% and 44%, respectively, at baseline and increased to 77% and 63%, respectively, after radiation therapy. For nearly all tumor types, the majority of patients had or developed mild to moderate anemia (hemoglobin 10.0 to 11.9 g/dL). Harrison, *et al.* (2001) *Semin Oncol.* 28(2 Suppl 8): 54-9.

**[0334]** Drug-induced immune hemolytic anemia may be caused by therapeutic regimes involving the administration of drugs, where three classes of drug predominate in drug-induced
immune hemolytic anemia (DIIHA), namely anti-microbial, anti-inflammatory, and anti-neoplastic drugs. Additionally, drugs that cause anemia include but are not limited to carboplatin, cefamandole, cefazolin, cefixime, cefotetan, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, cephalixin, cephalosporins (a class of antibiotics), cephalothin, chlorpropamide, cimetidine, dapsone, diclofenac, erythromycin, fludarabine, hydrochlorothiazide, levodopa, levofoxcin, mefloquine, methylodopa, nafcillin, nitrofurantoin, nonsteroidal anti-inflammatory drugs (NSAIDs), oxaliplatin, penicillin (and its derivatives), phenacetin, phenazopyridine (pyridium), piperacillin, probenecid, procainamide, quinidine, rifampin, sulfamethoxazole, ticarcillin, tolectin, trimethoprim, and β-lactamase inhibitors. Garratty (2009) Hematology 1: 73-79; MedlinePlus website "Drug-induced immune hemolytic anemia." (2011).

[0335] The invention described herein provides a method of treating or preventing anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to treat anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used to prevent anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to the onset of anemia.

[0336] In methods for treating or preventing anemia the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be an IL-6 antagonist is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab1O, Ab1 1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab1O, Ab1 1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0337] The invention described herein provides compositions of treating or preventing anemia comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used in compositions for treating anemia comprising an effective amount of an IL-
6 antagonist. The IL-6 antagonists described herein may be used in compositions for preventing anemia comprising an effective amount of an IL-6 antagonist, optionally prior to the onset of anemia.

[0338] In compositions for treating or preventing anemia the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avenirim, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbIO, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0339] The invention described herein provides a method of treating or preventing anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to treat anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used to prevent anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to beginning chemotherapy.

[0340] In methods for treating or preventing anemia associated with chemotherapy the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avenirim, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6
antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbIO, Abl 1, Abl 2, Abl 3, Abl 4, Abl 5, Abl 6, Abl 7, Abl 8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0341] The invention described herein provides compositions for treating or preventing anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used in compositions for treating anemia associated with chemotherapy comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used in compositions for preventing anemia associated with chemotherapy comprising an effective amount of an IL-6 antagonist, optionally prior to beginning chemotherapy.

[0342] In compositions for treating or preventing anemia associated with chemotherapy the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avenir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbIO, Abl 1, Abl 2, Abl 3, Abl 4, Abl 5, Abl 6, Abl 7, Abl 8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0343] The invention described herein provides a method of treating or preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to treat anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used to
prevent anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to beginning radiotherapy.

**[0344]** In methods for treating or preventing anemia associated with radiotherapy the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gp30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gp30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

**[0345]** The invention described herein provides compositions for treating or preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used in compositions for treating anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be in compositions for preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to beginning radiotherapy.

**[0346]** In compositions for treating or preventing anemia associated with radiotherapy the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gp30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gp30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6
antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbIO, AbI 1, Ab2 2, Ab3 3, Ab4 4, Ab5 5, Ab6 6, Ab7 7, Ab8 8, Ab9 9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0347] The invention described herein provides a method of treating or preventing cancer-related anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to treat cancer-related anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used to prevent cancer-related anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to diagnosis of cancer or the diagnosis of anemia as an effect of cancer. The patient may suffer from a benign tumor, a malignant tumor, a non-malignant tumor, or a metastatic tumor.

[0348] In the methods of treating or preventing cancer-related anemia the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avenin, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AbIO, AbI 1, AbI 2, AbI 3, AbI 4, AbI 5, AbI 6, AbI 7, AbI 8, AbI 9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0349] The invention described herein provides compositions of treating or preventing cancer-related anemia comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used in compositions for treating cancer-related anemia comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used in compositions for preventing cancer-related anemia comprising an effective amount of an IL-6
antagonist, optionally the composition may be administered prior to diagnosis of cancer or the diagnosis of anemia as an effect of cancer. The patient may suffer from a benign tumor, a malignant tumor, a non-malignant tumor, or a metastatic tumor.

[0350] In compositions for treating or preventing cancer-related anemia the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an a vemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab1, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0351] The invention described herein provides a method of treating or preventing drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to treat drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used to prevent drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an IL-6 antagonist, optionally prior to beginning the drug therapy that may cause DIIHA.

[0352] In the methods of treating or preventing drug-induced immune hemolytic anemia (DIIHA) the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an a vemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment),
wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

[0353] The invention described herein provides a compositions of treating or preventing drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an IL-6 antagonist. Also, the IL-6 antagonists described herein may be used to in compositions for treating drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an IL-6 antagonist. The IL-6 antagonists described herein may be used in compositions for prevention of drug-induced immune hemolytic anemia (DIIHA) comprising an effective amount of an IL-6 antagonist, optionally for administration prior to beginning the drug therapy that may cause DIIHA.

[0354] In compositions for treating or preventing drug-induced immune hemolytic anemia (DIIHA), the IL-6 antagonists may target IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. Further, the IL-6 antagonist may be an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an aemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, or anti-SYK antibody or antibody fragment. The IL-6 antagonist may be a small molecule comprising thalidomide, lenalidomide, or any combination thereof. The IL-6 antagonist may be IL-6 antagonists is an anti-IL-6 antibody or antibody fragment (e.g., antigen-binding fragment), wherein the anti-IL-6 antibody or antibody fragment thereof (e.g., antigen-binding fragment) may be Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, AblO, Abl 1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab3 1, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

TREATMENT OF RHEUMATOID ARTHRITIS

[0355] This invention also relates to the use of IL-6 antagonists including anti-IL-6 antibodies described herein, such as Ab l or humanized forms thereof for treating or preventing rheumatoid arthritis. This application provides results of clinical studies showing safety, pharmacokinetics, and pharmacodynamics for subcutaneous and intravenous administration of an exemplary anti-IL-
The clinical data demonstrates that an anti-IL-6 antibody decreases disease severity in rheumatoid arthritis patients which have been subcutaneously (SC) or intravenously (IV) administered ALD-518, including improvement in mental and physical components of disease.

The anti-IL-6 antibody (e.g., ALD518) was well tolerated when administered in a single subcutaneous (SC) dose; injection site reactions were generally mild. The bioavailability of SC ALD518 was -60% of IV ALD518, and the half life was -30 days. Rapid and significant reductions in CRP (C-reactive protein) were observed, which were sustained over 24 weeks of assessment. The half-life of ALD518 when administered subcutaneously (approximately 30 days) is similar to the half-life previously observed with IV administration. Additionally, subcutaneous ALD518 led to rapid and large reductions in serum CRP and the reductions in CRP observed during the first 12 weeks of the study were sustained over 24 weeks of assessment. These results are also similar to those observed with IV administration. Together, these results suggest that anti-IL-6 antibodies, such as Abl (ALD518) may be used for the treatment of RA, as well as prevention or treatment of other IL-6 associated conditions. These therapeutic regimens may be combined with other RA therapeutics, including methotrexate or other RA drugs identified herein and generally known in the art, including analgesics, disease-modifying antirheumatic drugs (DMARDS), anti-inflammatory agents, and others.

The invention further provides specific dosage regimens and dosage formulations for treating rheumatoid arthritis by subcutaneous or intravenous administration of anti-IL-6 antibodies or antibody fragments according to the invention such as humanized Abl antibodies. For example, a subject may be administered 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl).

The anti-IL-6 antibodies may be used to subcutaneously administer antibodies of the invention, including Abl, for rheumatoid arthritis indications, the administration formulation comprises, or alternatively consists of, about 50 or 100 mg/mL of antibody, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80. In another embodiment of the invention that may be used to subcutaneously administer antibodies of the invention, including Ab1, for rheumatoid arthritis indications, the administration formulation comprises, or alternatively consists of, about 20 or 100 mg/mL of antibody, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 to 280 mM sorbitol (or sorbitol in combination with sucrose), and 0.015% (w/w) Polysorbate 80, said formulation having a nitrogen headspace in the shipping vials.
Therapeutic regimens for the prevention or treatment of RA may be combined with other RA therapeutics, including analgesics, DMARDs, anti-inflammatories, and others. For example, analgesics and anti-inflammatory drugs, including steroids, may provide relief of disease symptoms, while disease-modifying antirheumatic drugs (DMARDs), may inhibit or halt the underlying immune process and prevent further long-term damage. In exemplary embodiments, ALD5 18 (or another antibody of the present disclosure) may be administered to a patient at approximately the same time as another RA therapeutic (which may or may not be formulated together) or may be administered to a patient who is also undergoing another therapeutic regimen but not necessarily at the same time. A regimen may be considered to provide a combination of therapeutics as long as the patient concurrently experiences the effects of the combined therapeutics. Due to possible differences in dosing schedule, a combination may include administration of different therapeutics at different times, e.g., a patient may receive a drug such as methotrexate on a weekly schedule (e.g., at least 10 mg per week) and may receive ALD518 (or another anti-IL-6 antibody of the present disclosure) less frequently (such as about every eight weeks, every twelve weeks, every three months). Exemplary DMARDs that may administered in combination with ALD5 18 (or another antibody of the present disclosure) include, but are not limited to Mycophenolate mofetil (CellCept®), calcineurin inhibitors (e.g., cyclosporine, sirolimus, everolimus), oral retinoids, azathioprine, fumeric acid esters, D-penicillamine, cyclophosphamide, immunoabsorption columns (e.g., Prosurba® columns), gold salts (e.g., Auranofin, sodium aurothiomalate (Myocrisin)), hydroxychloroquine, chloroquine, leflunomide, methotrexate (MTX), minocycline, sulfasalazine (SSZ), tumor necrosis factor alpha (TNFa) blockers (e.g., etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi)), Interleukin 1 (IL-1) blockers (e.g., anakinra (Kineret)), monoclonal antibodies against B cells (e.g., rituximab (Rituxan)), T cell costimulation blockers (e.g., abatacept (Ocrevus)), Interleukin 6 (IL-6) blockers (e.g., tocilizumab (an anti-IL-6 receptor antibody), RoActemra, Actemra). Exemplary anti-inflammatory agents that may administered in combination with ALD5 18 (or another antibody of the present disclosure) include, but are not limited to, anti-inflammatory steroids such as Cortisone, Glucocorticoids, prednisone, prednisolone, Hydrocortisone (Cortisol), Cortisone acetate, Methylprednisolone, Dexamethasone, Betamethasone, Triamcinolone, Beclometasone, and Fluadrocortisone acetate, and non-steroidal anti-inflammatory drug (NSAIDs) (which may also act as analgesics), such as ibuprofen, naproxen, meloxicam, etodolac, nabumetone, sulindac, toletenant, choline magnesium salicylate, diclofenac, diflunisal, indomethacin, Ketoprofen, Oxaprozin, piroxicam, and nimesulide, Salicylates, Aspirin (acetylsalicylic acid), Diflunisal,
Salsalate, p-amino phenol derivatives, Paracetamol, phenacetin, Propionic acid derivatives, Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Acetic acid derivatives, Indomethacin, Sulindac, Etdolac, Ketorolac, Diclofenac, Nabumetone, Enolic acid (Oxicam) derivatives, Piroxicam, Meloxicam, Tenoxicam, Droxid, Lornoxicam, Isoxicam, Fenamic acid derivatives (Fenamates), Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Selective COX-2 inhibitors (Coxibs), Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumaracoxib, Etoricoxib, Firocoxib, Sulphonanilides, Nimesulide, and Licofelone. Exemplary analgesics include that may administered in combination with ALD5 18 (or another antibody of the present disclosure) include, but are not limited to, NSAIDs, COX-2 inhibitors (including Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumaracoxib, Etoricoxib, and Firocoxib), acetaminophen, opiates (e.g., Dextropropoxyphene, Codeine, Tramadol, Anileridine, Pethidine, Hydrocodone, Morphine [e.g., oral, intravenous (IV), or intramuscular (IM)], Oxycodone, Methadone, Diacetylmorphine, Hydromorphone, Oxymorphone, Levorphanol, Buprenorphine, Fentanyl, Sufentanil, Etorphine, Carfentanyl, dihydromorphine, dihydrocodeine, Thebaine, and Papaverine), diproqualone, Flupirtine, Tricyclic antidepressants, and lidocaine (topical).

ANTI-IL-6 ANTAGONISTS

[0360] The IL-6 antagonist may comprise an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof. The IL-6 antagonist may be an agent that blocks signal transmission by IL-6, blocks IL-6 binding to its receptor, suppresses/interferes with IL-6 expression, and/or inhibits the biological activity of IL-6. The IL-6 antagonists may be attached directly or indirectly to immunoglobulin polypeptides or effector moieties such as therapeutic or detectable entities.

[0361] Examples of IL-6 antagonists include but are not limited to anti-IL-6 antibody, anti-IL-6R antibody, anti-gpl30 antibody, IL-6 mutant, IL-6R antisense oligonucleotide, and partial peptides of IL-6 or IL-6R. An example of the IL-6 mutant used in the present invention is disclosed in Brakenhoff, et al. (1994) J. Biol. Chem. 269: 86-93 or Savino, et al. (1994) EMBO J. 13: 1357-1367. The IL-6 mutant polypeptide or fragment thereof does not possess the signal transmission effects of IL-6 but retains the binding activity with IL-6R, and is produced by introducing a mutation in the form of a substitution, deletion or insertion into the amino acid sequence of IL6. While there are no limitations on the animal species used, it is preferable to use an IL6 of human origin. Similarly, any IL-6 partial peptides or IL-6R partial peptides used in the present invention provided they prevent IL6 or IL6R (gp80) or gpl30 from affecting signal transduction and thereby prevent IL-6 associated biological activity. For details regarding IL-6...


**[0363]** Further, oligonucleotides capable of IL6 or IL6R RNA silencing or antisense mechanisms can be used in the method of the present invention (JP5-300338 for details regarding IL-6R antisense oligonucleotide).

**[0364]** Additionally, the IL-6 antagonist may target IL-6, IL-6 receptor, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, or any combination thereof. For example, SANT-7 is an IL-6 receptor antagonist that interferes with the formation of IL-6/IL-6R/gpl30 heteromers. *See* Honemann, *et al.* (2001) *Int J Cancer* 93: 674-680.


**[0366]** The IL-6 antagonist may comprise a small molecule including but not limited to thalidomide, lenalidomide, aryl hydrocarbon receptor agonists (*e.g.*, 7,12-dimethylbenz[a]anthracene (DMBA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)) or any combination thereof. *See* Jensen, *et al.* (2003) *Environmental Health: A Global Access Science Source* 2:16.

**[0367]** IL-6 antagonist may be an IL-6 antagonist peptide. *See*, *e.g.*, U.S. Patent No. 6,838,433. For example, a truncated IL-6 molecule may act as an IL-6 antagonist. *See* Alberti, *et al.* (2005) *J Cancer Res* 65: 2-5.

**[0368]** The IL-6 antagonist may be an anti-IL-6 antibody. *See also* U.S. Patent Application Publication No. 2007/0292420. The IL-6 antagonist may comprise an anti-IL-6 antibody or antibody fragment as described in further detail herein. The invention includes antibodies having binding specificity to IL-6 and possessing a variable light chain sequence comprising the sequence set forth in the polypeptide sequence of SEQ ID NO: 2 or SEQ ID NO: 709 and
humanized versions and variants thereof including those set forth in FIGS. 1-5, and those identified in Table 4.

**ANTI-IL-6 ANTIBODIES AND ANTIBODY FRAGMENTS THEREOF**

[0369] Antibodies consist of two identical light polypeptide chains of molecular weight approximately 23,000 daltons (the "light chain"), and two identical heavy chains of molecular weight 53,000-70,000 (the "heavy chain"). The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" configuration. The "branch" portion of the "Y" configuration is designated the Fab region; the stem portion of the "Y" configuration is designated the Fc region. The amino acid sequence orientation runs from the N-terminal end at the top of the "Y" configuration to the C-terminal end at the bottom of each chain. The N-terminal end possesses the variable region having specificity for the antigen that elicited it, and is approximately 100 amino acids in length, there being slight variations between light and heavy chain and from antibody to antibody.

[0370] The variable region is linked in each chain to a constant region that extends the remaining length of the chain and that within a particular class of antibody does not vary with the specificity of the antibody (i.e., the antigen eliciting it). There are five known major classes of constant regions that determine the class of the immunoglobulin molecule (IgG, IgM, IgA, IgD, and IgE corresponding to γ, µ, α, δ, and ε (gamma, mu, alpha, delta, or epsilon) heavy chain constant regions). The constant region or class determines subsequent effector function of the antibody, including activation of complement (Kabat, E. A. (1976) Structural Concepts in Immunology and Immunochemistry [2nd Ed.] pages 413-436, Holt, Rinehart, Winston), and other cellular responses (Andrews, et al. (1980) Clinical Immunobiology pages 1-18, W. B. Sanders; Kohl, et al. (1983) Immunology 48: 187); while the variable region determines the antigen with which it will react. Light chains are classified as either κ (kappa) or λ (lambda). Each heavy chain class can be paired with either kappa or lambda light chain. The light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages when the immunoglobulins are generated either by hybridomas or by B cells.

[0371] For example, antibodies or antigen binding fragments or variants thereof may be produced by genetic engineering. In this technique, as with other methods, antibody-producing cells are sensitized to the desired antigen or immunogen. The messenger RNA isolated from antibody producing cells is used as a template to make cDNA using PCR amplification. A library of vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, is produced by insertion of appropriate sections of the amplified

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immunoglobulin cDNA into the expression vectors. A combinatorial library is constructed by combining the heavy chain gene library with the light chain gene library. This results in a library of clones which co-express a heavy and light chain (resembling the Fab fragment or antigen binding fragment of an antibody molecule). The vectors that carry these genes are co-transfected into a host cell. When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by screening with the antigen or immunogen.

[0372] Antibody coding sequences of interest include those encoded by native sequences, as well as nucleic acids that, by virtue of the degeneracy of the genetic code, are not identical in sequence to the disclosed nucleic acids, and variants thereof. Variant polypeptides can include amino acid (aa) substitutions, additions or deletions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acids, such as to alter a glycosylation site, or to minimize misfolding by substitution or deletion of at least one cysteine residues that are not necessary for function. Variants can be designed so as to retain or have enhanced biological activity of a particular region of the protein (e.g., a functional domain, catalytic amino acid residues). Variants also include fragments of the polypeptides disclosed herein, particularly biologically active fragments and/or fragments corresponding to functional domains. Techniques for in vitro mutagenesis of cloned genes are known. Also included in the subject invention are polypeptides that have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent.

[0373] Chimeric antibodies may be made by recombinant means by combining the variable light and heavy chain regions (V_L and V_H), obtained from antibody producing cells of one species with the constant light and heavy chain regions from another. Typically chimeric antibodies utilize rodent or rabbit variable regions and human constant regions, in order to produce an antibody with predominantly human domains. The production of such chimeric antibodies is well known in the art, and may be achieved by standard means (as described, e.g., in U.S. Patent No. 5,624,659, incorporated herein by reference in its entirety). It is further contemplated that the human constant regions of chimeric antibodies of the invention may be selected from IgGl, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgGlO, IgGl 1, IgG12, IgG13, IgG14, IgG15, IgGl 6, IgGl 7, IgGl 8 or IgGl 9 constant regions.

[0374] Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This is accomplished by carefully examining the sequence of the
hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. Although facially complex, the process is straightforward in practice. See, e.g., U.S. Patent No. 6,187,287. In a preferred embodiment, humanization may be effected as disclosed in detail infra. This scheme grafts CDRs onto human FRs highly homologous to the parent antibody that is being humanized.

[0375] Immunoglobulins and fragments thereof may be modified post-translationally, e.g. to add effector moieties such as chemical linkers, detectable moieties, such as fluorescent dyes, enzymes, toxins, substrates, bioluminescent materials, radioactive materials, chemiluminescent moieties and the like, or specific binding moieties, such as streptavidin, avidin, or biotin, and the like may be utilized in the methods and compositions of the present invention.

Exemplary Anti-IL-6 Antibodies

[0376] The invention also includes antibodies having binding specificity to IL-6 and possessing a variable heavy chain sequence comprising the sequence set forth in the polypeptide sequences of SEQ ID NO: 3 and SEQ ID NO: 657 and humanized versions and variants thereof including those set forth in FIGS. 1-5, and those identified in Table 4.

[0377] The invention further includes antibodies having binding specificity to IL-6 and possessing a variable heavy chain sequence which is a modified version of SEQ ID NO: 3 wherein the tryptophan residue in CDR2 is changed to a serine as set forth in the polypeptide sequence of SEQ ID NO: 658 and humanized versions and variants thereof including those set forth in FIGS. 1-5, and those identified in Table 4.

[0378] The invention further contemplates antibodies comprising at least one of the polypeptide sequences of SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 2, and/or at least one of the polypeptide sequences of SEQ ID NO: 7; SEQ ID NO: 8 or 120; and SEQ ID NO: 9 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or 19, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention include combinations of the CDRs and the variable heavy and light chain sequences set forth herein.

[0379] In another embodiment, the invention contemplates other antibodies, such as for example chimeric antibodies, comprising at least one of the polypeptide sequences of SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 2, and/or at least one of the polypeptide sequences of SEQ ID NO: 7; SEQ ID NO: 8 or 120; and
SEQ ID NO: 9 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or 19, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention include combinations of the CDRs and humanized versions of the variable heavy and light chain sequences set forth above.

[0380] The invention also contemplates fragments of the antibody having binding specificity to IL-6. In one embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, humanized versions of the polypeptide sequence of SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, or 709. In another embodiment of the invention, antibody fragments of the invention comprise, or alternatively consist of, humanized versions of the polypeptide sequence of SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, or 708.

[0381] In a further embodiment of the invention, fragments of the antibody having binding specificity to IL-6 comprise, or alternatively consist of, at least one of the polypeptide sequences of SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 2 or SEQ ID NO: 709.

[0382] In a further embodiment of the invention, fragments of the antibody having binding specificity to IL-6 comprise, or alternatively consist of, at least one of the polypeptide sequences of SEQ ID NO: 7; SEQ ID NO: 8 or SEQ ID NO: 120; and SEQ ID NO: 9 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 and 657 or 19.

[0383] The invention also contemplates antibody fragments which include at least one of the antibody fragments described herein. In one embodiment of the invention, fragments of the antibodies having binding specificity to IL-6 comprise, or alternatively consist of, one, two, three or more, including all of the following antibody fragments: the variable light chain region of SEQ ID NO: 2; the variable heavy chain region of SEQ ID NO: 3; the complementarity-determining regions (SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6) of the variable light chain region of SEQ ID NO: 2; and the complementarity-determining regions (SEQ ID NO: 7; SEQ ID NO: 8 or SEQ ID NO: 120; and SEQ ID NO: 9) of the variable heavy chain region of SEQ ID NO: 3 and 657 or 19.

[0384] The invention also contemplates variants wherein either of the heavy chain polypeptide sequences of SEQ ID NO: 18 or SEQ ID NO: 19 is substituted for the heavy chain polypeptide sequence of SEQ ID NO: 3 or 657; the light chain polypeptide sequence of SEQ ID NO: 20 is substituted for the light chain polypeptide sequence of SEQ ID NO: 2 or SEQ ID NO: 709; and
the heavy chain CDR sequence of SEQ ID NO: 120 is substituted for the heavy chain CDR sequence of SEQ ID NO: 8.

[0385] In a preferred embodiment of the invention, the anti-IL-6 antibody is Ab 1, comprising SEQ ID NO: 2 and SEQ ID NO: 3, or more particularly an antibody comprising SEQ ID NO: 657 and SEQ ID NO: 709 (which are respectively encoded by the nucleic acid sequences in SEQ ID NO: 700 and SEQ ID NO: 723) or one comprised of the alternative SEQ ID NOs set forth in the preceding paragraph, and having at least one of the biological activities set forth herein. In a preferred embodiment the anti-IL-6 antibody will comprise a humanized sequence as shown in Figures 1-5.

[0386] Sequences of anti-IL-6 antibodies of the present invention are shown in Table 4. Exemplary sequence variants other alternative forms of the heavy and light chains of Ab 1 through Ab 36 are shown. The antibodies of the present invention encompass additional sequence variants, including conservative substitutions, substitution of at least one CDR sequences and/or FR sequences.

[0387] Exemplary Ab 1 embodiments include an antibody comprising a variant of the light chain and/or heavy chain. Exemplary variants of the light chain of Abl include the sequence of any of the Abl light chains shown (i.e., any of SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, or 709) wherein the entire CDR1 sequence is replaced or wherein at least one residues in the CDR1 sequence is substituted by the residue in the corresponding position of any of the other light chain CDR1 sequences set forth (i.e., any of SEQ ID NO: 23, 39, 55, 71, 87, 103, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, or 572); and/or wherein the entire CDR2 sequence is replaced or wherein at least one residues in the CDR2 sequence is substituted by the residue in the corresponding position of any of the other light chain CDR2 sequences set forth (i.e., any of SEQ ID NO: 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, or 573); and/or wherein the entire CDR3 sequence is replaced or wherein at least one residues in the CDR3 sequence is substituted by the residue in the corresponding position of any of the other light chain CDR3 sequences set forth (i.e., any of SEQ ID NO: 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574).

[0388] Exemplary variants of the heavy chain of Ab 1 include the sequence of any of the Ab 1 heavy chains shown (i.e., any of SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, or 708) wherein the entire CDR1 sequence is replaced or wherein at least one residues in the
CDR1 sequence is substituted by the residue in the corresponding position of any of the other heavy chain CDR1 sequences set forth /i.e., any of SEQ ID NO: 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, or 575); and/or wherein the entire CDR2 sequence is replaced or wherein at least one residues in the CDR2 sequence is substituted by the residue in the corresponding position of an Ab1 heavy chain CDR2, such as those set forth in Table 4 /i.e., any of SEQ ID NO: 8, or 120) or any of the other heavy chain CDR2 sequences set forth /i.e., any of SEQ ID NO: 27, 43, 59, 75, 91, 107, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, or 576); and/or wherein the entire CDR3 sequence is replaced or wherein at least one residues in the CDR3 sequence is substituted by the residue in the corresponding position of any of the other heavy chain CDR3 sequences set forth /i.e., any of SEQ ID NO: 28, 44, 60, 76, 92, 108, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, or 577).

[0389] In another embodiment, the invention contemplates other antibodies, such as for example chimeric or humanized antibodies, comprising at least one of the polypeptide sequences of SEQ ID NO: 4; SEQ ID NO: 5; and SEQ ID NO: 6 which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable light chain sequence of SEQ ID NO: 2, and/or at least one of the polypeptide sequences of SEQ ID NO: 7 (CDR1); SEQ ID NO: 8 (CDR2); SEQ ID NO: 120 (CDR2); and SEQ ID NO: 9 (CDR3) which correspond to the complementarity-determining regions (CDRs, or hypervariable regions) of the variable heavy chain sequence of SEQ ID NO: 3 or SEQ ID NO: 19, or combinations of these polypeptide sequences. In another embodiment of the invention, the antibodies of the invention include combinations of the CDRs and the variable heavy and light chain sequences set forth above including those set forth in FIGS. 1-5, and those identified in Table 4.

[0390] In another embodiment the anti-IL-6 antibody of the invention is one comprising at least one of the following: a CDR1 light chain encoded by the sequence in SEQ ID NO: 12 or SEQ ID NO: 694; a light chain CDR2 encoded by the sequence in SEQ ID NO: 13; a light chain CDR3 encoded by the sequence in SEQ ID NO: 14 or SEQ ID NO: 695; a heavy chain CDR1 encoded by the sequence in SEQ ID NO: 15, a heavy chain CDR2 encoded by SEQ ID NO: 16 or SEQ ID NO: 696 and a heavy chain CDR3 encoded by SEQ ID NO: 17 or SEQ ID NO: 697. In addition the invention embraces such nucleic acid sequences and variants thereof.

[0391] In another embodiment the invention is directed to amino acid sequences corresponding to the CDRs of said anti-IL-6 antibody which are selected from SEQ ID NO: 4
In another embodiment the anti-IL-6 antibody of the invention comprises a light chain nucleic acid sequence of SEQ ID NO: 10, 662, 698, 701, 705, 720, 721, 722, or 723; and/or a heavy chain nucleic acid sequence of SEQ ID NO: 11, 663, 700, 703, 707, 724, or 725. In addition the invention is directed to the corresponding polypeptides encoded by any of the foregoing nucleic acid sequences and combinations thereof.

In a specific embodiment of the invention the anti-IL-6 antibodies or a portion thereof will be encoded by a nucleic acid sequence selected from those comprised in SEQ ID NO: 10, 12, 13, 14, 662, 694, 695, 698, 701, 705, 720, 721, 722, 723, 11, 15, 16, 17, 663, 696, 697, 700, 703, 707, 724, and 725. For example the CDR1 in the light chain may be encoded by SEQ ID NO: 12 or 694, the CDR2 in the light chain may be encoded by SEQ ID NO: 13, the CDR3 in the light chain may be encoded by SEQ ID NO: 14 or 695; the CDR1 in the heavy chain may be encoded by SEQ ID NO: 15, the CDR2 in the heavy chain may be encoded by SEQ ID NO: 16 or 696, the CDR3 in the heavy chain may be encoded by SEQ ID NO: 17 or 697. As discussed infra antibodies containing these CDRs may be constructed using appropriate human frameworks based on the humanization methods disclosed herein.

In another specific embodiment of the invention the variable light chain will be encoded by SEQ ID NO: 10, 662, 698, 701, 705, 720, 721, 722, or 723 and the variable heavy chain of the anti-IL-6 antibodies will be encoded by SEQ ID NO: 11, 663, 700, 703, 707, 724, or 725.

In a more specific embodiment variable light and heavy chains of the anti-IL-6 antibody respectively will be encoded by SEQ ID NO: 10 and 11, or SEQ ID NO: 698 and SEQ ID NO: 700, or SEQ ID NO: 701 and SEQ ID NO: 703 or SEQ ID NO: 705 and SEQ ID NO: 707.

In another specific embodiment the invention covers nucleic acid constructs containing any of the foregoing nucleic acid sequences and combinations thereof as well as recombinant cells containing these nucleic acid sequences and constructs containing wherein these nucleic acid sequences or constructs may be extrachromosomal or integrated into the host cell genome.

In another specific embodiment the invention covers polypeptides containing any of the CDRs or combinations thereof recited in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 120, SEQ ID NO: 9 or polypeptides comprising any of the variable light polypeptides comprised in SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, or
709 and/or the variable heavy polypeptides comprised in SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, ... 4; a polypeptide having 100%. (i.e., 1 1 out of 11 amino acids) identity to the light chain CDR1 of SEQ ID NO: 4;

[0398] In another embodiment the anti-IL-6 antibody is one comprising at least one of the following: a variable light chain encoded by the sequence in SEQ ID NO: 10 or SEQ ID NO: 698 or SEQ ID NO: 701 or SEQ ID NO: 705 and a variable chain encoded by the sequence in SEQ ID NO: 11 or SEQ ID NO: 700 or SEQ ID NO: 703 or SEQ ID NO: 707.

[0399] In another embodiment the anti-IL-6 antibody is a variant of the foregoing sequences that includes at least one substitution in the framework and/or CDR sequences and which has at least one of the properties of Abl in vitro and/or upon in vivo administration.

[0400] These in vitro and in vivo properties are described in more detail in the examples below and include: competing with Abl for binding to IL-6 and/or peptides thereof; having a binding affinity (Kd) for IL-6 of less than about 50 picomolar, and/or a rate of dissociation (Koff) from IL-6 of less than or equal to 10^(-4) S^-1; having an in-vivo half-life of at least about 22 days in a healthy human subject; ability to prevent or treat hypoalbuminemia; ability to prevent or treat elevated CRP; ability to prevent or treat abnormal coagulation; and/or ability to decrease the risk of thrombosis in an individual having a disease or condition associated with increased risk of thrombosis.

Additional non-limiting examples of anti-IL-6 activity are set forth herein, for example, under the heading "Anti-IL-6 Activity."

[0401] In another embodiment the anti-IL-6 antibody includes at least one of the Abl light-chain and/or heavy chain CDR sequences (see Table 4) or variant(s) thereof which has at least one of the properties of Abl in vitro and/or upon in vivo administration (examples of such properties are discussed in the preceding paragraph). One of skill in the art would understand how to combine these CDR sequences to form an antigen-binding surface, e.g. by linkage to at least one scaffold which may comprise human or other mammalian framework sequences, or their functional orthologs derived from a SMIP (Small Modular ImmunoPharmaceutical), camelbody, nanobody, IgNAR, other immunoglobulin, or other engineered antibody. See, e.g., Robak & Robak (2011) BioDrugs 25(1): 13-25 and Wesolowski, et al. (2009) Med Microbiol Immunol 198: 157-174. For example, embodiments may specifically bind to human IL-6 and include one, two, three, four, five, six, or more of the following CDR sequences or variants thereof: a polypeptide having at least 72.7% sequence identity (i.e., 8 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polypeptide having at least 81.8% (i.e., 9 out of 11 amino acids) identity to the light chain CDR1 of SEQ ID NO: 4; a polypeptide having at least 90.9% (i.e., 10 out of 11 amino acids) identity to the light chain CDR1 of SEQ ID NO: 4; a polypeptide having 100%. (i.e., 11 out of 11 amino acids) identity to the light chain CDR1 of SEQ ID NO: 4;
a polypeptide having at least 85.7% sequence identity (i.e., 6 out of 7 amino acids) to the light chain CDR2 of SEQ ID NO: 5; a polypeptide having 100% (i.e., 7 out of 7 amino acids) identity to the light chain CDR2 of SEQ ID NO: 5; a polypeptide having at least 50% sequence identity (i.e., 6 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 58.3% sequence identity (i.e., 7 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6;

[0402] a polypeptide having at least 66.6% (i.e., 8 out of 12 amino acids) identity to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 75% (i.e., 9 out of 12 amino acids) identity to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having 100% (i.e., 12 out of 12 amino acids) identity to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 80% sequence identity (i.e., 4 out of 5 amino acids) to the heavy chain CDR1 of SEQ ID NO: 7; a polypeptide having 100% (i.e., 5 out of 5 amino acids) identity to the heavy chain CDR1 of SEQ ID NO: 7; a polypeptide having at least 50% sequence identity (i.e., 8 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 56.2% sequence identity (i.e., 9 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 62.5% sequence identity (i.e., 10 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 68.7% sequence identity (i.e., 11 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 75% sequence identity (i.e., 12 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120;

[0403] a polypeptide having at least 81.2% sequence identity (i.e., 13 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 87.5% sequence identity (i.e., 14 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 93.7% sequence identity (i.e., 15 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having 100% (i.e., 16 out of 16 amino acids) identity to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 33.3% sequence identity (i.e., 4 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 41.6% (i.e., 5 out of 12 amino acids) identity to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 50% sequence identity (i.e., 6 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 58.3% sequence identity (i.e., 7 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 66.6% sequence identity (i.e., 8 out of 12 amino acids) to the heavy
chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 75% sequence identity (i.e., 9 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having 100% (i.e., 12 out of 12 amino acids) identity to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 90.9% sequence identity (i.e., 10 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polypeptide having 100% (i.e., 11 out of 11 amino acids) similarity to the light chain CDR1 of SEQ ID NO: 4; a polypeptide having at least 85.7% sequence identity (i.e., 6 out of 7 amino acids) to the light chain CDR2 of SEQ ID NO: 5; a polypeptide having 100% (i.e., 7 out of 7 amino acids) similarity to the light chain CDR2 of SEQ ID NO: 5; a polypeptide having at least 66.6% sequence identity (i.e., 8 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 75% sequence identity (i.e., 9 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having 100% (i.e., 12 out of 12 amino acids) similarity to the light chain CDR3 of SEQ ID NO: 6; a polypeptide having at least 80% sequence identity (i.e., 4 out of 5 amino acids) to the heavy chain CDR1 of SEQ ID NO: 7; a polypeptide having 100% (i.e., 5 out of 5 amino acids) similarity to the heavy chain CDR1 of SEQ ID NO: 7; a polypeptide having at least 56.2% sequence identity (i.e., 9 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 62.5% sequence identity (i.e., 10 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 68.7% sequence identity (i.e., 11 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 75% sequence identity (i.e., 12 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 81.2% sequence identity (i.e., 13 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 87.5% sequence identity (i.e., 14 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 93.7% sequence identity (i.e., 15 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having 100% (i.e., 16 out of 16 amino acids) similarity to the heavy chain CDR2 of SEQ ID NO: 120; a polypeptide having at least 50% sequence similarity (i.e., 6 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 58.3% sequence identity (i.e., 7 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 66.6% sequence identity (i.e., 8 out
of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 75% sequence identity (i.e., 9 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polypeptide having at least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; or a polypeptide having 100% (i.e., 12 out of 12 amino acids) similarity to the heavy chain CDR3 of SEQ ID NO: 9.

[0404] Other exemplary embodiments include at least one polynucleotides encoding any of the foregoing, e.g., a polynucleotide encoding a polypeptide that specifically binds to human IL-6 and includes one, two, three, four, five, six, or more of the following CDRs or variants thereof:

[0405] a polynucleotide encoding a polypeptide having at least 72.7% sequence identity (i.e., 8 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having at least 81.8% sequence identity (i.e., 9 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having at least 90.9% sequence identity (i.e., 10 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having 100% sequence identity to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having at least 85.7% sequence identity (i.e., 6 out of 7 amino acids) to the light chain CDR2 of SEQ ID NO: 5; a polynucleotide encoding a polypeptide having 100% sequence identity to the light chain CDR2 of SEQ ID NO: 5; a polynucleotide encoding a polypeptide having at least 50% sequence identity (i.e., 6 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 58.3% sequence identity (i.e., 7 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 66.6% sequence identity (i.e., 8 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 75% sequence identity (i.e., 9 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having 100% identity to the light chain CDR3 of SEQ ID NO: 6; a polynucleotide encoding a polypeptide having at least 80% sequence identity (i.e., 4 out of 5 amino acids) to the heavy chain CDR1 of SEQ ID NO: 7; a polynucleotide encoding a polypeptide having 100% identity to the heavy chain CDR1 of SEQ ID NO: 7; a polynucleotide encoding a polypeptide having at least 50% sequence identity (i.e., 8 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least
56.2% sequence identity (i.e., 9 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 62.5% sequence identity (i.e., 10 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 68.7% sequence identity (i.e., 11 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 75% sequence identity (i.e., 12 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 81.2% sequence identity (i.e., 13 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 87.5% sequence identity (i.e., 14 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 93.7% sequence identity (i.e., 15 out of 16 amino acids) to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having 100% identity to the heavy chain CDR2 of SEQ ID NO: 120; a polynucleotide encoding a polypeptide having at least 33.3% sequence identity (i.e., 4 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 41.6% (i.e., 5 out of 12 amino acids) identity to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 50% sequence identity (i.e., 6 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 58.3% sequence identity (i.e., 7 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 66.6% sequence identity (i.e., 8 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 75% sequence identity (i.e., 9 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 83.3% sequence identity (i.e., 10 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 91.6% sequence identity (i.e., 11 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9; a polynucleotide encoding a polypeptide having at least 90.9% sequence identity (i.e., 10 out of 11 amino acids) to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having 100% sequence similarity to the light chain CDR1 of SEQ ID NO: 4; a polynucleotide encoding a polypeptide having at least 85.7% sequence identity (i.e., 6 out of 7 amino acids) to the light chain CDR2 of SEQ ID NO: 5; a polynucleotide encoding a polypeptide having 100% sequence similarity to the light chain CDR2 of SEQ ID NO: 5; a polynucleotide encoding a polypeptide having at least 66.6% sequence identity (i.e., 8 out of 12 amino acids) to the light chain CDR3 of
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polypeptide having 100% sequence similarity (i.e., 12 out of 12 amino acids) to the heavy chain CDR3 of SEQ ID NO: 9.
Table 4: Sequences of exemplary anti-IL-6 antibodies.

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<tr>
<td>Ab24 heavy chain</td>
<td>379</td>
<td>387</td>
<td>383</td>
<td>391</td>
</tr>
<tr>
<td>Ab25 light chain</td>
<td>394</td>
<td>402</td>
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<td>404</td>
</tr>
<tr>
<td>Ab25 heavy chain</td>
<td>395</td>
<td>403</td>
<td>399</td>
<td>407</td>
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<tr>
<td>Ab26 light chain</td>
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</tr>
<tr>
<td>Ab26 heavy chain</td>
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<td>415</td>
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</tr>
<tr>
<td>Ab27 light chain</td>
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<td>434</td>
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<td>436</td>
</tr>
<tr>
<td>Ab27 heavy chain</td>
<td>427</td>
<td>435</td>
<td>431</td>
<td>439</td>
</tr>
<tr>
<td>Ab28 light chain</td>
<td>442</td>
<td>450</td>
<td>444</td>
<td>452</td>
</tr>
<tr>
<td>Ab28 heavy chain</td>
<td>443</td>
<td>451</td>
<td>447</td>
<td>455</td>
</tr>
<tr>
<td>Ab29 light chain</td>
<td>458</td>
<td>466</td>
<td>460</td>
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<tr>
<td>Ab29 heavy chain</td>
<td>459</td>
<td>467</td>
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<tr>
<td>Ab30 light chain</td>
<td>474</td>
<td>482</td>
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<td>484</td>
</tr>
<tr>
<td>Ab30 heavy chain</td>
<td>475</td>
<td>483</td>
<td>479</td>
<td>487</td>
</tr>
</tbody>
</table>
Exemplary sequence variant forms of heavy and light chains are shown on separate lines.

(PRT.: Polypeptide sequence  
Nuc.: Exemplary coding sequence)

[0407] For reference, sequence identifiers other than those included in Table 4 are summarized in Table 5.

**Table 5: Summary of sequence identifiers in this application.**

<table>
<thead>
<tr>
<th>SEQ ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Human IL-6</td>
</tr>
<tr>
<td>586</td>
<td>kappa constant light chain polypeptide sequence</td>
</tr>
<tr>
<td>587</td>
<td>kappa constant light chain polynucleotide sequence</td>
</tr>
<tr>
<td>588</td>
<td>gamma-1 constant heavy chain polypeptide sequence</td>
</tr>
<tr>
<td>589</td>
<td>gamma-1 constant heavy chain polynucleotide sequence</td>
</tr>
<tr>
<td>590-646</td>
<td>Human IL-6 peptides (Example 14)</td>
</tr>
<tr>
<td>719</td>
<td>gamma-1 constant heavy chain polypeptide sequence (differs from SEQ ID NO: 518 at two positions)</td>
</tr>
<tr>
<td>726</td>
<td>C-reactive protein polypeptide sequence</td>
</tr>
<tr>
<td>727</td>
<td>IL-6 receptor alpha</td>
</tr>
<tr>
<td>728</td>
<td>IL-6 receptor beta/gp130</td>
</tr>
</tbody>
</table>
Such antibody fragments or variants thereof may be present in at least one of the following non-limiting forms: Fab, Fab', F(ab')2, Fv and single chain Fv antibody forms. In a preferred embodiment, the anti-IL-6 antibodies described herein further comprises the kappa constant light chain sequence comprising the sequence set forth in the polypeptide sequence of SEQ ID NO: 586.

In another preferred embodiment, the anti-IL-6 antibodies described herein further comprises the gamma-1 constant heavy chain polypeptide sequence comprising one of the sequences set forth in the polypeptide sequence of SEQ ID NO: 588 and SEQ ID NO: 719.

Embodiments of antibodies described herein may include a leader sequence, such as a rabbit Ig leader, albumin pre-peptide, a yeast mating factor pre pro secretion leader sequence (such as P. pastoris or Saccharomyces cerevisiae a or alpha factor), or human HAS leader. Exemplary leader sequences are shown offset from FR1 at the N-terminus of polypeptides shown in Figs. 4A-B and 5A-B as follows: rabbit Ig leader sequences in SEQ ID NOS: 2 and 660 and SEQ ID NOS: 3 and 661; and an albumin prepeptide in SEQ ID NOS: 706 and 708, which facilitates secretion. Other leader sequences known in the art to confer desired properties, such as secretion, improved stability or half-life, may also be used, either alone or in combinations with one another, on the heavy and/or light chains, which may optionally be cleaved prior to administration to a subject. For example, a polypeptide may be expressed in a cell or cell-free expression system that also expresses or includes (or is modified to express or include) a protease, e.g., a membrane-bound signal peptidase, that cleaves a leader sequence.

In another embodiment, the invention contemplates an isolated anti-IL-6 antibody comprising a \( V_H \) polypeptide sequence comprising: SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86, 102, 117, 118, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 652, 656, 657, 658, 661, 664, 665, 668, 672, 676, 680, 684, 688, 691, 692, 704, or 708; and further comprising a \( V_L \) polypeptide sequence comprising: SEQ ID NO: 2, 20, 21, 37, 53, 69, 85, 101, 119, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 647, 651, 660, 666, 667, 671, 675, 679, 683, 687, 693, 699, 702, 706, or 709 or a variant thereof wherein at least one of the framework residues (FR residues) or CDR residues in said \( V_H \)\( V_L \) polypeptide has been substituted with another amino acid residue resulting in an anti-IL-6 antibody that specifically binds IL-6. The invention contemplates humanized and chimeric forms of these antibodies wherein preferably the FR will comprise human FRs highly homologous to the parent antibody. The chimeric antibodies may include an
Fc derived from IgGl, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgGlO, IgGl 1, IgG12, IgG13, IgG14, IgG15, IgG16, IgG17, IgG18 or IgG19 constant regions and in particular a variable heavy and light chain constant region as set forth in SEQ ID NO: 588 and SEQ ID NO: 586.

[0412] In one embodiment of the invention, the antibodies or V_H or V_L polypeptides originate or are selected from at least one rabbit B cell populations prior to initiation of the humanization process referenced herein.

[0413] In another embodiment of the invention, the anti-IL-6 antibodies and fragments and variants thereof have binding specificity for primate homologs of the human IL-6 protein. Non-limiting examples of primate homologs of the human IL-6 protein are IL-6 obtained from Macaca fascicularis (cynomolgus monkey) and the Rhesus monkey. In another embodiment of the invention, the anti-IL-6 antibodies and fragments and variants thereof inhibits the association of IL-6 with IL-6R, and/or the production of IL-6/IL-6R/gpl30 complexes and/or the production of IL-6/IL-6R/gpl30 multimers and/or antagonizes the biological effects of at least one of the foregoing.

Polyclonal Antibody

[0414] Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. Polyclonal antibodies which selectively bind the IL-6 may be made by methods well-known in the art. See, e.g., Howard & Kaser (2007) Making and Using Antibodies: A Practical Handbook CRC Press.

Monoclonal Antibody

Chimeric Antibody


Humanized Antibody

[0417] Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This may be accomplished by examining the sequence of the hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. See, e.g., U.S. Patent No. 6,187,287. Likewise, other methods of producing humanized antibodies are now well known in the art. See, e.g., U.S. Patent Nos. 5,225,539; 5,530,101; 5,585,089; 5,693,762; 6,054,297; 6,180,370; 6,407,213; 6,548,640; 6,632,927; and 6,639,055; Jones, et al. (1986) Nature 321: 522-525; Reichmann, et al. (1988) Nature 332: 323-327; Verhoyen, et al. (1988) Science 239: 1534-36; and Zhiqiang An (2009) [Ed.] Therapeutic Monoclonal Antibodies: From Bench to Clinic John Wiley & Sons, Inc.

Antibody Fragments (antigen-binding fragments)

[0418] In addition to entire immunoglobulins (or their recombinant counterparts), immunoglobulin fragments comprising the epitope binding site (e.g., Fab’, F(ab’)2, or other fragments) may be synthesized. "Fragment," or minimal immunoglobulins may be designed utilizing recombinant immunoglobulin techniques. For instance "Fv" immunoglobulins for use in the present invention may be produced by synthesizing a fused variable light chain region and a variable heavy chain region. Combinations of antibodies are also of interest, e.g. diabodies, which comprise two distinct Fv specificities. Antigen-binding fragments of immunoglobulins
include but are not limited to SMIPs (small molecule immunopharmaceuticals), camelbodies, nanobodies, and IgNAR. Further, antigen-binding fragments may comprise the epitope binding site and have the same antigen binding selectivity as the antibody.

**Anti-idiotypic Antibody**

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody may be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the antibody with the antibody to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See e.g., U.S. Patent No. 4,699,880. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original antibody which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of an antibody it is possible to identify other clones expressing antibodies of identical specificity.

**Engineered And Modified Antibodies**

An antibody of the invention further may be prepared using an antibody having at least one of the VH and/or VL sequences derived from an antibody starting material to engineer a modified antibody, which modified antibody may have altered properties from the starting antibody. An antibody may be engineered by modifying at least one residues within one or both variable regions (i.e., VH and/or VL), for example within at least one CDR regions and/or within at least one framework regions. Additionally or alternatively, an antibody may be engineered by
modifying residues within the constant region(s), for example to alter the effector function(s) of the antibody.

One type of variable region engineering that may be performed is CDR grafting. Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties. See, e.g., Riechmann, et al. (1998) Nature 332: 323-327; Jones, et al. (1986) Nature 321: 522-525; Queen, et al. (1989) Proc. Natl. Acad. U.S.A. 86: 10029-10033; U.S. Patent Nos. 5,225,539; 5,530,101; 5,585,089; 5,693,762; and 6,180,370.


Another type of variable region modification is to mutate amino acid residues within the VH and/or VL CDR 1, CDR2 and/or CDR3 regions to thereby improve at least one binding property (e.g., affinity) of the antibody of interest. Site-directed mutagenesis or PCR-mediated mutagenesis may be performed to introduce the mutation(s) and the effect on antibody binding, or other functional property of interest, may be evaluated in appropriate in vitro or in vivo assays. Preferably conservative modifications (as discussed herein) may be introduced. The mutations may be amino acid substitutions, additions or deletions, but are preferably substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are altered.

Engineered antibodies of the invention include those in which modifications have been made to framework residues within VH and/or VL, e.g. to improve the properties of the antibody. Typically such framework modifications are made to decrease the immunogenicity of the
antibody. For example, one approach is to "backmutate" at least one framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues may be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived.

In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter at least one functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (e.g., at least one chemical moieties may be attached to the antibody) or be modified to alter its glycosylation, again to alter at least one functional properties of the antibody. Such embodiments are described further below. The numbering of residues in the Fc region is that of the EU index of Kabat.

The hinge region of CHI may be modified such that the number of cysteine residues in the hinge region is altered, e.g., increased or decreased. See U.S. Patent No. 5,677,425. The number of cysteine residues in the hinge region of CHI may be altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody. The Fc hinge region of an antibody may be mutated to decrease the biological half life of the antibody. More specifically, at least one amino acid mutations may be introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired *Staphylococcus* protein A (SpA) binding relative to native Fc-hinge domain SpA binding. See, e.g., U.S. Patent No. 6,165,745.

The antibody may be modified to increase its biological half life. Various approaches are possible. For example, at least one of the following mutations may be introduced: T252L, T254S, T256F. See U.S. Patent No. 6,277,375. Alternatively, to increase the biological half life, the antibody may be altered within the CHI or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG. See U.S. Patent Nos. 5,869,046 and 6,121,022.

The Fc region may be altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector function(s) of the antibody. For example, at least one amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322 may be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector
ligand to which affinity may be altered may be, for example, an Fc receptor or the C1 component of complement. See U.S. Patent Nos. 5,624,821 and 5,648,260.

[0430] The Fc region may be modified to increase the affinity of the antibody for an Fcy receptor by modifying at least one amino acids at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 301, 303, 305, 307, 312, 315, 320, 322, 324, 326, 327, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438 or 439. See WO 00/42072. Moreover, the binding sites on human IgGl for FcyRI, FcyRII, FcyRIII and FcRn have been mapped and variants with improved binding. See Shields, et al. (2001) J. Biol. Chem. 276: 6591-6604. Specific mutations at positions 256, 290, 298, 333, 334 and 339 are shown to improve binding to FcyRII. Additionally, the following combination mutants are shown to improve FcyRIII binding: T256A/S298A, S298A/E333A, S298A/K224A and S298A/E333A/K334A.

[0431] The glycosylation of an antibody may be modified. For example, an aglycoslated antibody may be made (i.e., the antibody lacks glycosylation). Glycosylation may be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications may be accomplished by, for example, altering at least one sites of glycosylation within the antibody sequence. For example, at least one amino acid substitutions may be made that result in elimination of at least one variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. See, e.g., U.S. Patent Nos. 5,714,350 and 6,350,861.

[0432] Additionally or alternatively, an antibody may be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such carbohydrate modifications may be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and may be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See U.S. Patent Application Publication No. 2004/01 10704 and Yamane-Ohnuki, et al. (2004) Biotechnol Bioeng. 87: 614-22; EP 1,176,195; WO 2003/035835; Shields, et al. (2002) J. Biol. Chem. 277: 26733-26740; WO 99/54342; Umana, et al. (1999) Nat. Biotech. 17: 176-180; and Tarentino, et al. (1975) Biochem. 14: 5516-23.

[0433] An antibody may be pegylated to, for example, increase the biological (e.g., serum) half life of the antibody. To pegylate an antibody, the antibody, or fragment thereof, typically is
reacted with polyethylene glycol (PEG), such as a reactive ester or aldehyde derivative of PEG, under conditions in which at least one PEG groups become attached to the antibody or antibody fragment. Preferably, the pegylation is carried out via an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer).

[0434] The invention also provides variants and equivalents that are substantially homologous to the antibodies, antibody fragments, diabodies, SMIPs, camelbodies, nanobodies, IgNAR, polypeptides, variable regions and CDRs set forth herein. These may contain, e.g., conservative substitution mutations, (i.e., the substitution of at least one amino acids by similar amino acids). For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, e.g., one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid. In another embodiment, the invention further contemplates the above-recited polypeptide homologs of the antibody fragments, variable regions and CDRs set forth herein further having anti-IL-6 activity. Non-limiting examples of anti-IL-6 activity are set forth herein, for example, under the heading "Anti-IL-6 Activity," infra.


Polypeptide Sequence Variants

[0436] For any anti-IL-6 antibodies sequence described herein, further characterization or optimization may be achieved by systematically either adding or removing amino acid residues to generate longer or shorter peptides, and testing those and sequences generated by walking a window of the longer or shorter size up or down the antigen from that point. Coupling this approach to generating new candidate targets with testing for effectiveness of antigenic molecules based on those sequences in an immunogenicity assay, as known in the art or as described herein, may lead to further manipulation of the antigen. Further still, such optimized sequences may be adjusted by, e.g., the addition, deletions, or other mutations as known in the art and/or discussed herein to further optimize the anti-IL-6 antibodies (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing delivery, enhance immunogenicity, increasing solubility, targeting to a particular in vivo location or cell type).
In another embodiment, the invention contemplates polypeptide sequences having at least about 90% sequence homology to any at least one of the polypeptide sequences of antibody fragments, variable regions and CDRs set forth herein. More preferably, the invention contemplates polypeptide sequences having at least about 95% sequence homology, even more preferably at least about 98% sequence homology, and still more preferably at least about 99% sequence homology to any at least one of the polypeptide sequences of antibody fragments, variable regions and CDRs set forth herein. Methods for determining homology between nucleic acid and amino acid sequences are well known to those of ordinary skill in the art.

The anti-IL-6 antibodies polypeptides described herein may comprise conservative substitution mutations, (i.e., the substitution of at least one amino acids by similar amino acids). For example, conservative substitution refers to the substitution of an amino acid with another within the same general class, e.g., one acidic amino acid with another acidic amino acid, one basic amino acid with another basic amino acid, or one neutral amino acid by another neutral amino acid.

Anti-IL-6 antibodies polypeptide sequences may have at least about 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.5, 99, 99.5, 99.8, 99.9, or 100% sequence homology to any at least one of the polypeptide sequences set forth herein. More preferably, the invention contemplates polypeptide sequences having at least about 95% sequence homology, even more preferably at least about 98% sequence homology, and still more preferably at least about 99% sequence homology to any at least one of the polypeptide sequences of Anti-IL-6 antibodies polypeptide sequences set forth herein. Methods for determining homology between amino acid sequences, as well as nucleic acid sequences, are well known to those of ordinary skill in the art. See, e.g., Nedelkov & Nelson (2006) New and Emerging Proteomic Techniques Humana Press. Thus, an anti-IL-6 antibodies polypeptide may have at least about 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 98.5, 99, 99.5, 99.8, 99.9, or 100% sequence homology with a polypeptide sequence.

The term homology, or identity, is understood as meaning the number of agreeing amino acids (identity) with other proteins, expressed in percent. The identity is preferably determined by comparing a given sequence with other proteins with the aid of computer programs. If sequences which are compared with each other are different in length, the identity is to be determined in such a way that the number of amino acids which the short sequence shares with the longer sequence determines the percentage identity. The identity can be determined routinely by means of known computer programs which are publicly available such as, for example, Clustal W. Thompson, et al. (1994) Nucleic Acids Research 22: 4673-4680. ClustalW
is publicly available from the European Molecular Biology Laboratory and may be downloaded
from various internet pages, *inter alia* the IGBMC (Institut de Genetique et de Biologie
Moleculaire et Cellulaire) and the EBI and all mirrored EBI internet pages (European
Bioinformatics Institute). If the ClustalW computer program Version 1.8 is used to determine
the identity between, for example, the reference protein of the present application and other proteins,
the following parameters are to be set: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3,
GAOPEN=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET,
ENDGAPS(OFF), NOPGAP, NOHGAP. *See also* European Bioinformatics Institute (EBI)

One possibility of finding similar sequences is to carry out sequence database
researches. Here, at least one sequences may be entered as what is known as a query. This query
sequence is then compared with sequences present in the selected databases using statistical
computer programs. Such database queries (blast searches) are known to the skilled worker and
may be carried out at different suppliers. If, for example, such a database query is carried out at the
NCBI (National Center for Biotechnology Information), the standard settings for the respective
comparison query should be used. For protein sequence comparisons (blastp), these settings are:
Limit entrez = not activated; Filter = low complexity activated; Expect value = 10; word size = 3;
Matrix = BLOSUM62; Gap costs: Existence = 11, Extension = 1. The result of such a query is,
among other parameters, the degree of identity between the query sequence and the similar
sequences found in the databases. Methods and materials for making fragments of Anti-IL-6
antibodies polypeptides are well known in the art. *See, e.g.*, Maniatis, *et al.* (2001) Molecular

Variant anti-IL-6 antibodies polypeptides may retain their antigenic specificity to bind
IL-6. Fully specific variants may contain only conservative variations or variations in non-critical
residues or in non-critical regions. Variants may also contain substitution of similar amino acids
that result in no change or an insignificant change in their specificity. Alternatively, such
substitutions may positively or negatively affect specificity to some degree. Non-specific
variants typically contain at least one non-conservative amino acid substitutions, deletions,
insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical
residue or critical region of an epitope. Molecular biology and biochemistry techniques for
modifying anti-IL-6 antibodies polypeptides while preserving specificity are well known in the
Amino acids that are essential for function may be identified by methods known in the
art, such as site-directed mutagenesis or alanine-scanning mutagenesis. Cunningham, et al.
(1989) Sci. 244: 1081-85. The latter procedure introduces single alanine mutations at every
residue in the molecule. The resulting mutant molecules are then tested for biological activity
such as epitope binding. Sites that are critical for ligand-receptor binding may also be determined
by structural analysis such as crystallography, nuclear magnetic resonance, or photoaffinity

For example, one class of substitutions is conserved amino acid substitutions. Such
substitutions are those that substitute a given amino acid in a Anti-IL-6 antibodies polypeptide
with another amino acid of like characteristics. Typically seen as conservative substitutions are
the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and He:
interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu,
substitution between the amide residues Asn and Gin, exchange of the basic residues Lys and
Arg, replacements among the aromatic residues Phe, Tyr. Guidance concerning which amino
acid changes are likely to be phenotypically silent is found in, for example, Bowie, et al. (1990)
Sci. 247: 1306-10. Hence, one of ordinary skill in the art appreciates that the inventors possess
peptide variants without delineation of all the specific variants. As to amino acid sequences, one
of skill will recognize that individual substitutions, deletions or additions to a nucleic acid,
peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a
small percentage of amino acids in the encoded sequence is a "conservatively modified variant"
where the alteration results in the substitution of an amino acid with a chemically similar amino
acid. Conservative substitution tables providing functionally similar amino acids are well known
in the art. Such conservatively modified variants are in addition to and do not exclude
polymorphic variants, interspecies homologs, and alleles of the invention. See, e.g., Creighton

Moreover, polypeptides often contain amino acids other than the twenty "naturally
occurring" amino acids. Further, many amino acids, including the terminal amino acids, may be
modified by natural processes, such as processing and other post-translational modifications, or
by chemical modification techniques well known in the art. Known modifications include, but
are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of
flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide
derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of

[0446] In another embodiment, the invention further contemplates the generation and use of anti-idiotypic antibodies that bind any of the foregoing sequences. In an exemplary embodiment, such an anti-idiotypic antibody could be administered to a subject who has received an anti-IL-6 antibody to modulate, reduce, or neutralize, the effect of the anti-IL-6 antibody. A further exemplary use of such anti-idiotypic antibodies is for detection of the anti-IL-6 antibodies of the present invention, for example to monitor the levels of the anti-IL-6 antibodies present in a subject's blood or other bodily fluids.

[0447] The present invention also contemplates anti-IL-6 antibodies comprising any of the polypeptide or polynucleotide sequences described herein substituted for any of the other polynucleotide sequences described herein. For example, without limitation thereto, the present invention contemplates antibodies comprising the combination of any of the variable light chain and variable heavy chain sequences described herein, and further contemplates antibodies resulting from substitution of any of the CDR sequences described herein for any of the other CDR sequences described herein. As noted preferred anti-IL-6 antibodies or fragments or variants thereof may contain a variable heavy and/or light sequence as shown in FIG. 2-5, such as SEQ ID NO: 651, 657, 709 or variants thereof wherein at least one CDR or FR residues are modified without adversely affecting antibody binding to IL-6 or other desired functional activity.

POLYNUCLEOTIDES ENCODING ANTI-IL-6 ANTIBODY POLYPEPTIDES

[0448] The invention is further directed to polynucleotides encoding polypeptides of the antibodies having binding specificity to IL-6. In one embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable light chain polypeptide sequence of SEQ ID NO: 2 which is encoded by the polynucleotide sequence of SEQ ID NO: 10 or the polynucleotide sequence of SEQ ID NO: 662, 698, 701, or 705.
In another embodiment of the invention, polynucleotides of the invention comprise, or alternatively consist of, the following polynucleotide sequence encoding the variable heavy chain polypeptide sequence of SEQ ID NO: 3 which is encoded by the polynucleotide sequence of SEQ ID NO: 11 or the polynucleotide sequence of SEQ ID NO: 663, 700, 703, or 707.

In a further embodiment of the invention, polynucleotides encoding fragments or variants of the antibody having binding specificity to IL-6 comprise, or alternatively consist of, at least one of the polynucleotide sequences of SEQ ID NO: 12 or 694; SEQ ID NO: 13; and SEQ ID NO: 14 or 695 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the light chain variable sequence of SEQ ID NO: 2.

In a further embodiment of the invention, polynucleotides encoding fragments or variants of the antibody having binding specificity to IL-6 comprise, or alternatively consist of, at least one of the polynucleotide sequences of SEQ ID NO: 15; SEQ ID NO: 16 or 696; and SEQ ID NO: 17 or 697 which correspond to polynucleotides encoding the complementarity-determining regions (CDRs, or hypervariable regions) of the heavy chain variable sequence of SEQ ID NO: 3 or SEQ ID NO: 661 or SEQ ID NO: 657 or others depicted in Figs. 4 or 5.

The invention also contemplates polynucleotide sequences including at least one of the polynucleotide sequences encoding antibody fragments or variants described herein. In one embodiment of the invention, polynucleotides encoding fragments or variants of the antibody having binding specificity to IL-6 comprise, or alternatively consist of, one, two, three or more, including all of the following polynucleotides encoding antibody fragments: the polynucleotide SEQ ID NO: 10 encoding the light chain variable region of SEQ ID NO: 2; the polynucleotide SEQ ID NO: 11 encoding the heavy chain variable region of SEQ ID NO: 3; the polynucleotide SEQ ID NO: 720 encoding the light chain polypeptide of SEQ ID NO: 20; the polynucleotide SEQ ID NO: 721 encoding the light chain polypeptide of SEQ ID NO: 647; the polynucleotide SEQ ID NO: 662 encoding the light chain polypeptide of SEQ ID NO: 660; the polynucleotide SEQ ID NO: 722 encoding the light chain polypeptide of SEQ ID NO: 666; the polynucleotide SEQ ID NO: 698 encoding the light chain polypeptide of SEQ ID NO: 699; the polynucleotide SEQ ID NO: 701 encoding the light chain polypeptide of SEQ ID NO: 702; the polynucleotide SEQ ID NO: 705 encoding the light chain polypeptide of SEQ ID NO: 706; the polynucleotide SEQ ID NO: 723 encoding the light chain polypeptide of SEQ ID NO: 709; the polynucleotide SEQ ID NO: 724 encoding the heavy chain polypeptide of SEQ ID NO: 19; the polynucleotide SEQ ID NO: 725 encoding the heavy chain polypeptide of SEQ ID NO: 652; the polynucleotide SEQ ID NO: 700 encoding the heavy chain polypeptide of SEQ ID NO: 657; the polynucleotide
SEQ ID NO: 663 encoding the heavy chain polypeptide of SEQ ID NO: 661; the polynucleotide
SEQ ID NO: 703 encoding the heavy chain polypeptide of SEQ ID NO: 704; the polynucleotide
SEQ ID NO: 707 encoding the heavy chain polypeptide of SEQ ID NO: 708; the polynucleotides
of SEQ ID NO: 12, 13, 14, 694 and 695 encoding the complementarity-determining regions of the
aforementioned light chain polypeptides; and the polynucleotides of SEQ ID NO: 15, 16, 17, 696
and 697 encoding the complementarity-determining regions of the aforementioned heavy chain
polypeptides, and polynucleotides encoding the variable heavy and light chain sequences in SEQ
ID NO: 657 and SEQ ID NO: 709 respectively, e.g., the nucleic acid sequences in SEQ ID NO:
700 and SEQ ID NO: 723 and fragments or variants thereof, e.g., based on codon degeneracy.
These nucleic acid sequences encoding variable heavy and light chain sequences may be
expressed alone or in combination and these sequences preferably are fused to suitable variable
constant sequences, e.g., those in SEQ ID NO: 589 and SEQ ID NO: 587.

[0453] Exemplary nucleotide sequences encoding anti-IL-6 antibodies of the present invention
are identified in Table 4. The polynucleotide sequences shown are to be understood to be
illustrative, rather than limiting. One of skill in the art can readily determine the polynucleotide
sequences that would encode a given polypeptide and can readily generate coding sequences
suitable for expression in a given expression system, such as by adapting the polynucleotide
sequences provided and/or by generating them de novo, and can readily produce codon-optimized
expression sequences, for example as described in published U.S. Patent Application No.
2008/0120732 or using other methods known in the art.

[0454] In another embodiment of the invention, polynucleotides of the invention further
comprise, the following polynucleotide sequence encoding the kappa constant light chain
sequence of SEQ ID NO: 586 which is encoded by the polynucleotide sequence of SEQ ID NO:
587.

[0455] In another embodiment of the invention, polynucleotides of the invention further
comprise, the following polynucleotide sequence encoding the gamma-1 constant heavy chain
polypeptide sequence of SEQ ID NO: 588 which is encoded by the polynucleotide sequence of
SEQ ID NO: 589.

[0456] In one embodiment, the invention is directed to an isolated polynucleotide comprising a
polynucleotide encoding an anti-IL-6 V_H antibody amino acid sequence selected from SEQ ID
NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, and 708 or encoding a variant thereof
wherein at least one framework residue (FR residue) has been substituted with an amino acid
present at the corresponding position in a rabbit anti-IL-6 antibody V_H polypeptide or a
conservative amino acid substitution. In addition, the invention specifically encompasses
humanized anti-IL-6 antibodies or humanized antibody binding fragments or variants thereof and nucleic acid sequences encoding the foregoing comprising the humanized variable heavy chain and/or light chain polypeptides depicted in the sequences contained in FIG. 1-5, or those identified in Table 4, or variants thereof wherein at least one framework or CDR residues may be modified. Preferably, if any modifications are introduced they will not affect adversely the binding affinity of the resulting anti-IL-6 antibody or fragment or variant thereof.

[0457] In another embodiment, the invention is directed to an isolated polynucleotide comprising the polynucleotide sequence encoding an anti-IL-6 V_{L} antibody amino acid sequence selected from SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, and 709 or encoding a variant thereof wherein at least one framework residue (FR residue) has been substituted with an amino acid present at the corresponding position in a rabbit anti-IL-6 antibody V_{L} polypeptide or a conservative amino acid substitution.

[0458] In yet another embodiment, the invention is directed to at least one heterologous polynucleotides comprising a sequence encoding the polypeptides set forth in SEQ ID NO: 2 and SEQ ID NO: 3; SEQ ID NO: 2 and SEQ ID NO: 18; SEQ ID NO: 2 and SEQ ID NO: 19; SEQ ID NO: 20 and SEQ ID NO: 3; SEQ ID NO: 20 and SEQ ID NO: 18; or SEQ ID NO: 20 and SEQ ID NO: 19.

[0459] In another embodiment, the invention is directed to an isolated polynucleotide that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-IL-6 antibody wherein said expressed polypeptide alone specifically binds IL-6 or specifically binds IL-6 when expressed in association with another polynucleotide sequence that expresses a polypeptide containing at least one CDR polypeptide derived from an anti-IL-6 antibody wherein said at least one CDR is selected from those contained in the V_{L} or V_{H} polypeptides set forth in SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, 708, 2, 20, 647, 651, 660, 666, 699, 702, 706, or 709.

[0460] Host cells and vectors comprising said polynucleotides are also contemplated.

[0461] In another specific embodiment the invention covers nucleic acid constructs containing any of the foregoing nucleic acid sequences and combinations thereof as well as recombinant cells containing these nucleic acid sequences and constructs containing wherein these nucleic acid sequences or constructs may be extrachromosomal or integrated into the host cell genome.

[0462] The invention further contemplates vectors comprising the polynucleotide sequences encoding the variable heavy and light chain polypeptide sequences, as well as the individual complementarity determining regions (CDRs, or hypervariable regions) set forth herein, as well as host cells comprising said sequences. In one embodiment of the invention, the host cell is a
yeast cell. In another embodiment of the invention, the yeast host cell belongs to the genus *Pichia*.

[0463] In some instances, more than one exemplary polynucleotide encoding a given polypeptide sequence is provided, as summarized in Table 5.

**Table 5** Multiple exemplary polynucleotides encoding particular polypeptides.

<table>
<thead>
<tr>
<th>Polypeptide SEQ ID NO</th>
<th>Exemplary coding SEQ ID NOs</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>12, 111, 694</td>
</tr>
<tr>
<td>5</td>
<td>13, 112, 389, 501</td>
</tr>
<tr>
<td>6</td>
<td>14, 113, 695</td>
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<tr>
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<td>72</td>
<td>80, 325, 565, 581</td>
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<td>89</td>
<td>97, 134, 166</td>
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<tr>
<td>103</td>
<td>12, 111, 694</td>
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<td>104</td>
<td>13, 112, 389, 501</td>
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<td>108</td>
<td>17, 116, 697</td>
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<td>126</td>
<td>97, 134, 166</td>
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<td>97, 134, 166</td>
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<td>198, 214</td>
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<td>484, 500</td>
</tr>
<tr>
<td>493</td>
<td>13, 112, 389, 501</td>
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</tbody>
</table>
In some instances, multiple sequence identifiers refer to the same polypeptide or polynucleotide sequence, as summarized in Table 6. References to these sequence identifiers are understood to be interchangeable, except where context indicates otherwise.

**Table 6** Repeated sequences. Each cell lists a group of repeated sequences included in the sequence listing.

<table>
<thead>
<tr>
<th>Polypeptide SEQ ID NO</th>
<th>Exemplary coding SEQ ID NOs</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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<td>556</td>
<td>564, 580</td>
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<tr>
<td>557</td>
<td>80, 325, 565, 581</td>
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<td>558</td>
<td>566, 582</td>
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<tr>
<td>570</td>
<td>562, 578</td>
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<tr>
<td>572</td>
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<tr>
<td>574</td>
<td>566, 582</td>
</tr>
<tr>
<td>577</td>
<td>553, 585</td>
</tr>
</tbody>
</table>

**SEQ ID NOs of repeated sequences**

- 4, 103
- 5, 104, 381, 493
- 6, 105
- 9, 108
- 12, 111
- 13, 112
- 14, 113
- 17, 116
- 39, 252
- 40, 253
- 48, 261
- 60, 257
- 68, 265
- 72, 317, 557, 573
- 80, 325, 565, 581
- 89, 126, 158
- 97, 134, 166
- 120, 659
- 190, 206
- 191, 207
- 198, 214
- 199, 215
Certain exemplary embodiments include polynucleotides that hybridize under moderately or highly stringent hybridization conditions to a polynucleotide having one of the exemplary coding sequences recited in Table 4, and also include polynucleotides that hybridize under moderately or highly stringent hybridization conditions to a polynucleotide encoding the same polypeptide as a polynucleotide having one of the exemplary coding sequences recited in Table 4, or polypeptide encoded by any of the foregoing polynucleotides.

The phrase "high stringency hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. High stringency conditions are sequence dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, high stringency conditions are selected to be about 5-10°C lower than the thermal melting point ($T_m$) for the specific sequence at a defined ionic strength pH. The $T_m$ is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the

<table>
<thead>
<tr>
<th>SEQ ID NOs of repeated sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>205, 461, 477</td>
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<tr>
<td>213, 469</td>
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<tr>
<td>564, 580</td>
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<tr>
<td>566, 582</td>
</tr>
</tbody>
</table>
probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). High stringency conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). High stringency conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary high stringency hybridization conditions can be as following: 50% formamide, 5×SSC, and 1% SDS, incubating at 42 °C, or, 5×SSC, 1% SDS, incubating at 65°C, with wash in 0.2×SSC, and 0.1% SDS at 65°C. Such hybridizations and wash steps can be carried out for, e.g., 1, 2, 5, 10, 15, 30, 60; or more minutes.

Nucleic acids that do not hybridize to each other under high stringency conditions are still substantially related if the polypeptides that they encode are substantially related. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderate stringency hybridization conditions. Exemplary "moderate stringency hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1×SSC at 45°C. Such hybridizations and wash steps can be carried out for, e.g., 1, 2, 5, 10, 15, 30, 60, or more minutes. A positive hybridization is a signal positive. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

Expression vectors for use in the methods of the invention will further include yeast specific sequences, including a selectable auxotrophic or drug marker for identifying transformed yeast strains. A drug marker may further be used to amplify copy number of the vector in a yeast host cell.

The polypeptide coding sequence of interest is operably linked to transcriptional and translational regulatory sequences that provide for expression of the polypeptide in yeast cells. These vector components may include, but are not limited to, at least one of the following: an enhancer element, a promoter, and a transcription termination sequence. Sequences for the secretion of the polypeptide may also be included, e.g. a signal sequence. A yeast origin of replication is optional, as expression vectors are often integrated into the yeast genome.

In one embodiment of the invention, the polypeptide of interest is operably linked, or fused, to sequences providing for optimized secretion of the polypeptide from yeast diploid cells.
Nucleic acids are "operably linked" when placed into a functional relationship with another nucleic acid sequence. For example, DNA for a signal sequence is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites or alternatively via a PCR/recombination method familiar to those skilled in the art (Gateway® Technology; Invitrogen, Carlsbad California). If such sites do not exist, the synthetic oligonucleotide adapters or linkers are used in accordance with conventional practice.

Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequences to which they are operably linked. Such promoters fall into several classes: inducible, constitutive, and repressible promoters (that increase levels of transcription in response to absence of a repressor). Inducible promoters may initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature.

The yeast promoter fragment may also serve as the site for homologous recombination and integration of the expression vector into the same site in the yeast genome; alternatively a selectable marker is used as the site for homologous recombination. Pichia transformation is described in Cregg, et al. (1985) Mol. Cell. Biol. 5:3376-3385.


Other yeast promoters include ADH1, alcohol dehydrogenase II, GAL4, PH03, PH05, Pyk, and chimeric promoters derived therefrom. Additionally, non-yeast promoters may be used in the invention such as mammalian, insect, plant, reptile, amphibian, viral, and avian promoters. Most typically the promoter will comprise a mammalian promoter (potentially endogenous to the
expressed genes) or will comprise a yeast or viral promoter that provides for efficient transcription in yeast systems.

[0476] The polypeptides of interest may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g., a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed through one of the standard pathways available within the host cell. The *S. cerevisiae* alpha factor pre-pro signal has proven effective in the secretion of a variety of recombinant proteins from *P. pastoris*. Other yeast signal sequences include the alpha mating factor signal sequence, the invertase signal sequence, and signal sequences derived from other secreted yeast polypeptides. Additionally, these signal peptide sequences may be engineered to provide for enhanced secretion in diploid yeast expression systems. Other secretion signals of interest also include mammalian signal sequences, which may be heterologous to the protein being secreted, or may be a native sequence for the protein being secreted. Signal sequences include pre-peptide sequences, and in some instances may include propeptide sequences. Many such signal sequences are known in the art, including the signal sequences found on immunoglobulin chains, e.g., K28 preprotoxin sequence, PHA-E, FACE, human MCP-1, human serum albumin signal sequences, human Ig heavy chain, human Ig light chain, and the like. See Hashimoto, *et al.* (1998) *Protein Eng.* 11(2): 75; and Kobayashi, *et al.* (1998) *Therapeutic Apheresis* 2(4): 257.

[0477] Transcription may be increased by inserting a transcriptional activator sequence into the vector. These activators are cis-acting elements of DNA, usually about from 10 to 300 bp, which act on a promoter to increase its transcription. Transcriptional enhancers are relatively orientation and position independent, having been found 5’ and 3’ to the transcription unit, within an intron, as well as within the coding sequence itself. The enhancer may be spliced into the expression vector at a position 5’ or 3’ to the coding sequence, but is preferably located at a site 5’ from the promoter.

[0478] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from 3’ to the translation termination codon, in untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA.
Construction of suitable vectors containing at least one of the above-listed components employs standard ligation techniques or PCR/recombination methods. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required or via recombination methods. For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform host cells, and successful transformants selected by antibiotic resistance (*e.g.* ampicillin or Zeocin® (phleomycin)) where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion and/or sequenced.

As an alternative to restriction and ligation of fragments, recombination methods based on att sites and recombination enzymes may be used to insert DNA sequences into a vector. Such methods are described, for example, by Landy (1989) *Ann. Rev. Biochem.* 58: 913-949; and are known to those of skill in the art. Such methods utilize intermolecular DNA recombination that is mediated by a mixture of lambda and *E.coli* -encoded recombination proteins. Recombination occurs between specific attachment (*att*) sites on the interacting DNA molecules. For a description of att sites see Weisberg and Landy (1983) *Site-Specific Recombination in Phage Lambda* Cold Spring Harbor, NY: Cold Spring Harbor Press, pages 211-250. The DNA segments flanking the recombination sites are switched, such that after recombination, the *att* sites are hybrid sequences comprised of sequences donated by each parental vector. The recombination can occur between DNAs of any topology.

*Att* sites may be introduced into a sequence of interest by ligating the sequence of interest into an appropriate vector; generating a PCR product containing *att B* sites through the use of specific primers; generating a cDNA library cloned into an appropriate vector containing *att* sites.

The expression host may be further modified by the introduction of sequences encoding at least one enzymes that enhance folding and disulfide bond formation, *i.e.* foldases, chaperonins. Such sequences may be constitutively or inducibly expressed in the yeast host cell, using vectors, markers, are known in the art. Preferably the sequences, including transcriptional regulatory elements sufficient for the desired pattern of expression, are stably integrated in the yeast genome through a targeted methodology.

For example, the eukaryotic PDI is not only an efficient catalyst of protein cysteine oxidation and disulfide bond isomerization, but also exhibits chaperone activity. Co-expression of PDI can facilitate the production of active proteins having multiple disulfide bonds. Also of interest is the expression of BIP (immunoglobulin heavy chain binding protein); cyclophilin; and the like. In one embodiment of the invention, each of the haploid parental strains expresses a
distinct folding enzyme, e.g. one strain may express BIP, and the other strain may express PDI or combinations thereof.

[0484] Vectors are used to introduce a foreign substance, such as DNA, RNA or protein, into an organism or host cell. Typical vectors include recombinant viruses (for polynucleotides) and liposomes or other lipid aggregates (for polypeptides and/or polynucleotides). A "DNA vector" is a replicon, such as plasmid, phage or cosmids, to which another polynucleotide segment may be attached so as to bring about the replication of the attached segment. An "expression vector" is a DNA vector which contains regulatory sequences which will direct polypeptide synthesis by an appropriate host cell. This usually means a promoter to bind RNA polymerase and initiate transcription of mRNA, as well as ribosome binding sites and initiation signals to direct translation of the mRNA into a polypeptide(s). Incorporation of a polynucleotide sequence into an expression vector at the proper site and in correct reading frame, followed by transformation of an appropriate host cell by the vector, enables the production of a polypeptide encoded by said polynucleotide sequence. Exemplary expression vectors and techniques for their use are described in the following publications: Old, et al. (1989) *Principles of Gene Manipulation: An Introduction to Genetic Engineering,* Blackwell Scientific Publications [4th Ed.]; Sambrook, et al. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press; Sambrook, et al. (2001) *Molecular Cloning: A Laboratory Manual* [3rd Ed.] Cold Spring Harbor Laboratory Press; Gorman, "High Efficiency Gene Transfer into Mammalian Cells," in DNA Cloning, Volume II, Glover, D. M., Ed., IRL Press, Washington, D.C., pages 143-190.

[0485] For example, a liposomes or other lipid aggregate may comprise a lipid such as phosphatidylcholines (lecithins) (PC), phosphatidylethanolamines (PE), lysolecithins, lysophosphatidylethanolamines, phosphatidylserines (PS), phosphatidylglycerols (PG), phosphatidylinositols (PI), sphingomyelins, cardiolipin, phosphatidic acids (PA), fatty acids, gangliosides, glucolipids, glycolipids, mono- di or triglycerides, ceramides, cerebrosides and combinations thereof; a cationic lipid (or other cationic amphiphile) such as 1,2-dioleoyloxy-3-trimethylamino) propane (DOTAP); N-cholesteryloxy-carbaryl-3,7,12-triazapentadecane-1,15-diamine (CTAP); N-[l-(2,3, -ditetradecyloxy)propyl]-N,N-dimethyl-N-hydroxyethylammonium bromide (DMRIE); N-[l-(2,3,-dioleloyloxy)propyl]-N,N-dimethyl-N-hydroxy ethylammonium bromide (DORIE); N-[l-(2,3-dioleloyloxy) propyl]-N,N,N-trimethylammonium chloride (DOTMA); 3 beta [N-(N',N'-dimethylaminoethane) carbamoly] cholesterol (DC-Choi); and dimethylidioctadecylammonium (DDAB); dioleylphosphatidyl ethanolamine (DOPE), cholesterol-containing DOPC; and combinations thereof; and/or a hydrophilic polymer such as polyvinylpyrrolidone, polyvinylmethylene, polymethyloxazoline, polyethyloxazoline,
polyhydroxypropyloxazoline, polyhydroxypropylmethacrylamide, polymethacrylamide, polydimethylacrylamide, polyhydroxypropylmethacrylate, polyhydroxyethylacrylate, hydroxyethylcellulose, hydroxyethylcellulose, polyethylene glycol, polyaspartamide and combinations thereof. Other suitable cationic lipids are described in Miller (1998) Angewandte Chemie International Edition 37(13-14): 1768-1785 and Cooper, et al. (1998) Chem. Eur. J. 4(1): 137-151. Liposomes can be crosslinked, partially crosslinked, or free from crosslinking. Crosslinked liposomes can include crosslinked as well as non-crosslinked components. Suitable cationic liposomes or cytofectins are commercially available and can also be prepared as described in Sipkins, et al. (1998) Nature Medicine 4(5): 623-626 or as described in Miller, supra. Exemplary liposomes include a polymerizable zwitterionic or neutral lipid, a polymerizable integrin targeting lipid and a polymerizable cationic lipid suitable for binding a nucleic acid. Liposomes can optionally include peptides that provide increased efficiency, for example as described in U.S. Patent No. 7,297,759. Additional exemplary liposomes and other lipid aggregates are described in U.S. Patent No. 7,166,298.

Methods of Producing Antibodies and Fragments thereof

[0486] The invention is also directed to the production of the antibodies described herein or fragments thereof. Recombinant polypeptides corresponding to the antibodies described herein or fragments thereof are secreted from polyploidal, preferably diploid or tetraploid strains of mating competent yeast. In an exemplary embodiment, the invention is directed to methods for producing these recombinant polypeptides in secreted form for prolonged periods using cultures comprising polyploid yeast, i.e., at least several days to a week, more preferably at least a month or several months, and even more preferably at least 6 months to a year or longer. These polyploid yeast cultures will express at least 10-25 mg/liter of the polypeptide, more preferably at least 50-250 mg/liter, still more preferably at least 500-1000 mg/liter, and most preferably a gram per liter or more of the recombinant polypeptide(s).

[0487] In one embodiment of the invention a pair of genetically marked yeast haploid cells are transformed with expression vectors comprising subunits of a desired heteromultimeric protein. One haploid cell comprises a first expression vector, and a second haploid cell comprises a second expression vector. In another embodiment diploid yeast cells will be transformed with at least one expression vectors that provide for the expression and secretion of at least one of the recombinant polypeptides. In still another embodiment a single haploid cell may be transformed with at least one vectors and used to produce a polyploid yeast by fusion or mating strategies. In yet another embodiment a diploid yeast culture may be transformed with at least one vectors providing for the expression and secretion of a desired polypeptide or polypeptides. These
vectors may comprise vectors e.g., linearized plasmids or other linear DNA products that integrate into the yeast cell's genome randomly, through homologous recombination, or using a recombinase such as Cre/Lox or Flp/Frt. Optionally, additional expression vectors may be introduced into the haploid or diploid cells; or the first or second expression vectors may comprise additional coding sequences; for the synthesis of heterotrimers; heterotetramers. The expression levels of the non-identical polypeptides may be individually calibrated, and adjusted through appropriate selection, vector copy number, promoter strength and/or induction and the like. The transformed haploid cells are genetically crossed or fused. The resulting diploid or tetraploid strains are utilized to produce and secrete fully assembled and biologically functional proteins, humanized antibodies described herein or fragments thereof.

[0488] The use of diploid or tetraploid cells for protein production provides for unexpected benefits. The cells can be grown for production purposes, i.e. scaled up, and for extended periods of time, in conditions that can be deleterious to the growth of haploid cells, which conditions may include high cell density; growth in minimal media; growth at low temperatures; stable growth in the absence of selective pressure; and which may provide for maintenance of heterologous gene sequence integrity and maintenance of high level expression over time. Without wishing to be bound thereby, the inventors theorize that these benefits may arise, at least in part, from the creation of diploid strains from two distinct parental haploid strains. Such haploid strains can comprise numerous minor autotrophic mutations, which mutations are complemented in the diploid or tetraploid, enabling growth and enhanced production under highly selective conditions.

[0489] Transformed mating competent haploid yeast cells provide a genetic method that enables subunit pairing of a desired protein. Haploid yeast strains are transformed with each of two expression vectors, a first vector to direct the synthesis of one polypeptide chain and a second vector to direct the synthesis of a second, non-identical polypeptide chain. The two haploid strains are mated to provide a diploid host where optimized target protein production can be obtained.

[0490] Optionally, additional non-identical coding sequence(s) are provided. Such sequences may be present on additional expression vectors or in the first or the second expression vectors. As is known in the art, multiple coding sequences may be independently expressed from individual promoters; or may be coordinately expressed through the inclusion of an "internal ribosome entry site" or "IRES", which is an element that promotes direct internal ribosome entry to the initiation codon, such as ATG, of a cistron (a protein encoding region), thereby leading to the cap-independent translation of the gene. IRES elements functional in yeast are described by Thompson, et al. (2001) PNAS 98: 12866-12868.
In one embodiment of the invention, antibody sequences are produced in combination with a secretory J chain, which provides for enhanced stability of IgA. See U.S. Patent Nos. 5,959,177 and 5,202,422.

In a preferred embodiment the two haploid yeast strains are each auxotrophic, and require supplementation of media for growth of the haploid cells. The pair of auxotrophs are complementary, such that the diploid product will grow in the absence of the supplements required for the haploid cells. Many such genetic markers are known in yeast, including requirements for amino acids (e.g. met, lys, his, arg), nucleosides (e.g. ura3, adel); and the like. Amino acid markers may be preferred for the methods of the invention. Alternatively diploid cells which contain the desired vectors can be selected by other means, e.g., by use of other markers, such as green fluorescent protein, antibiotic resistance genes, various dominant selectable markers, and the like.

Two transformed haploid cells may be genetically crossed and diploid strains arising from this mating event selected by their hybrid nutritional requirements and/or antibiotic resistance spectra. Alternatively, populations of the two transformed haploid strains are spheroplasted and fused, and diploid progeny regenerated and selected. By either method, diploid strains can be identified and selectively grown based on their ability to grow in different media than their parents. For example, the diploid cells may be grown in minimal medium that may include antibiotics. The diploid synthesis strategy has certain advantages. Diploid strains have the potential to produce enhanced levels of heterologous protein through broader complementation to underlying mutations, which may impact the production and/or secretion of recombinant protein. Furthermore, once stable strains have been obtained, any antibiotics used to select those strains do not necessarily need to be continuously present in the growth media.

As noted above, in some embodiments a haploid yeast may be transformed with a single or multiple vectors and mated or fused with a non-transformed cell to produce a diploid cell containing the vector or vectors. In other embodiments, a diploid yeast cell may be transformed with at least one vectors that provide for the expression and secretion of a desired heterologous polypeptide by the diploid yeast cell.

In one embodiment of the invention, two haploid strains are transformed with a library of polypeptides, e.g. a library of antibody heavy or light chains. Transformed haploid cells that synthesize the polypeptides are mated with the complementary haploid cells. The resulting diploid cells are screened for functional protein. The diploid cells provide a means of rapidly, conveniently and inexpensively bringing together a large number of combinations of polypeptides for functional testing. This technology is especially applicable for the generation of
heterodimeric protein products, where optimized subunit synthesis levels are critical for functional protein expression and secretion.

[0496] In another embodiment of the invention, the expression level ratio of the two subunits is regulated in order to maximize product generation. Heterodimer subunit protein levels have been shown previously to impact the final product generation. Simmons (2002) J Immunol Methods, 263(1-2): 133-47. Regulation can be achieved prior to the mating step by selection for a marker present on the expression vector. By stably increasing the copy number of the vector, the expression level can be increased. In some cases, it may be desirable to increase the level of one chain relative to the other, so as to reach a balanced proportion between the subunits of the polypeptide. Antibiotic resistance markers are useful for this purpose, e.g. Zeocin® (phleomycin) resistance marker, G418 resistance and provide a means of enrichment for strains that contain multiple integrated copies of an expression vector in a strain by selecting for transformants that are resistant to higher levels of Zeocin® (phleomycin) or G418. The proper ratio (e.g. 1:1; 1:2) of the subunit genes may be important for efficient protein production. Even when the same promoter is used to transcribe both subunits, many other factors contribute to the final level of protein expressed and therefore, it can be useful to increase the number of copies of one encoded gene relative to the other. Alternatively, diploid strains that produce higher levels of a polypeptide, relative to single copy vector strains, are created by mating two haploid strains, both of which have multiple copies of the expression vectors.

[0497] Host cells are transformed with the above-described expression vectors, mated to form diploid strains, and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants or amplifying the genes encoding the desired sequences. A number of minimal media suitable for the growth of yeast are known in the art. Any of these media may be supplemented as necessary with salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as phosphate, HEPES), nucleosides (such as adenosine and thymidine), antibiotics, trace elements, and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[0498] Secreted proteins are recovered from the culture medium. A protease inhibitor, such as phenyl methyl sulfonyl fluoride (PMSF) may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. The composition may be concentrated, filtered, dialyzed, using methods known in the art.
The diploid cells of the invention are grown for production purposes. Such production purposes desirably include growth in minimal media, which media lacks pre-formed amino acids and other complex biomolecules, e.g., media comprising ammonia as a nitrogen source, and glucose as an energy and carbon source, and salts as a source of phosphate, calcium and the like. Preferably such production media lacks selective agents such as antibiotics, amino acids, purines, pyrimidines. The diploid cells can be grown to high cell density, for example at least about 50 g/L; more usually at least about 100 g/L; and may be at least about 300, about 400, about 500 g/L or more.

In one embodiment of the invention, the growth of the subject cells for production purposes is performed at low temperatures, which temperatures may be lowered during log phase, during stationary phase, or both. The term "low temperature" refers to temperatures of at least about 15°C, more usually at least about 17°C, and may be about 20°C, and is usually not more than about 25°C, more usually not more than about 22°C. In another embodiment of the invention, the low temperature is usually not more than about 28°C. Growth temperature can impact the production of full-length secreted proteins in production cultures, and decreasing the culture growth temperature can strongly enhance the intact product yield. The decreased temperature appears to assist intracellular trafficking through the folding and post-translational processing pathways used by the host to generate the target product, along with reduction of cellular protease degradation.

The methods of the invention provide for expression of secreted, active protein, preferably a mammalian protein. In one embodiment, secreted, "active antibodies", as used herein, refers to a correctly folded multimer of at least two properly paired chains, which accurately binds to its cognate antigen. Expression levels of active protein are usually at least about 10-50 mg/liter culture, more usually at least about 100 mg/liter, preferably at least about 500 mg/liter, and may be 1000 mg/liter or more.

The methods of the invention can provide for increased stability of the host and heterologous coding sequences during production. The stability is evidenced, for example, by maintenance of high levels of expression of time, where the starting level of expression is decreased by not more than about 20%, usually not more than 10%, and may be decreased by not more than about 5% over about 20 doublings, 50 doublings, 100 doublings, or more.

The strain stability also provides for maintenance of heterologous gene sequence integrity over time, where the sequence of the active coding sequence and requisite transcriptional regulatory elements are maintained in at least about 99% of the diploid cells, usually in at least about 99.9% of the diploid cells, and preferably in at least about 99.99% of the diploid cells over
about 20 doublings, 50 doublings, 100 doublings, or more. Preferably, substantially all of the diploid cells maintain the sequence of the active coding sequence and requisite transcriptional regulatory elements.


[0506] Antibody polypeptides of the invention having IL-6 binding specificity may also be produced by constructing, using conventional techniques well known to those of ordinary skill in the art, an expression vector containing an operon and a DNA sequence encoding an antibody heavy chain in which the DNA sequence encoding the CDRs required for antibody specificity is derived from a non-human cell source, preferably a rabbit B-cell source, while the DNA sequence encoding the remaining parts of the antibody chain is derived from a human cell source.

[0507] A second expression vector is produced using the same conventional means well known to those of ordinary skill in the art, said expression vector containing an operon and a DNA sequence encoding an antibody light chain in which the DNA sequence encoding the CDRs required for antibody specificity is derived from a non-human cell source, preferably a rabbit B-cell source, while the DNA sequence encoding the remaining parts of the antibody chain is derived from a human cell source.

[0508] The expression vectors are transfected into a host cell by convention techniques well known to those of ordinary skill in the art to produce a transfected host cell, said transfected host cell cultured by conventional techniques well known to those of ordinary skill in the art to produce said antibody polypeptides.

[0509] The host cell may be co-transfected with the two expression vectors described above, the first expression vector containing DNA encoding an operon and a light chain-derived polypeptide and the second vector containing DNA encoding an operon and a heavy chain-derived polypeptide. The two vectors contain different selectable markers, but preferably achieve substantially equal expression of the heavy and light chain polypeptides. Alternatively, a single vector may be used, the vector including DNA encoding both the heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA.
The host cells used to express the antibody polypeptides may be either a bacterial cell such as *E. coli*, or a eukaryotic cell. In a particularly preferred embodiment of the invention, a mammalian cell of a well-defined type for this purpose, such as a myeloma cell or a Chinese hamster ovary (CHO) cell line may be used.

The general methods by which the vectors may be constructed, transfection methods required to produce the host cell and culturing methods required to produce the antibody polypeptides from said host cells all include conventional techniques. Although preferably the cell line used to produce the antibody is a mammalian cell line, any other suitable cell line, such as a bacterial cell line such as an *E. coli*-derived bacterial strain, or a yeast cell line, may alternatively be used.

Similarly, once produced the antibody polypeptides may be purified according to standard procedures in the art, such as for example cross-flow filtration, ammonium sulphate precipitation, affinity column chromatography and the like.

The antibody polypeptides described herein may also be used for the design and synthesis of either peptide or non-peptide mimetics that would be useful for the same therapeutic applications as the antibody polypeptides of the invention. See, e.g., Saragobi et al. (1991) Science 253: 792-795.

**B-Cell Screening and Isolation**

The present invention provides methods of isolating a clonal population of antigen-specific B cells that may be used for isolating at least one antigen-specific cell. As described and exemplified infra, these methods contain a series of culture and selection steps that can be used separately, in combination, sequentially, repetitively, or periodically. Preferably, these methods are used for isolating at least one antigen-specific cell, which can be used to produce a monoclonal antibody, which is specific to a desired antigen, or a nucleic acid sequence corresponding to such an antibody.

The present invention provides a method comprising the steps of:

(a) preparing a cell population comprising at least one antigen-specific B cell;

(b) enriching the cell population, e.g., by chromatography, to form an enriched cell population comprising at least one antigen-specific B cell;

(c) isolating a single B cell from the enriched B cell population; and

(d) determining whether the single B cell produces an antibody specific to the antigen.

The present invention provides an improvement to a method of isolating a single, antibody-producing B cell, the improvement comprising enriching a B cell population obtained from a host that has been immunized or naturally exposed to an antigen, wherein the enriching
step precedes any selection steps, comprises at least one culturing step, and results in a clonal population of B cells that produces a single monoclonal antibody specific to said antigen.

[0517] Throughout this application, a "clonal population of B cells" refers to a population of B cells that only secrete a single antibody specific to a desired antigen. That is to say that these cells produce only one type of monoclonal antibody specific to the desired antigen.

[0518] In the present application, "enriching" a cell population cells means increasing the frequency of desired cells, typically antigen-specific cells, contained in a mixed cell population, e.g., a B cell-containing isolate derived from a host that is immunized against a desired antigen. Thus, an enriched cell population encompasses a cell population having a higher frequency of antigen-specific cells as a result of an enrichment step, but this population of cells may contain and produce different antibodies.

[0519] The general term "cell population" encompasses pre- and a post-enrichment cell populations, keeping in mind that when multiple enrichment steps are performed, a cell population can be both pre- and post-enrichment. For example, in one embodiment, the present invention provides a method:

(a) harvesting a cell population from an immunized host to obtain a harvested cell population;

(b) creating at least one single cell suspension from the harvested cell population;

(c) enriching at least one single cell suspension to form a first enriched cell population;

(d) enriching the first enriched cell population to form a second enriched cell population;

(e) enriching the second enriched cell population to form a third enriched cell population; and

(f) selecting an antibody produced by an antigen-specific cell of the third enriched cell population.

[0520] Each cell population may be used directly in the next step, or it can be partially or wholly frozen for long- or short- term storage or for later steps. Also, cells from a cell population can be individually suspended to yield single cell suspensions. The single cell suspension can be enriched, such that a single cell suspension serves as the pre-enrichment cell population. Then, at least one antigen-specific single cell suspensions together form the enriched cell population; the antigen-specific single cell suspensions can be grouped together, e.g., re-plated for further analysis and/or antibody production.

[0521] In one embodiment, the present invention provides a method of enriching a cell population to yield an enriched cell population having an antigen-specific cell frequency that is about 50% to about 100%, or increments therein. Preferably, the enriched cell population has an
antigen-specific cell frequency at least about 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99%, or 100%.

In another embodiment, the present invention provides a method of enriching a cell population whereby the frequency of antigen-specific cells is increased by at least about 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, or increments therein.

Throughout this application, the term "increment" is used to define a numerical value in varying degrees of precision, e.g., to the nearest 10, 1, 0.1, 0.01. The increment can be rounded to any measurable degree of precision, and the increment need not be rounded to the same degree of precision on both sides of a range. For example, the range 1 to 100 or increments therein includes ranges such as 20 to 80, 5 to 50, and 0.4 to 98. When a range is open-ended, e.g., a range of less than 100, increments therein means increments between 100 and the measurable limit. For example, less than 100 or increments therein means 0 to 100 or increments therein unless the feature, e.g., temperature, is not limited by 0.

Antigen-specificity can be measured with respect to any antigen. The antigen can be any substance to which an antibody can bind including, but not limited to, peptides, proteins or fragments thereof; carbohydrates; organic and inorganic molecules; receptors produced by animal cells, bacterial cells, and viruses; enzymes; agonists and antagonists of biological pathways; hormones; and cytokines. Exemplary antigens include, but are not limited to, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-α, IFN-γ, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF) and Hepcidin. Preferred antigens include IL-6, IL-13, TNF-α, VEGF-a, Hepatocyte Growth Factor (HGF) and Hepcidin. In a method utilizing more than one enrichment step, the antigen used in each enrichment step can be the same as or different from one another. Multiple enrichment steps with the same antigen may yield a large and/or diverse population of antigen-specific cells; multiple enrichment steps with different antigens may yield an enriched cell population with cross-specificity to the different antigens.

Enriching a cell population can be performed by any cell-selection means known in the art for isolating antigen-specific cells. For example, a cell population can be enriched by chromatographic techniques, e.g., Miltenyi bead or magnetic bead technology. The beads can be directly or indirectly attached to the antigen of interest. In a preferred embodiment, the method of enriching a cell population includes at least one chromatographic enrichment step.

A cell population can also be enriched by performed by any antigen-specificity assay technique known in the art, e.g., an ELISA assay or a halo assay. ELISA assays include, but are not limited to, selective antigen immobilization (e.g., biotinylated antigen capture by streptavidin, avidin, or neutravidin coated plate), non-specific antigen plate coating, and through an antigen
build-up strategy (e.g., selective antigen capture followed by binding partner addition to generate a heteromeric protein-antigen complex). The antigen can be directly or indirectly attached to a solid matrix or support, e.g., a column. A halo assay comprises contacting the cells with antigen-loaded beads and labeled anti-host antibody specific to the host used to harvest the B cells. The label can be, e.g., a fluorophore. In one embodiment, at least one assay enrichment step is performed on at least one single cell suspension. In another embodiment, the method of enriching a cell population includes at least one chromatographic enrichment step and at least one assay enrichment step.

[0527] Methods of "enriching" a cell population by size or density are known in the art. See, e.g., U.S. Patent 5,627,052. These steps can be used in the present method in addition to enriching the cell population by antigen-specificity.

[0528] The cell populations of the present invention contain at least one cell capable of recognizing an antigen. Antigen-recognizing cells include, but are not limited to, B cells, plasma cells, and progeny thereof. In one embodiment, the present invention provides a clonal cell population containing a single type of antigen-specific B-cell, i.e., the cell population produces a single monoclonal antibody specific to a desired antigen.

[0529] In such embodiment, it is believed that the clonal antigen-specific population of B cells consists predominantly of antigen-specific, antibody-secreting cells, which are obtained by the novel culture and selection protocol provided herein. Accordingly, the present invention also provides methods for obtaining an enriched cell population containing at least one antigen-specific, antibody-secreting cell. In one embodiment, the present invention provides an enriched cell population containing about 50% to about 100%, or increments therein, at least about 60%, 70%, 80%, 90%, or 100% of antigen-specific, antibody-secreting cells.

[0530] In one embodiment, the present invention provides a method of isolating a single B cell by enriching a cell population obtained from a host before any selection steps, e.g., selecting a particular B cell from a cell population and/or selecting an antibody produced by a particular cell. The enrichment step can be performed as one, two, three, or more steps. In one embodiment, a single B cell is isolated from an enriched cell population before confirming whether the single B cell secretes an antibody with antigen-specificity and/or a desired property.

[0531] In one embodiment, a method of enriching a cell population is used in a method for antibody production and/or selection. Thus, the present invention provides a method comprising enriching a cell population before selecting an antibody. The method can include the steps of: preparing a cell population comprising at least one antigen-specific cell, enriching the cell population by isolating at least one antigen-specific cell to form an enriched cell population, and
inducing antibody production from at least one antigen-specific cell. In a preferred embodiment, the enriched cell population contains more than one antigen-specific cell. In one embodiment, each antigen-specific cell of the enriched population is cultured under conditions that yield a clonal antigen-specific B cell population before isolating an antibody producing cell therefrom and/or producing an antibody using said B cell, or a nucleic acid sequence corresponding to such an antibody. In contrast to prior techniques where antibodies are produced from a cell population with a low frequency of antigen-specific cells, the present invention allows antibody selection from among a high frequency of antigen-specific cells. Because an enrichment step is used prior to antibody selection, the majority of the cells, preferably virtually all of the cells, used for antibody production are antigen-specific. By producing antibodies from a population of cells with an increased frequency of antigen specificity, the quantity and variety of antibodies are increased.

[0532] In the antibody selection methods of the present invention, an antibody is preferably selected after an enrichment step and a culture step that results in a clonal population of antigen-specific B cells. The methods can further comprise a step of sequencing a selected antibody or portions thereof from at least one isolated, antigen-specific cell. Any method known in the art for sequencing can be employed and can include sequencing the heavy chain, light chain, variable region(s), and/or complementarity determining region(s) (CDR).

[0533] In addition to the enrichment step, the method for antibody selection can also include at least one steps of screening a cell population for antigen recognition and/or antibody functionality. For example, the desired antibodies may have specific structural features, such as binding to a particular epitope or mimicry of a particular structure; antagonist or agonist activity; or neutralizing activity, e.g., inhibiting binding between the antigen and a ligand. In one embodiment, the antibody functionality screen is ligand-dependent. Screening for antibody functionality includes, but is not limited to, an in vitro protein-protein interaction assay that recreates the natural interaction of the antigen ligand with recombinant receptor protein; and a cell-based response that is ligand dependent and easily monitored (e.g., proliferation response).

In one embodiment, the method for antibody selection includes a step of screening the cell population for antibody functionality by measuring the inhibitory concentration (IC50). In one embodiment, at least one of the isolated, antigen-specific cells produces an antibody having an IC50 of less than about 100, 50, 30, 25, 10 µg/mL, or increments therein.

[0534] In addition to the enrichment step, the method for antibody selection can also include at least one steps of screening a cell population for antibody binding strength. Antibody binding strength can be measured by any method known in the art (e.g., Biacore®). In one embodiment,
at least one of the isolated, antigen-specific cells produces an antibody having a high antigen
affinity, e.g., a dissociation constant (Kd) of less than about 5×10^{-10} M\text{-}1, preferably about 1×10^{-13}
to 5×10^{-10}, 1×10^{-4.2} to 1×10^{-4.0}, 1×10^{-4.2} to 7.5×10^{-4.1}, 1×10^{-4.1} to 2×10^{-4.1}, about 1.5×10^{-4.1} or less, or
increments therein. In this embodiment, the antibodies are said to be affinity mature. In a
preferred embodiment, the affinity of the antibodies is comparable to or higher than the affinity of
any one of Panorex® (edrecolomab), Rituxan® (rituximab), Herceptin® (traztuzumab),
Mylotarg® (gentuzumab), Campath® (alemtuzumab), Zevalin® (ibrutinumab), Erbitux®
(cetuximab), Avastin® (bevacizumab), Raptiva® (efalizumab), Remicade® (infliximab),
Humira® (adalimumab), or Xolair® (omalizumab). Preferably, the affinity of the antibodies is
comparable to or higher than the affinity of Humira®. The affinity of an antibody can also be
increased by known affinity maturation techniques. In one embodiment, at least one cell
population is screened for at least one of, preferably both, antibody functionality and antibody
binding strength.

[0535] In addition to the enrichment step, the method for antibody selection can also include at
least one steps of screening a cell population for antibody sequence homology, especially human
homology. In one embodiment, at least one of the isolated, antigen-specific cells produces an
antibody that has a homology to a human antibody of at least about 50% to about 100%, or
increments therein, or at least about 60%, 70%, 80%, 85%, 90%, or 95% homologous. The
antibodies can be humanized to increase the homology to a human sequence by techniques known
in the art such as CDR grafting or selectivity determining residue grafting (SDR).

[0536] In another embodiment, the present invention also provides the antibodies themselves
according to any of the embodiments described above in terms of IC50, Kd, and/or homology.

Methods of Humanizing Antibodies

[0537] The invention also provides a method for humanizing antibody heavy and light chains.
In this embodiment, the following method may be followed for the humanization of the heavy
and light chains:

Light Chain

[0538] 1. Identify the amino acid that is the first one following the signal peptide sequence.
This is the start of Framework 1. The signal peptide starts at the first initiation methionine and is
typically, but not necessarily 22 amino acids in length for rabbit light chain protein sequences.
The start of the mature polypeptide can also be determined experimentally by N-terminal protein
sequencing, or can be predicted using a prediction algorithm. This is also the start of Framework
1 as classically defined by those in the field.
Example: RbtVL Amino acid residue 1 in Fig. 1, starting 'AYDM...' (SEQ ID NO: 733)

2. Identify the end of Framework

3. This is typically 86-90 amino acids following the start of Framework 1 and is typically a cysteine residue preceded by two tyrosine residues. This is the end of the Framework 3 as classically defined by those in the field.

Example: RbtVL amino acid residue 88 in Fig. 1, ending as 'TYYC' (SEQ ID NO: 733)

3. Use the rabbit light chain sequence of the polypeptide starting from the beginning of Framework 1 to the end of Framework 3 as defined above and perform a sequence homology search for the most similar human antibody protein sequences. This will typically be a search against human germline sequences prior to antibody maturation in order to reduce the possibility of immunogenicity, however any human sequences can be used. Typically a program like BLAST can be used to search a database of sequences for the most homologous. Databases of human antibody sequences can be found from various sources such as NCBI (National Center for Biotechnology Information).

Example: RbtVL amino acid sequence from residues numbered 1 through 88 in Fig. 1 is BLASTed against a human antibody germline database. The top three unique returned sequences are shown in Fig. 1 as L12A (SEQ ID NO: 734), V1 (SEQ ID NO: 735), and Vx02 (SEQ ID NO: 736).

4. Generally the most homologous human germline variable light chain sequence is then used as the basis for humanization. However those skilled in the art may decide to use another sequence that wasn't the highest homology as determined by the homology algorithm, based on other factors including sequence gaps and framework similarities.

Example: In Fig. 1, L12A (SEQ ID NO: 734) was the most homologous human germline variable light chain sequence and is used as the basis for the humanization of RbtVL.

5. Determine the framework and CDR arrangement (FR1, FR2, FR3, CDR1 & CDR2) for the human homolog being used for the light chain humanization. This is using the traditional layout as described in the field. Align the rabbit variable light chain sequence with the human homolog, while maintaining the layout of the framework and CDR regions.

Example: In Fig. 1, the RbtVL sequence is aligned with the human homologous sequence L12A, and the framework and CDR domains are indicated.

6. Replace the human homologous light chain sequence CDR1 and CDR2 regions with the CDR1 and CDR2 sequences from the rabbit sequence. If there are differences in length
between the rabbit and human CDR sequences then use the entire rabbit CDR sequences and their lengths. It is possible that the specificity, affinity and/or immunogenicity of the resulting humanized antibody may be unaltered if smaller or larger sequence exchanges are performed, or if specific residue(s) are altered, however the exchanges as described have been used successfully, but do not exclude the possibility that other changes may be permitted.

Example: In Fig. 1, the CDR1 and CDR2 amino acid residues of the human homologous variable light chain L12A are replaced with the CDR1 and CDR2 amino acid sequences from the RbtVL rabbit antibody light chain sequence. The human L12A frameworks 1, 2 and 3 are unaltered. The resulting humanized sequence is shown below as VLh from residues numbered 1 through 88. Note that the only residues that are different from the L12A human sequence are underlined, and are thus rabbit-derived amino acid residues. In this example only 8 of the 88 residues are different than the human sequence.

After framework 3 of the new hybrid sequence created in Step 6, attach the entire CDR3 of the rabbit light chain antibody sequence. The CDR3 sequence can be of various lengths, but is typically 9 to 15 amino acid residues in length. The CDR3 region and the beginning of the following framework 4 region are defined classically and identifiable by those skilled in the art. Typically the beginning of Framework 4, and thus after the end of CDR3 consists of the sequence ‘FGGG…’ (SEQ ID NO: 743), however some variation may exist in these residues.

Example: In Fig. 1, the CDR3 of RbtVL (amino acid residues numbered 89-100) is added after the end of framework 3 in the humanized sequence indicated as VLh.

The rabbit light chain framework 4, which is typically the final 11 amino acid residues of the variable light chain and begins as indicated in Step 7 above and typically ends with the amino acid sequence ‘…VVKR’ (SEQ ID NO: 744) is replaced with the nearest human light chain framework 4 homolog, usually from germline sequence. Frequently this human light chain framework 4 is of the sequence ‘FGGGTKVEIKR’ (SEQ ID NO: 745). It is possible that other human light chain framework 4 sequences that are not the most homologous or otherwise different may be used without affecting the specificity, affinity and/or immunogenicity of the resulting humanized antibody. This human light chain framework 4 sequence is added to the end of the variable light chain humanized sequence immediately following the CDR3 sequence from Step 7 above. This is now the end of the variable light chain humanized amino acid sequence.

Example: In Fig. 1, Framework 4 (FR4) of the RbtVL rabbit light chain sequence is shown above a homologous human FR4 sequence. The human FR4 sequence is added to the
humanized variable light chain sequence (VLh) right after the end of the CD3 region added in Step 7 above.

In addition, Figs. 4 and 5 depict preferred humanized anti-IL-6 variable heavy and variable light chain sequences humanized from the variable heavy and light regions in Ab 1 according to the invention. These humanized light and heavy chain regions are respectively contained in the polypeptides set forth in SEQ ID NO: 647, or 651 and in SEQ ID NO: 652, 656, 657 or 658. The CDR2 of the humanized variable heavy region in SEQ ID NO: 657 (containing a serine substitution in CDR2) is set forth in SEQ ID NO: 658. Alignments illustrating variants of the light and heavy chains are shown in Figs. 2 and 3, respectively, with sequence differences within the CDR regions highlighted. Sequence identifiers of CDR sequences and of exemplary coding sequences are summarized in Table 4, above.

**Heavy Chain**

1. Identify the amino acid that is the first one following the signal peptide sequence. This is the start of Framework 1. The signal peptide starts at the first initiation methionine and is typically 19 amino acids in length for rabbit heavy chain protein sequences. Typically, but not necessarily always, the final 3 amino acid residues of a rabbit heavy chain signal peptide are '...VQC, followed by the start of Framework 1. The start of the mature polypeptide can also be determined experimentally by N-terminal protein sequencing, or can be predicted using a prediction algorithm. This is also the start of Framework 1 as classically defined by those in the field.

Example: RbtVH Amino acid residue 1 in Fig. 1, starting 'QEQL...' (SEQ ID NO: 738)

2. Identify the end of Framework 3. This is typically 95-100 amino acids following the start of Framework 1 and typically has the final sequence of '...CAR' (although the alanine can also be a valine). This is the end of the Framework 3 as classically defined by those in the field.

Example: RbtVH amino acid residue 98 in Fig. 1, ending as '...FCVR' (SEQ ID NO: 738).

3. Use the rabbit heavy chain sequence of the polypeptide starting from the beginning of Framework 1 to the end of Framework 3 as defined above and perform a sequence homology search for the most similar human antibody protein sequences. This will typically be against a database of human germline sequences prior to antibody maturation in order to reduce the possibility of immunogenicity, however any human sequences can be used. Typically a program like BLAST can be used to search a database of sequences for the most homologous. Databases
of human antibody sequences can be found from various sources such as NCBI (National Center for Biotechnology Information).

[0561] Example: RbtVH amino acid sequence from residues numbered 1 through 98 in Fig. 1 is BLASTed against a human antibody germline database. The top three unique returned sequences are shown in Fig. 1 as 3-64-04 (SEQ ID NO: 739), 3-66-04 (SEQ ID NO: 740), and 3-53-02 (SEQ ID NO: 741).

[0562] 4. Generally the most homologous human germline variable heavy chain sequence is then used as the basis for humanization. However those skilled in the art may decide to use another sequence that wasn't the most homologous as determined by the homology algorithm, based on other factors including sequence gaps and framework similarities.

[0563] Example: 3-64-04 in Fig. 1 was the most homologous human germline variable heavy chain sequence and is used as the basis for the humanization of RbtVH.

[0564] 5. Determine the framework and CDR arrangement (FR1, FR2, FR3, CDR1 & CDR2) for the human homolog being used for the heavy chain humanization. This is using the traditional layout as described in the field. Align the rabbit variable heavy chain sequence with the human homolog, while maintaining the layout of the framework and CDR regions.

[0565] Example: In Fig. 1, the RbtVH sequence is aligned with the human homologous sequence 3-64-04, and the framework and CDR domains are indicated.

[0566] 6. Replace the human homologous heavy chain sequence CDR1 and CDR2 regions with the CDR1 and CDR2 sequences from the rabbit sequence. If there are differences in length between the rabbit and human CDR sequences then use the entire rabbit CDR sequences and their lengths. In addition, it may be necessary to replace the final three amino acids of the human heavy chain Framework 1 region with the final three amino acids of the rabbit heavy chain Framework 1. Typically but not always, in rabbit heavy chain Framework 1 these three residues follow a Glycine residue preceded by a Serine residue. In addition, it may be necessary replace the final amino acid of the human heavy chain Framework 2 region with the final amino acid of the rabbit heavy chain Framework 2. Typically, but not necessarily always, this is a Glycine residue preceded by an Isoleucine residue in the rabbit heavy chain Framework 2. It is possible that the specificity, affinity and/or immunogenicity of the resulting humanized antibody may be unaltered if smaller or larger sequence exchanges are performed, or if specific residue(s) are altered, however the exchanges as described have been used successfully, but do not exclude the possibility that other changes may be permitted. For example, a tryptophan amino acid residue typically occurs four residues prior to the end of the rabbit heavy chain CDR2 region, whereas in human heavy chain CDR2 this residue is typically a Serine residue. Changing this rabbit
tryptophan residue to a the human Serine residue at this position has been demonstrated to have minimal to no effect on the humanized antibody's specificity or affinity, and thus further minimizes the content of rabbit sequence-derived amino acid residues in the humanized sequence. **[0567]** Example: In Fig. 1, The CDR1 and CDR2 amino acid residues of the human homologous variable heavy chain are replaced with the CDR1 and CDR2 amino acid sequences from the RbtVH rabbit antibody light chain sequence, except for the boxed residue, which is tryptophan in the rabbit sequence (position number 63) and Serine at the same position in the human sequence, and is kept as the human Serine residue. In addition to the CDR1 and CDR2 changes, the final three amino acids of Framework 1 (positions 28-30) as well as the final residue of Framework 2 (position 49) are retained as rabbit amino acid residues instead of human. The resulting humanized sequence is shown below as VHh from residues numbered 1 through 98. Note that the only residues that are different from the 3-64-04 human sequence are underlined, and are thus rabbit-derived amino acid residues. In this example only 15 of the 98 residues are different than the human sequence. **[0568]** 7. After framework 3 of the new hybrid sequence created in Step 6, attach the entire CDR3 of the rabbit heavy chain antibody sequence. The CDR3 sequence can be of various lengths, but is typically 5 to 19 amino acid residues in length. The CDR3 region and the beginning of the following framework 4 region are defined classically and are identifiable by those skilled in the art. Typically the beginning of framework 4, and thus after the end of CDR3 consists of the sequence WGPX... (where X is usually Q or P) (SEQ ID NO: 746), however some variation may exist in these residues. **[0569]** Example: The CDR3 of RbtVH (amino acid residues numbered 99-110) is added after the end of framework 3 in the humanized sequence indicated as VHh. **[0570]** 8. The rabbit heavy chain framework 4, which is typically the final 11 amino acid residues of the variable heavy chain and begins as indicated in Step 7 above and typically ends with the amino acid sequence '...TVSS' (SEQ ID NO: 747) is replaced with the nearest human heavy chain framework 4 homolog, usually from germline sequence. Frequently this human heavy chain framework 4 is of the sequence 'WGQGTVTVSS' (SEQ ID NO: 748). It is possible that other human heavy chain framework 4 sequences that are not the most homologous or otherwise different may be used without affecting the specificity, affinity and/or immunogenicity of the resulting humanized antibody. This human heavy chain framework 4 sequence is added to the end of the variable heavy chain humanized sequence immediately following the CDR3 sequence from Step 7 above. This is now the end of the variable heavy chain humanized amino acid sequence.
Example: In Fig. 1, framework 4 (FR4) of the RbtVH rabbit heavy chain sequence is shown above a homologous human heavy FR4 sequence. The human FR4 sequence is added to the humanized variable heavy chain sequence (VHh) right after the end of the CD3 region added in Step 7 above.

Additional Exemplary Embodiments of the Invention

In another embodiment, the invention contemplates at least one anti-IL-6 antibodies or antibody fragments or variants thereof which may specifically bind to the same linear or conformational epitope(s) and/or compete for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof as an anti-IL-6 antibody comprising Ab1 and chimeric, humanized, single chain antibodies and fragments thereof (containing at least one CDRs of the afore-identified antibodies) that specifically bind IL-6, which preferably are aglycosylated. In a preferred embodiment, the anti-IL-6 antibody or fragment or variant thereof may specifically bind to the same linear or conformational epitope(s) and/or compete for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or a fragment thereof as Ab1 and chimeric, humanized, single chain antibodies and fragments thereof (containing at least one CDRs of the afore-mentioned antibody) that specifically bind IL-6, which preferably are aglycosylated.

In another embodiment of the invention, the anti-IL-6 antibody which may specifically bind to the same linear or conformational epitopes on an intact IL-6 polypeptide or fragment thereof that is (are) specifically bound by Ab1 may bind to an IL-6 epitope(s) ascertained by epitopic mapping using overlapping linear peptide fragments which span the full length of the native human IL-6 polypeptide. In one embodiment of the invention, the IL-6 epitope comprises, or alternatively consists of, at least one residues comprised in IL-6 fragments selected from those respectively encompassing amino acid residues 37-51, amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45 and/or amino acid residues 58-72.

The invention is also directed to an anti-IL-6 antibody that binds with the same IL-6 epitope and/or competes with an anti-IL-6 antibody for binding to IL-6 as an antibody or antibody fragment disclosed herein, including but not limited to an anti-IL-6 antibody selected from Ab1 and chimeric, humanized, single chain antibodies and fragments thereof (containing at least one CDRs of the afore-mentioned antibody) that specifically bind IL-6, which preferably are aglycosylated.

In another embodiment, the invention is also directed to an isolated anti-IL-6 antibody or antibody fragment or variant thereof comprising at least one of the CDRs contained in the $V_{\text{H}}$ polypeptide sequences comprising: SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86, 102, 117, 118, 123,

[0576] In one embodiment of the invention, the anti-IL-6 antibody described herein may comprise at least 2 complementarity determining regions (CDRs) in each the variable light and the variable heavy regions which are identical to those contained in an anti-IL-6 antibody comprising Ab1 and chimeric, humanized, single chain antibodies and fragments thereof (containing at least one CDRs of the afore-mentioned antibody) that specifically bind IL-6, which preferably are aglycosylated.

[0577] In a preferred embodiment, the anti-IL-6 antibody described herein may comprise at least 2 complementarity determining regions (CDRs) in each the variable light and the variable heavy regions which are identical to those contained in Ab1. In another embodiment, all of the CDRs of the anti-IL-6 antibody discussed above are identical to the CDRs contained in an anti-IL-6 antibody comprising Abl and chimeric, humanized, single chain antibodies and fragments thereof (containing at least one CDRs of the afore-mentioned antibody) that specifically bind IL-6, which preferably are aglycosylated. In a preferred embodiment of the invention, all of the CDRs of the anti-IL-6 antibody discussed above are identical to the CDRs contained in Abl, e.g., an antibody comprised of the VH and VL sequences comprised in SEQ ID NO: 657 and SEQ ID NO: 709 respectively.

[0578] The invention further contemplates that the one or more anti-IL-6 antibodies discussed above are aglycosylated or substantially non-glycosylated (e.g., may contain one or more, e.g., 1-5 mannose residues); that contain an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation; are human, humanized, single chain or chimeric; and are a humanized antibody derived from a rabbit (parent) anti-IL-6 antibody. Exemplary constant regions that provide for the production of aglycosylated antibodies in Pichia are comprised in SEQ ID NO: 588 and SEQ ID NO: 586 which respectively are encoded by the nucleic acid sequences in SEQ ID NO: 589 and SEQ ID NO: 587.

[0579] The invention further contemplates at least one anti-IL-6 antibodies wherein the framework regions (FRs) in the variable light region and the variable heavy regions of said
antibody respectively are human FRs which are unmodified or which have been modified by the substitution of at most 2 or 3 human FR residues in the variable light or heavy chain region with the corresponding FR residues of the parent rabbit antibody, and wherein said human FRs have been derived from human variable heavy and light chain antibody sequences which have been selected from a library of human germline antibody sequences based on their high level of homology to the corresponding rabbit variable heavy or light chain regions relative to other human germline antibody sequences contained in the library.

[0580] In one embodiment of the invention, the anti-IL-6 antibody or fragment or variant thereof may specifically bind to IL-6 expressing human cells and/or to circulating soluble IL-6 molecules in vivo, including IL-6 expressed on or by human cells in a patient with a disease associated with cells that express IL-6.

[0581] The invention further contemplates anti-IL-6 antibodies or fragments or variants thereof directly or indirectly attached to a detectable label or therapeutic agent.

[0582] The invention also contemplates at least one nucleic acid sequences which result in the expression of an anti-IL-6 antibody or antibody fragment or variant thereof as set forth above, including those comprising, or alternatively consisting of, yeast or human preferred codons. The invention also contemplates vectors (including plasmids or recombinant viral vectors) comprising said nucleic acid sequence(s). The invention also contemplates host cells or recombinant host cells expressing at least one of the antibodies set forth above, including a mammalian, yeast, bacterial, and insect cells. In a preferred embodiment, the host cell is a yeast cell. In a further preferred embodiment, the yeast cell is a diploidal yeast cell. In a more preferred embodiment, the yeast cell is a Pichia yeast.

[0583] The invention also contemplates a method of treatment comprising administering to a patient with a disease or condition associated with anemia a therapeutically effective amount of at least one anti-IL-6 antibody or antigen-binding fragment or variant thereof. The diseases that may be treated are presented in the non-limiting list set forth above. In another embodiment the treatment further includes the administration of another therapeutic agent or regimen selected from chemotherapy, radiotherapy, cytokine administration or gene therapy agent. For example, TNF-a inhibitors including but not limited to glycocorticoids, triamcinolone, dexamethasone, prednisone, may also be administered sequentially or subsequently with at least one anti-IL-6 antibody or antigen-binding fragment or variant thereof described herein. Further examples of drugs that may be included with the IL-6 antagonists include but are not limited to ARISTOCORT® (triamcinolone), BAYCADROM® (dexamethasone), DECADRON® (dexamethasone), DELTASONE® (prednisone), DEXAMETHASONE INTENSOL®
(dexamethasone), ENBREL® (etanercept), HUMIRA® (adalimumab), REMICADE® (infliximab), RIDUARA® (aruaofm), and SIMPONI® (golimumab).

**Exemplary Embodiments of Heavy and Light Chain Polypeptides and Polynucleotides**

[0584] This section recites exemplary embodiments of heavy and light chain polypeptides, as well as exemplary polynucleotides encoding such polypeptides. These exemplary polynucleotides are suitable for expression in the disclosed *Pichia* expression system.

[0585] In certain embodiments, the present invention encompasses polynucleotides having at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity (sequence homology) to the polynucleotides recited in this application or that encode polypeptides recited in this application, or that hybridize to said polynucleotides under conditions of low-stringency, moderate-stringency, or high-stringency conditions, preferably those that encode polypeptides (e.g. an immunoglobulin heavy and light chain, a single-chain antibody, an antibody fragment) that have at least one of the biological activities set forth herein, including without limitation thereto specific binding to an IL-6 polypeptide. In another aspect, the invention encompasses a composition comprising such a polynucleotide and/or a polypeptide encoded by such a polynucleotide. In yet another aspect, the invention encompasses a method of treatment of a disease or condition associated with IL-6 or that may be prevented, treated, or ameliorated with an IL-6 antagonist such as Abl (e.g. anemia) comprising administration of a composition comprising such a polynucleotide and/or polypeptide.

[0586] In certain preferred embodiments, a heavy chain polypeptide will comprise at least one of the CDR sequences of the heavy and/or light chain polypeptides recited herein (including those contained in the heavy and light chain polypeptides recited herein) and at least one of the framework region polypeptides recited herein, including those depicted in FIGS. 1-5 or Table 4, and contained in the heavy and light chain polypeptide sequences recited herein. In certain preferred embodiments, a heavy chain polypeptide will comprise at least one Framework 4 region sequences as depicted in FIGS. 1-5 or Table 4, or as contained in a heavy or light chain polypeptide recited herein.

[0587] In certain preferred embodiments, a light chain polypeptide will comprise at least one of the CDR sequences of the heavy and/or light chain polypeptides recited herein (including those contained in the heavy and light chain polypeptides recited herein) and at least one of the Framework region polypeptides recited herein, including those depicted in FIGS. 1-5 or Table 4, and contained in the heavy and light chain polypeptide sequences recited herein. In certain preferred embodiments, a light chain polypeptide will comprise at least one Framework 4 region sequences as depicted in FIGS. 1-5 or Table 4, or as contained in a heavy or light chain polypeptide recited herein.
sequences as depicted in FIGS. 1-5 or Table 4, or as contained in a heavy or light chain polypeptide recited herein.

[0588] In any of the embodiments recited herein, certain of the sequences recited may be substituted for each other, unless the context indicates otherwise. The recitation that particular sequences may be substituted for one another, where such recitations are made, are understood to be illustrative rather than limiting, and it is also understood that such substitutions are encompassed even when no illustrative examples of substitutions are recited. For example, wherever at least one of the Abl light chain polypeptides is recited, e.g. any of SEQ ID NO: 2, 20, 647, 651, 660, 666, 699, 702, 706, or 709, another Abl light chain polypeptide may be substituted unless the context indicates otherwise. Similarly, wherever one of the Ab1 heavy chain polypeptides is recited, e.g. any of SEQ ID NO: 3, 18, 19, 652, 656, 657, 658, 661, 664, 665, 704, or 708, another Ab1 heavy chain polypeptide may be substituted unless the context indicates otherwise. Likewise, wherever one of the Abl light chain polynucleotides is recited, e.g. any of SEQ ID NO: 10, 662, 698, 701, or 705, another Abl light chain polynucleotide may be substituted unless the context indicates otherwise. Similarly, wherever one of the Ab1 heavy chain polynucleotides is recited, e.g. any of SEQ ID NO: 11, 663, 700, 703, or 707, another Ab1 heavy chain polynucleotide may be substituted unless the context indicates otherwise.

[0589] Additionally, recitation of any member of any of the following groups is understood to encompass substitution by any other member of the group, as follows: Ab2 Light chain polypeptides (SEQ ID NO: 21 and 667); Ab2 Light chain polynucleotides (SEQ ID NO: 29 and 669); Ab2 Heavy chain polypeptides (SEQ ID NO: 22 and 668); Ab2 Heavy chain polynucleotides (SEQ ID NO: 30 and 670); Ab3 Light chain polypeptides (SEQ ID NO: 37 and 671); Ab3 Light chain polynucleotides (SEQ ID NO: 45 and 673); Ab3 Heavy chain polypeptides (SEQ ID NO: 38 and 672); Ab3 Heavy chain polynucleotides (SEQ ID NO: 46 and 674); Ab4 Light chain polypeptides (SEQ ID NO: 53 and 675); Ab4 Light chain polynucleotides (SEQ ID NO: 61 and 677); Ab4 Heavy chain polypeptides (SEQ ID NO: 54 and 676); Ab4 Heavy chain polynucleotides (SEQ ID NO: 62 and 678); Ab5 Light chain polypeptides (SEQ ID NO: 69 and 679); Ab5 Light chain polynucleotides (SEQ ID NO: 77 and 681); Ab5 Heavy chain polypeptides (SEQ ID NO: 70 and 680); Ab5 Heavy chain polynucleotides (SEQ ID NO: 78 and 682); Ab6 Light chain polypeptides (SEQ ID NO: 85 and 683); Ab6 Light chain polynucleotides (SEQ ID NO: 93 and 685); Ab6 Heavy chain polypeptides (SEQ ID NO: 86 and 684); Ab6 Heavy chain polynucleotides (SEQ ID NO: 94 and 686); Ab7 Light chain polypeptides (SEQ ID NO: 101, 119, 687, 693); Ab7 Light chain polynucleotides (SEQ ID NO: 109 and 689); Ab7 Heavy chain polypeptides (SEQ ID NO: 102, 117, 118, 688, 691, and 692); Ab7 Heavy chain polynucleotides
(SEQ ID NO: 110 and 690); Abl Light Chain CDR1 polynucleotides (SEQ ID NO: 12 and 694); Abl Light Chain CDR3 polynucleotides (SEQ ID NO: 14 and 695); Abl Heavy Chain CDR2 polynucleotides (SEQ ID NO: 16 and 696) and Abl Heavy Chain CDR3 polynucleotides (SEQ ID NO: 17 and 697). Exemplary Abl-encoding polynucleotide sequences include but are not limited to SEQ ID NO: 662, 663, 698, 700, 701, 703, 705, 707, 720, 721, 722, 723, 724, and 725.

**ANTI-IL-6 ACTIVITY**

[0590] As stated previously, IL-6 is a member of a family of cytokines that promote cellular responses through a receptor complex consisting of at least one subunit of the signal-transducing glycoprotein gp130 and the IL-6 receptor (IL-6R). The IL-6R may also be present in a soluble form (sIL-6R). IL-6 binds to IL-6R, which then dimerizes the signal-transducing receptor gp130.

[0591] It is believed that the anti-IL-6 antibodies of the invention, or IL-6 binding fragments or variants thereof, are useful by exhibiting anti-IL-6 activity. In one non-limiting embodiment of the invention, the anti-IL-6 antibodies of the invention, or IL-6 binding fragments or variants thereof, exhibit anti-IL-6 activity by binding to IL-6 which may be soluble IL-6 or cell surface expressed IL-6 and/or may prevent or inhibit the binding of IL-6 to IL-6R and/or activation (dimerization) of the gp130 signal-transducing glycoprotein and the formation of IL-6/IL-6R/gp130 multimers and the biological effects of any of the foregoing. The subject anti-IL-6 antibodies may possess different antagonistic activities based on where (i.e., epitope) the particular antibody binds IL-6 and/or how it affects the formation of the foregoing IL-6 complexes and/or multimers and the biological effects thereof. Consequently, different anti-IL-6 antibodies according to the invention e.g., may be better suited for preventing or treating conditions involving the formation and accumulation of substantial soluble IL-6 such as rheumatoid arthritis whereas other antibodies may be favored in treatments wherein the prevention of IL-6/IL-6R/gp130 or IL-6/IL-6R/gp130 multimers is a desired therapeutic outcome. This can be determined in binding and other assays.

[0592] The anti-IL-6 activity of the anti-IL-6 antibody of the present invention, and fragments and variants thereof having binding specificity to IL-6, may also be described by their strength of binding or their affinity for IL-6. This also may affect their therapeutic properties. In one embodiment of the invention, the anti-IL-6 antibodies of the present invention, and fragments thereof having binding specificity to IL-6, bind to IL-6 with a dissociation constant \( (K_d) \) of less than or equal to \( 5 \times 10^{-7}, 10^{-7}, 5 \times 10^{-8}, 10^{-8}, 5 \times 10^{-9}, 10^{-9}, 5 \times 10^{-10}, 10^{-10}, 5 \times 10^{-11}, 10^{-11}, 5 \times 10^{-12}, 10^{-12}, 5 \times 10^{-13}, 10^{-13}, 5 \times 10^{-14}, 10^{-14}, 5 \times 10^{-15} \) or \( 10^{-15} \). Preferably, the anti-IL-6 antibodies and fragments and variants thereof bind IL-6 with a dissociation constant of less than or equal to \( 5 \times 10^{-10} \).
In another embodiment of the invention, the anti-IL-6 activity of the anti-IL-6 antibodies of the present invention, and fragments and variants thereof having binding specificity to IL-6, bind to IL-6 with an off-rate of less than or equal to $10^4$ S$^{-1}$, $5\times 10^{-4}$ S$^{-1}$, $10^{-5}$ S$^{-1}$, $5\times 10^{-6}$ S$^{-1}$, or $10^{-7}$ S$^{-1}$. In one embodiment of the invention, the anti-IL-6 antibodies of the invention, and fragments and variants thereof having binding specificity to IL-6, bind to a linear or conformational IL-6 epitope.

In a further embodiment of the invention, the anti-IL-6 activity of the anti-IL-6 antibodies of the present invention, and fragments and variants thereof having binding specificity to IL-6, exhibit anti-IL-6 activity by ameliorating or reducing the symptoms of, or alternatively treating, or preventing, diseases and disorders associated with IL-6. Non-limiting examples of diseases and disorders associated with IL-6 are set forth infra. In another embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments and variants thereof, do not have binding specificity for IL-6R or the gp-130 signal-transducing glycoprotein.

**SCREENING ASSAYS**

The invention also includes screening assays designed to assist in the identification of diseases and disorders associated with IL-6 in patients exhibiting symptoms of an IL-6 associated disease or disorder, especially anemia.

In one embodiment of the invention, the anti-IL-6 antibodies of the invention, or IL-6 binding fragments or variants thereof, are used to detect the presence of IL-6 in a biological sample obtained from a patient exhibiting symptoms of a disease or disorder associated with IL-6. The presence of IL-6, or elevated levels thereof when compared to pre-disease levels of IL-6 in a comparable biological sample, may be beneficial in diagnosing a disease or disorder associated with IL-6.

Another embodiment of the invention provides a diagnostic or screening assay to assist in diagnosis of diseases or disorders associated with IL-6 in patients exhibiting symptoms of an IL-6 associated disease or disorder identified herein, comprising assaying the level of IL-6 expression in a biological sample from said patient using a post-translationally modified anti-IL-6 antibody or binding fragment or variant thereof. The anti-IL-6 antibody or binding fragment or variant thereof may be post-translationally modified to include a detectable moiety such as set forth previously in the disclosure.

The IL-6 level in the biological sample is determined using a modified anti-IL-6 antibody or binding fragment or variant thereof as set forth herein, and comparing the level of IL-6 in the biological sample against a standard level of IL-6 (e.g., the level in normal biological
samples). The skilled clinician would understand that some variability may exist between normal biological samples, and would take that into consideration when evaluating results.

[0599] The above-mentioned assay may also be useful in monitoring a disease or disorder, where the level of IL-6 obtained in a biological sample from a patient believed to have an IL-6 associated disease or disorder is compared with the level of IL-6 in prior biological samples from the same patient, in order to ascertain whether the IL-6 level in said patient has changed with, for example, a treatment regimen. A skilled clinician would understand that a biological sample includes, but is not limited to, sera, plasma, urine, saliva, mucous, pleural fluid, synovial fluid and spinal fluid.

**FUSION PROTEINS**

[0600] Fusion proteins comprising IL-6 antagonists are also provided by the present invention. Fusions comprising the anti-IL-6 antibodies polypeptides are also within the scope of the present invention. For example, the fusion protein may be linked to a GST fusion protein in which the anti-IL-6 antibodies polypeptide sequences are fused to the C-terminus of the GST sequences. Such fusion proteins may facilitate the purification of the recombinant Anti-IL-6 antibodies polypeptides. Alternatively, anti-IL-6 antibodies polypeptides may be fused with a protein that binds B-cell follicles, thus initiating both a humoral immune response and activation of T cells. Berney, et al. (1999) *J. Exp. Med.*, 190: 851-60. Alternatively, for example, the Anti-IL-6 antibodies polypeptides may be genetically coupled with and anti-dendritic cell antibody to deliver the antigen to the immune system and stimulate a cellular immune response. He, et al. (2004) *Clin. Cancer Res.*, 10: 1920-27. A chimeric or fusion protein of the invention may be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, e.g., by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. The fusion gene may be synthesized by conventional techniques including automated DNA synthesizers.

[0601] Fusion proteins may include C-terminal or N-terminal translocation sequences. Further, fusion proteins can comprise additional elements, e.g., for protein detection, purification, or other applications. Detection and purification facilitating domains including but not limited to metal chelating peptides such as polyhistidine tracts, histidine-tryptophan modules, or other domains that allow purification on immobilized metals; maltose binding protein; protein A domains that allow purification on immobilized immunoglobulin; or the domain utilized in the FLAG extension/affinity purification system (Immunex Corp, Seattle WA.)
A fusion protein may be prepared from a protein of the invention by fusion with a portion of an immunoglobin comprising a constant region of an immunoglobin. More preferably, the portion of the immunoglobin comprises a heavy chain constant region which is optionally and more preferably a human heavy chain constant region. The heavy chain constant region is most preferably an IgG heavy chain constant region, and optionally and most preferably is an Fc chain, most preferably an IgG Fc fragment that comprises CH2 and CH3 domains. Although any IgG subtype may optionally be used, the IgGl subtype is preferred. The Fc chain may optionally be a known or "wild type" Fc chain, or alternatively may be mutated. See, e.g., U.S. Patent Application Publication No. 2006/0034852. The term "Fc chain" also optionally comprises any type of Fc fragment. Several of the specific amino acid residues that are involved in antibody constant region-mediated activity in the IgG subclass have been identified. Inclusion, substitution or exclusion of these specific amino acids therefore allows for inclusion or exclusion of specific immunoglobin constant region-mediated activity. Furthermore, specific changes may result in aglycosylation for example and/or other desired changes to the Fc chain. At least some changes may optionally be made to block a function of Fc which is considered to be undesirable, such as an undesirable immune system effect. See McCafferty, et al. (2002) Antibody Engineering: A Practical Approach (Eds.) Oxford University Press.

The inclusion of a cleavable linker sequences such as Factor Xa (see, e.g., Ottavi, (1998) Biochimie 80: 289-93), subtilisin protease recognition motif (see, e.g., Polyak (1997) Protein Eng. 10: 615-19); enterokinase (Invitrogen, San Diego, CA.), between the translocation domain (for efficient plasma membrane expression) and the rest of the newly translated polypeptide may be useful to facilitate purification. For example, one construct can include a polypeptide encoding a nucleic acid sequence linked to six histidine residues followed by a thioredoxin, an enterokinase cleavage site (see, e.g., Williams (1995) Biochemistry 34: 1787-97), and an C-terminal translocation domain. The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the desired protein(s) from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the art. See, e.g., Kroll (1993) DNA Cell. Biol. 12: 441-53.

CONJUGATES

IL-6 antagonists may be conjugated to other moieties (e.g., conjugates). Further, the anti-IL-6 antibodies, antibodies that bind the Anti-IL-6 antibodies and fragments thereof, may be conjugated to other moieties. Such conjugates are often used in the preparation of vaccines. The anti-IL-6 antibodies polypeptide may be conjugated to a carbohydrate (e.g., mannose, fucose,
glucose, GlcNAs, maltose), which is recognized by the mannose receptor present on dendritic cells and macrophages. The ensuing binding, aggregation, and receptor-mediated endocytosis and phagocytosis functions provide enhanced innate and adaptive immunity. See Mahnke, et al. (2000) J. Cell Biol. 151: 673-84; Dong, et al. (1999) J. Immunol. 163: 5427-34. Other moieties suitable for conjugation to elicit an immune response includes but not limited to Keyhole Limpit Hemocyanin (KLH), diphtheria toxoid, cholera toxoid, Pseudomonas exoprotein A, and microbial outer membrane proteins (OMPS).

LABELS

[0605] As stated above, antibodies and fragments and variants thereof may be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent moieties, or functional moieties such as for example streptavidin, avidin, biotin, a cytotoxic agent, and radioactive materials.

[0606] The anti-IL-6 antibodies and antigen-binding fragments thereof described herein may be modified post-translationally to add effector moieties such as chemical linkers, detectable moieties such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, chemiluminescent moieties, a cytotoxic agent, radioactive materials, or functional moieties.

[0607] A wide variety of entities, e.g., ligands, may be coupled to the oligonucleotides as known in the art. Ligands may include naturally occurring molecules, or recombinant or synthetic molecules. Exemplary ligands include, but are not limited to, avadin, biotin, peptides, peptidomimetics, polylysine (PLL), polyethylene glycol (PEG), mPEG, cationic groups, spermine, spermidine, polyamine, thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, mucin, glycosylated polyminoacids, transferrin, aptamer, immunoglobulins (e.g., antibodies), insulin, transferrin, albumin, sugar, lipophilic molecules (e.g., steroids, bile acids, cholesterol, cholic acid, and fatty acids), vitamin A, vitamin E, vitamin K, vitamin B, folic acid, B12, riboflavin, biotin, pyridoxal, vitamin cofactors, lipopolysaccharide, hormones and hormone receptors, lectins, carbohydrates, multivalent carbohydrates, radiolabeled markers, fluorescent dyes, and derivatives thereof. See, e.g., U.S. Patent Nos. 6,153,737; 6,172,208; 6,300,319; 6,335,434; 6,335,437; 6,395,437; 6,444,806; 6,486,308; 6,525,031; 6,528,631; and 6,559,279.

[0608] Additionally, moieties may be added to the antigen or epitope to increase half-life in vivo (e.g., by lengthening the time to clearance from the blood stream. Such techniques include, for example, adding PEG moieties (also termed pegilation), and are well-known in the art. See U.S. Patent Application Publication No. 2003/0031671.
[0609] An IL-6 antagonist, such as an anti-IL-6 antibody or antigen binding fragment thereof, described herein may be "attached" to a substrate when it is associated with the solid label through a non-random chemical or physical interaction. The attachment may be through a covalent bond. However, attachments need not be covalent or permanent. Materials may be attached to a label through a "spacer molecule" or "linker group." Such spacer molecules are molecules that have a first portion that attaches to the biological material and a second portion that attaches to the label. Thus, when attached to the label, the spacer molecule separates the label and the biological materials, but is attached to both. Methods of attaching biological material (e.g., label) to a label are well known in the art, and include but are not limited to chemical coupling.

Detectable Labels
[0610] The anti-IL-6 antibody or antigen-binding fragments described herein may be modified post-translationally to add effector labels such as chemical linkers, detectable labels such as for example fluorescent dyes, enzymes, substrates, bioluminescent materials, radioactive materials, and chemiluminescent labels, or functional labels such as for example streptavidin, avidin, biotin, a cytotoxin, a cytotoxic agent, and radioactive materials. Further exemplary enzymes include, but are not limited to, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, β-galactosidase and luciferase. Further exemplary fluorescent materials include, but are not limited to, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin and dansyl chloride. Further exemplary chemiluminescent labels include, but are not limited to, luminol. Further exemplary bioluminescent materials include, but are not limited to, luciferin and aequorin. Further exemplary radioactive materials include, but are not limited to, bismuth-213 (213Bs), carbon-14 (14C), carbon-11 (11C), chlorine-18 (18Cl), chromium-51 (51Cr), cobalt-57 (57Co), cobalt-60 (60Co), copper-64 (64Cu), copper-67 (67Cu), dysprosium-165 (165Dy), erbium-169 (169Er), fluorine-18 (18F), gallium-67 (67Ga), gallium-68 (68Ga), germanium-68 (68Ge), holmium-166 (166Ho), indium-111 (111In), iodine-125 (125I), iodine-123 (123I), iodine-124 (124I), iodine-131 (131I), iridium-192 (192Ir), iron-59 (59Fe), krypton-81 (81Kr), lead-212 (212Pb), lutetium-177 (177Lu), molybdenum-99 (99Mo), nitrogen-13 (13N), oxygen-15 (15O), palladium-103 (103Pd), phosphorus-32 (32P), potassium-42 (42K), rhenium-186 (186Re), rhenium-188 (188Re), rubidium-81 (81Rb), rubidium-82 (82Rb), samarium-153 (153Sm), selenium-75 (75Se), sodium-24 (24Na), strontium-82 (82Sr), strontium-89 (89Sr), sulfur-35 (35S), technetium-99m (99mTc), thallium-201 (201Tl), tritium (3H), xenon-133 (133Xe), ytterbium-169 (169Yb), ytterbium-177 (177Yb), and yttrium-90 (90Y).
Cytotoxic Agents

[0611] The anti-IL-6 antibodies and antigen-binding fragments described herein may be conjugated to cytotoxic agents including, but are not limited to, methotrexate, aminopterin, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine; alkylating agents such as mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BCNU), mitomycin C, lomustine (CCNU), 1-methylN-nitrosourea, cyclophosphamide, mechlorethamine, busulfan, dibromomannitol, streptozotocin, mitomycin C, cis-dichlorodiamine platinum (II) (DDP) cisplatin and carboplatin (paraplatin); anthracyclines include daunorubicin (formerly daunomycin), doxorubicin (adriamycin), detorubicin, carminomycin, idarubicin, mitoxantrone and bisantrene; antibiotics include dactinomycin (actinomycin D), bleomycin, calicheamicin, mithramycin, and anthramycin (AMC); and antimitotic agents such as the vinca alkaloids, vincristine and vinblastine. Other cytotoxic agents include paclitaxel (TAXOL®), ricin, pseudomonas exotoxin, gemcitabine, cytochalasin B, gramicidin D, ethidium bromide, emetine, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, glucocorticoids, procarine, tetracaine, lidocaine, propranolol, puromycin, procarbazine, hydroxyurea, asparaginase, corticosteroids, myotone (0,P’-(DDD)), interferons, and mixtures of these cytotoxic agents.

[0612] Further cytotoxic agents include, but are not limited to, chemotherapeutic agents such as carboplatin, cisplatin, paclitaxel, gemcitabine, calicheamicin, doxorubicin, 5-fluorouracil, mitomycin C, actinomycin D, cyclophosphamide, vincristine, bleomycin, VEGF antagonists, EGFR antagonists, platins, taxols, irinotecan, 5-fluorouracil, gemcitabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids (e.g., vinblastine, vincristine, vindesine and vinorelbine), mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins (e.g. IL-12 or IL-2), IL-12R antagonists, Erbitux®, Avastin®, Pertuzumab, anti-CD20 antibodies, Rituxan®, ocrelizumab, ofatumumab, DXL625, Herceptin®, or any combination thereof. Toxic enzymes from plants and bacteria such as ricin, diphtheria toxin and Pseudomonas toxin may be conjugated to the humanized antibodies, or binding fragments thereof, to generate cell-type-specific -killing reagents. Youle, et al. (1980) Proc. NatT Acad. Sci. USA 77: 5483; Gilliland, et al. (1980) Proc. NatT Acad. Sci. USA 77: 4539; Kroll, et al. (1980) Proc. NatT Acad. Sci. USA 77: 5419.

Other cytotoxic agents include cytotoxic ribonucleases. See U.S. Patent No. 6,653,104.

[0613] The anti-IL-6 antibodies and antigen-binding fragments described herein may be conjugated to a radionuclide that emits alpha or beta particles (e.g., radioimmunonoconjugates). Such radioactive isotopes include but are not limited to beta-emitters such as phosphorus-32 (32P),...
scandium-47 ($^{47}\text{Sc}$), copper-67 ($^{67}\text{Cu}$), gallium-67 ($^{67}\text{Ga}$), yttrium-88 ($^{88}\text{Y}$), yttrium-90 ($^{90}\text{Y}$), iodine-125 ($^{125}\text{I}$), iodine-131 ($^{131}\text{I}$), samarium-153 ($^{153}\text{Sm}$), lutetium-177 ($^{177}\text{Lu}$), rhenium-186 ($^{186}\text{Re}$), rhenium-188 ($^{188}\text{Re}$), and alpha-emitters such as astatine-211 ($^{211}\text{At}$), lead-212 ($^{212}\text{Pb}$), bismuth-212 ($^{212}\text{Bi}$), bismuth-213 ($^{213}\text{Bi}$) or actinium-225 ($^{225}\text{Ac}$).

**Methods**


**SUBSTRATES**

The anti-IL-6 antibodies and antigen-binding fragments thereof described herein may be attached to a substrate. A number of substrates (*e.g.*, solid supports) known in the art are suitable for use with the anti-IL-6 antibody described herein. The substrate may be modified to contain channels or other configurations. See Fung (2004) [Ed.] *Protein Arrays: Methods and Protocols* Humana Press and Kambhampti (2004) [Ed.] *Protein Microarray Technology* John Wiley & Sons.

Substrate materials include, but are not limited to acrylics, agarose, borosilicate glass, carbon (*e.g.*, carbon nanofiber sheets or pellets), cellulose acetate, cellulose, ceramics, gels, glass (*e.g.*, inorganic, controlled-pore, modified, soda-lime, or functionalized glass), latex, magnetic beads, membranes, metal, metalloids, nitrocellulose, NYLON®, optical fiber bundles, organic polymers, paper, plastics, polycryloylmorphohilde, poly(4-methylbutene), poly(ethylene terephthalate), poly(vinyl butyrate), polyacrylamide, polybutylene, polycarbonate, polyethylene, polyethyleneglycol terephthalate, polyformaldehyde, polymethacrylate, polymethylmethacrylate, polypropylene, polysaccharides, polystyrene, polyurethanes, polyvinylacetate, polyvinylchloride, polyvinylidene difluoride (PVDF), polyvinylpyrrolidinone, rayon, resins, rubbers, semiconductor materials, sepharose®, silica, silicon, styrene copolymers, TEFLOW®, and variety of other polymers.

Substrates need not be flat and can include any type of shape including spherical shapes (*e.g.*, beads) or cylindrical shapes (*e.g.*, fibers). Materials attached to solid supports may be attached to any portion of the solid support (*e.g.*, may be attached to an interior portion of a porous solid support material).

The substrate body may be in the form of a bead, box, column, cylinder, disc, dish (*e.g.*, glass dish, PETRI dish), fiber, film, filter, microtiter plate (*e.g.*, 96-well microtiter plate), multi-bladed stick, net, pellet, plate, ring, rod, roll, sheet, slide, stick, tray, tube, or vial. The substrate may be a singular discrete body (*e.g.*, a single tube, a single bead), any number of a
plurality of substrate bodies (e.g., a rack of 10 tubes, several beads), or combinations thereof
(e.g., a tray comprises a plurality of microtiter plates, a column filled with beads, a microtiter plate filled with beads).

[0619] An anti-IL-6 antibody or antigen-binding fragment thereof may be "attached" to a substrate when it is associated with the solid substrate through a non-random chemical or physical interaction. The attachment may be through a covalent bond. However, attachments need not be covalent or permanent. Materials may be attached to a substrate through a "spacer molecule" or "linker group." Such spacer molecules are molecules that have a first portion that attaches to the biological material and a second portion that attaches to the substrate. Thus, when attached to the substrate, the spacer molecule separates the substrate and the biological materials, but is attached to both. Methods of attaching biological material (e.g., label) to a substrate are well known in the art, and include but are not limited to chemical coupling.

[0620] Plates, such as microtiter plates, which support and contain the solid-phase for solid-phase synthetic reactions may be used. Microtiter plates may house beads that are used as the solid-phase. By "particle" or "microparticle" or "nanoparticle" or "bead" or "microbead" or "microsphere" herein is meant microparticulate matter having any of a variety of shapes or sizes. The shape may be generally spherical but need not be spherical, being, for example, cylindrical or polyhedral. As will be appreciated by those in the art, the particles may comprise a wide variety of materials depending on their use, including, but not limited to, cross-linked starch, dextrans, cellulose, proteins, organic polymers including styrene polymers such as polystyrene and methylstyrene as well as other styrene co-polymers, plastics, glass, ceramics, acrylic polymers, magnetically responsive materials, colloids, thioriasol, carbon graphite, titanium dioxide, nylon, latex, and TEFлон®. See e.g., "Microsphere Detection Guide" from Bangs Laboratories, Fishers, IN.

[0621] The anti-IL-6 antibody or antigen-binding fragment may be attached to any of the forms of substrates described herein (e.g., bead, box, column, cylinder, disc, dish (e.g., glass dish, PETRI dish), fiber, film, filter, microtiter plate (e.g., 96-well microtiter plate), multi-bladed stick, net, pellet, plate, ring, rod, roll, sheet, slide, stick, tray, tube, or vial). In particular, particles or beads may be a component of a gelling material or may be separate components such as latex beads made of a variety of synthetic plastics (e.g., polystyrene). The label (e.g., streptavidin) may be bound to a substrate (e.g., bead).
**ASSESSMENT OF INFLAMMATORY MARKERS**

[0622] Known inflammatory markers (e.g., IL-6) may be measured to assess the risk for anemia or the severity of anemia. These markers may be measured from serum, synovial fluid, or skin biopsies using known methods in the art (e.g., immunassays).

**IL-6 Serum Levels**

[0623] Serum IL-6 levels may be measured as a pharmacodynamic marker evaluate the effect of neutralization of IL-6 levels. Serum IL-6 levels may be measured using an immunoassay (e.g., ELISA assay). A decrease of serum IL-6 levels may be indicative of a lessening of inflammation.

**Serum Inflammatory Biomarkers**

[0624] Serum biomarkers may be measured to determine the expression of pro-inflammatory cytokines and other soluble biomarkers that may correlate with anemia (e.g., anemia associated with chemotherapy or radiotherapy) disease activity including but not limited to acute phase reactants, serum pro-inflammatory cytokines (e.g., IL-1, TNF-a, IFN-γ, IL-12p40, IL-17), chemokines (e.g., RANTES, MIP-la, MCP-1), matrix metalloproteinases (e.g., MMP-2, MMP-3, MMP-9) and other biomarkers associated with inflammation and autoimmune pathways that are known in the art. Soluble biomarkers of bone and cartilage metabolism (e.g., osteocalcin and other collagen degradation products) may also be assessed by an immunoassay (e.g., ELISA). A decrease in a serum inflammatory biomarker may be indicative of a lessening of inflammation.

**Immunohistochemistry of Skin Biopsies**

[0625] Skin biopsies may be collected for biomarker analysis including whole genome array analysis and immunohistochemistry (IHC). Immunohistochemical analysis may include the measurement of epidermal thickness, frequency of resident and inflammatory cell populations (e.g., T cells, macrophages, keratinocytes) and other inflammatory markers related to the IL-6 pathway known in the art. Specifically, the following specific antigens may be assessed per standard IHC procedure using the formalin-fixed samples: CD3, CD68, keratin 16, FoxP3, IL-6R and MMP-3. A decrease in an inflammatory biomarker in a skin biopsy may be indicative of a lessening of inflammation.

**Anemia Markers**

[0626] Anemia may be assessed by assays well-known in the art such as a Complete Blood Count (CBC) test that measures the red blood cell (RBC) count, hematocrit, hemoglobin levels, white blood cell count (CBC), differential blood count, and platelet count. The first three parameters, the RBC, hematocrit, and hemoglobin levels are the most commonly used in determining whether or not the patient is suffering from anemia. Other anemia marker include the measurement of the levels of serum ferritin and serum iron.
[0627] Hematocrit levels below about 42-52% for men or about 36-48% for women are indicative of anemia. Serum ferritin levels below about 30-400 ng/mL for men or about 13-150 ng/mL for women are indicative of anemia. Serum iron levels below about 60-170 µg/dL is indicative of anemia. A reticulocyte count below about 0.5%—1.5% is indicative of anemia. A white blood cell (WBC) count of below about 5,000-1 0,000/mL is indicative of anemia and a red blood cell (RBC) count of below about 4.5-5.5x10^6/mL for men and below about 4.0-5.0x10^6/mL for women are indicative of anemia. Further, a platelet count below about 1.4-4.0x10^5/mL is indicative of anemia. Also, Additionally, total iron binding capacity (TIBC) measures the level for transferring in the blood and the normal levels are about 250-370 µg/dL. Transferrin is a protein that carries iron in the blood and a higher than normal TIBC value is a sign of iron-deficiency anemia and a lower than normal level indicates chronic anemia, pernicious anemia, or hemolytic anemia. Additionally, tests for anemia include direct or indirect Coombs' test, indirect bilirubin levels, serum haptoglobin, vitamin B12 levels, folate levels, and urine hemoglobin. MedlinePlus website "Drug-induced immune hemolytic anemia." (2011) & D Medical Center (2011) "Anemia- Diagnosis".

ADMINISTRATION

[0628] In one embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations of said antibody fragments or variants, are administered to a subject at a concentration of between about 0.1 and 20 mg/kg, such as about 0.4 mg/kg, about 0.8 mg/kg, about 1.6 mg/kg, or about 4 mg/kg, of body weight of recipient subject. For example, compositions comprising the IL-6 antagonists described herein may comprise at least about 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, or 500 mg. For example, compositions comprising the anti-IL-6 antibodies described herein may comprise at least about 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, or 500 mg.

[0629] For example, a composition for treating anemia may comprise 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl). A composition for treating drug-induced immune hemolytic anemia may comprise 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl). A composition for treating anemia associated with chemotherapy may comprise 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl). A composition for treating anemia associated with radiotherapy may comprise 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl). A composition for treating
anemia associated with cancer may comprise 80, 160, or 320 mg of an anti-IL-6 antibody (e.g., Abl). For example, compositions comprising the anti-IL-6 antibodies described herein may comprise at least about 0.5-10 mg/kg of the anti-IL-6 antibody. In a preferred embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations of said antibody fragments or variants, are administered to a subject at a concentration of about 0.4 mg/kg of body weight of recipient subject. In a preferred embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations of said antibody fragments or variants, are administered to a recipient subject with a frequency of once every twenty-six weeks or less, such as once every sixteen weeks or less, once every eight weeks or less, or once every four weeks, or less. In another preferred embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations thereof, are administered to a recipient subject with a frequency at most once per period of approximately one week, such as at most once per period of approximately two weeks, such as at most once per period of approximately four weeks, such as at most once per period of approximately eight weeks, such as at most once per period of approximately twelve weeks, such as at most once per period of approximately sixteen weeks, such as at most once per period of approximately twenty-four weeks.

[0630] The compositions described herein may be administered in any of the following routes: buccal, epicutaneous, epidural, infusion, inhalation, intraarterial, intracardial, intracerebroventricular, intradermal, intramuscular, intranasal, intraocular, intraperitoneal, intraspinal, intrathecal, intravenous, oral, parenteral, pulmonary, rectally via an enema or suppository, subcutaneous, subdermal, sublingual, transdermal, and transmucosal. The preferred routes of administration are intravenous injection or infusion. The administration can be local, where the composition is administered directly, close to, in the locality, near, at, about, or in the vicinity of, the site(s) of disease, e.g., local (joint) or systemic, wherein the composition is given to the patient and passes through the body widely, thereby reaching the site(s) of disease. Local administration (e.g., subcutaneous injection) may be accomplished by administration to the cell, tissue, organ, and/or organ system, which encompasses and/or is affected by the disease, and/or where the disease signs and/or symptoms are active or are likely to occur (e.g., swollen joint). Administration can be topical with a local effect, composition is applied directly where its action is desired (e.g., joint). Further, administration of a composition comprising an effective amount of an anti-IL-6 antibody selected from the group consisting of Abl-Ab36 or an antigen-binding fragment thereof, may be subcutaneous.
For each of the recited embodiments, the compounds can be administered by a variety of dosage forms as known in the art. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, caplets, lozenges, chewable lozenges, beads, powders, gum, granules, particles, microparticles, dispersible granules, cachets, douches, suppositories, creams, topicals, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, ingestibles, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, and combinations thereof.

Other compounds which can be included by admixture are, for example, medically inert ingredients (e.g., solid and liquid diluent), such as lactose, dextrose, saccharose, cellulose, starch or calcium phosphate for tablets or capsules, olive oil or ethyl oleate for soft capsules and water or vegetable oil for suspensions or emulsions; lubricating agents such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; gelling agents such as colloidal clays; thickening agents such as gum tragacanth or sodium alginate, binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinylpyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescent mixtures; dyestuff; sweeteners; wetting agents such as lecithin, polysorbates or laurylsulphates; and other therapeutically acceptable accessory ingredients, such as humectants, preservatives, buffers and antioxidants, which are known additives for such formulations.

Liquid dispersions for oral administration can be syrups, emulsions, solutions, or suspensions. The syrups can contain as a carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. The suspensions and the emulsions can contain a carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol.

In further embodiments, the present invention provides kits including at least one containers comprising pharmaceutical dosage units comprising an effective amount of at least one antibodies and fragments thereof of the present invention. Kits may include instructions, directions, labels, marketing information, warnings, or information pamphlets.

Dosages

The amount of anti-IL-6 antibodies in a therapeutic composition according to any embodiments of this invention may vary according to factors such as the disease state, age, gender, weight, patient history, risk factors, predisposition to disease, administration route, pre-
existing treatment regime (e.g., possible interactions with other medications), and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of therapeutic situation.

[0636] For example, for the treatment of anemia a composition comprising at least about 80, 160, or 320 mg IL-6 antagonists may be administered to a patient in need thereof. In another embodiment, for the treatment of anemia associated with chemotherapy a composition comprising at least about 80, 160, or 320 mg IL-6 antagonists may be administered to a patient in need thereof. Further, for the treatment of anemia a composition comprising at least about 80, 160, or 320 mg anti-IL-6 antibody (e.g., Abl) may be administered to a patient in need thereof. In another embodiment, for the treatment of anemia associated with chemotherapy a composition comprising at least about 80, 160, or 320 mg anti-IL-6 antibody (e.g., Abl) may be administered to a patient in need thereof. The dosage of IL-6 antagonist, may depend upon the mode of administration. For example, for subcutaneous administration of a composition comprising an IL-6 antagonist, the composition may comprise at least about 1-500 mg/mL, 10-250 mg/mL, 10-100 mg/mL, or 40-100 mg/mL of an IL-antagonist. For example, a composition for subcutaneous administration may comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 mg/mL of an IL-6 antagonist. Thus, a composition for subcutaneous administration may comprise at least about at least about 1-500 mg/mL, 10-250 mg/mL, 10-100 mg/mL, or 40-100 mg/mL of an anti-IL-6 antibody (e.g., Abl). For intravenous administration of a composition comprising an IL-6 antagonist, the composition may comprise at least about 1-500 mg/mL, 10-250 mg/mL, 10-100 mg/mL, or 40-100 mg/mL of an anti-IL-6 antibody (e.g., Abl). For intravenous administration of a composition comprising an IL-6 antagonist, the composition may comprise at least about 1-500 mg/mL, 10-250 mg/mL, 10-100 mg/mL, or 40-100 mg/mL of an anti-IL-6 antagonist. For example, a composition for intravenous administration may comprise at least about at least about 1-500 mg/mL, 10-250 mg/mL, 10-100 mg/mL, or 40-100 mg/mL of an anti-IL-6 antibody (e.g., Abl). Further, an intravenous formulation of an Ab1 anti-IL-6 antibody may comprise at least about 10 mg/mL or 40 mg/L for the treatment of rheumatoid arthritis and a subcutaneous formulation of an Abl anti-IL-6 antibody may comprise at least about 100 mg/mL for the treatment of rheumatoid arthritis.

[0637] It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically
discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of antibodies, or antigen-binding fragments thereof, calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the antibodies, and fragments thereof, and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an antibodies, and fragments thereof, for the treatment of sensitivity in individuals. In therapeutic use for treatment of conditions in mammals (e.g., humans) for which the antibodies and fragments thereof of the present invention or an appropriate pharmaceutical composition thereof are effective, the antibodies and fragments thereof of the present invention may be administered in an effective amount. The dosages as suitable for this invention may be a composition, a pharmaceutical composition or any other compositions described herein.

[0638] The dosage may be administered as a single dose, a double dose, a triple dose, a quadruple dose, and/or a quintuple dose. The dosages may be administered singularly, simultaneously, and sequentially. For example, two doses may be administered on the same day followed by subsequent two doses four weeks later.

[0639] The dosage form may be any form of release known to persons of ordinary skill in the art. The compositions of the present invention may be formulated to provide immediate release of the active ingredient or sustained or controlled release of the active ingredient. In a sustained release or controlled release preparation, release of the active ingredient may occur at a rate such that blood levels are maintained within a therapeutic range but below toxic levels over an extended period of time (e.g., 4 to 24 hours). The preferred dosage forms include immediate release, extended release, pulse release, variable release, controlled release, timed release, sustained release, delayed release, long acting, and combinations thereof, and are known in the art.

[0640] It will be appreciated that the pharmacological activity of the compositions may be monitored using standard pharmacological models that are known in the art. Furthermore, it will be appreciated that the compositions comprising an anti-IL-6 antibodies or antigen-binding fragments thereof may be incorporated or encapsulated in a suitable polymer matrix or membrane for site-specific delivery, or may be functionalized with specific targeting agents capable of effecting site specific delivery. These techniques, as well as other drug delivery techniques are well known in the art. Determination of optimal dosages for a particular situation is within the capabilities of those skilled in the art. See, e.g., Grennaro (2005) [Ed.] Remington: The Science and Practice of Pharmacy [2 th Ed.]
[0641] In another embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations of said antibody fragments or variants, are administered to a subject in a pharmaceutical formulation.

[0642] A "pharmaceutical composition" refers to a chemical or biological composition suitable for administration to a mammal. Such compositions may be specifically formulated for administration via at least one of a number of routes, including but not limited to buccal, epicutaneous, epidural, inhalation, intraarterial, intracardial, intracerebroventricular, intradermal, intramuscular, intranasal, intraocular, intraperitoneal, intraspinal, intrathecal, intravenous, oral, parenteral, rectally via an enema or suppository, subcutaneous, subdermal, sublingual, transdermal, and transmucosal. In addition, administration can occur by means of injection, powder, liquid, gel, drops, or other means of administration. Further, a pharmaceutical composition comprising an anti-IL-6 antibody described herein (e.g., ALD5 18) may be administered subcutaneously.

[0643] In one embodiment of the invention, the anti-IL-6 antibodies described herein, or IL-6 binding fragments or variants thereof, as well as combinations of said antibody fragments or variants, may be optionally administered in combination with at least one active agents. Such active agents include analgesic, antipyretic, anti-inflammatory, antibiotic, antiviral, and anti-cytokine agents. Active agents include agonists, antagonists, and modulators of TNF-alpha, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, WN-alpha, IFN-gamma, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, including antibodies reactive against any of the foregoing, and antibodies reactive against any of their receptors. Active agents also include 2-Arylpropionic acids, Aceclofenac, Acemetacin, Acetylsalicylic acid (Aspirin), Alclofenac, Alminoprofen, Amoxiprin, Ampyrone, Arylalkanoic acids, Azapropazone, Benorylate/Benorilate, Benoxaprofen, Bromfenac, Carprofen, Celecoxib, Choline magnesium salicylate, Clofzone, COX-2 inhibitors, Desibuprofen, Dexketoprofen, Diclofenac, Diflunisal, Drixicam, Ethenamide, Etodolac, Etoricoxib, Faislamine, fenamic acids, Fenbufen, Fenoprofen, Flufenamic acid, Flunoxaprofen, Flurbiprofen, Ibuprofen, Ibuprocam, Indometacin, Indoprofen, Kebuzone, Ketoprofen, Ketorolac, Lornoxicam, Loxoprofen, Lumiracoxib, Magnesium salicylate, Meclofenamic acid, Mefenamic acid, Meloxicam, Metamizole, Methyl salicylate, Mofebutazone, Nabumetone, Naproxen, N-Arylanthranilic acids, Oxametacin, Oxaprozin, Oxicams, Oxyphenbutazone, Parecoxib, Phenazone, Phenylbutazone, Phenylbutazone, Piroxicam, Pirprofen, profens, Proglumetacin, Pyrazolidine derivatives, Rofecoxib, Salicyl salicylate, Salicylamide, Salicylates, Sulfinpyrazone, Sulindac, Suprofen, Tenoxicam, Tiaprofenic acid, Tolfenamic acid, Tolmetin, and Valdecoxib. Antibiotics include Amikacin,
Aminoglycosides, Amoxicillin, Ampicillin, Ansamycins, Arsphenamine, Azithromycin, Azlocillin, Aztreonam, Bacitracin, Carbacephem, Carbapenems, Carbenicillin, Cefaclor, Cefadroxil, Cefalexin, Cefalothin, Cefamandole, Cefazolin, Cefdinir, Cefditoren, Cefepime, Cefixime, Cefoperazone, Cefotaxime, Cefuroxime, Cephalosporins, Chloramphenicol, Clindamycin, Cloxacillin, Colistin, Co-trimoxazole, Dalfopristin, Demeclocycline, Dicloxacillin, Dirithromycin, Doripenem, Doxycycline, Enoxacin, Ertapenem, Erythromycin, Ethambutol, Flucloxacillin, Fosfomycin, Furazolidone, Fusidic acid, Gatifloxacin, Geldanamycin, Gentamicin, Glycopeptides, Herbimycin, Imipenem, Isoniazid, Kanamycin, Levofloxacin, Lincomycin, Linezolid, Lomefloxacin, Loracarbef, Macrolides, Mafenide, Meropenem, Meticillin, Metronidazole, Metzlocillin, Minocycline, Monobactams, Moxifloxacin, Mupirocin, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Oxytetracycline, Paromomycin, Penicillin, Penicillins, Piperacillin, Platensimycin, Polymyxin B, Polypeptides, Prontosil, Pyrazinamide, Quinolones, Quinupristin, Rifampicin, Rifampin, Roxithromycin, Spectinomycin, Streptomycin, Sulfacetamide, Sulfamethizole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Sulfonamides, Teicoplanin, Telithromycin, Tetracycline, Tetracyclines, Ticarcillin, Tinidazole, Tobramycin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Troleandomycin, Trovafloxacin, and Vancomycin. Active agents also include Aldosterone, Beclometasone, Betamethasone, Corticosteroids, Cortisol, Cortisone acetate, Deoxycorticosterone acetate, Dexamethasone, Fludrocortisone acetate, Glucocorticoids, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone, Steroids, and Triamcinolone. Antiviral agents include but are not limited to abacavir, aciclovir, acyclovir, adefovir, amantadine, ampmavir, an antiretroviral fixed dose combination, an antiretroviral synergistic enhancer, arbidol, atazanavir, atiprala, brivudine, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, fanciclovir, fomivirsen, fosamprenavir, foscarnet, fosfonet, fusion inhibitor, ganciclovir, gardasil, ibacitabine, idoxuridine, imiquimod, imunovir, indinavir, inosine, integrase inhibitor, interferon, interferon type I, interferon type II, interferon type III, lamivudine, lopinavir, loviride, maraviroc, MK-0518, moroxydine, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, and zidovudine. Any suitable combination of these active agents is also contemplated.
A "pharmaceutical excipient" or a "pharmaceutically acceptable excipient" is a carrier, usually a liquid, in which an active therapeutic agent is formulated. In one embodiment of the invention, the active therapeutic agent is a humanized antibody described herein, or at least one fragments or variants thereof. The excipient generally does not provide any pharmacological activity to the formulation, though it may provide chemical and/or biological stability, and release characteristics. Exemplary formulations can be found, for example, in Grennaro (2005) [Ed.] Remington: The Science and Practice of Pharmacy [2 1st Ed.]

As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, or sublingual administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The invention contemplates that the pharmaceutical composition is present in lyophilized form. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The invention further contemplates the inclusion of a stabilizer in the pharmaceutical composition.

The antibodies and fragments thereof, of the present invention thereof may be formulated into pharmaceutical compositions of various dosage forms. For example, the antibody may be ALD518, a humanized anti-interleukin-6 (anti-IL-6) monoclonal immunoglobulin 1 (IgGl) antibody manufactured in the yeast Pichia pastoris. ALD518 may be supplied as a pH 6.0 frozen injection in single-use vials (80 mg or 160 mg) for intravenous administration. Exemplary non-active excipients include but are not limited to histidine (e.g., 25 mM) and sorbitol (e.g., 250 mM). For example, a 160 mg formulation may comprise as non-active excipients, 25 mM histidine, 250 mM sorbitol, and 0.015% polysorbate 80. To prepare the pharmaceutical compositions of the invention, at least one anti-IL-6 antibodies or binding
fragments thereof, as the active ingredient may be intimately mixed with appropriate carriers and additives according to techniques well known to those skilled in the art of pharmaceutical formulations. See Gennaro (2005) [Ed.] Remington: The Science and Practice of Pharmacy [21st Ed.] For example, the antibodies described herein may be formulated in phosphate buffered saline pH 7.2 and supplied as a 5.0 mg/mL clear colorless liquid solution.

Similarly, compositions for liquid preparations include solutions, emulsions, dispersions, suspensions, syrups, and elixirs, with suitable carriers and additives including but not limited to water, alcohols, oils, glycols, preservatives, flavoring agents, coloring agents, and suspending agents. Typical preparations for parenteral administration comprise the active ingredient with a carrier such as sterile water or parenterally acceptable oil including but not limited to polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil, with other additives for aiding solubility or preservation may also be included. In the case of a solution, it may be lyophilized to a powder and then reconstituted immediately prior to use. For dispersions and suspensions, appropriate carriers and additives include aqueous gums, celluloses, silicates, or oils.

For each of the recited embodiments, the anti-IL-6 antibodies or binding fragments thereof, may be administered by a variety of dosage forms. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, powders, granules, particles, microparticles, dispersible granules, cachets, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, and combinations thereof.

In many cases, it will be preferable to include isotonic agents, e.g., sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, e.g., monostearate salts and gelatin. Moreover, the compounds described herein may be formulated in a time release formulation, e.g. in a composition that includes a slow release polymer. The anti-IL-6 antibodies may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are known to those skilled in the art.
In one embodiment of the invention that may be used to intravenously administer antibodies of the invention, including ALD518, for anemia, the administration formulation comprises, or alternatively consists of, about 10.5 mg/mL of antibody, 25 mM Histidine base, Phosphoric acid q.s. to pH 6, and 250 mM sorbitol.

In another embodiment of the invention that may be used to intravenously administer antibodies of the invention, including ALD581, for anemia, the administration formulation comprises, or alternatively consists of, about 10.5 mg/mL of antibody, 12.5 mM Histidine base, 12.5 mM Histidine HCl (or 25 mM Histidine base and Hydrochloric acid q.s. to pH 6), 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

In one embodiment of the invention that may be used to subcutaneously administer antibodies of the invention, including ALD518, for anemia, the administration formulation comprises, or alternatively consists of, about 50 or 100 mg/mL of antibody, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80. In another embodiment of the invention that may be used to subcutaneously administer antibodies of the invention, including Abl, for anemia, the administration formulation comprises, or alternatively consists of, about 20 or 100 mg/mL of antibody, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 to 280 mM sorbitol (or sorbitol in combination with sucrose), and 0.015% (w/w) Polysorbate 80, said formulation having a nitrogen headspace in the shipping vials.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The invention contemplates that the pharmaceutical composition is present in lyophilized form. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The invention further contemplates the inclusion of a stabilizer in the pharmaceutical composition.

In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the alkaline polypeptide can be formulated in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable,
biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are known to those skilled in the art.

[0656] For each of the recited embodiments, the compounds can be administered by a variety of dosage forms. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, powders, granules, particles, microparticles, dispersible granules, cachets, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, and combinations thereof.


[0658] The above description of various illustrated embodiments of the invention is not intended to be exhaustive or to limit the invention to the precise form disclosed. While specific embodiments of, and examples for, the invention are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the invention, as those skilled in the relevant art will recognize. The teachings provided herein of the invention can be applied to other purposes, other than the examples described above.

[0659] These and other changes can be made to the invention in light of the above detailed description. In general, in the following claims, the terms used should not be construed to limit the invention to the specific embodiments disclosed in the specification and the claims. Accordingly, the invention is not limited by the disclosure, but instead the scope of the invention is to be determined entirely by the following claims.

[0660] The invention may be practiced in ways other than those particularly described in the foregoing description and examples. Numerous modifications and variations of the invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[0662] Certain teachings related to humanization of rabbit-derived monoclonal antibodies and preferred sequence modifications to maintain antigen binding affinity were disclosed in U.S. Patent Application Publication No. 2009/0104187.

[0663] Certain teachings related to producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent Application Publication No. 2006/0270045.

[0664] Certain teachings related to anti-IL-6 antibodies, methods of producing antibodies or fragments thereof using mating competent yeast and corresponding methods were disclosed in U.S. Patent Application Publication No. 2009/0104187.

[0665] Certain teachings related to anti-IL-6 antibodies and methods of using those antibodies or fragments thereof to address certain diseases and/or disorders were disclosed in U.S. Patent Application Publication No. 2010/0150829.

[0666] Certain anti-IL-6 antibody polynucleotides and polypeptides are disclosed in the sequence listing accompanying this patent application filing, and the disclosure of said sequence listing is herein incorporated by reference in its entirety.

[0667] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g., amounts, temperature, concentrations) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

EXAMPLES

[0668] In the following examples, the term "Ab1" refers to an antibody comprising the light chain sequence of SEQ ID NO: 702 and the heavy chain sequence of SEQ ID NO: 704, except where the context indicates otherwise. The laboratory designation "Ab1" also encompasses an anti-IL-6 antibody also known as "ALD518" and "BMS-945429" comprising the light chain sequence of SEQ ID NO: 19 and the heavy chain sequence of SEQ ID NO: 20.

Example 1
Production of Enriched Antigen-Specific B Cell Antibody Culture

[0669] Panels of antibodies are derived by immunizing traditional antibody host animals to exploit the native immune response to a target antigen of interest. Typically, the host used for immunization is a rabbit or other host that produces antibodies using a similar maturation process and provides for a population of antigen-specific B cells producing antibodies of comparable
diversity, e.g., epitopic diversity. The initial antigen immunization can be conducted using complete Freund's adjuvant (CFA), and the subsequent boosts effected with incomplete adjuvant. At about 50-60 days after immunization, preferably at day 55, antibody titers are tested, and the Antibody Selection (ABS) process is initiated if appropriate titers are established. The two key criteria for ABS initiation are potent antigen recognition and function-modifying activity in the polyclonal sera.

[0670] At the time positive antibody titers are established, animals are sacrificed and B cell sources isolated. These sources include: the spleen, lymph nodes, bone marrow, and peripheral blood mononuclear cells (PBMCs). Single cell suspensions are generated, and the cell suspensions are washed to make them compatible for low temperature long term storage. The cells are then typically frozen.

[0671] To initiate the antibody identification process, a small fraction of the frozen cell suspensions are thawed, washed, and placed in tissue culture media. These suspensions are then mixed with a biotinylated form of the antigen that was used to generate the animal immune response, and antigen-specific cells are recovered using the Miltenyi magnetic bead cell selection methodology. Specific enrichment is conducted using streptavidin beads. The enriched population is recovered and progressed in the next phase of specific B cell isolation.

**Example 2**

**Production of Clonal, Antigen-Specific B Cell-Containing Culture**

[0672] Enriched B cells produced according to Example 1 are then plated at varying cell densities per well in a 96 well microtiter plate. Generally, this is at 50, 100, 250, or 500 cells per well with 10 plates per group. The media is supplemented with 4% activated rabbit T cell conditioned media along with 50K frozen irradiated EL4B feeder cells. These cultures are left undisturbed for 5-7 days at which time supernatant-containing secreted antibody is collected and evaluated for target properties in a separate assay setting. The remaining supernatant is left intact, and the plate is frozen at -70°C. Under these conditions, the culture process typically results in wells containing a mixed cell population that comprises a clonal population of antigen-specific B cells, i.e., a single well will only contain a single monoclonal antibody specific to the desired antigen.

**Example 3**

**Screening of Antibody Supernatants for Monoclonal Antibody of Desired Specificity and/or Functional Properties**

[0673] Antibody-containing supernatants derived from the well containing a clonal antigen-specific B cell population produced according to Example 2 are initially screened for antigen
recognition using ELISA methods. This includes selective antigen immobilization (e.g., biotinylated antigen capture by streptavidin coated plate), non-specific antigen plate coating, or alternatively, through an antigen build-up strategy (e.g., selective antigen capture followed by binding partner addition to generate a heteromeric protein-antigen complex). Antigen-positive well supernatants are then optionally tested in a function-modifying assay that is strictly dependant on the ligand. One such example is an in vitro protein-protein interaction assay that recreates the natural interaction of the antigen ligand with recombinant receptor protein. Alternatively, a cell-based response that is ligand dependent and easily monitored (e.g., proliferation response) is utilized. Supernatant that displays significant antigen recognition and potency is deemed a positive well. Cells derived from the original positive well are then transitioned to the antibody recovery phase.

Example 4
Recovery of Single, Antibody-Producing B Cell of Desired Antigen Specificity

[0674] Cells are isolated from a well that contains a clonal population of antigen-specific B cells (produced according to Example 2 or 3), which secrete a single antibody sequence. The isolated cells are then assayed to isolate a single, antibody-secreting cell. Dynal® (magnetic beads) streptavidin beads are coated with biotinylated target antigen under buffered medium to prepare antigen-containing microbeads compatible with cell viability. Next antigen-loaded beads, antibody-producing cells from the positive well, and a fluorescein isothiocyanate (FITC)-labeled anti-host H&L IgG antibody (as noted, the host can be any mammalian host, e.g., rabbit, mouse, rat) are incubated together at 37°C. This mixture is then re-pipetted in aliquots onto a glass slide such that each aliquot has on average a single, antibody-producing B-cell. The antigen-specific, antibody-secreting cells are then detected through fluorescence microscopy. Secreted antibody is locally concentrated onto the adjacent beads due to the bound antigen and provides localization information based on the strong fluorescent signal. Antibody-secreting cells are identified via FITC detection of antibody-antigen complexes formed adjacent to the secreting cell. The single cell found in the center of this complex is then recovered using a micromanipulator. The cell is snap-frozen in an eppendorf PCR tube for storage at -80°C until antibody sequence recovery is initiated.

Example 5
Isolation of Antibody Sequences From Antigen-Specific B Cell

[0675] Antibody sequences are recovered using a combined RT-PCR based method from a single isolated B-cell produced according to Example 4 or an antigenic specific B cell isolated from the clonal B cell population obtained according to Example 2. Primers are designed to
anneal in conserved and constant regions of the target immunoglobulin genes (heavy and light), such as rabbit immunoglobulin sequences, and a two-step nested PCR recovery step is used to obtain the antibody sequence. Amplicons from each well are analyzed for recovery and size integrity. The resulting fragments are then digested with Alul to fingerprint the sequence clonality. Identical sequences display a common fragmentation pattern in their electrophoretic analysis. Significantly, this common fragmentation pattern which proves cell clonality is generally observed even in the wells originally plated up to 1000 cells/well. The original heavy and light chain amplicon fragments are then restriction enzyme digested with HindIII and Xhol or HindIII and BsiWI to prepare the respective pieces of DNA for cloning. The resulting digestions are then ligated into an expression vector and transformed into bacteria for plasmid propagation and production. Colonies are selected for sequence characterization.

Example 6
Recombinant Production of Monoclonal Antibody of Desired Antigen Specificity and/or Functional Properties

[0676] Correct full-length antibody sequences for each well containing a single monoclonal antibody is established and miniprep DNA is prepared using Qiagen solid-phase methodology. This DNA is then used to transfect mammalian cells to produce recombinant full-length antibody. Crude antibody product is tested for antigen recognition and functional properties to confirm the original characteristics are found in the recombinant antibody protein. Where appropriate, large-scale transient mammalian transfections are completed, and antibody is purified through Protein A affinity chromatography. Kd is assessed using standard methods (e.g., Biacore®) as well as IC50 in a potency assay.

Example 7
Preparation of Antibodies that Bind Human IL-6

[0677] By using the antibody selection protocol described herein, one can generate an extensive panel of antibodies. The antibodies have high affinity towards IL-6 (single to double digit pM Kd) and demonstrate potent antagonism of IL-6 in multiple cell-based screening systems (T1 165 and HepG2). Furthermore, the collection of antibodies displays distinct modes of antagonism toward IL-6-driven processes.

Immunization Strategy

[0678] Rabbits were immunized with huIL-6 (R&R). Immunization consisted of a first subcutaneous (sc) injection of 100 µg in complete Freund's adjuvant (CFA) (Sigma) followed by two boosts, two weeks apart, of 50 µg each in incomplete Freund's adjuvant (IFA) (Sigma).
Animals were bled on day 55, and serum titers were determined by ELISA (antigen recognition) and by non-radioactive proliferation assay (Promega) using the T1165 cell line.

Antibody Selection Titer Assessment

Antigen recognition was determined by coating Immulon 4 plates (Thermo) with 1 µg/mL of huIL-6 (50 µE/well) in phosphate buffered saline (PBS, Hyclone) overnight at 4 °C. On the day of the assay, plates were washed 3 times with PBS/Tween 20 (PBST tablets, Calbiochem). Plates were then blocked with 200 µE/well of 0.5% fish skin gelatin (FSG, Sigma) in PBS for 30 minutes at 37°C. Blocking solution was removed, and plates were blotted. Serum samples were made (bleeds and pre-bleeds) at a starting dilution of 1:100 (all dilutions were made in FSG 50 µL/well) followed by 1:10 dilutions across the plate (column 12 was left blank for background control). Plates were incubated for 30 minutes at 37°C. Plates were washed 3 times with PBS/Tween 20. Goat anti-rabbit Fc-HRP (Pierce) diluted 1:5000 was added to all wells (50 µE/well), and plates were incubated for 30 minutes at 37°C. Plates were washed as described above. 50 µE/well of TMB-Stable stop (Fitzgerald Industries) was added to plates, and color was allowed to develop, generally for 3 to 5 minutes. The development reaction was stopped with 50 µE/well 0.5 M HCl. Plates were read at 450 nm. Optical density (OD) versus dilution was plotted using Graph Pad Prizm software, and titers were determined.

Functional Titer Assessment

The functional activity of the samples was determined by a T1165 proliferation assay. T1165 cells were routinely maintained in modified RPMI medium (Hyclone) supplemented with HEPES, sodium pyruvate, sodium bicarbonate, L-glutamine, high glucose, penicillin/streptomycin, 10% heat inactivated fetal bovine serum (FBS) (all supplements from Hyclone), 2-mercaptoethanol (Sigma), and 10 ng/mL of huIL-6 (R&D). On the day of the assay, cell viability was determined by trypan blue (Invitrogen), and cells were seeded at a fixed density of 20,000 cells/well. Prior to seeding, cells were washed twice in the medium described above without human-IL-6 (by centrifuging at 13000 rpm for 5 minutes and discarding the supernatant). After the last wash, cells were resuspended in the same medium used for washing in a volume equivalent to 50 µE/well. Cells were set aside at room temperature.

In a round-bottom, 96-well plate (Costar), serum samples were added starting at 1:100, followed by a 1:10 dilution across the plate (columns 2 to 10) at 30 µE/well in replicates of 5 (rows B to F: dilution made in the medium described above with no huIL-6). Column 11 was medium only for IL-6 control. 30 µE/well of huIL-6 at 4x concentration of the final EC50 (concentration previously determined) were added to all wells (huIL-6 was diluted in the medium described above). Wells were incubated for 1 hour at 37°C to allow antibody binding to occur.
After 1 hour, 50 µL/well of antibody-antigen (Ab-Ag) complex were transferred to a flat-bottom, 96-well plate (Costar) following the plate map format laid out in the round-bottom plate. On Row G, 50 µL/well of medium were added to all wells (columns 2 to 11) for background control. 50 µL/well of the cell suspension set aside were added to all wells (columns 2 to 11, rows B to G). On Columns 1 and 12 and on rows A and H, 200 µL/well of medium was added to prevent evaporation of test wells and to minimize edge effect. Plates were incubated for 72 hours at 37°C in 4% CO₂. At 72 hours, 20 µL/well of CellTiter96 (Promega) reagents was added to all test wells per manufacturer protocol, and plates were incubated for 2 hours at 37°C. At 2 hours, plates were gently mixed on an orbital shaker to disperse cells and to allow homogeneity in the test wells. Plates were read at 490 nm wavelength. Optical density (OD) versus dilution was plotted using Graph Pad Prizm software, and functional titer was determined. A positive assay control plate was conducted as described above using MAB2061 (R&D Systems) at a starting concentration of 1 µg/mL (final concentration) followed by 1:3 dilutions across the plate.

Tissue Harvesting

[0682] Once acceptable titers were established, the rabbit(s) were sacrificed. Spleen, lymph nodes, and whole blood were harvested and processed as follows:

[0683] Spleen and lymph nodes were processed into a single cell suspension by disassociating the tissue and pushing through sterile wire mesh at 70 µH (Fisher) with a plunger of a 20 cc syringe. Cells were collected in the modified RPMI medium described above without huIL-6, but with low glucose. Cells were washed twice by centrifugation. After the last wash, cell density was determined by trypan blue. Cells were centrifuged at 1500 rpm for 10 minutes; the supernatant was discarded. Cells were resuspended in the appropriate volume of 10% dimethyl sulfoxide (DMSO, Sigma) in FBS (HyClone) and dispensed at 1 mL/vial. Vials were then stored at -70 °C for 24 h prior to being placed in a liquid nitrogen (LN2) tank for long-term storage.

[0684] Peripheral blood mononuclear cells (PBMCs) were isolated by mixing whole blood with equal parts of the low glucose medium described above without FBS. 35 mL of the whole blood mixture was carefully layered onto 8 mL of Lympholyte Rabbit (Cedarlane) into a 45 mL conical tube (Corning) and centrifuged 30 minutes at 2500 rpm at room temperature without brakes. After centrifugation, the PBMC layers were carefully removed using a glass Pasteur pipette (VWR), combined, and placed into a clean 50 mL vial. Cells were washed twice with the modified medium described above by centrifugation at 1500 rpm for 10 minutes at room temperature, and cell density was determined by trypan blue staining. After the last wash, cells were resuspended in an appropriate volume of 10% DMSO/FBS medium and frozen as described herein.
B cell culture

On the day of setting up B cell culture, PBMC, splenocyte, or lymph node vials were thawed for use. Vials were removed from LN2 tank and placed in a 37°C water bath until thawed. Contents of vials were transferred into 15 mL conical centrifuge tube (Corning) and 10 mL of modified RPMI described above was slowly added to the tube. Cells were centrifuged for 5 minutes at 1.5K RPM, and the supernatant was discarded. Cells were resuspended in 10 mL of fresh media. Cell density and viability was determined by trypan blue. Cells were washed again and resuspended at 1E07 cells/80 µL medium. Biotinylated huIL-6 (B huIL-6) was added to the cell suspension at the final concentration of 3 µg/mL and incubated for 30 minutes at 4°C.

Unbound B huIL-6 was removed with two 10 mL washes of phosphate-buffered (PBF):Ca/Mg free PBS (Hyclone), 2 mM ethylenediamine tetraacetic acid (EDTA), 0.5% bovine serum albumin (BSA) (Sigma-biotin free). After the second wash, cells were resuspended at 1E07 cells/80 µL PBF. 20 µL of MACS® streptavidin beads (Milteni)/10E7 cells were added to the cell suspension. Cells were incubated at 4°C for 15 minutes. Cells were washed once with 2 mL of PBF/10E7 cells. After washing, the cells were resuspended at 1E08 cells/500 µL of PBF and set aside. A MACS® MS column (Milteni) was pre-rinsed with 500 mL of PBF on a magnetic stand (Milteni). Cell suspension was applied to the column through a pre-filter, and unbound fraction was collected. The column was washed with 1.5 mL of PBF buffer. The column was removed from the magnet stand and placed onto a clean, sterile 5 mL Polypropylene Falcon tube. 1 mL of PBF buffer was added to the top of the column, and positive selected cells were collected. The yield and viability of positive and negative cell fraction was determined by trypan blue staining. Positive selection yielded an average of 1% of the starting cell concentration.

A pilot cell screen was established to provide information on seeding levels for the culture. Three 10-plate groups (a total of 30 plates) were seeded at 50, 100, and 200 enriched B cells/well. In addition, each well contained 50K cells/well of irradiated EL-4.B5 cells (5,000 Rads) and an appropriate level of T cell supernatant (ranging from 1-5% depending on preparation) in high glucose modified RPMI medium at a final volume of 250 pL/well. Cultures were incubated for 5 to 7 days at 37 °C in 4% CO₂.

Identification of Selective Antibody Secreting B Cells

Cultures were tested for antigen recognition and functional activity between days 5 and 7.

Antigen Recognition Screening

The ELISA format used is as described above except 50 µL of supernatant from the B cell cultures (BCC) wells (all 30 plates) was used as the source of the antibody. The conditioned
medium was transferred to antigen-coated plates. After positive wells were identified, the supernatant was removed and transferred to a 96-well master plate(s). The original culture plates were then frozen by removing all the supernatant except 40 μL/well and adding 60 μL/well of 16% DMSO in FBS. Plates were wrapped in paper towels to slow freezing and placed at -70 °C.

Functional Activity Screening

Master plates were then screened for functional activity in the T1 165 proliferation assay as described before, except row B was media only for background control, row C was media + IL-6 for positive proliferation control, and rows D-G and columns 2-11 were the wells from the BCC (50 μL/well, single points). 40 μL/well of IL-6 was added to all wells except the media row at 2.5 times the EC50 concentration determined for the assay. After 1 hour incubation, the Ab/Ag complex was transferred to a tissue culture (TC) treated, 96-well, flat-bottom plate. 20 μL/well of cell suspension in modified RPMI medium without huIL-6 (T1 165 at 20,000 cells/well) was added to all wells (100 μL final volume per well). Background was subtracted, and observed OD values were transformed into % of inhibition.

B cell recovery

Plates containing wells of interest were removed from -70°C, and the cells from each well were recovered with 5-200 μL, washes of medium/well. The washes were pooled in a 1.5 mL sterile centrifuge tube, and cells were pelleted for 2 minutes at 1500 rpm.

The tube was inverted, the spin repeated, and the supernatant carefully removed. Cells were resuspended in 100 μL/tube of medium. 100 μL biotinylated IL-6 coated streptavidin M280 dynabeads (Invitrogen) and 16 μL of goat anti-rabbit H&L IgG-FITC diluted 1:100 in medium was added to the cell suspension.

20 μL of cell/beads/FITC suspension was removed, and 5 μL droplets were prepared on a glass slide (Corning) previously treated with Sigmacote (Sigma), 35 to 40 droplets/slide. An impermeable barrier of paraffin oil (JT Baker) was added to submerge the droplets, and the slide was incubated for 90 minutes at 37°C, 4% CO2 in the dark.

Specific B cells that produce antibody can be identified by the fluorescent ring around them due to antibody secretion, recognition of the bead-associated biotinylated antigen, and subsequent detection by the fluorescent-IgG detection reagent. Once a cell of interest was identified, the cell in the center of the fluorescent ring was recovered via a micromanipulator (Eppendorf). The single cell synthesizing and exporting the antibody was transferred into a 250 μL microcentrifuge tube and placed in dry ice. After recovering all cells of interest, these were transferred to -70°C for long-term storage.
Example 8
Yeast Cell Expression

Antibody genes: Genes were cloned and constructed that directed the synthesis of a chimeric humanized rabbit monoclonal antibody.

Expression vector: The vector contains the following functional components: 1) a mutant ColEl origin of replication, which facilitates the replication of the plasmid vector in cells of the bacterium *Escherichia coli*; 2) a bacterial Sh ble gene, which confers resistance to the antibiotic Zeocin® (phleomycin) and serves as the selectable marker for transformations of both *E. coli* and *P. pastoris*; 3) an expression cassette composed of the glyceraldehyde dehydrogenase gene (GAP gene) promoter, fused to sequences encoding the *Saccharomyces cerevisiae* alpha mating factor pre pro secretion leader sequence, followed by sequences encoding a *P. pastoris* transcriptional termination signal from the *P. pastoris* alcohol oxidase I gene (AOX1). The Zeocin® (phleomycin) resistance marker gene provides a means of enrichment for strains that contain multiple integrated copies of an expression vector in a strain by selecting for transformants that are resistant to higher levels of Zeocin® (phleomycin).

*Pichia pastoris* strains: *Pichia pastoris* strains metl, lys3, ura3 and adel may be used. Although any two complementing sets of auxotrophic strains could be used for the construction and maintenance of diploid strains, these two strains are especially suited for this method for two reasons. First, they grow more slowly than diploid strains that are the result of their mating or fusion. Thus, if a small number of haploid adel or ura3 cells remain present in a culture or arise through meiosis or other mechanism, the diploid strain should outgrow them in culture.

The second is that it is easy to monitor the sexual state of these strains since diploid Ade+ colonies arising from their mating are a normal white or cream color, whereas cells of any strains that are haploid adel mutants will form a colony with a distinct pink color. In addition, any strains that are haploid ura3 mutants are resistant to the drug 5-fluoro-orotic acid (FOA) and can be sensitively identified by plating samples of a culture on minimal medium + uracil plates with FOA. On these plates, only uracil-requiring ura3 mutant (presumably haploid) strains can grow and form colonies. Thus, with haploid parent strains marked with adel and ura3, one can readily monitor the sexual state of the resulting antibody-producing diploid strains (haploid versus diploid).

Methods

Construction of pGAPZ-alpha expression vectors for transcription of light and heavy chain antibody genes. The humanized light and heavy chain fragments were cloned into the pGAPZ expression vectors through a PCR directed process. The recovered humanized constructs
were subjected to amplification under standard KOD polymerase (Novagen) kit conditions ((1) 94°C, 2 minutes; (2) 94°C, 30 seconds (3) 55°C, 30 seconds; (4) 72°C, 30 seconds-cycling through steps 2-4 for 35 times; (5) 72°C 2 minutes) employing the following primers (1) light chain forward AGCGCTTATTCCGCTATCCAGATGACCCAGTC-the Afel site is single underlined (SEQ ID NO: 729). The end of the HSA signal sequence is double underlined, followed by the sequence for the mature variable light chain (not underlined); the reverse CGTACGTTTGTATTTCCACCTTG (SEQ ID NO: 730).

**0699** Variable light chain reverse primer. BsiWI site is underlined, followed by the reverse complement for the 3’ end of the variable light chain. Upon restriction enzyme digest with Afel and BsiWI this enable insertion in-frame with the pGAPZ vector using the human HAS leader sequence in frame with the human kapp light chain constant region for export. (2) A similar strategy is performed for the heavy chain. The forward primer employed is AGCGCTTATTCCGAGGTGCAGCTGTGAGTC (SEQ ID NO: 731). The Afel site is single underlined. The end of the HSA signal sequence is double underlined, followed by the sequence for the mature variable heavy chain (not underlined). The reverse heavy chain primer is CTGCAGACCGTGACGAGGT (SEQ ID NO: 732). The Xhol site is underlined, followed by the reverse complement for the 3’ end of the variable heavy chain. This enables cloning of the heavy chain in-frame with IgG-γ1 CH1-CH2-CH3 region previous inserted within pGAPZ using a comparable directional cloning strategy.


**0701** Prior to transformation, each expression vector is linearized within the GAP promoter sequences with Avrll to direct the integration of the vectors into the GAP promoter locus of the P. pastoris genome. Samples of each vector are then individually transformed into electrocompetent cultures of the adel, ura3, metl and lys3 strains by electroporation and successful transformants are selected on YPD Zeocin® (phleomycin) plates by their resistance to this antibiotic. Resulting colonies are selected, streaked for single colonies on YPD Zeocin® (phleomycin) plates and then examined for the presence of the antibody gene insert by a PCR assay on genomic DNA extracted from each strain for the proper antibody gene insert and/or by the ability of each strain to synthesize an antibody chain by a colony lift/immunoblot method. Wung, *et al.* (1996) *Biotechniques* 21: 808-812. Haploid adel, metl and lys3 strains expressing one of the three
heavy chain constructs are collected for diploid constructions along with haploid ura3 strain expressing light chain gene. The haploid expressing heavy chain genes are mated with the appropriate light chain haploid ura3 to generate diploid secreting protein.

[0702] Mating of haploid strains synthesizing a single antibody chain and selection of diploid derivatives synthesizing tetrameric functional antibodies. To mate *P. pastoris* haploid strains, each adel (or metl or lys3) heavy chain producing strain to be crossed is streaked across a rich YPD plate and the ura3 light chain producing strain is streaked across a second YPD plate (-10 streaks per plate). After one or two days incubation at 30°C, cells from one plate containing heavy chain strains and one plate containing ura3 light chain strains are transferred to a sterile velvet cloth on a replica-plating block in a cross hatched pattern so that each heavy chain strain contain a patch of cells mixed with each light chain strain. The cross-streaked replica plated cells are then transferred to a mating plate and incubated at 25°C to stimulate the initiation of mating between strains. After two days, the cells on the mating plates are transferred again to a sterile velvet on a replica-plating block and then transferred to minimal medium plates. These plates are incubated at 30°C for three days to allow for the selective growth of colonies of prototrophic diploid strains. Colonies that arose are picked and streaked onto a second minimal medium plate to single colony isolate and purify each diploid strain. The resulting diploid cell lines are then examined for antibody production.

[0703] Putative diploid strains are tested to demonstrate that they are diploid and contain both expression vectors for antibody production. For diploidy, samples of a strain are spread on mating plates to stimulate them to go through meiosis and form spores. Haploid spore products are collected and tested for phenotype. If a significant percentage of the resulting spore products are single or double auxotrophs it may be concluded that the original strain must have been diploid. Diploid strains are examined for the presence of both antibody genes by extracting genomic DNA from each and utilizing this DNA in PCR reactions specific for each gene.

[0704] Fusion of haploid strains synthesizing a single antibody chain and selection of diploid derivatives synthesizing tetrameric functional antibodies. As an alternative to the mating procedure described above, individual cultures of single-chain antibody producing haploid adel and ura3 strains are spheroplasted and their resulting spheroplasts fused using polyethylene glycol/CaCl2. The fused haploid strains are then embedded in agar containing 1 M sorbitol and minimal medium to allow diploid strains to regenerate their cell wall and grow into visible colonies. Resulting colonies are picked from the agar, streaked onto a minimal medium plate, and the plates are incubated for two days at 30°C to generate colonies from single cells of diploid
cell lines. The resulting putative diploid cell lines are then examined for diploidy and antibody production as described above.

[0705] Purification and analysis of antibodies. A diploid strain for the production of full length antibody is derived through the mating of metl light chain and lys3 heavy chain using the methods described above. Culture media from shake-flask or fermenter cultures of diploid P. pastoris expression strains are collected and examined for the presence of antibody protein via SDS-PAGE and immunoblotting using antibodies directed against heavy and light chains of human IgG, or specifically against the heavy chain of IgG.

[0706] To purify the yeast secreted antibodies, clarified media from antibody producing cultures are passed through a protein A column and after washing with 20 mM sodium phosphate, pH 7.0, binding buffer, protein A bound protein is eluted using 0.1 M glycine HCl buffer, pH 3.0. Fractions containing the most total protein are examined by Coomassie blue stained SDS-PAGE and immunoblotting for antibody protein. Antibody is characterized using the ELISA described above for IL-6 recognition.

[0707] Assay for antibody activity. The recombinant yeast-derived humanized antibody is evaluated for functional activity through the IL-6 driven T1165 cell proliferation assay and IL-6 stimulated HepG2 haptoglobin assay described above.

Example 9
Acute Phase Response Neutralization by Intravenous Administration of Anti-IL-6 Antibody Abl

[0708] Human IL-6 can provoke an acute phase response in rats, and one of the major acute phase proteins that is stimulated in the rat is alpha-2 macroglobulin (A2M). A study was designed to assess the dose of antibody Ab1 required to ablate the A2M response to a single subcutaneous injection of 100 µg of human IL-6 given one hour after different doses (0.03, 0.1, 0.3, 1, and 3 mg/kg) of antibody Ab1 administered intravenously (n=10 rats/dose level) or polyclonal human IgGl as the control (n=10 rats). Plasma was recovered and the A2M was quantitated via a commercial sandwich ELISA kit (ICL Inc., Newberg OR; cat. no.- E-25A2M). The endpoint was the difference in the plasma concentration of A2M at the 24 hour time point (post-Abl).

[0709] The ID50 for antibody Ab1 was 0.1 mg/kg with complete suppression of the A2M response at the 0.3 mg/kg. See Figure 6. This demonstrates that the IL-6 may be neutralized in vivo by anti-IL-6 antibodies described herein.
Example 10
RXF393 Cachexia Model Study 1

Introduction

[0710] The human renal cell cancer cell line, RXF393 produces profound weight loss when transplanted into athymic nude mice. Weight loss begins around day 15 after transplantation with 80% of all animals losing at least 30% of their total body weight by day 18-20 after transplantation. RXF393 secretes human IL-6 and the plasma concentration of human IL-6 in these animals is very high at around 10ng/ml. Human IL-6 can bind murine soluble IL-6 receptor and activate IL-6 responses in the mouse. Human IL-6 is approximately 10 times less potent than murine IL-6 at activating IL-6 responses in the mouse. The objectives of this study were to determine the effect of antibody Abl, on survival, body weight, serum amyloid A protein, hematology parameters, and tumor growth in athymic nude mice transplanted with the human renal cell cancer cell line, RXF393.

Methods

[0711] Eighty, 6 week old, male athymic nude mice were implanted with RXF393 tumor fragments (30-40 mg) subcutaneously in the right flank. Animals were then divided into eight groups of ten mice. Three groups were given either antibody Abl at 3 mg/kg, 10 mg/kg, or 30 mg/kg intravenously weekly on day 1, day 8, day 15 and day 22 after transplantation (progression groups). Another three groups were given either antibody Abl at 3 mg/kg, or 10 mg/kg, or 30 mg/kg intravenously weekly on day 8, day 15 and day 22 after transplantation (regression groups). Finally, one control group was given polyclonal human IgG 30 mg/kg and a second control group was given phosphate buffered saline intravenously weekly on day 1, day 8, day 15 and day 22 after transplantation.

[0712] Animals were euthanized at either day 28, when the tumor reached 4,000 mm³ or if they became debilitated (>30% loss of body weight). Animals were weighed on days 1, 6 and then daily from days 9 to 28 after transplantation. Mean Percent Body Weight (MPBW) was used as the primary parameter to monitor weight loss during the study. It was calculated as follows: (Body Weight - Tumor Weight)/Baseline Body Weight x 100. Tumor weight was measured on days 1, 6, 9, 12, 15, 18, 22, 25 and 28 after transplantation. Blood was taken under anesthesia from five mice in each group on days 5 and 13 and all ten mice in each group when euthanized (day 28 in most cases). Blood was analyzed for hematology and serum amyloid A protein (SAA) concentration. An additional group of 10 non-tumor bearing 6 week old, athymic nude male mice had blood samples taken for hematology and SAA concentration estimation to act as a baseline set of values.
Results - Survival

[0713] No animals were euthanized or died in any of the antibody Abl groups prior to the study termination date of day 28. In the two control groups, 15 animals (7/9 in the polyclonal human IgG group and 8/10 in the phosphate buffered saline group) were found dead or were euthanized because they were very debilitated (>30% loss of body weight). Median survival time in both control groups was 20 days.

[0714] The survival curves for the two control groups and the antibody Abl progression (dosed from day 1 of the study) groups are presented in FIGURE 7.

[0715] The survival curves for the two control groups and the antibody Abl regression (dosed from day 8 of the study) groups are presented in FIGURE 8.

[0716] There was a statistically significant difference between the survival curves for the polyclonal human IgG (p=0.0038) and phosphate buffered saline (p=0.0003) control groups and the survival curve for the six antibody Abl groups. There was no statistically significant difference between the two control groups (p=0.97).

Results - Tumor Size

[0717] Tumor size in surviving mice was estimated by palpation. For the first 15 days of the study, none of the mice in any group were found dead or were euthanized, and so comparison of tumor sizes between groups on these days was free from sampling bias. No difference in tumor size was observed between the antibody Ab 1 progression or regression groups and the control groups through day 15. Comparison of the tumor size between surviving mice in the control and treatment groups subsequent to the onset of mortality in the controls (on day 15) was not undertaken because tumor size the surviving control mice was presumed to be biased and accordingly the results of such comparison would not be meaningful.

[0718] As administration of antibody Abl promoted survival without any apparent reduction in tumor size, elevated serum IL-6 may contribute to mortality through mechanisms independent of tumor growth. These observations supports the hypothesis that antibody Ab 1 can promote cancer patient survivability without directly affecting tumor growth, possibly by enhancing general patient well-being.

Results - Weight Loss

[0719] Compared to controls, mice dosed with Abl were protected from weight loss. On day 18, MPBW in control mice was 75%, corresponding to an average weight loss of 25%. In contrast, on the same day, MPBW in Ab-1 treatment groups was minimally changed (between 97% and 103%). There was a statistically significant difference between the MPBW curves for the controls (receiving polyclonal human IgG or PBS) and the 10 mg/kg dosage group (p<0.0001).
or 3 mg/kg and 30 mg/kg dosage groups (p<0.0005). There was no statistically significant
difference between the two control groups.

[0720] Control mice are emaciated compared to the normal appearance of the Ab 1-treated mouse. These results suggest that Ab 1 may be useful to prevent or treat cachexia caused by elevated IL-6 in humans.

Results - Plasma Serum Amyloid A

[0721] The mean (± SEM) plasma serum amyloid A concentration versus time for the two control groups and the antibody Ab 1 progression (dosed from day 1 of the study) and regression (dosed from day 8 of the study) groups are presented in Table 7.

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Mean Plasma SAA—antibody Abl, all groups versus control groups</th>
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<tbody>
<tr>
<td></td>
<td>Mean Plasma SAA±SEM Day 5 (µg/ml)</td>
</tr>
<tr>
<td></td>
<td>Mean Plasma SAA±SEM Day 13 (µg/ml)</td>
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<tr>
<td></td>
<td>Mean Plasma SAA±SEM Terminal Bleed (µg/ml)</td>
</tr>
<tr>
<td>Polyclonal IgG 30 mg/kg iv weekly from day 1</td>
<td>675 ± 240 (n=5)</td>
</tr>
<tr>
<td>PBS iv weekly from day 1</td>
<td>355 ± 207 (n=5)</td>
</tr>
<tr>
<td>Ab 1 30 mg/kg iv weekly from day 1</td>
<td>246 ± 100 (n=5)</td>
</tr>
<tr>
<td>Ab 1 3 mg/kg iv weekly from day 1</td>
<td>106 ± 9 (n=5)</td>
</tr>
<tr>
<td>Ab 1 30 mg/kg iv weekly from day 8</td>
<td>375 ± 177 (n=5)</td>
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<tr>
<td>Ab 1 10 mg/kg iv weekly from day 8</td>
<td>487 ± 170 (n=5)</td>
</tr>
<tr>
<td>Ab 1 3 mg/kg iv weekly from day 8</td>
<td>1255 ± 516 (n=5)</td>
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</tbody>
</table>

[0722] SAA is up-regulated via the stimulation of hIL-6 and this response is directly correlated with circulating levels of hIL-6 derived from the implanted tumor. The surrogate marker provides an indirect readout for active hIL-6. Thus in the two treatment groups described above there are significantly decreased levels of SAA due to the neutralization of tumor-derived hIL-6. This further supports the contention that antibody Ab 1 displays in vivo efficacy.

Example 11
RXF393 Cachexia Model Study 2

Introduction

[0723] A second study was performed in the RXF-393 cachexia model where treatment with antibody Abl was started at a later stage (days 10 and 13 post-transplantation) and with a more prolonged treatment phase (out to 49 days post transplantation). The dosing interval with
antibody Ab 1 was shortened to 3 days from 7 and also daily food consumption was measured. There was also an attempt to standardize the tumor sizes at the time of initiating dosing with antibody Ab 1.

Methods

Eighty, 6 week old, male athymic nude mice were implanted with RXF393 tumor fragments (30-40 mg) subcutaneously in the right flank. 20 mice were selected whose tumors had reached between 270-320 mg in size and divided into two groups. One group received antibody Ab 1 at 10 mg/kg i.v. every three days and the other group received polyclonal human IgG 10 mg/kg every 3 days from that time-point (day 10 after transplantation). Another 20 mice were selected when their tumor size had reached 400-527 mg in size and divided into two groups. One group received antibody Ab 1 at 10 mg/kg i.v. every three days and the other group received polyclonal human IgG 10 mg/kg every 3 days from that time-point (day 13 after transplantation). The remaining 40 mice took no further part in the study and were euthanized at either day 49, when the tumor reached 4,000 mm³ or if they became very debilitated (>30% loss of body weight).

Animals were weighed every 3-4 days from day 1 to day 49 after transplantation. Mean Percent Body Weight (MPBW) was used as the primary parameter to monitor weight loss during the study. It was calculated as follows: ((Body Weight - Tumor Weight)/Baseline Body Weight) x 100. Tumor weight was measured every 3-4 days from day 5 to day 49 after transplantation. Food consumption was measured (amount consumed in 24 hours by weight (g) by each treatment group) every day from day 10 for the 270-320 mg tumor groups and day 13 for the 400-527 mg tumor groups.

Results -survival

The survival curves for antibody Ab 1 at 10 mg/kg i.v. every three days (270-320 mg tumor size) and for the polyclonal human IgG 10 mg/kg i.v. every three days (270-320 mg tumor size) are presented in Figure 9.

Median survival for the antibody Ab 1 at 10 mg/kg i.v. every three days (270-320 mg tumor size) was 46 days and for the polyclonal human IgG at 10 mg/kg i.v. every three days (270-320 mg tumor size) was 32.5 days (p=0.0071).

The survival curves for the antibody Ab 1 at 10 mg/kg i.v. every three days (400-527 mg tumor size) and for the polyclonal human IgG at 10 mg/kg i.v. every three days (400-527 mg tumor size) are presented in Figure 10. Median survival for the antibody Ab 1 at 10 mg/kg i.v. every three days (400-527 mg tumor size) was 46.5 days and for the polyclonal human IgG at 10 mg/kg i.v. every three days (400-527 mg tumor size) was 27 days (p=0.0481).
Example 12
Multi-dose Pharmacokinetic Evaluation of Antibody Abl in Non-human Primates

Antibody Abl was dosed in a single bolus infusion to a single male and single female cynomologous monkey in phosphate buffered saline. Plasma samples were removed at fixed time intervals and the level of antibody Abl was quantitated through the use of an antigen capture ELISA assay. Biotinylated IL-6 (50 μl of 3 μg/mL) was captured on Streptavidin coated 96 well microtiter plates. The plates were washed and blocked with 0.5% Fish skin gelatin. Appropriately diluted plasma samples were added and incubated for 1 hour at room temperature. The supematants removed and an anti-hFc-HRP conjugated secondary antibody applied and left at room temperature.

The plates were then aspirated and TMB added to visualize the amount of antibody. The specific levels were then determined through the use of a standard curve. A second dose of antibody Abl was administered at day 35 to the same two cynomologous monkeys and the experiment replicated using an identical sampling plan.

This humanized full length aglycosylated antibody expressed and purified Pichia pastoris displays comparable characteristics to mammalian expressed protein. In addition, multiple doses of this product display reproducible half-lives inferring that this production platform does not generate products that display enhanced immunogenicity.

Example 13
Octet Mechanistic Characterization of Antibody Proteins

IL-6 signaling is dependent upon interactions between IL-6 and two receptors, IL-6R1 (CD 126) and gpl30 (IL-6 signal transducer). To determine the antibody mechanism of action, mechanistic studies were performed using bio-layer interferometry with an Octet QK instrument (ForteBio; Menlo Park, CA). Studies were performed in two different configurations. In the first orientation, biotinylated IL-6 (R&D systems part number 206-IL-OOlMG/CF, biotinylated using Pierce EZ-link sulfo-NHS-LC-LC-biotin product number 21338 according to manufacturer's protocols) was initially bound to a streptavidin coated biosensor (ForteBio part number 18-5006). Binding is monitored as an increase in signal.

The IL-6 bound to the sensor was then incubated either with the antibody in question or diluent solution alone. The sensor was then incubated with soluble IL-6R1 (R&D systems product number 227-SR-025/CF) molecule. If the IL-6R1 molecule failed to bind, the antibody was deemed to block IL-6/IL-6R1 interactions. These complexes were incubated with gpl30 (R&D systems 228-GP-OIO/CF) in the presence of IL-6R1 for stability purposes. If gpl30 did not bind, it was concluded that the antibody blocked gpl30 interactions with IL-6.
In the second orientation, the antibody was bound to a biosensor coated with an anti-human IgG1 Fc-specific reagent (ForteBio part number 18-5001). The IL-6 was bound to the immobilized antibody and the sensor was incubated with IL-6R1. If the IL-6R1 did not interact with the IL-6, then it was concluded that the IL-6 binding antibody blocked IL-6/IL-6R1 interactions. In those situations where antibody/IL-6/IL-6R1 was observed, the complex was incubated with gpl30 in the presence of IL-6R1. If gpl30 did not interact, then it was concluded that the antibody blocked IL-6/gpl30 interactions. All studies were performed in a 200 µL final volume, at 30°C and 1000 rpm. For these studies, all proteins were diluted using ForteBio's sample diluent buffer (part number 18-5028). Results are presented in TABLE 8.

**TABLE 8 Anti-IL6 Antibodies binding to R1 or GP130**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Blocks IL6 binding to R1</th>
<th>Blocks IL6 Binding to GP130</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab1</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ab2</td>
<td>No</td>
<td>Partial</td>
</tr>
<tr>
<td>Ab3</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ab4</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ab6</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ab7</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ab8</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Example 14**

**Peptide Mapping**

In order to determine the epitope recognized by Abl on human IL-6, the antibody was employed in a western-blot based assay. The form of human IL-6 utilized in this example had a sequence of 183 amino acids in length. A 57-member library of overlapping 15 amino acid peptides encompassing this sequence was commercially synthesized and covalently bound to a PepSpots nitrocellulose membrane (JPT Peptide technologies, Berlin, Germany). The sequences of the overlapping 15 amino acid peptides is in SEQ ID NOs: 590-646. Blots were prepared and probed according to the manufacturer's recommendations.

Briefly, blots were pre-wet in methanol, rinsed in PBS, and blocked for over 2 hours in 10% non-fat milk in PBS/0.05% Tween (Blocking Solution). The Abl antibody was used at 1 mg/mL final dilution, and the HRP-conjugated Mouse Anti-Human-Kappa secondary antibody (Southern BioTech #9220-05) was used at a 1:5000 dilution. Antibody dilutions/incubations were performed in blocking solution. Blots were developed using Amersham ECL advance reagents (GE# RPN2135) and chemiluminescent signal documented using a CCD camera (Alphalnnotec). The sequence of the form of human IL-6 utilized to generate peptide library is set forth in SEQ ID NO: 1.
Example 15
Abl has high affinity for IL-6

Surface plasmon resonance was used to measure association rate (Ka), dissociation rate (Kd) and dissociation constant (KD) for Abl to IL-6 from rat, mouse, dog, human, and cynomolgus monkey at 25°C (TABLE 5). The dissociation constant for human IL-6 was 4 pM, indicating very high affinity. As expected, affinity generally decreased with phylogenetic distance from human. The dissociation constants of Abl for IL-6 of cynomolgus monkey, rat, and mouse were 31 pM, 1.4 nM, and 0.4 nM, respectively. Abl affinity for dog IL-6 below the limit of quantitation of the experiment.

The high affinity of Abl for mouse, rat, and cynomolgus monkey IL-6 suggest that Abl may be used to inhibit IL-6 of these species. This hypothesis was tested using a cell proliferation assay. In brief, each species's IL-6 was used to stimulate proliferation of T1165 cells, and the concentration at which Abl could inhibit 50% of proliferation (IC50) was measured. Inhibition was consistent with the measured dissociation constants (TABLE 6). These results demonstrate that Abl can inhibit the native IL-6 of these species, and suggest the use of these organisms for in vitro or in vivo modeling of IL-6 inhibition by Abl. Further, other IL-6 antibodies described herein may have similar properties.

Table 9 Surface Plasmon Resonance: Averaged binding constants determined at 25°C for Abl to IL-6.

<table>
<thead>
<tr>
<th>Species (IL-6)</th>
<th>$K_a$ (M$^{-1}$s$^{-1}$)</th>
<th>$K_d$ (s$^{-1}$)</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1.6e6</td>
<td>2.2e-3</td>
<td>1.4nM</td>
</tr>
<tr>
<td>Mouse</td>
<td>1.1e6</td>
<td>4.0e-4</td>
<td>0.4nM</td>
</tr>
<tr>
<td>Dog</td>
<td>Below LOQ$^a$</td>
<td>Below LOQ$^a$</td>
<td>Below LOQ$^a$</td>
</tr>
<tr>
<td>Human</td>
<td>1.6e5</td>
<td>5e-3</td>
<td>4 pM</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>9.6e4</td>
<td>3e-6</td>
<td>31 pM</td>
</tr>
</tbody>
</table>

*a. Below Limit of Quantitation

Table 10 IC50 values for Abl against human, cynomolgus monkey, mouse, rat and dog IL-6 in the T1165 assay.

<table>
<thead>
<tr>
<th>Species</th>
<th>IC50 (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>13</td>
</tr>
<tr>
<td>Cynomolgus monkey</td>
<td>12</td>
</tr>
<tr>
<td>Mouse</td>
<td>1840</td>
</tr>
<tr>
<td>Rat</td>
<td>2060</td>
</tr>
<tr>
<td>Dog</td>
<td>No inhibition of cell proliferation</td>
</tr>
</tbody>
</table>
Example 16
Multi-dose Pharmacokinetic Evaluation of Antibody Abl in Healthy Human Volunteers

Antibody Abl was dosed in a single bolus infusion in histidine and sorbitol to healthy human volunteers. Dosages of 1 mg, 3 mg, 10 mg, 30 mg or 100 mg were administered to each individual in dosage groups containing five to six individuals. Plasma samples were removed at fixed time intervals for up to twelve weeks. Human plasma was collected via venipuncture into a vacuum collection tube containing EDTA. Plasma was separated and used to assess the circulating levels of Abl using a monoclonal antibody specific for Abl, as follows. A 96 well microtiter plate was coated overnight with the monoclonal antibody specific for Abl in 1X PBS overnight at 4°C. The remaining steps were conducted at room temperature. The wells were aspirated and subsequently blocked using 0.5% Fish Skin Gelatin (FSG) (Sigma) in IX PBS for 60 minutes. Human plasma samples were then added and incubated for 60 minutes, then aspirated, then 50 μL of 1 μg/mL biotinylated IL-6 was then added to each well and incubated for 60 minutes. The wells were aspirated, and 50 μL streptavidin-HRP (Pharmingen), diluted 1:5,000 in 0.5% FSG/PBS, was added and incubated for 45 minutes. Development was conducted using standard methods employing TMB for detection. Levels were then determined via comparison to a standard curve prepared in a comparable format.

Average plasma concentration of Abl for each dosage group was examined. Mean AUC and Cmax increased linearly with dosage. For dosages of 30 mg and above, the average Abl half-life in each dosage group was between approximately 25 and 30 days. The pharmacokinetics is shown in Table 11.

Table 11 Summary of Abl Pharmacokinetics in Health Human Volunteers

<table>
<thead>
<tr>
<th>Dose of Abl</th>
<th>T1/2 (days)</th>
<th>AUC (μg • h / mL)</th>
<th>Cmax (μg / mL)</th>
<th>Tmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mg</td>
<td>10.3</td>
<td>35</td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td>3mg</td>
<td>11.6</td>
<td>229</td>
<td>0.7</td>
<td>4</td>
</tr>
<tr>
<td>10mg</td>
<td>22.4</td>
<td>1473</td>
<td>4.0</td>
<td>4</td>
</tr>
<tr>
<td>30mg</td>
<td>25.1</td>
<td>9076</td>
<td>19.7</td>
<td>4</td>
</tr>
<tr>
<td>100mg</td>
<td>30.3</td>
<td>26128</td>
<td>48.0</td>
<td>12</td>
</tr>
<tr>
<td>300mg</td>
<td>26.2</td>
<td>92891</td>
<td>188.0</td>
<td>12</td>
</tr>
<tr>
<td>640mg</td>
<td>30.2</td>
<td>175684</td>
<td>306.0</td>
<td>12</td>
</tr>
</tbody>
</table>

Example 17
Pharmacokinetics of Abl in patients with advanced cancer

Antibody Abl was dosed in a single bolus infusion in phosphate buffered saline to five individuals with advanced cancer. Each individual received a dosage of 80 mg (n=2) or 160 mg (n=3) of Abl. Plasma samples were drawn weekly, and the level of antibody Abl was quantitated as in Example 16. Average plasma concentration of Abl in these individuals as a
function of time was examined. The average Abl half-life was approximately 31 days. The anti-IL-6 antibodies described herein may have similarly long half-lives.

**Example 18**

*Abl has an unexpectedly long half-life*

[0742] Overall, the average half-life of Abl was approximately 31 days in humans (for dosages of 10 mg and above), and approximately 15-21 days in cynomolgus monkey. The Abl half-life in humans and cynomolgus monkeys are unprecedented when compared with the half-lives of other anti-IL-6 antibodies (TABLE 11). As described above, Abl was derived from humanization of a rabbit antibody, and is produced from *Pichia pastoris* in an aglycosylated form. These characteristics results in an antibody with very low immunogenicity in humans. Moreover, the lack of glycosylation prevents Abl from interacting with the Fc receptor or complement. Without intent to be limited by theory, it is believed that the unexpectedly long half-life of Ab 1 is at least partially attributable to the humanization and/or the lack of glycosylation. The particular sequence and/or structure of the antigen binding surfaces may also contribute to Abl ’s half-life.

*See also* WO 2011/066369.

**TABLE 12 Elimination Half-life of Abl**

<table>
<thead>
<tr>
<th>Dose of Abl</th>
<th>Cynomolgus Monkey (days)</th>
<th>Human (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abl</td>
<td>15-21</td>
<td>~31</td>
</tr>
<tr>
<td>Acemra (Tocilizumab)</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Remicade</td>
<td>5</td>
<td>8-9.5</td>
</tr>
<tr>
<td>Synagis</td>
<td>8.6</td>
<td>20</td>
</tr>
<tr>
<td>Erbitux</td>
<td>3-7</td>
<td>5</td>
</tr>
<tr>
<td>Zenapax</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Avastin</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Pertuzumab</td>
<td>10</td>
<td>18-22</td>
</tr>
</tbody>
</table>

**Example 19**

*Abl Effect on Hemoglobin Concentration, Plasma Lipid Concentration, and Neutrophil Counts in Patients with Advanced Cancer.*

[0743] Antibody Abl was dosed in a single bolus infusion in phosphate buffered saline to eight individuals with advanced cancer (NSCLC, colorectal cancer, cholangiocarcinoma, or mesothelioma). Each individual received a dosage of 80 mg, 160 mg, or 320 mg of Abl. Blood samples were removed just prior to infusion and at fixed time intervals for six weeks, and the hemoglobin concentration, plasma lipid concentration, and neutrophil counts were determined. Average hemoglobin concentration rose slightly (Figure 11), as did total cholesterol and triglycerides (Figure 12), while mean neutrophil counts fell slightly (Figure 13).
These results further demonstrate some of the beneficial effects of administration of Abl to chronically ill individuals. Because IL-6 is the main cytokine responsible for the anemia of chronic disease (including cancer-related anemia), neutralization of IL-6 by Abl increases hemoglobin concentration in these individuals. Similarly, as IL-6 is centrally important in increasing neutrophil counts in inflammation, the observed slight reduction in neutrophil counts further confirms that Abl inhibits IL-6. Finally, IL-6 causes anorexia as well as cachexia in these patients; neutralization of IL-6 by Abl results in the return of appetite and reversal of cachexia. The increase in plasma lipid concentrations reflects the improved nutritional status of the patients. Taken together, these results further demonstrate that Abl effectively reverses these adverse consequences of IL-6 in these patients.

Example 20

Abl Suppresses Serum CRP in Healthy Volunteers and in Patients with Advanced Cancer

Introduction

Serum CRP concentrations have been identified as a strong prognostic indicator in patients with certain forms of cancer. For example, Hashimoto et al. performed univariate and multivariate analysis of preoperative serum CRP concentrations in patients with hepatocellular carcinoma in order to identify factors affecting survival and disease recurrence. Hashimoto, et al. (2005) Cancer 103(9): 1856-1864. Patients were classified into two groups, those with serum CRP levels > 1.0 mg/dL ("the CRP positive group") and those with serum CRP levels < 1.0 mg/dL ("the CRP negative group"). The authors identified "a significant correlation between preoperative serum CRP level and tumor size." Id. Furthermore, the authors found that "[f]he overall survival and recurrence-free survival rates in the CRP-positive group were significantly lower compared with the rates in the CRP-negative group." Id. The authors concluded that the preoperative CRP level of patients is an independent and significant predictive indicator or poor prognosis and early recurrence in patients with hepatocellular carcinoma.

Similar correlations have been identified by other investigators. For example, Karakiewicz et al. determined that serum CRP was an independent and informative predictor of renal cell carcinoma-specific mortality. Karakiewicz, et al. (2007) Cancer. 110(6): 1241-1247. Accordingly, there remains a need in the art for methods and/or treatments that reduce serum C-Reactive Protein (CRP) concentrations in cancer patients, and particularly those with advanced cancers.

Methods

Healthy volunteers received a single 1-hour intravenous (IV) infusion of either 100 mg (5 patients), 30 mg (5 patients), 10 mg (6 patients), 3 mg (6 patients) or 1 mg (6 patients) of the
Abl monoclonal antibody, while another 14 healthy volunteers received intravenous placebo. Comparatively, 2 patients with advanced forms of colorectal cancer received a single 1-hour intravenous (IV) infusion of 80 mg of the Abl monoclonal antibody. No further dosages of the Abl monoclonal antibody were administered to the test population.

[0748] Patients were evaluated prior to administration of the dosage, and thereafter on a weekly basis for at least 5 weeks post dose. At the time of each evaluation, patients were screened for serum CRP concentration.

**Results—Healthy Volunteers**

[0749] As noted above, serum CRP levels are a marker of inflammation; accordingly, baseline CRP levels are typically low in healthy individuals. The low baseline CRP levels can make a further reduction in CRP levels difficult to detect. Nonetheless, a substantial reduction in serum CRP concentrations was detectable in healthy volunteers receiving all concentrations of the Abl monoclonal antibody, compared to controls (Figure 14A). The reduction in serum CRP levels was rapid, occurring within one week of antibody administration, and prolonged, continuing at least through the final measurement was taken (8 or 12 weeks from antibody administration).

**Results—Cancer Patients**

[0750] Five advanced cancer patients (colorectal cancer, cholangiocarcinoma, or NSCLC) having elevated serum CRP levels were dosed with 80 mg or 160 mg of Abl. Serum CRP levels were greatly reduced in these patients (Figure 14B). The reduction in serum CRP levels was rapid, with 90% of the decrease occurring within one week of Ab1 administration, and prolonged, continuing at least until the final measurement was taken (up to twelve weeks). In two representative individuals, the CRP levels were lowered to below the normal reference range (less than 5-6 mg/l) within one week. Thus, administration of Abl to patients can cause a rapid and sustained suppression of serum CRP levels.

**Example 21**

**Abl Improved Muscular Strength, Improved Weight, and Reduced Fatigue in Patients with Advanced Cancer**

**Introduction**

[0751] Weight loss and fatigue (and accompanying muscular weakness) are very common symptoms of patients with advanced forms of cancer, and these symptoms can worsen as the cancer continues to progress. Fatigue, weight loss and muscular weakness can have significant negative effects on the recovery of patients with advanced forms of cancer, for example by disrupting lifestyles and relationships and affecting the willingness or ability of patients to continue cancer treatments. Known methods of addressing fatigue, weight loss and muscular
weakness include regular routines of fitness and exercise, methods of conserving the patient's energy, and treatments that address anemia-induced fatigue and muscular weakness. Nevertheless, there remains a need in the art for methods and/or treatments that improve fatigue, weight loss and muscular weakness in cancer patients.

Methods

Four patients with advanced forms of cancer [(colorectal cancer (2), NSCLC (1), cholangiocarcinoma (1)] received a single 1-hour intravenous (IV) infusion of either 80 mg or 160 mg of the Abl monoclonal antibody. No further dosages of the Abl monoclonal antibody were administered to the test population.

Patients were evaluated prior to administration of the dosage, and thereafter for at least 6 weeks post dose. At the time of each evaluation, patients were screened for the following: a.) any change in weight; b.) fatigue as measured using the Facit-F Fatigue Subscale questionnaire a medically recognized test for evaluating fatigue. See, e.g., Cella, et al. (2002) Cancer 94(2): 528-538; Cella, et al. (2002) Journal of Pain & Symptom Management 24(6): 547-561); and hand-grip strength (a medically recognized test for evaluating muscle strength, typically employing a handgrip dynamometer).

Results—Weight Change

The averaged data for both dosage concentrations (80 mg and 160 mg) of the Abl monoclonal antibody demonstrated an increase of about 2 kilograms of weight per patient over the period of 6 weeks.

Fatigue

The averaged data for both dosage concentrations (80 mg and 160 mg) of the Abl monoclonal antibody demonstrated an increase in the mean Facit-F FS subscale score of at least about 10 points in the patient population over the period of 6 weeks.

Hand-Grip Strength

The averaged data for both dosage concentrations (80 mg and 160 mg) of the Abl monoclonal antibody demonstrated an increase in the mean hand-grip strength of at least about 10 percent in the patient population over the period of 6 weeks. See, e.g., WO 201 1/066371.

Example 22

Abl For Prevention of Thrombosis

Prior studies have shown that administration of an anti-IL-6 antibody can cause decreased platelet counts. Emilie, et al. (1994) Blood 84(8): 2472-9; Blay, et al. (1997) Int J Cancer 72(3): 424-30. These results have apparently been viewed as an indicator of potential danger, because further decreases in platelet counts could cause complications such as bleeding.
However, Applicants have now discerned that inhibiting IL-6 restores a normal coagulation profile, which Applicants predict will prevent thrombosis. Decreased platelet counts resulting from inhibition of IL-6 is not a sign of potential danger but rather reflects the beneficial restoration of normal coagulation.

[0758] The mechanism by which normal coagulation is restored is believed to result from the interplay between IL-6 and the acute phase reaction. In response to elevated IL-6 levels, as for example in a cancer patient, the liver produces acute phase proteins. These acute phase proteins include coagulation factors, such as Factor II, Factor V, Factor VIII, Factor IX, Factor XI, Factor XII, F/fibrin degradation products, thrombin-antithrombin III complex, fibrinogen, plasminogen, prothrombin, and von Willebrand factor. This increase in coagulation factors may be measured directly, or may be inferred from functional measurements of clotting ability. Antagonists of IL-6, such as Abl, suppresses acute phase proteins, e.g., Serum Amyloid (Example 23). Applicants now predict that this suppression of acute phase proteins will restore the normal coagulation profile, and thereby prevent thrombosis. The restoration of normal coagulation may cause a slight drop in platelet counts, but the patient will nonetheless retain normal coagulation ability and thus will not have an increased risk of bleeding. Such a treatment will represent a vast improvement over the available anticoagulation therapies whose usefulness is limited by the risk of adverse side-effects, such as major bleeding. See, e.g., WO 201 1/066371.

[0759] Applicants contemplate that the same beneficial effects of inhibiting IL-6 will be obtained regardless of the method of inhibition. Suitable methods of inhibiting IL-6 include administration of anti-IL-6 antibodies, antisense therapy, soluble IL-6 receptor, either individually or in combinations.

Example 23

Abl Increases Plasma Albumin Concentration in Patients with Advanced Cancer

Introduction

[0760] Serum albumin concentrations are recognized as predictive indicators of survival and/or recovery success of cancer patients. Hypoalbuminemia correlates strongly with poor patient performance in numerous forms of cancer. For example, in one study no patients undergoing systemic chemotherapy for metastatic pancreatic adenocarcinoma and having serum albumin levels less than 3.5 g/dL successfully responded to systemic chemotherapy. Fujishiro, et al. (2000) Hepatogastroenterology 47(36): 1744-46 and Senior and Maroni (1999) Am. Soc. Nutr. Sci. 129: 313S-3 14S. In at least one study, attempts to rectify hypoalbuminemia in 27 patients with metastatic cancer by daily intravenous albumin infusion of 20 g until normal serum albumin levels (>3.5 g/dL) were achieved had little success. Demirkazik, et al. (2002) Proc. Am. Soc.
Clin. Oncol. 21:Abstr 2892. Accordingly, there remains a need in the art for methods and/or treatments that improve serum albumin concentrations in cancer patients and address hypoalbuminemic states in cancer patients, particularly those with advanced cancers.

Methods

Four patients with advanced forms of cancer [(colorectal cancer (2), NSCLC (1), cholangiocarcinoma (1)] received a single 1-hour intravenous (IV) infusion of either 80 mg or 160 mg of the Ab1 monoclonal antibody. No further dosages of the Ab1 monoclonal antibody were administered to the test population.

Patients were evaluated prior to administration of the dosage, and thereafter for at least 6 weeks post dose. At the time of each evaluation, patients were screened for plasma albumin concentration.

Results

The averaged data for both dosage concentrations (80 mg and 160 mg) of the Ab1 monoclonal antibody demonstrated an increase of about 5 g/L of plasma albumin concentration per patient over the period of 6 weeks. See, e.g., WO 2011/066371.

Example 24

Abl Suppresses Serum CRP in Patients with Advanced Cancer

Introduction

Serum CRP concentrations have been identified as a strong prognostic indicator in patients with certain forms of cancer. For example, Hashimoto et al. performed univariate and multivariate analysis of preoperative serum CRP concentrations in patients with hepatocellular carcinoma in order to identify factors affecting survival and disease recurrence. Hashimoto, et al. (2005) Cancer 103(9): 1856-1864. Patients were classified into two groups, those with serum CRP levels > 1.0 mg/dL (“the CRP positive group”) and those with serum CRP levels < 1.0 mg/dL (“the CRP negative group”). The authors identified "a significant correlation between preoperative serum CRP level and tumor size." Id. Furthermore, the authors found that "[t]he overall survival and recurrence-free survival rates in the CRP-positive group were significantly lower compared with the rates in the CRP-negative group." Id. The authors concluded that the preoperative CRP level of patients is an independent and significant predictive indicator of poor prognosis and early recurrence in patients with hepatocellular carcinoma.

Similar correlations have been identified by other investigators. For example, Karakiewicz et al. determined that serum CRP was an independent and informative predictor of renal cell carcinoma-specific mortality. Karakiewicz, et al. (2007) Cancer 110(6): 1241-1247.
Accordingly, there remains a need in the art for methods and/or treatments that reduce serum C-Reactive Protein (CRP) concentrations in cancer patients, and particularly those with advanced cancers.

Methods

One-hundred twenty-four patients with non-small cell lung cancer (NSCLC) were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV) infusion of either placebo (n=31), 80 mg (n=29), 160 mg (n=32), or 320 mg (n=32) of the Abl monoclonal antibody every 8 weeks over a 24 week duration for a total of 3 doses. CRP concentration was quantitated by a C-reactive protein particle-enhanced immunoturbidimetric assay using latex-attached anti-CRP antibodies (i.e. Roche CRP Tinaquant®). Briefly, about 1.0 mL of patient sample serum was collected and stored in a plastic collection tube. Sample was placed into appropriate buffer, and anti-CRP antibody coupled to latex microparticles was added to the sample to start the reaction. These anti-CRP antibodies with conjugated latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this was measured turbidimetrically using a Roche/Hitachi Modular P analyzer.

Patients were evaluated prior to administration of the dosage, and thereafter at weeks 2, 4, 8, and 12. At the time of each evaluation, patients were screened for serum CRP concentration.

Results

The averaged data for each dosage concentrations (placebo, 80 mg, 160 mg, and 320 mg) of the Abl monoclonal antibody are plotted in Figure 15A. All dosage levels of Abl antibody demonstrated an immediate drop in CRP concentrations relative to placebo over the period of 12 weeks. CRP levels displayed breakthrough at 8 weeks post-dosing. The CRP levels fell below 5 mg/L by week 12. Median values of CRP demonstrated rapid and sustained decreases for all dosage concentrations relative to placebo (Fig. 15B). Thus, administration of Ab1 to advanced cancer patients can cause a rapid and sustained suppression of serum CRP levels.

Example 25
Abl Suppresses Serum CRP in Patients with Advanced Cancers

Introduction

Serum CRP concentrations have been identified as a strong prognostic indicator in patients with certain forms of cancer. For example, Hashimoto et al. performed univariate and multivariate analysis of preoperative serum CRP concentrations in patients with hepatocellular carcinoma in order to identify factors affecting survival and disease recurrence. Hashimoto, et al. (2005) Cancer 103(9): 1856-1864. Patients were classified into two groups, those with serum
CRP levels > 1.0 mg/dL ("the CRP positive group") and those with serum CRP levels < 1.0 mg/dL ("the CRP negative group"). The authors identified "a significant correlation between preoperative serum CRP level and tumor size." Id. Furthermore, the authors found that "[t]he overall survival and recurrence-free survival rates in the CRP-positive group were significantly lower compared with the rates in the CRP-negative group." Id. The authors concluded that the preoperative CRP level of patients is an independent and significant predictive indicator of poor prognosis and early recurrence in patients with hepatocellular carcinoma.

Similar correlations have been identified by other investigators. For example, Karakiewicz et al. determined that serum CRP was an independent and informative predictor of renal cell carcinoma-specific mortality. Karakiewicz, et al. (2007) Cancer 110(6): 1241-1247. Accordingly, there remains a need in the art for methods and/or treatments that reduce serum C-Reactive Protein (CRP) concentrations in cancer patients, and particularly those with advanced cancers.

**Methods**

Eight patients with various forms of advanced cancer [(colorectal (3), NSCLC (1), cholangio (1), and mesothelioma (2)] received a single 1-hour intravenous infusion of either 80 mg (2 patients), 160 mg (3 patients) or 320 mg (3 patients) of the Abl monoclonal antibody. No further dosages of the Ab1 monoclonal antibody were administered to the test population.

Patients were evaluated prior to administration of the dosage and thereafter on a weekly basis for at least 8 weeks post dose. At the time of each evaluation, patients were screened for serum CRP concentration. CRP concentration was quantitated by a C-reactive protein particle-enhanced immunoturbidimetric assay using latex-attached anti-CRP antibodies (i.e. Roche CRP Tinaquant®). Briefly, about 1.0 mL of patient sample serum was collected and stored in a plastic collection tube. Sample was placed into appropriate buffer, and anti-CRP antibody coupled to latex microparticles was added to the sample to start the reaction. These anti-CRP antibodies with conjugated latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this was measured turbidimetrically using a Roche/Hitachi Modular P analizer.

**Results**

Serum CRP levels were greatly reduced in all patients studied (Fig. 16). The reduction in serum CRP levels was rapid, with approximately 90% of the decrease occurring within one week of Ab1 administration, and prolonged diminished levels continued at least until the final measurement was taken (up to twelve weeks). In all cases except one patient with colorectal cancer, CRP levels fell to at or below the normal reference range (less than 5-6 mg/L) within one
week. The colorectal cancer patient achieved similar normal levels by week 4 of the study. Thus, administration of Ab1 to advanced cancer patients can cause a rapid and sustained suppression of serum CRP levels.

Example 26

Abl Suppresses Serum CRP in Patients with Rheumatoid Arthritis.

Introduction

[0774] Serum CRP concentrations have been identified as a strong prognostic indicator in patients with rheumatoid arthritis. Patients suffering from rheumatoid arthritis with high levels of CRP demonstrated almost universal deterioration. Amos, et al. (1977) Br. Med. J. 1: 195-97. Conversely, patients with low CRP levels showed no disease progression, suggesting that sustaining low levels of CRP is necessary for effectively treating rheumatoid arthritis. Id.

Tracking of CRP during rheumatoid arthritis treatment regimes of gold, D-penicillamine, chloroquine, or dapsone indicated that radiological deterioration was impeded after the first 6 months of treatment when CRP levels were consistently controlled. Dawes et al. (1986) Rheumatology 25: 44-49. A highly significant correlation between CRP production and radiological progression was identif ed, van Leeuwen, et al. (1997) Rheumatology 32 (Supp. 3): 9-13. Another study revealed that for patients with active rheumatoid arthritis, suppression of abnormally elevated CRP led to improvement in functional testing metrics, whereas sustained CRP elevation associated with deterioration in the same metrics. Devlin, et al. (1997) J. Rheumatol, 24: 9-13. No further deterioration was observed without CRP re-elevation, indicating CRP suppression as a viable candidate for rheumatoid arthritis treatment. Id.

Accordingly, there remains a need in the art for methods and/or treatments that reduce serum C-Reactive Protein (CRP) concentrations in rheumatoid arthritis patients.

Methods

[0775] One-hundred twenty-seven patients with active rheumatoid arthritis and CRP >10 mg/L were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV) infusion of either placebo (n=33), 80 mg (n=32), 160 mg (n=34), or 320 mg (n=28) of the Abl monoclonal antibody, once at the start of the 16 week trial and again at week 8. CRP concentration was quantitated by a C-reactive protein particle-enhanced immunoturbidimetric assay using latex-attached anti-CRP antibodies (i.e., Roche CRP Tinaquant®). Briefly, about 1.0 mL of patient sample serum was collected and stored in a plastic collection tube. Sample was placed into appropriate buffer, and anti-CRP antibody coupled to latex microparticles was added to the sample to start the reaction. These anti-CRP antibodies with conjugated latex microparticles react with antigen in the sample to form an antigen/antibody complex. Following
agglutination, this was measured turbidimetrically using a Roche/Hitachi Modular P analyser. Data on CRP concentration was collected every week for the first 4 weeks, every two weeks between weeks 4 and 12, and at the conclusion of the test at week 16.

Results

[0776] Serum CRP levels were greatly reduced in all patients studied (Fig. 17). The reduction in serum CRP levels was rapid, with immediate reduction in CRP levels relative to placebo within one week of Ab1 administration, and prolonged diminished levels continued at least until the final measurement was taken (up to sixteen weeks). In all cases, CRP levels fell to at or below the normal reference range (less than 5-6 mg/L) within one week. Thus, administration of Ab1 to rheumatoid arthritis patients can cause a rapid and sustained suppression of serum CRP levels and presents an effective treatment regime.

Example 27
Abl Increases Hemoglobin in Patients with Advanced Cancer

[0777] Antibody Abl was dosed at 80 mg, 160 mg, or 320 mg of Abl in phosphate buffered saline to 93 individuals with non-small cell lung carcinoma. The placebo group of 31 individuals with non-small cell lung carcinoma was dosed with phosphate buffered saline only. Blood samples were removed just prior to dosing (zero week), and at two, four, eight and twelve weeks, and the hemoglobin concentration was determined. Mean hemoglobin concentration rose for those receiving antibody Ab1, while mean hemoglobin concentration of those receiving placebo did not rise after twelve weeks when compared to the concentration just prior to dosing (zero week) (Figs. 18A and 18B).

[0778] A subset of the study population began the study with low levels of hemoglobin, defined as a baseline hemoglobin concentration below 11 g/l. Mean hemoglobin concentration rose above 11 g/l after eight weeks for those receiving antibody Ab1 at dosages of 160 mg and 320 mg, while mean hemoglobin concentration of those receiving antibody Abl at dosages of 80 mg or placebo did not rise above 11 g/l after eight weeks (Fig. 18C).

[0779] These results further demonstrate some of the beneficial effects of administration of Abl to chronically ill individuals. Because IL-6 is the main cytokine responsible for the anemia of chronic disease (including cancer-related anemia), neutralization of IL-6 by Abl increases hemoglobin concentration in these individuals.

Example 28
Abl Increases Hemoglobin in Patients with Rheumatoid Arthritis

[0780] Hemoglobin levels were analyzed in patients with rheumatoid arthritis during treatment with Abl antibody. Abl antibody was dosed at 80 mg, 160 mg, or 320 mg in phosphate buffered
saline to 94 individuals with rheumatoid arthritis. The placebo group of 33 individuals with rheumatoid arthritis was dosed with phosphate buffered saline only. Blood samples were removed just prior to dosing (zero week), and at one, two, three, four, six, eight, ten, twelve, and sixteen weeks, and the hemoglobin concentration was determined. Mean hemoglobin concentration rose for those receiving antibody Ab 1, while mean hemoglobin concentration of those receiving placebo did not appreciably rise after sixteen weeks when compared to the concentration just prior to dosing (zero week) (Fig. 19).

These results further demonstrate some of the beneficial effects of administration of Abl to chronically ill individuals. Because IL-6 is the main cytokine responsible for the anemia of chronic disease (including cancer-related anemia), neutralization of IL-6 by Abl increases hemoglobin concentration.

Example 29
Abl Increases Albumin in Patients with Advanced Cancer

Introduction

Serum albumin concentrations are recognized as predictive indicators of survival and/or recovery success of cancer patients. Hypoalbumemia correlates strongly with poor patient performance in numerous forms of cancer. For example, in one study no patients undergoing systemic chemotherapy for metastatic pancreatic adenocarcinoma and having serum albumin levels less than 3.5 g/dL successfully responded to systemic chemotherapy. Fujishiro, et al. (2000) Hepatogastroenterology 47(36): 1744-46. The authors conclude that "[p]atients with … hypoalbuminemia … might be inappropriate candidates for systemic chemotherapy and might be treated with other experimental approaches or supportive care." Id.

Similarly, Senior and Maroni state that "[t]he recent appreciation that hypoalbuminemia is the most powerful predictor of mortality in end-stage renal disease highlights the critical importance of ensuring adequate protein intake in this patient population." Senior & Maroni (1999) Am. Soc. Nutr. Sci. 129: 313S-314S.

In at least one study, attempts to rectify hypoalbuminemia in 27 patients with metastatic cancer by daily intravenous albumin infusion of 20 g until normal serum albumin levels (>3.5 g/dL) were achieved had little success. The authors note that "[a]lbumin infusion for the advanced stage cancer patients has limited value in clinical practice. Patients with PS 4 and hypoalbuminemia have poorer prognosis." Demirkazik, et al. (2002) Proc. Am. Soc. Clin. Oncol. 21: Abstr 2892.
Accordingly, there remains a need in the art for methods and/or treatments that improve serum albumin concentrations in cancer patients and address hypoalbuminemic states in cancer patients, particularly those with advanced cancers.

Methods

Antibody Abl was dosed at 80 mg, 160 mg, or 320 mg of Abl in phosphate buffered saline to 93 individuals with non-small cell lung carcinoma. Each individual received a dosage of. The placebo group of 31 individuals with non-small cell lung carcinoma was dosed with phosphate buffered saline only. Blood samples were removed just prior to dosing (zero week), and at two, four, eight and twelve weeks, and the albumin concentration was determined.

Results

Mean albumin concentration rose for those receiving antibody Ab 1, while mean albumin concentration of those receiving placebo did not rise after twelve weeks when compared to the concentration just prior to dosing (zero week) (Fig. 20A). The change from baseline albumin values for all dosage concentration groups is plotted in Figure 20B.

A subset of the study population began the study with low levels of albumin, defined as a baseline albumin concentration less than or equal to 35 g/L. Mean albumin concentration initially rose with all dosages of antibody Ab 1 over placebo, but only patients receiving 160 mg or 320 mg demonstrated sustained albumin levels above 35 g/L over 8 weeks of the study (Fig. 20C). The 80 mg dosage group demonstrated an initial increase, but gradually declined after week 2 and never rose above 35 g/L during the 8 weeks where data was available. Id.

Example 30

Abl Improved Weight and Reduced Fatigue in Patients with Advanced Cancer

Introduction

Weight loss and fatigue are very common symptoms of patients with advanced forms of cancer, and these symptoms can worsen as the cancer continues to progress. Fatigue and weight loss can have significant negative effects on the recovery of patients with advanced forms of cancer, for example by disrupting lifestyles and relationships and affecting the willingness or ability of patients to continue cancer treatments. Known methods of addressing fatigue and weight loss include regular routines of fitness and exercise, methods of conserving the patient's energy, and treatments that address anemia-induced fatigue. Nevertheless, there remains a need in the art for methods and/or treatments that improve fatigue and weight loss in cancer patients.

Methods

One-hundred twenty-four patients with non-small cell lung cancer (NSCLC) were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV)
infusion of either placebo (n=31), 80 mg (n=29), 160 mg (n=32), or 320 mg (n=32) of the Abl monoclonal antibody every 8 weeks over a 24 week duration for a total of 3 doses.

[0791] Patients were evaluated prior to administration of the dosage, and thereafter for at least 12 weeks post dose. At the time of each evaluation, patients were screened for the following: any change in weight; and fatigue as measured using the Facit-F Fatigue Subscale questionnaire a medically recognized test for evaluating fatigue. See, e.g., Cella, et al. (2002) Cancer 94(2): 528-538; Cella, et al. (2002) Journal of Pain & Symptom Management 24(6): 547-561.

Results

Weight Change

[0792] The averaged weight change data from each dosage concentration group (placebo, 80 mg, 160 mg, and 320 mg) of the Abl monoclonal antibody over 12 weeks. The average percent change in body weight from each dosage concentration. The averaged lean body mass data for the dosage concentration groups.

Fatigue

[0793] The averaged fatigue from each dosage concentration group (placebo, 80 mg, 160 mg, and 320 mg) of the Abl monoclonal antibody demonstrated increases in the mean Facit-F FS subscale score for some of the dosage concentration groups in the patient population over the period of 8 weeks.

Example 31

Abl Decreases D-dimer Levels in Patients with Advanced Cancer

Introduction


Accordingly, there remains a need in the art for methods and/or treatments of thrombosis that improve D-dimer concentrations in cancer patients and address elevated D-dimer states in cancer patients, particularly those with advanced cancers.

Methods

One-hundred twenty-four patients with non-small cell lung cancer (NSCLC) were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV) infusion of either placebo (n=31), 80 mg (n=29), 160 mg (n=32), or 320 mg (n=32) of the Abl monoclonal antibody every 8 weeks over a 24 week duration for a total of 3 doses. Data on D-dimer concentration was collected for the first 8 weeks of treatment. D-dimer data concentration was quantitated by a D-dimer immunoturbidimetric assay. Briefly, the assay is based on the change in turbidity of a microparticle suspension that is measured by photometry. About 1.5 mL of patient sample sodium citrate plasma was collected and stored in a plastic collection tube. A suspension of latex microparticles, coated by covalent bonding with monoclonal antibodies specific for D-dimer, was mixed with the test plasma whose D-dimer level was to be assayed. Antigen-antibody reactions leading to an agglutination of the latex microparticles induced an increase in turbidity of the reaction medium. This increase in turbidity was reflected by an increase in absorbance, the latter being measured photometrically using a STAGO STA analyzer. The increase in absorbance was a function of the D-dimer level present in the test sample.

Results

The averaged data for each dosage concentrations (placebo, 80 mg, 160 mg, and 320 mg) of the Ab 1 monoclonal antibody. All dosage levels of Ab 1 antibody demonstrated a drop in D-dimer levels over placebo over the period of 8 weeks. See WO 2011/066371.
Abl Efficacy and Safety in Patients with Advanced NSCLC

The primary objective of this study was to determine the efficacy and safety of ALD518 or humanized Ab 1 in patients with advanced NSCLC.

Methods

124 patients (pts) with NSCLC, ECOG 0-3, weight loss in the preceding 3 months of >5% body weight, hemoglobin (Hb) >7g/dL, and C-reactive protein (CRP) > 10mg/L were dosed. Pts were randomized to 1 of 4 groups (n~30/group). Placebo or ALD518 80mg, 160mg, or 320mg was administered intravenously every 8 weeks. Pts were followed up for 24 weeks. Data included hematology, clinical chemistry, CRP and adverse events (AEs).

Results

29 pts completed the study treatments and evaluations, 38 failed to complete every visit, 52 died of progressive disease, and 5 withdrew because of adverse events. There were no dose limiting toxicities (DLTs) or infusion reactions. 84 pts had serious AEs of which 1 was deemed to be possibly related to administration of ALD518 (rectal hemorrhage). The mean (±SD) values for Hb, hematocrit (Hct), mean corpuscular Hb (MCH), and albumin are below:

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>MCH (pg)</th>
<th>Albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD518</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pooled)</td>
<td>93</td>
<td>11.5 (±2.1)</td>
<td>37.9 (±6.2)</td>
<td>28.4 (±2.8)</td>
<td>37.3 (±5.3)</td>
</tr>
<tr>
<td>Week 4</td>
<td>69</td>
<td>13.1 (±1.6)a</td>
<td>42.5 (±5.0)a</td>
<td>29.2 (±2.5)a</td>
<td>43.6 (±4.7)a</td>
</tr>
<tr>
<td>Week 12</td>
<td>39</td>
<td>13.4 (±1.6)a</td>
<td>42.5 (±4.7)b</td>
<td>29.8 (±2.8)a</td>
<td>45.2 (±4.5)a</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-dose</td>
<td>31</td>
<td>12.2 (±1.8)</td>
<td>39.0 (±5.9)</td>
<td>29.0 (±2.8)</td>
<td>37.5 (±5.7)</td>
</tr>
<tr>
<td>Week 4</td>
<td>29</td>
<td>11.8 (±2.0)</td>
<td>39.5 (±6.4)</td>
<td>28.0 (±2.8)c</td>
<td>37.3 (±6.8)</td>
</tr>
<tr>
<td>Week 12</td>
<td>21</td>
<td>12.0 (±2.5)</td>
<td>39.6 (±7.4)</td>
<td>27.8 (±3.0)c</td>
<td>37.0 (±7.5)</td>
</tr>
</tbody>
</table>

*p<0.0001   ^p<0.0002   °p<0.001 (paired t-test compared to pre-dose)

38/93 pts treated ALD518 and 10/31 given placebo has a pre-dose Hb ≤ 11g/dL. 24 of these pts on ALD518 and 7 of these pts on placebo remained in the study at week 4. 14/24 pts on ALD518 and 0/7 on placebo had raised their Hb from ≤ 11g/dL to ≥ 12g/dL.

Conclusion

ALD518 increased Hb, Hct, MCH and albumin in NSCLC pts and raised Hb to ≥ 12g/dL in 58% of pts with a Hb ≤ 11g/dL at baseline. This further indicates that ALD518 can be administered as a non-erythropoietic stimulating agent for treating cancer-related anemia.
Example 33
Abl achieved ACR 20/50/70 in Patients with Rheumatoid Arthritis.

Introduction

Rheumatoid arthritis is a chronic, systemic inflammatory disorder that principally
attack synovium of joints. The disease causes painful and potentially disabling inflammation,
with onset typically occurring between 40 and 50 years of age. Interpretation of drug treatment
efficacy in rheumatoid arthritis is made difficult by the myriad of subjective and objective
assessment tools made available over the years. The American College of Rheumatology
(“ACR”) released a standardized set of rheumatoid arthritis measures to facilitate evaluation of
729-40.

Methods

One-hundred twenty-seven patients with active rheumatoid arthritis and CRP >10 mg/L
were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV)
infusion of either placebo (n=33), 80 mg (n=32), 160 mg (n=34), or 320 mg (n=28) of the Abl
monoclonal antibody, once at the start of the 16 week trial and again at week 8. Data on CRP
concentration was collected every week for the first 4 weeks, every two weeks between weeks 4
and 12, and at the conclusion of the test at week 16.

Assessment under the standardized protocols from the American College of
Rheumatology were employed in determining the percentage of improvement of patients during
the clinical trial and conducted by a person trained in the ordinary art of evaluating rheumatoid
arthritis. The evaluation was based upon activity measures, including tender joint count, swollen
joint count, the patient’s assessment of pain, the patient’s and physician’s global assessments of
disease activity, and laboratory evaluation of either erythrocyte sedimentation rate or CRP level.

The patient’s assessment of pain was based upon the Stanford Health Assessment
Questionnaire Disability Index (HAQ DI). Patients that achieve a 20% increase in activity
measures for rheumatoid arthritis during a clinical trial are categorized as achieving ACR 20.
Similarly, patients achieving 50% and 70% improvements are categorized as ACR 50 and ACR
70, respectively.

Results

A significant portion of patients suffering from rheumatoid arthritis achieved ACR 20
or greater during the course of the study. See Table 14. Patients observed rapid improvement in
systems within the first 4 weeks of the study, as well as continued, steady improvement
throughout the course of the 16 week evaluation. The greatest results were exhibited by patients receiving the 320 mg dosage level, with 43% achieving ACR 70 status during the study.

TABLE 14: Percentage patients achieving ACR 20/50/70 at week 16 - MITT non responder imputation

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=33)</th>
<th>Ab1 80mg (n=32)</th>
<th>Ab1 160mg (n=34)</th>
<th>Ab1 320mg (n=28)</th>
<th>Ab1 Pooled (n=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR 20</td>
<td>36%</td>
<td>75% (p=0.0026)</td>
<td>65% (p=0.0283)</td>
<td>82% (p=0.0005)</td>
<td>73% (p=0.0002)</td>
</tr>
<tr>
<td>ACR 50</td>
<td>15%</td>
<td>41% (p=0.0281)</td>
<td>41% (p=0.0291)</td>
<td>50% (p=0.0052)</td>
<td>44% (p=0.0032)</td>
</tr>
<tr>
<td>ACR 70</td>
<td>6%</td>
<td>22% (p=0.0824)</td>
<td>18% (p=0.2585)</td>
<td>43% (p=0.0015)</td>
<td>27% (p=0.0130)</td>
</tr>
</tbody>
</table>

[0809] Analysis of the individual components of the ACR evaluation demonstrated gains in every component. HAQ DI scores demonstrated clinically meaningful change over placebo during the course of the evaluation. Serum CRP levels were greatly reduced in all patients studied. The reduction in serum CRP levels was rapid, with immediate reduction in CRP levels relative to placebo within one week of Ab1 administration, and prolonged diminished levels continued at least until the final measurement was taken (up to sixteen weeks). In all cases, CRP levels fell to at or below the normal reference range (less than 5-6 mg/L) within one week. Thus, administration of Ab1 can cause a rapid and sustained improvement rheumatoid arthritis patients, as evidenced by the significant improvement in ACR scores during clinical evaluation, and presents an effective treatment regime. See also WO 201 1/066371.

Example 34

Ab1 Achieved Improved DAS28 and EULAR Scores in Patients with Rheumatoid Arthritis

Introduction

[0810] Rheumatoid arthritis is a chronic, systemic inflammatory disorder that principally attack synovium of joints. The disease causes painful and potentially disabling inflammation, with onset typically occurring between 40 and 50 years of age. Interpretation of drug treatment efficacy in rheumatoid arthritis is made difficult by the myriad of subjective and objective assessment tools made available over the years. The American College of Rheumatology ("ACR") released a standardized set of rheumatoid arthritis measures to facilitate evaluation of improvement of the disease in clinical trials. Felson, et al. (1993) Arthritis & Rheumatism 36: 729-40.

[0811] Inflammatory activity associated with rheumatoid arthritis is measured using numerous variables through validated response criteria such as Disease Activity Score (DAS), DAS28 and EULAR. The DAS is a clinical index of rheumatoid arthritis disease activity that combines
information from swollen joints, tender joints, the acute phase response, and general health. Fransen, et al. (2005) Clin. Exp. Rheumatol. 23(Suppl. 39): S93-S99. The DAS 28 is an index similar to the original DAS, but utilizes a 28 tender joint count (range 0-28), a 28 swollen joint count (range 0-28), ESR (erythrocyte sedimentation rate), and an optional general health assessment on a visual analogue scale (range 0-100). Id. The European League against Rheumatism (EULAR) response criteria classify patients using the individual amount of change in the DAS and the DAS value (low, moderate, high) reached into one of the following classifications: Good; Moderate; or Non-Responders. Id.

Methods

One hundred twenty-seven patients with active rheumatoid arthritis were divided into 4 treatment groups. Patients in one group received one 1-hour intravenous (IV) infusion of either placebo (n=33), 80 mg (n=32), 160 mg (n=34), or 320 mg (n=28) of the Abl monoclonal antibody, once at the start of the 16 week trial and again at week 8. Data on the DAS28 and EULAR scores was collected every week for the first 4 weeks, every two weeks between weeks 4 and 12, and at the conclusion of the test at week 16. Assessment under the standardized DAS28 and EULAR protocols were employed in determining the respective scores of patients during the clinical trial and conducted by a person trained in the ordinary art of evaluating rheumatoid arthritis.

Results

Patients receiving 80 mg, 160 mg or 320 mg of Abl demonstrated improved DAS28 scores relative to those patients receiving placebo over the course of 16 weeks, as presented in Fig. 62 as a mean change from the baseline DAS28 score. Furthermore, a significant percentage of patients receiving 80 mg, 160 mg or 320 mg of Abl achieved "Good" or "Moderate" classifications relative to those patients receiving placebo over the course of 16 weeks. Thus, administration of Abl can result in improved DAS28 and EULAR scores in rheumatoid arthritis when compared to those patients receiving placebo. See WO 2011/066371.

EXAMPLE 35

Safety, Pharmacokinetics (PK), and Pharmacodynamics (PD) of Abl in Human Subjects

Background

A humanized antibody derived from Ab I (humanized Ab I or ALD5 18) containing the variable heavy and light sequences in SEQ ID NO: 19 and 20 was administered to rheumatoid arthritis patients. This antibody is a humanized, asialated, IgGl monoclonal antibody against IL-6 which has been shown to have a half-life (t½) of approximately 30 days in humans. In studies in patients with RA, intravenous (IV) with this antibody (humanized Abl) has demonstrated:

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efficacy over 16 weeks with rapid American College of Rheumatology (ACR) responses;
Complete and durable suppression of C-reactive protein (CRP); Good tolerability, and a safety
profile consistent with the biology of IL-6 blockade. This humanized antibody binds to IL-6 with
high affinity, preventing interaction and signaling mediated via IL-6R. Rapid and significant
treatment responses have been demonstrated with intravenous (IV) administration of humanized
Ab l in patients with RA. In this example we study the safety, pharmacokinetics and
pharmacodynamics of subcutaneous (SC) administration of humanized Abl in healthy subjects.

The objective of this study was to assess the safety, pharmacokinetics (PK) and
pharmacodynamics (PD) of a single SC injection of this humanized antibody in healthy male
subjects.

Methods

In this Phase I, double-blind, placebo-controlled study, 27 subjects were randomized
2:1 to receive a single dose of humanized Abl or placebo in the following groups: humanized
Abl 50 mg SC, humanized Abl 100 mg SC or humanized Abl 100 mg IV (n=6 active and n=3
placebo per group). The primary objective was to assess safety of SC humanized Abl versus
placebo over 12 weeks. Plasma concentrations of humanized Abl and serum concentrations of C-
reactive protein (CRP) were assessed as secondary objectives. Assessments were performed daily
in Week 1 and then on Day 10, Weeks 2, 4, 6 and 8, and then monthly to Week 12. The study was
unblinded at Week 12, and humanized Abl subjects were monitored to Week 24.

Study design and population

The study included 27 healthy male subjects (aged 18-65 years). Subjects were dosed
in three treatment groups of nine subjects each, randomized 2:1 to receive a single dose of
humanized Ab l or placebo on Day 1. Humanized Ab l treatments per group were: humanized
Abl IV 100 mg infusion over 60 minutes; humanized Abl SC 50 mg injection (1 mL); or
humanized Abl 100 mg injection (1 mL). The study was unblinded at Week 12, after which
placebo subjects discontinued the trial and ALD518 subjects were monitored to Week 24.

Safety and immunogenicity assessments

The primary objective of the study was to assess the safety of SC humanized Abl
compared with placebo over 12 weeks. Safety was monitored over 12 weeks for all subjects.
The study was unblinded at Week 12, and Humanized Ab l subjects were monitored to Week 24.
Laboratory safety tests were performed pre-dose at screening and Day -1, and post dose on Days
2 and 7, Weeks 2, 4, 6, 8 and 12 for all subjects, and Weeks 16, 20 and 24 post-dose for those
randomized to Humanized Ab l. Anti-Humanized Ab l antibodies were measured by enzyme-
linked immunosorbent assay (ELISA). Blood samples were collected at Day 1 (pre-dose) and
Week 12 post-dose for all subjects, and Week 24 post-dose for those randomized to Humanized Abl.

Pharmacokinetic and pharmacodynamic assessments

[0819] Plasma Humanized AB1 and serum CRP concentrations were assessed by ELISA. For all subjects, samples were collected at screening, pre-dose on Day 1, and post-dose on Days 2 and 7 and Weeks 2, 4, 6, 8 and 12. For subjects randomized to Humanized AB1, further samples were collected at Weeks 16, 20 and 24 post-dose.

Statistical analysis

[0820] All subjects who received a dose of Humanized AB1 or placebo were included in the safety analysis. All subjects who received a dose of Humanized AB1 or placebo were included in PD and immunogenicity analyses. All subjects who received a dose of Humanized AB1 were included in PK analyses (n=18). All PK samples for placebo subjects were confirmed as below quantification. Descriptive statistics were generated for baseline demographics, safety data, plasma Humanized AB1 parameters and serum CRP concentrations. Wilcoxon Rank Sum test was used to compare CRP concentrations for Humanized AB1 treatments versus placebo.

Results — Summary

[0821] Over 24 weeks, there were no deaths or serious AEs, and no withdrawals due to AEs. Nearly all subjects (89%) experienced AEs, which were mild or moderate except one event of severe gastroenteritis in the Humanized Abl SC 50 mg group. Injection site reactions occurred in 5/12 Humanized Abl SC subjects, 1/6 placebo SC subjects and 1/3 placebo IV subjects (none were reported in Humanized Abl IV subjects). These were mild except one case of moderate erythema and pruritis in the Humanized Abl 100 mg SC group. Increases in direct bilirubin and neutrophil counts below the limit of normal were more common in subjects receiving Humanized Abl than placebo; all were CTC Grade 1 or 2. The half life of Humanized Abl was similar across all groups (mean range: 30.7-33.6 days). The median Tmax of Humanized Abl was longer after SC (~1 week) than after IV administration (end of infusion). The PK of SC Humanized Abl was dose-proportional in terms of AUC and Cmax at doses of 50 mg and 100 mg. Based on AUC0-∞ (day*µg/mL) of 237, 452 and 764 for the Humanized Abl 50 mg SC, 100 mg SC and 100 mg IV groups, respectively, the bioavailability of Humanized Abl was ~60% for the SC versus IV groups. Subjects receiving Humanized Abl experienced rapid and sustained reductions in serum CRP (Fig. 21A), similar results were seen when the antibody was administered either intravenous or subcutaneously (Fig. 21B).
Subject disposition and baseline demographics

A total of 27 subjects were enrolled and completed the study (n=18 Humanized Abl and n=9 placebo). No subjects were withdrawn for any reason. All subjects were male; 23/27 subjects were Caucasian and 4/27 were Asian. Mean age was 29 (range 20-59) and was similar across the groups. Mean height and weight were also generally comparable across groups, although the IV placebo group were slightly lighter.

Safety and immunogenicity to Week 12 for Humanized Abl and placebo

A summary of safety is presented in TABLE 15. For the SC Humanized Abl groups, a total of 11/12 (91%) patients experienced an adverse event (AE) compared with: 6/6 (100%) for the IV Humanized Abl group; 4/6 (66.6%) for the SC placebo group; and 3/3 (100%) for the IV placebo group.

TABLE 15 Adverse Events

<table>
<thead>
<tr>
<th>MedR A Preferred Term</th>
<th>Up to Week 12</th>
<th>Week 12 – Week 24*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SC 50 mg n=6</td>
<td>SC 100 mg n=6</td>
</tr>
<tr>
<td>Subjects with an AE</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>AE severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Discontinuations Due to AEs</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Deaths</td>
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<td>0</td>
</tr>
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</table>

AEs reported in ≥ 2 subjects in any group

<table>
<thead>
<tr>
<th>Injection site erythema</th>
<th>1</th>
<th>2</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection site pruritis</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>URTI</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Skin laceration</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Patients randomized to placebo (IV or SC) discontinued at Week 12 and are not included in Week 24 analyses; AE=adverse event; SC=subcutaneous; IV=intravenous; URTI=upper respiratory tract infection.

Across groups: No deaths or serious AEs were reported and there were no withdrawals due to AEs. Most AEs were mild or moderate in intensity. One case of gastroenteritis in a SC
Humanized ABI 50 mg subject was considered severe, but not serious, and not related to study medication. No anti-Humanized ABI antibodies were detected in any subject during this period.

Injection site reactions

Injection site reactions were reported in 26% (7/27) of subjects, and all occurred prior to Week 12 (TABLE 40). Injection site reactions occurred in 5/12 SC Humanized ABI subjects and 1/6 SC placebo subjects. In the IV groups, 0/6 Humanized ABI subjects and 1/3 placebo subjects experienced injection site reactions. All injection site reactions were mild except in one SC Humanized ABI 100 mg subject with moderate injection site erythema and pruritis. No injection site reactions occurred after Week 12 in any of the Humanized ABI groups. Infusion site reactions were reported in 0/6 subjects receiving IV Humanized ABI and 1/3 IV placebo subjects (infusion site pruritis)

TABLE 16 Abl Injection Site Reactions to Week 12*

<table>
<thead>
<tr>
<th></th>
<th>50 mg n=6</th>
<th>100 mg n=6</th>
<th>100 mg n=6</th>
<th>Placebo SC n=6</th>
<th>Placebo IV n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects with injection site reaction</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Injection site erythema</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Injection site pain</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Injection site pruritus</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Injection site rash</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*A11 injection site reactions were reported in the first 12 weeks of the study. SC=subcutaneous; IV=intravenous

Clinical laboratory evaluations

TABLE 43 shows incidences of increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and bilirubin levels across the Humanized ABI and placebo groups. All ALT and AST levels were Grade 1 by the Common Terminology Criteria for Adverse Events (CTCAE), and no levels were >3 times the upper limit of normal (ULN). All increases in total and direct bilirubin were CTCAE Grade 1 or 2 and no subject met criteria for drug-induced liver damage. Only one subject (SC Humanized ABI 100 mg group) had total bilirubin out of range (26 μmol/L, range 0-24 μmol/L), at Week 24.

TABLE 16 Clinical Laboratory Evaluations Over 24 Weeks (Abl)

<table>
<thead>
<tr>
<th></th>
<th>SC 50 mg n=6</th>
<th>SC 100 mg n=6</th>
<th>IV 100mg n=6</th>
<th>Placebo n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated ALT</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Elevated total bilirubin</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Elevated direct bilirubin</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Low neutrophil count</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Low platelet count</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

211
SC and IV groups combined up to Week 12 only, after which placebo-treated patients discontinued; below the lower limit of normal; SC=subcutaneous; IV=intravenous; ALT=alanine aminotransferase; AST=aspartate aminotransferase

[0827] Sporadic decreases in neutrophil and platelet counts were also observed in the Humanized AB1 and placebo groups. Neutrophil counts below the lower limit of normal were more common in subjects receiving Humanized AB1 than placebo but all decreases were CTCAE Grade 1 or 2. Only one subject (SC Humanized AB1 50 mg group) had consistent mild neutropenia to Week 24 (1.6 x 10^9/L at Week 24). Reductions in platelet counts were all CTCAE Grade 1 (lowest level 134 x 10^9/L) and no subject had a low platelet count past Week 8.

**Pharmacokinetics**

[0828] Bioavailability of Humanized AB1 was 60% for SC Humanized AB1 50 and 100 mg versus IV Humanized AB1 100 mg groups based on the mean AUC₀-∞ (TABLE 44). The half-life of Humanized AB1 was similar across all groups (mean range: 30.7-33.6 days) (Table 17). Peak plasma concentration (Cₘₐₓ) of SC Humanized AB1 was reduced as compared to IV (Fig. 15). Median time to maximum plasma concentration (Tₘₐₓ) of Humanized Ab1 was longer after SC Humanized AB1 (at approximately one week) than after IV Humanized AB1 administration (at approximately the end of infusion).

**TABLE 17 Ab1 Plasma Pharmacokinetic Parameters to Week 24**

<table>
<thead>
<tr>
<th></th>
<th>SC 50 mg</th>
<th>SC 100 mg</th>
<th>IV 100mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
</tr>
<tr>
<td>Cₘₐₓ (µg/mL) (CV)*</td>
<td>5.57 (24%)</td>
<td>9.19 (34%)</td>
<td>33.6 (30%)</td>
</tr>
<tr>
<td>Tₘₐₓ (days) (min, max)†</td>
<td>6 (6, 14)</td>
<td>5.5 (2, 28)</td>
<td>0.17 (0, 17, 0.34)</td>
</tr>
<tr>
<td>AUC₀-24 (day•µg/mL) (CV)*</td>
<td>218 (34%)</td>
<td>435 (19%)</td>
<td>732 (22%)</td>
</tr>
<tr>
<td>AUC₂₄ (day•µg/mL) (CV)*</td>
<td>224 (39%)</td>
<td>444 (20%)</td>
<td>746 (22%)</td>
</tr>
<tr>
<td>t₁/₂ (days+SD)‡</td>
<td>33.6±21.7</td>
<td>31.1±9.0</td>
<td>30.7±5.9</td>
</tr>
<tr>
<td>CL (mL/day) (CV)*</td>
<td>223 (32%)</td>
<td>225 (21%)</td>
<td>134 (27%)</td>
</tr>
</tbody>
</table>

*Data are geometric mean (coefficient of variation %, CV%). †Data are median (minimum, maximum). ‡Data are mean (±SD). CV=coefficient of variation; Cₘₐₓ=maximum plasma concentration; AUC=area under curve; SD=standard deviation; CL=apparent total body clearance for IV and apparent total body clearance divided by bioavailability for SC; IV=intravenous; SC=subcutaneous; Tₘₐₓ=time to maximum plasma concentration; t₁/₂=terminal plasma half-life

**Pharmacodynamics**

[0829] CRP levels were reduced in all subjects who received Humanized AB1 irrespective of dose or administration route. From Weeks 4 to 12, CRP levels were significantly lower in subjects who received Humanized Ab1 compared with placebo (unadjusted p-value <0.05). A high correlation between the IgG produced and antigen specificity for an exemplary IL-6 protocol was observed with 9 of 11 wells showed specific IgG correlation with antigen recognition. In Humanized Ab1 subjects, CRP levels were lowered to <20% of pre-dose levels in: 72% (13/18)
of subjects at Week 1: 73% (11/15) of subjects at Week 12; and 56% (10/18) of subjects at Week 24.

Conclusions

In this Phase I study, the anti-IL-6 antibody Humanized Abl was generally well tolerated when administered in a single SC dose in healthy male subjects. Injection site reactions were generally mild. No anti-Humanized Ab1 antibodies were detected. Changes in liver enzymes, neutrophil and platelet counts were reversible. The bioavailability of SC Humanized AB1 was approximately 60% of that observed with IV Humanized Ab1. The half-life of Humanized Ab1 was approximately 30 days, irrespective of route of administration. These data concur with previous data using IV Humanized Abl 2. Subcutaneous Humanized AB1 led to rapid and large reductions in serum CRP. Reductions in CRP observed during the first 12 weeks of the study were sustained over 24 weeks of assessment. These preliminary data support the continued development and evaluation of subcutaneous Humanized Ab1 for the treatment of patients with mucositis.

In summary, in this Phase I study, the anti-IL-6 antibody Humanized Abl was well tolerated when administered in a single SC dose; injection site reactions were generally mild. The bioavailability of SC Humanized Abl was -60% of IV Humanized Abl, and the half-life was -30 days. Rapid and significant reductions in CRP were observed, which were sustained over 24 weeks of assessment.

Example 36

Effect of Abl on DAS28-Assessed Disease Activity

ALD518* is an asialated, humanized anti-IL-6 monoclonal antibody with a half-life of -30 days containing the humanized variable heavy and light sequences contained in SEQ ID NO: 19 and 20. These humanized heavy and light sequences are derived from a parent rabbit antibody that specifically binds human IL-6 which antibody is referred to in said incorporated application as Ab1. ALD518 binds to IL-6 with high affinity, preventing interaction and signalling mediated via soluble and membrane-bound IL-6R. Rapid and significant ACR responses have been demonstrated with ALD518* in patients with RA. In this example we report the impact of ALD518 on DAS28-assessed disease activity over 16 weeks.

Methods

Patients with active RA and an inadequate response to methotrexate (MTX) were randomized 1:1:1:1 to intravenous ALD518* 80, 160 or 320 mg or placebo during this 16-week, double-blind, placebo-controlled Phase II study. Patients received two IV infusions of ALD518 (Day 1 and Week 8), while continuing on stable doses of methotrexate (MTX). The primary
efficacy endpoint was the proportion of patients achieving ACR20 at Week 12; disease activity was assessed via Disease Activity Score (DAS28) based on C-reactive protein (CRP) as a secondary endpoint. The proportion of patients achieving DAS28-defined remission (score <2.6), low disease activity state (LDAS; score <3.2) and good EULAR responses (current DAS28 <3.2 and improvement from baseline >1.2) were assessed for the modified intent-to-treat population, and are presented for patients with available data (as observed). P-values are based on Chi-square tests.

Results

Of 127 randomized and treated patients, 116 completed the trial. At baseline, mean age was 52.3 years and RA duration was 6.8 years. At Weeks 4, 12 and 16, the proportion of patients achieving LDAS and remission was greater than placebo for all ALD518* doses; differences were significant versus placebo (p<0.05) for all assessments except ALD518* 80 mg at Week 4 (p=0.056). Similarly, EULAR responses were significantly better for all ALD518* doses versus placebo (p<0.01) at Weeks 4, 12 and 16. There was a trend toward greater responses with higher ALD518* doses.

TABLE 18 Proportion of patients achieving DAS28-defined remission, LDAS and good EULAR responses

<table>
<thead>
<tr>
<th></th>
<th>ALD518* 80 mg (N=32)</th>
<th>ALD518* 160 mg (N=34)</th>
<th>ALD518* 320 mg (N=28)</th>
<th>Placebo - (N=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 4</td>
<td>10.0</td>
<td>8.8</td>
<td>17.9</td>
<td>0</td>
</tr>
<tr>
<td>Week 12</td>
<td>17.2</td>
<td>21.2</td>
<td>34.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Week 16</td>
<td>13.8</td>
<td>28.1</td>
<td>44.0</td>
<td>0</td>
</tr>
<tr>
<td>LDAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>10.0</td>
<td>23.5</td>
<td>28.6</td>
<td>0</td>
</tr>
<tr>
<td>Week 12</td>
<td>20.6</td>
<td>33.3</td>
<td>46.1</td>
<td>6.6</td>
</tr>
<tr>
<td>Week 16</td>
<td>20.7</td>
<td>50.0</td>
<td>52.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Good EULAR response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>10.0</td>
<td>23.5</td>
<td>28.6</td>
<td>0</td>
</tr>
<tr>
<td>Week 12</td>
<td>20.7</td>
<td>33.3</td>
<td>46.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Week 16</td>
<td>20.7</td>
<td>50.0</td>
<td>52.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

DAS28=Disease Activity Score 28; LDAS=low disease activity state

SAEs were reported in two ALD518 patients (both had significant increases in liver enzymes, and discontinued treatment). Overall, elevations in liver enzymes >2xULN occurred in 17% of ALD518*- versus 0% placebo-treated patients; the frequency was highest in the 320 mg dose group. Modest increases in total cholesterol were observed (mean increase by Week 16=1.1 mmol/L for ALD518* versus 0.2 mmol/L for placebo). Nine ALD518 patients had transient
Grade II and two had transient Grade III neutropenias. There were no serious infections or infusion reactions in any treatment group, and no evident immunogenicity.

Conclusions

In this Phase II study, the novel IL-6 inhibitor ALD518 resulted in rapid and significant improvements in disease activity sustained over 16 weeks of assessment in patients with RA and an inadequate response to methotrexate (MTX). ALD518 was well tolerated, with a safety profile consistent with the biology of IL-6 blockade.

EXAMPLE 37
Abl Administration

Methods

Patients with active RA were randomized into a 16 week, double-blind, placebo-controlled trial comparing multiple iv infusions of ALD518 (80, 160 or 320mg). Patients received an infusion every 8 weeks and were maintained on a stable dose of MTX throughout the trial. Assessments included ACR 20/50/70 responses and DAS28. All patients were evaluated for safety. For early withdrawals, LOCF analysis was used for continuous variables and non-responder imputation for categorical variables.

Results

132 patients were randomized; 127 were dosed. Mean disease duration was 6.6 years; mean DAS28 score was 6.2 and mean HAQ-DI was 1.72. 11 patients did not complete the 16-week trial: 320mg-3, 160mg-0, 80mg-3, placebo-4: 4 discontinued due to adverse events (80mg-2, 320mg-2), with 2 SAEs (80mg-0, 320mg-0). Elevations in liver enzymes (LFTs) >2xULN were observed in 17% ALD518 versus 0% placebo. There were modest increases in total cholesterol (mean increase by week 16 = 1.1 mmol/L ALD518 versus 0.2 mmol/L placebo). 9 patients on ALD518 had transient grade 2 neutropenias; 2 pts transient grade 3 neutropenias. There were no serious infections reported in any treatment group. Infusions of ALD518 were well tolerated without infusion reactions or evident immunogenicity. At weeks 4 and 16, ACR responses (non responder imputation analysis) and improvements in DAS28 scores were:

| TABLE 19: Week 4 DAS28 Scores for Abl 80, 160, and 320 dosages |
|---------------------------------|-----------------|-----------------|-----------------|
| Week 4 DAS28 Scores for Abl 80, 160, and 320 dosages |
| ACR20 50% (16)*                  | 56% (19)*        | 71% (20)*        | 23% (8)         |
| ACR50 9% (3)                     | 15% (5)          | 29% (8)†         | 3% (1)          |
| ACR70 6% (2)                     | 0% (0)           | 11% (3)          | 0% (0)          |
| Mean Δ DAS28                    | -1.8             | -2.1             | -2              |
| *p≤0.05; †p=0.009                |                 |                 |                 |
TABLE 20: Week 16 DAS28 Scores for Abl 80, 160, and 320 dosages

<table>
<thead>
<tr>
<th>Week 16</th>
<th>80mg (n=32)</th>
<th>160mg (n=34)</th>
<th>320mg (n=28)</th>
<th>PBO+MTX (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR20</td>
<td>75% (24)*</td>
<td>65% (22)*</td>
<td>82% (23)*</td>
<td>36% (12)</td>
</tr>
<tr>
<td>ACR50</td>
<td>41% (13)*</td>
<td>41% (14)*</td>
<td>50% (140*)</td>
<td>15% (5)</td>
</tr>
<tr>
<td>ACR70</td>
<td>22% (7)♀</td>
<td>18% (6)♀</td>
<td>43% (12)*</td>
<td>6% (2)</td>
</tr>
<tr>
<td>Mean Δ DAS28</td>
<td>-2.7</td>
<td>-2.7</td>
<td>-3.2</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

*p<0.03 ♀p=0.08 ♀p=0.26

Conclusion

ALD518 is the first mAb to IL-6, as opposed to an anti-IL-6 receptor mAb, to show a significant, rapid and sustained improvement in disease activity in RA. ALD518 in doses ranging from 80 to 320mg given as 2 IV infusions to pts with active RA was well tolerated with increases in LFTs and total cholesterol and transient neutropenia observed in some patients. There were no infusion reactions associated with administration of ALD518 and no detectible immunogenicity.

EXAMPLE 38

Treatment of oral mucositis with head and neck cancer receiving concurrent chemotherapy and radiotherapy.

Subjects suffering from oral mucositis with head and neck cancer receiving concurrent chemotherapy and radiotherapy may receive a regimen of a 160 mg or 320 mg doses of a composition comprising a humanized monoclonal antibody that selectively binds IL-6.

Subjects will be assessed using tumor staging (standard TNM system) during the screening period, which may occur within 30 days prior to radiotherapy (RT) start. The RT treatment period will be approximately 7 weeks, depending on the subject's prescribed radiation plan. Post-RT treatment period visits will be at Weeks 1, 2, 3, and 4 following the treatment period. Long term follow-up visits will occur at 3, 6, 9, and 12 months following the end of RT to determine if there is an effect of ALD518 on the tumor response to CRT.

Subjects may have recently diagnosed, pathologically confirmed, non-metastatic SCC of the oral cavity, oropharynx, hypopharynx or larynx. Subjects may be scheduled to receive a continuous course of intensity-modulated radiotherapy (IMRT), with a minimum cumulative dose of 55 Gy and maximum dose of 72 Gy. Planned radiation treatment fields may include at least 2 oral sites (e.g., buccal mucosa, floor of oral cavity, tongue or soft palate) with each site receiving a total dose of ≥ 55 Gy. The treatment plan may include monotherapy with cisplatin administered in standard weekly (30 to 40 mg/m²) or tri-weekly (80 to 100 mg/m², given on Days 0, 21 and 42) regimens or monotherapy with carboplatin administered weekly (100 mg/m²).

A composition comprising a humanized monoclonal antibody that selectively binds IL-6 may be given within 2 hours prior to the subjects' radiation every 4 weeks for a total of 2 doses.
A baseline visit will occur on the first day of ALD518 and RT. Safety, PK, PD, and markers of IL-6 biology (e.g., total IL-6, sIL-6r, soluble gp130, sIL-6 Complex) will be monitored during the RT treatment and Post-RT treatment period. The long term follow-up period of the treatment may include long term follow-up visits, primarily for the assessment of tumor response and survival. These assessments will take place at Months 3, 6, 9 and 12 following the last dose of RT. At Months 3, 6, 9, and 12 tumors will be assessed clinically. At the Month 6 and Month 12 follow-up visits, tumor status will be assessed using RECIST criteria and the same imaging modality (CAT, PET or MRI) that was used to evaluate tumor status prior to RT start (at the time of staging) may be used.

Following a treatment regimen comprising the administration of a humanized monoclonal antibody that selectively binds IL-6, patients may show improvement in their oral mucositis (e.g., a reduction in symptoms).

**EXAMPLE 39**

**Oral Mucositis Study 1: Single Acute Radiation Dose (40 Gy) Study**


**Methods**: 36 male C3H mice were exposed to a single dose of 40 Gy radiation directed to the underside of the tongue on Day 0. Animals were dosed with a rodent anti-IL-6 antibody (monoclonal rat IgGl clone MP5-20F3, R&D Systems), control antibody (monoclonal rat IgGl clone 43414, R&D Systems), or vehicle on Days - 1, 2, 6, 9, and 13, via intravenous injection at 10 mg/kg into the tail vein. Animals were weighed daily, and food and water consumption were monitored in each treatment group.

**Images of the tongue were captured daily from Days 4 to 16. An oral mucositis score was assigned to each animal based on a defined scoring scale per protocol design. The scoring scale is presented in Table 21. Following completion of the study, the tongue images were scored by blinded observers to establish the values used to determine the degree and duration of oral mucositis and any treatment effects. A score of 1-2 is considered to represent a mild stage of disease, whereas a score of 3-5 is considered to indicate moderate to severe mucositis.**

**Table 21: Rodent Model Oral Mucositis Scoring Scale**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Tongue completely healthy. No erythema or vasodilation.</td>
</tr>
<tr>
<td>1</td>
<td>Light to severe erythema and vasodilation. No erosion of mucosa.</td>
</tr>
<tr>
<td>Score</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.</td>
</tr>
<tr>
<td>3</td>
<td>Formation of off-white ulcers in at least one places. Ulcers may have a yellow/gray appearance due to pseudomembrane. Cumulative size of ulcers should equal about ¼ of the tongue. Severe erythema and vasodilation.</td>
</tr>
<tr>
<td>4</td>
<td>Cumulative size of ulcers should equal ¼ to ½ of the tongue. Loss of pliability. Severe erythema and vasodilation.</td>
</tr>
<tr>
<td>5</td>
<td>Virtually all of tongue is ulcerated.</td>
</tr>
</tbody>
</table>

[0848] Results: The onset of mucositis was the same for all 3 groups with peak mucositis scores occurring on Day 10. An analysis of the number of days mice presented with scores of 3+ during the study demonstrated no statistical difference among the 3 groups (mean days of 3.3, 4 and 3.6 for vehicle, isotype control and anti-IL-6, respectively).

[0849] On Day 10, 100% of the mice in the vehicle and control antibody groups developed ulcers while 67% of the anti-IL-6 group developed ulcers (Figure 22). There was no statistical difference in ulceration scores at Day 10 between the anti-IL-6 antibody and control antibody or vehicle groups. On Days 12 and 13, a numerically larger (not statistically different) number of mice in the anti-IL-6 group had ulceration compared to the mice in the vehicle or control groups.

[0850] Weight loss was seen in all 3 groups with peak weight loss occurring between Days 11 and 12. There were no statistically significant differences in weight change between the three groups. No general toxicities were noted in this study that could be attributed to treatment with the control or anti-IL-6 antibodies or the vehicle. No treatment-related deaths occurred during the study.

EXAMPLE 40
Ascending Radiation Dose Levels Study in Mouse Model of Radiation-Induced Oral Mucositis

Introduction


Methods

[0852] 120 male C3H mice (12 per treatment group per radiation dose) were exposed to a single dose of radiation, totaling 25, 30, 35, 40, or 45 Gy directed to the underside of the tongue on Day 0. Animals were dosed with a rodent anti-IL-6 antibody (monoclonal rat IgGl clone
MP5-20F3, R&D Systems) or control antibody (monoclonal rat IgG1 clone 42414, R&D Systems) on Days -1, 2, 6, 9, and 13, via intravenous injection at 10 mg/kg into the tail vein. Animals were weighed daily; and food and water consumption was monitored in each treatment group.

Images of the tongue were captured daily from Days 4 to 16. An oral mucositis score was assigned to each animal based on a defined scoring scale per protocol design. The scoring scale is presented in Table 21. Following completion of the study, the tongue images were scored by blinded observers to establish the values used to determine the degree and duration of oral mucositis and any treatment effects. A score of 1-2 is considered to represent a mild stage of disease, whereas a score of 3-5 is considered to indicate moderate to severe mucositis.

Conclusions

Mice treated with the anti-IL-6 antibody at 25 Gy showed a statistically significant decrease in the median number of days with ulceration compared to mice treated with the control antibody (p=0.0134). There was no difference between the treatment groups at 30 and 35 Gy. Mice treated with the anti-IL-6 antibody at 40 and 45 Gy showed a statistically significant increase in the median number of days with ulceration compared to mice treated with the control antibody (p=0.0237 and 0.0037, respectively). These data are shown in Figure 23.

The anti-IL-6 treated group had a numerically lower percentage of mice that were ulcerated at any timepoint over the course of the study compared to control antibody treated group at the 25 and 30 Gy radiation levels (45% vs. 82%; 67% vs. 92%). See Figure 24. At higher radiation dose levels the percentage of mice that were ulcerated over the course of the study in the two treatment groups were similar.

Over the course of the study, the anti-IL-6 treatment group receiving 25 Gy had statistically significant positive median percentage changes from baseline body weight compared to the control antibody group at all timepoints. Additionally, at Day 4, the anti-IL-6 group at 30 and 35 Gy radiation dose levels had statistically significant positive median percentage changes from baseline body weight compared to the control antibody group. At the 40 and 45 Gy radiation dose levels, there were no differences in median percent change from baseline between the anti-IL-6 and control antibody groups.

No general toxicities were noted in this study that could be attributed to treatment with the anti-IL-6 antibody or control antibody. No treatment-related deaths were observed during the study.
Conclusions

In conclusion, at the lowest dose (25 Gy) of radiation there was a lower incidence and duration of ulcerated oral mucositis (scores 3-5) in the anti-IL-6 treated group compared to controls. Additionally, the mice treated with the anti-IL-6 antibody did not lose body weight compared to controls. At the 30 Gy radiation dose level, there was lower incidence of ulcerated oral mucositis in the anti-IL-6 treated group compared to controls. Mice receiving higher single doses of radiation (40 Gy and 45 Gy) had a longer duration of ulcerated oral mucositis in the anti-IL-6 antibody treated group compared to controls. The radiation dose levels administered as single doses in this study are much higher than the daily doses (approximately 2 Gy) given in IMRT for the treatment of head and neck cancer. These data support with the use of a humanized monoclonal antibody (e.g., ALD518) in the prevention of CRT-induced oral mucositis in head and neck cancer patients.

EXAMPLE 41

Effect of Anti-IL-6 Treatment on Tumor Growth in a Xenograft Model

Introduction

The human pharynx squamous cell carcinoma cell line (FaDu) has been utilized as a model for head and neck cancers in mouse xenograft studies. Alderson, et al (2002) Cancer Chemother. Pharmacol, 50: 202-212. FaDu expresses both IL-6 and the IL-6 receptor and IL-6 levels are induced in response to radiation treatment. Chen, et al. (2010) Int. J. Radiation Oncology Biol. Phys. 76:1214-1224 The effect of anti-IL-6 treatment on the growth of FaDu tumors in the presence or absence of radiation treatment was studied in an established mouse xenograft model. Study endpoints included tumor volume and body weights.

Methods

120, six week old, female athymic nude mice were implanted with ten million FaDu tumor cells subcutaneously. When tumors reached the weight range of 125-250mg (Day 10), animals were divided into 3 groups of 40 mice. One group was given vehicle twice weekly via intravenous injection into the tail vein. The second group was given 10mg/kg each of ALD518 and an anti-mouse IL-6 antibody (monoclonal rat IgGl, R&D Systems). The third treatment group was given 10mg/kg each of isotype control antibodies (monoclonal human IgGl, R&D Systems). In each of the treatment groups, half of the animals (N=20) were irradiated with 2Gy/day for 5 days and the other 20 animals were not irradiated. Animals were euthanized when tumor volume reached 4,000 mm³ or ulceration of the tumor occurred. All animals were weighed and tumor volumes measured three times a week for the duration of the study.
Results

The tumor volumes for each animal were measured three times a week starting on the first day of treatment (Day 10). The study was completed on Day 29. FaDu tumors have a high rate of ulceration; in this study, between 9 and 13 animals were sacrificed in each group by Day 29 due to tumor ulceration. No animals were euthanized due to tumor burden. The median tumor volume for each group is presented in Figure 25. All groups had median tumor volumes between 162-167 mm$^3$ at the start of treatment (Day 10). Groups treated with vehicle, isotype control antibodies or anti-IL-6 antibodies but not irradiated displayed very similar median tumor volumes throughout the study. These groups were not statistically different. Groups treated with vehicle, isotype control antibodies or anti-IL-6 antibodies plus radiation had reduced median tumor volumes of roughly 50% compared to the non-irradiated groups post Day 22. Median tumor volumes of the irradiated groups were similar and not statistically different. Thus, treatment with anti-IL-6 antibodies had no effect on tumor growth in either the non-irradiated or irradiated groups.

Additional conclusions from the study include: no differences in weight were observed between the six groups; no general toxicities were noted that could be attributed to treatment with the vehicle, control antibodies or anti-IL-6 antibodies; and there were no treatment-related deaths.

EXAMPLE 43

Clinical Trial Design

A phase 2, double-blind, placebo-controlled trial evaluating the safety, efficacy, pharmacokinetics and pharmacodynamics of ALD518, and the health and economic outcomes in subjects receiving CRT for the treatment of squamous cell carcinomas (SCCs) of the oral cavity, oropharynx, hypopharynx or larynx may be conducted. Up to 96 subjects may be enrolled into this trial. Initially 3 open-label subjects will be enrolled into a safety run-in of the 160 mg dose. Approximately 90 subjects will be randomized (1:1:1) into 1 of 2 dose levels of ALD518 (160 mg and 320 mg) or placebo during the double-blind portion of the trial. Safety, PK, PD, and markers of IL-6 biology (e.g., total IL-6, sIL-6r, soluble gpl30, sIL-6 Complex) will be monitored during the RT treatment and Post-RT treatment period. Additionally, exploratory analyses of IL-6 biology including cytokine biomarkers may be performed in a subset of subjects and will require separate consent.

Subject eligibility, including tumor staging (standard TNM system), will be assessed during the screening period, which may occur within 30 days prior to radiotherapy (RT) start. The RT treatment period will be approximately 7 weeks, depending on the subject's prescribed radiation plan. Post-RT follow-up visits will be at Weeks 1, 2, 3, and 4. Long term follow-up
visits will occur at 3, 6, 9, and 12 months following the end of RT to determine if there is an
effect of ALD518 on the tumor response to CRT.

[0865] Eligible subjects will have recently diagnosed, pathologically confirmed, non-
metastatic SCC of the oral cavity, oropharynx, hypopharynx or larynx. Subjects must be
scheduled to receive a continuous course of intensity-modulated radiotherapy (IMRT), with a
minimum cumulative dose of 55 Gy and maximum dose of 72 Gy. Planned radiation treatment
fields must include at least 2 oral sites (e.g., buccal mucosa, floor of oral cavity, tongue or soft
palate) with each site receiving a total dose of ≥ 55 Gy. The treatment plan must include
monotherapy with cisplatin administered in standard weekly (30 to 40 mg/m²) or tri-weekly (80
to 100 mg/m², given on Days 0, 21 and 42) regimens or monotherapy with carboplatin
administered weekly (100 mg/ m²).

[0866] ALD518 or placebo will be given every 4 weeks within 2 hours prior to the subjects’
radiation for a total of 2 doses. A baseline visit will occur on the first day of RT. During the RT
treatment period, subjects will be assessed twice weekly for the presence and severity of OM by
treatment-blinded, trained evaluators using the World Health Organization (WHO) grading scale
for OM. Subjects will also complete a daily diary, containing the Oral Mucositis Daily
Questionnaire (OMDQ) and a listing of analgesic use, and on a weekly basis the FACT-HN and
FACIT-F subscale PRO instruments.

[0867] All subjects will return to the clinic for 4 weekly visits after RT completion for
assessment of OM. During this time, subjects will also continue to complete the OMDQ and the
FACT-HN and FACIT-F subscale PRO instruments. The long term follow-up period of the
clinical trial will include quarterly visits, primarily for the assessment of tumor response. These
assessments will take place at Months 3, 6, 9 and 12 following the last dose of RT. At Months 3,
6, 9, and 12 tumors will be assessed clinically. At the Month 6 and Month 12 follow-up visits,
tumor status will be assessed using RECIST criteria and the same imaging modality (CAT, PET,
or MRI) that was used to evaluate tumor status prior to RT start (at the time of staging).

Example 44

Additional Evaluation of ALD518 in RA Clinical Trials

[0868] This example describes further Phase II clinical trial results for administration of
ALD518 to patients with active RA. For purposes of inclusion in this study, a patient was
considered to have active RA if the patient exhibited at least 6 swollen/6 tender joints, CRP >10
mg/dL, and had been treated with a stable dose of methotrexate (MTX) (>10 mg/ week) for at
least 3 months and stable use of NSAIDs or steroids (if any).
ALD518 was administered in a double-blind, placebo-controlled study in which patients with active RA were randomized 1:1:1:1 to receive either 80 mg (n=32), 160 mg (n=34), or 320 mg (n=28) ALD518, or placebo (n=33). ALD518 or placebo were given as an intravenous infusion over 60 minutes on Day 1 and then again 8 weeks later. Patients were maintained on stable doses of methotrexate (MTX) (at least 10 mg/week). Disease-modifying antirheumatic drugs (DMAPvDs) other than MTX were discontinued at least 4 months prior to study entry. Efficacy endpoints were assessed at weeks 12 (primary endpoint) and week 16. HRQoL was evaluated by the Medical Outcomes Survey Short Form-36 (SF-36). Analyses were performed on the modified intent-to-treat population for patients with data available at the visit of interest (as observed).

127 active RA patients were randomized and treated, and 116 completed the trial (80 mg, 29/32; 160 mg, 33/34; 320 mg, 25/28; placebo, 29/33). Patient disposition is summarized in FIG. 26.

At baseline, mean age was 52.3 years; mean RA duration was 6.8 years; mean tender and swollen joint counts were 26.1 and 16.7, and mean Physical (PCS) and Mental component summary (MCS) scores were 31.0 and 35.0, respectively. Mean changes from baseline to week 12 in MCS were significantly greater in each ALD518 dose group vs placebo, and mean changes in both PCS and MCS scores exceeded MCID in each ALD518 group. At week 12, mean changes from baseline in one or more SF-36 domains were significantly greater in ALD518 dose groups vs placebo. Changes >MCID were observed in all domains and in SF-6D in patients receiving ALD518. Improvements at week 12 were sustained at week 16.

Results

Short Form-36 Component Summary Scores: HRQoL was assessed by the patient-reported Short Form-36 (SF-36) questionnaire. The SF-36 includes 36 questions divided into eight domains and summarized into the physical and mental component summary scores (PCS and MCS, respectively). Scores range from 0 to 100, with higher scores indicating better health. The observed Minimum Clinically Important Differences (MCID) are 2.5-5.0 for the PCS and MCS, and 5.0-10.0 for domain scores.

Short Form-6D: The SF-6D is a validated preference-based measure of health utilities. The SF-6D was calculated using mean changes within treatment groups across all eight SF-36 domains to yield a single utility measure. The Minimum Important Difference (MID) is 0.041.

Analysis

Analysis was performed on the modified intent-to-treat population for patients with available data at the visit of interest (as observed). Changes from baseline in SF-36 PCS, MCS
and domain scores were summarized as descriptive statistics by treatment group and visit. ALD518 treatment groups were also compared with placebo at Week 12 using a two-sample t-test.

For Weeks 12 and 16, spydergrams were used to present results across all domains of the SF-36 in a single figure, and to compare with age- and gender-matched normative data from a US population. Demarcations along the domain axes of the spydergrams represent changes of 10 in domain score, and patient disposition and baseline demographics and characteristics.

As shown in FIG. 26, a total of 127 patients were randomized and received ≥1 dose of ALD518; 91.3% of patients completed the study and eleven (8.7%) patients discontinued the study.

The individual SF-36 domain scores at Baseline and Week 12 are shown in Table 22 and illustrated graphically in FIG. 27. Baseline domain scores were generally well balanced across the treatment groups. At baseline, patients had impaired HRQoL. Combined mean baseline PCS and MCS scores were 31.0 and 35.0, respectively, and 1.5-2.0 standard deviations less than normative values of 50. Scores for each of the individual subscales of the SF-36 were also considerably lower than age- and gender-matched US norms.

For all ALD518 treatment groups, mean improvements from baseline to Week 12 were large across the eight domains of the SF-36 and exceeded those observed with placebo (See the Table 22 and FIG. 27). Mean improvements were significantly greater than those observed with placebo (p<0.05; Table 22) at Week 12 in the following domains: Role physical (ALD518 320 mg group); bodily pain, general health, social functioning and mental health (ALD518 80 and 320 mg treatment groups); vitality (all ALD518 groups) ; role emotional (ALD518 80 mg group).

At all doses of ALD518, mean improvements in all eight SF-36 domains exceeded the MCID at Week 12. See Table 22. After adjustment for the change from baseline in the placebo group, improvements from baseline observed with ALD518 were greater than, and in some cases at least twice, that observed in the placebo group. There was observed dose-dependent changes (improvements) in the domains of role physical, bodily pain and mental health. Treatment with ALD518 resulted in improvements in SF-36 scores toward those observed in the ‘normal’ comparative population. See FIG. 29.

TABLE 22: SF-36 PCS and MCS Domains at Baseline and at Week 12

<table>
<thead>
<tr>
<th>Domain* (+age/gender norm)</th>
<th>Time point</th>
<th>ALD518 80 mg (n=32)</th>
<th>ALD518 160 mg (n=34)</th>
<th>ALD518 320 mg (n=28)</th>
<th>Placebo (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS Domains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Result Summary:** 172 active RA patients were randomized and treated, and 116 completed the trial (80 mg, 29/32; 160 mg, 33/34; 320 mg, 25/28; placebo, 29/33). At baseline, mean age was 52.3 years; mean RA duration was 6.8 years; mean tender and swollen joint counts were 26.1 and 16.7, and mean Physical (PCS) and Mental component summary (MCS) scores were 31.0 and 35.0, respectively. Mean changes from baseline to week 12 in MCS were significantly greater in each ALD518 dose group vs placebo, and mean changes in both PCS and MCS scores exceeded MCID in each ALD518 group. At week 12, mean changes from baseline in one or more SF-36 domains were significantly greater in ALD518 dose groups than the placebo group (Table 23). Improvements in SF-6D were 3-4 times the MID in the ALD-518 groups compared with less than 2 times the MID in the placebo group (as noted above, the MID is 0.041). Changes exceeding the MCID were observed in all domains and in SF-6D in patients receiving ALD518. Improvements at week 12 were sustained at week 16.

<table>
<thead>
<tr>
<th>Domain* (+age/gender norm)</th>
<th>Time point</th>
<th>ALD518 80 mg (n=32)</th>
<th>ALD518 160 mg (n=34)</th>
<th>ALD518 320 mg (n=28)</th>
<th>Placebo (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical functioning</td>
<td>Baseline</td>
<td>48.3</td>
<td>42.1</td>
<td>49.3</td>
<td>42.8</td>
</tr>
<tr>
<td>(79.6)</td>
<td>Mean at Week 12</td>
<td>61.0</td>
<td>61.6</td>
<td>70.4</td>
<td>55.0</td>
</tr>
<tr>
<td>Role physical (80.1)</td>
<td>Baseline</td>
<td>27.9</td>
<td>26.0</td>
<td>36.7</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>50.0</td>
<td>53.5</td>
<td>59.7†</td>
<td>47.1</td>
</tr>
<tr>
<td>Bodily pain (68.3)</td>
<td>Baseline</td>
<td>26.4</td>
<td>22.1</td>
<td>33.6</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>47.8†</td>
<td>50.5</td>
<td>56.9†</td>
<td>39.5</td>
</tr>
<tr>
<td>General health (69.5)</td>
<td>Baseline</td>
<td>36.5</td>
<td>33.4</td>
<td>38.7</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>45.1†</td>
<td>45.6</td>
<td>49.5†</td>
<td>39.4</td>
</tr>
<tr>
<td>Vitality (58.2)</td>
<td>Baseline</td>
<td>32.5</td>
<td>26.2</td>
<td>38.8</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>50.9†</td>
<td>50.8†</td>
<td>60.9†</td>
<td>46.3</td>
</tr>
<tr>
<td>Social functioning (83.6)</td>
<td>Baseline</td>
<td>47.7</td>
<td>31.6</td>
<td>42.1</td>
<td>48.8</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>66.8†</td>
<td>59.4</td>
<td>73.1†</td>
<td>57.5</td>
</tr>
<tr>
<td>Role emotional (86.8)</td>
<td>Baseline</td>
<td>44.5</td>
<td>40.8</td>
<td>37.3</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>60.3†</td>
<td>63.0</td>
<td>61.7</td>
<td>51.9</td>
</tr>
<tr>
<td>Mental health (74.9)</td>
<td>Baseline</td>
<td>48.4</td>
<td>34.7</td>
<td>51.1</td>
<td>52.7</td>
</tr>
<tr>
<td></td>
<td>Mean at Week 12</td>
<td>61.0†</td>
<td>61.6</td>
<td>70.4†</td>
<td>55.0</td>
</tr>
</tbody>
</table>

*0–100 scores are presented for each domain to enable interpretation within the context of the MCIDs; shading highlights changes ≥MCID in domain scores; Baseline scores are mean, based on patients with available data at visit of interest; PCS=Physical Component Score; MCS=Mental Component Score; MCID=Minimum Clinically Important Differences; †represents p<0.05 associated with comparison of changes from baseline between a ALD518 arm versus placebo based on an ANCOVA model, adjusted for age at baseline and sex.
Table 23. SF-6D Scores at Baseline and Weeks 12 and 16. Shading highlights changes that exceeded the MID (minimum important difference).

<table>
<thead>
<tr>
<th>SF-6D (+age/gender norm)</th>
<th>ALD518 80 mg (n=32)</th>
<th>ALD518 160 mg (n=34)</th>
<th>ALD518 320 mg (n=28)</th>
<th>Placebo (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n =</td>
<td>32</td>
<td>33</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.582</td>
<td>0.522</td>
<td>0.612</td>
<td>0.603</td>
</tr>
<tr>
<td>Mean at Week 12</td>
<td>0.714</td>
<td>0.715</td>
<td>0.785</td>
<td>0.664</td>
</tr>
<tr>
<td>Mean change to Week 12</td>
<td>0.132</td>
<td>0.193</td>
<td>0.172</td>
<td>0.062</td>
</tr>
<tr>
<td>Week 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n =</td>
<td>32</td>
<td>33</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.556</td>
<td>0.584</td>
<td>0.579</td>
<td>0.592</td>
</tr>
<tr>
<td>Mean at Week 16</td>
<td>0.692</td>
<td>0.736</td>
<td>0.751</td>
<td>0.662</td>
</tr>
<tr>
<td>Mean change to Week 16</td>
<td>0.140</td>
<td>0.150</td>
<td>0.170</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Conclusions: Treatment with the IL-6 inhibitor ALD5 18 resulted in statistically significant and clinically meaningful improvements in physical and mental aspects of HRQoL. These data further support the clinical efficacy of ALD5 18 for treatment of patients with active RA and inadequate responses to methotrexate (MTX).

EXAMPLE 45

ORAL MUCOSITIS CLINICAL TRIAL IN PROGRESS

Subjects suffering from oral mucositis with head and neck cancer receiving concurrent chemotherapy and radiotherapy are being treated with regimen of a 160 mg doses of a composition comprising a humanized monoclonal antibody that selectively binds IL-6 (ALD518, also known as Abl which contains the variable sequences in SEQ ID NO: 19 and SEQ ID NO:20).

Subjects are being assessed using tumor staging (standard TNM system) during the screening period, which occurs within 30 days prior to radiotherapy (RT) start. The RT treatment period is approximately 7 weeks, depending on the subject's prescribed radiation plan. Post-RT treatment period visits are scheduled at weeks 1, 2, 3, and 4 following the treatment period. Long term follow-up visits are scheduled at 3, 6, 9, and 12 months following the end of RT to determine if there is an effect of ALD5 18 on the tumor response to CRT.

Subjects were recently diagnosed and pathologically confirmed with non-metastatic SCC of the oral cavity, oropharynx, hypopharynx or larynx. Subjects are scheduled to receive a continuous course of intensity-modulated radiotherapy (IMRT) with a minimum cumulative dose of 55 Gy and maximum dose of 72 Gy. Planned radiation treatment fields include at least 2 oral sites (e.g., buccal mucosa, floor of oral cavity, tongue or soft palate) with each site receiving a total dose of ≥ 55 Gy. The treatment plan include monotherapy with cisplatin administered in
standard weekly (30 to 40 mg/m²) or tri-weekly (80 to 100 mg/m², given on Days 0, 1 and 42) regimens or monotherapy with carboplatin administered weekly (100 mg/m²).

[0885] A composition comprising a humanized monoclonal antibody that selectively binds IL-6 (ALD518 also known as Abl) is being given within 2 hours prior to the subjects' radiation every 4 weeks for a total of 2 doses. A baseline visit occurred on the first day of ALD518 and RT. Safety, PK, PD, and markers of IL-6 biology (e.g., total IL-6, sIL-6R, soluble gpl30, sIL-6 Complex) are being monitored during the RT treatment and Post-RT treatment period. The long term follow-up period of the treatment includes scheduled long term follow-up visits, primarily for the assessment of tumor response and survival. These assessments are scheduled at months 3, 6, 9 and 12 following the last dose of RT. At months 3, 6, 9, and 12 tumors will be assessed clinically. At the Month 6 and Month 12 follow-up visits, tumor status will be assessed using RECIST criteria and the same imaging modality (CAT, PET or MRI) that was used to evaluate tumor status prior to RT start (at the time of staging) may be used.

[0886] Following a treatment regimen comprising the administration of a humanized monoclonal antibody that selectively binds IL-6 ALD-518 (Abl) the patients show improvement in their oral mucositis (e.g., a reduction in symptoms) after only 4 weeks of treatment.

[0887] As assessed using the WHO (World Health Organization) oral mucositis scale (Table 2) 3 patients receiving 160 mg intravenous administration of ALD518 (Abl) were assessed. The first subject (circles) has not shown any signs of developing oral mucositis, maintaining a Grade 0 for the entire 4 weeks. This is indicative of ALD518 acting to prevent the development of oral mucositis. The second patient (squares) developed Grade 2 oral mucositis, but this appears to have lessened in severity. This is indicative of ALD518 acting to prevent the development of severe oral mucositis (e.g., Grade 4) and even lessen the severity of oral mucositis. The third patient (triangles) developed Grade 2/3 oral mucositis. This is indicative of ALD518 acting to prevent the development of severe oral mucositis (e.g., Grade 4). In this patient population, it is expected that about 60% of patients to develop at least Grade 3 or Grade 4 oral mucositis with this type of IMRT + chemotherapy and over 80% of the patients to develop at least Grade 2 and above oral mucositis. Thus, this data suggests that a humanized monoclonal antibody that selectively binds IL-6 (e.g., ALD518 also known as Abl) is effective in treating and preventing oral mucositis resulting from the combination of chemotherapy and radiotherapy.

[0888] We further conclude based on these results that other IL-6 antagonists, including those identified in this application, e.g., the exemplified anti-IL-6 antibodies and antibody fragments, as well as the identified non-antibody IL-6 antagonists, will have clinical application in treating and preventing mucositis, e.g., oral and gastrointestinal or alimentary mucositis.
EXAMPLE 46
ONGOING ANEMIA CLINICAL TRIAL

[0889] Three cancer patients which were to be treated with cisplatin were treated with ALD-518 prior to cisplatin chemotherapy in order to prevent or lessen anemia, and in particular to prevent the onset of severe anemia which is a very common side effect of cisplatin therapy, i.e., when administered alone or in conjunction with radiotherapy.

[0890] All three patients received cisplatin every 3 weeks at a dosage of 100mg/m². Particularly, said dosage of chemo was administered at week 0, at week 3 and in one patient another dose was administered at week 6. In these same patients, 160 mg of ALD5 18 (Ab1), a humanized anti-IL-6 monoclonal antibody containing the variable sequences in SEQ ID NO: 19 and SEQ ID NO:20, was administered intravenously at week 0 and week 4. Radiotherapy (RT) was also administered to these patients at a dosage of 2-2.2 Gray per day from week 0 and will continue until the end of the planned RT for each patient every day 5 days a week.

[0891] All 3 patients are now post-therapy (between week 8 and week 12 of the treatment regimen). The last blood count was at the end of RT about week 8. None of these patients as of week 8 after treatment shows signs of severe anemia. All three patients will be monitored at least until week 12 and are expected to show no or less severe anemia resulting from the combination of cisplatin and radiotherapy as compared to the severe anemia typically seen in patients receiving cisplatin alone or when administered in a clinical regimen also including radiation. This will be confirmed by assaying hemoglobin and/or RBC counts and other clinical indicators of anemia in these patients.

[0892] Although the invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications will practiced within the scope of the appended claims. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in medicine, pharmacology, microbiology, and/or related fields are intended to be within the scope of the following claims.

[0893] All publications (e.g. Non-Patent Literature), patent application publications, and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All such publications (e.g., Non-Patent Literature), patent application publications, and patent applications are herein incorporated by reference to the same extent as if each individual publication, patent, patent application
publication, or patent application is specifically and individually indicated to be incorporated by reference.
CLAIMS

What we claim is:

1. A method of treating or preventing anemia comprising administration of a composition comprising an effective amount of an IL-6 antagonist.

2. A method of treating or preventing drug-induced immune hemolytic anemia (DIIHA) comprising administration of a composition comprising an effective amount of an IL-6 antagonist.

3. A method of treating or preventing anemia associated with chemotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist.

4. A method of treating or preventing anemia associated with radiotherapy comprising administration of a composition comprising an effective amount of an IL-6 antagonist.

5. A method of treating or preventing anemia associated with cancer comprising administration of a composition comprising an effective amount of an IL-6 antagonist.

6. Use of an effective amount of an IL-6 antagonist for the preparation of a medicament for treating or preventing anemia.

7. Use of an effective amount of an IL-6 antagonist for the preparation of a medicament for treating or preventing drug-induced immune hemolytic anemia (DIIHA).

8. Use of an effective amount of an IL-6 antagonist for the preparation of a medicament for treating or preventing anemia associated with chemotherapy.

9. Use of an effective amount of an IL-6 antagonist for the preparation of a medicament for treating or preventing anemia associated with radiotherapy.

10. Use of an effective amount of an IL-6 antagonist for the preparation of a medicament for treating or preventing anemia associated with cancer.

11. The method or use of any one of claims 1-10, wherein said IL-6 antagonist targets IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, STAT3, or any combination thereof.

12. The method or use of any one of claims 1-10, wherein said IL-6 antagonist is an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof.

13. The method or use of any one of claims 1-10, wherein said IL-6 antagonist is an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, anti-SYK, or anti-STAT3 antibody or antibody fragment.

14. The method or use of any one of claims 1-10, wherein said IL-6 antagonist is thalidomide, lenalidomide, or any combination thereof.
15. The method or use of any one of claims 1-10, wherein said IL-6 antagonist is an anti-IL-6 antibody or antibody fragment.

16. The method or use of claim 15, wherein said anti-IL-6 antibody or antibody fragment thereof, is Abl1, Abl2, Abl3, Abl4, Abl5, Abl6, Abl7, Abl8, Abl9, Abl20, Abl21, Abl22, Abl23, Abl24, Abl25, Abl26, Abl27, Abl28, Abl29, Abl30, Abl31, Abl32, Abl33, Abl34, Abl35, or Abl36 antibody, or an antigen-binding fragment thereof, to a subject in need thereof, wherein the antibody, or antigen-binding fragment thereof, specifically binds to IL-6.

17. The method or use of claim 15, wherein said antibody comprises at least one light chain selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 2, 20, 21, 37, 53, 69, 85, 101, 119, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 647, 648, 649, 650, 651, 655, 660, 666, 667, 671, 675, 679, 683, 687, 693, 699, 702, 706, or 709.

18. The method or use of claim 15, wherein said antibody comprises at least one light chain selected from the group consisting of nucleic acid sequences with at least about 90% sequence identity to a nucleic acid sequence of SEQ ID NO: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723, wherein said nucleic acid sequence encodes said light chain.

19. The method or use of claim 15, wherein said antibody comprises at least one heavy chain selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86, 102, 117, 118, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 668, 672, 676, 680, 684, 688, 691, 692, 704, or 708.

20. The method or use of claim 15, wherein said antibody comprises at least one heavy chain selected from the group consisting of nucleic acid sequences with at least about 90% sequence identity to a nucleic acid sequence of SEQ ID NO: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725, wherein said nucleic acid sequence encodes said heavy chain.


23. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises at least two light chain CDR polypeptide sequences selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an

24. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a light chain CDR1 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 4, 23, 39, 55, 71, 74, 87, 103, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 710, 711, or 712.

25. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a light chain CDR2 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 5, 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 713, 714, 715, or 718.

26. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a light chain CDR3 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 6, 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574.

27. The method or use of any one of claims 24-26, wherein said antibody or antigen-binding fragment thereof comprises three light chain CDR polypeptides.

28. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises at least two heavy chain CDR polypeptide sequences selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 7, 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, 575, 716, 8, 27, 43, 59, 75, 91, 107, 120, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464,

29. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR1 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 7, 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, 575, or 716.

30. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR2 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 8, 27, 43, 59, 75, 91, 107, 120, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, 659, 717, or 718.

31. The method or use of claim 15, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain CDR3 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 9, 28, 44, 60, 76, 92, 108, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, or 577.

32. The method or use of any one of claims 29-31, wherein said antibody or antigen-binding fragment thereof comprises three heavy chain CDR polypeptides.

33. The method or use of any one of claims 1-10, wherein the light chain of said antibody or antigen-binding fragment thereof is selected from the amino acid sequences of light chains listed in TABLE 4.

34. The method or use of claim 15, wherein at least one heavy chain of said antibody or antigen-binding fragment thereof is selected from the amino acid sequences of heavy chains listed in TABLE 4.

35. The method or use of claim 15, wherein at least one CDR of said antibody or antigen-binding fragment thereof is selected from the amino acid sequences of CDRs listed in TABLE 4.

36. The method or use of any one of claims 15-35, wherein said light chain comprises at least about 95% sequence identity said amino acid sequence.
37. The method or use of any one of claims 15-35, wherein said heavy chain comprises at least about 95% sequence identity said amino acid sequence.

38. The method or use of any one of claims 15-35, wherein said CDR sequence comprises at least about 95% sequence identity said amino acid sequence.


40. The method or use of claim 15, wherein said antibody is an Ab<sub>1</sub> antibody.

41. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID NO: 2, 20, 647, 648, 649, 650, 651, 660, 666, 699, 702, 706, or 709.

42. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises a humanized light chain comprising the amino acid sequence of SEQ ID NO: 648, 649, and 650.

43. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises at least one light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 4, 5, 6, 710, 711, 712, 713, 714, and 715.

44. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises at least one humanized light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 710, 711, 712, 713, 714, and 715.

45. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 3, 18, 19, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 704, 708.

46. The method or use of claim 40, wherein said antibody or antigen-binding fragment thereof comprises a humanized heavy chain comprising the amino acid sequence of SEQ ID NO: 653, 654, and 655.
47. The method or use of of claim 40, wherein said antibody or antigen-binding fragment thereof comprises at least one heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 7, 9, 74, 716, 8, 120, 659, 717, and 718.

48. The method or use of of claim 40, wherein said antibody or antigen-binding fragment thereof comprises at least one humanized heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 74, 716, 717, and 718.

49. The method or use of of claim 15, wherein the light chain polypeptide comprises at least one Ab 1 light chain CDR polypeptide comprising

(a) a light chain CDR1 having at least 72.7% sequence identity to SEQ ID NO: 4;
(b) a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5;
(c) a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6;
(d) a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4;
(e) a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or
(f) a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6;

and wherein the heavy chain polypeptide comprises at least one Ab 1 heavy chain CDR polypeptide comprising

(g) a heavy chain CDR1 having at least 80% sequence identity to SEQ ID NO: 7;
(h) a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120;
(i) a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9;
(j) a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7;
(k) a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or
(l) a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9.

50. The method or use of claim 15, wherein the light chain polypeptide comprises at least one Ab 1 light chain CDR polypeptide comprising

(a) a light chain CDR1 having at least 81.8% sequence identity to SEQ ID NO: 4;
(b) a light chain CDR2 having at least 71.4% sequence identity to SEQ ID NO: 5; or
(c) a light chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 6;

and wherein the heavy chain polypeptide comprises at least one Ab 1 heavy chain CDR polypeptide comprising

(d) a heavy chain CDR1 having at least 60% sequence identity to SEQ ID NO: 7;
(e) a heavy chain CDR2 having at least 87.5% sequence identity to SEQ ID NO: 120; or
(f) a heavy chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 9.

51. The method or use of either of claims 50 or 51, wherein the Ab1 antibody or antibody fragment comprises at least two of said light chain CDR polypeptides and at least two of said heavy chain CDR polypeptides.

52. The method or use of claim 15, wherein the Ab1 antibody or antibody fragment comprises
   (a) two or more Ab1 light chain CDR polypeptides comprising
   (b) a light chain CDR1 having at least 72.7% sequence identity to SEQ ID NO: 4;
   (c) a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5; or
   (d) a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6;
and two or more Ab1 heavy chain CDR polypeptide comprising
(e) a heavy chain CDR1 having at least 80% sequence identity (identical to at least 4 out of 5 residues) to SEQ ID NO: 7;
(f) a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120; or
(g) a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9;
wherein the Ab1 antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.

53. The method or use of claim 15, wherein the Ab1 antibody or antibody fragment comprises
   (a) two or more Ab1 light chain CDR polypeptides comprising
   (b) a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4;
   (c) a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or
   (d) a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6;
and two or more Ab1 heavy chain CDR polypeptide comprising
(e) a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7;
(f) a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or
(g) a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9;
wherein the Ab1 antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.
54. The method or use of either claim 52 or 53, wherein said Abl antibody or antibody fragment comprises said light chain CDR1, said light chain CDR3, said heavy chain CDR2, and said heavy chain CDR3.

55. The method or use of any one of claims 15-54, wherein the antibody or antibody fragment thereof is administered to the subject in the form of at least one nucleic acids that encode the antibody or antibody fragment thereof.

56. The method or use of claim 55, wherein the light chain of said antibody or antibody fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723.

57. The method or use of claim 55, wherein the heavy chain of said antibody or antibody fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ ID NOs: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725.


59. The method or use of claim 55, wherein at least one of the nucleic acids comprise the heavy and light chain polynucleotide sequences of SEQ ID NO: 723 and SEQ ID NO: 700;
SEQ ID NO: 701 and SEQ ID NO: 703; SEQ ID NO: 705 and SEQ ID NO: 707; SEQ ID NO: 720 and SEQ ID NO: 724; and SEQ ID NO: 10 and SEQ ID NO: 11.

60. The method or use of any one of claims 15-59, wherein said antibody comprises the humanized variable light sequence of amino acid sequence of SEQ ID NO: 20 or 709.

61. The method or use of any one of claims 15-59, wherein said antibody comprises the humanized variable heavy sequence of amino acid sequence of SEQ ID NO: 19 or 657.

62. The method or use of any one of claims 15-59, wherein said antibody comprises at least one light chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 4, 5, or 6.

63. The method or use of any one of claims 15-59, wherein said antibody comprises at least one heavy chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 7, 120, or 9.

64. The method or use of any one of claims 15-59, wherein said antibody is an asialilated, humanized anti-IL-6 monoclonal antibody with a half-life of 30 days comprising the humanized variable light and heavy sequences as set forth in SEQ ID NO: 20 and 19 or 709 and 657.

65. The method or use of any one of claims 15-59, wherein the antibody, or antibody fragment thereof, is expressed from a recombinant cell.

66. The method or use of claim 65, wherein the cell is selected from a mammalian, yeast, bacterial, and insect cell.

67. The method or use of claim 66, wherein the cell is a yeast cell.

68. The method or use of claim 67, wherein the cell is a diploid yeast cell.

69. The method or use of claim 68, wherein the yeast cell is a Pichia yeast.

70. The method or use of any one of claims 15-69, wherein said antibody is asialilated.

71. The method or use of any one of claims 15-69, wherein said antibody is humanized.

72. The method or use of any of one claims 15-71, wherein said antibody or antibody fragment comprises a Fab, Fab', F(ab')2, Fv, scFv, IgNAR, SMIP, camelbody, or nanobody.

73. The method or use of any one of claims 15-71, wherein the antibody, or antibody fragment thereof, has an in vivo half-life of at least about 30 days in a healthy human subject.

74. The method or use of any of claims 15-71, wherein the antibody or antibody fragment thereof, has a binding affinity (Kd) for IL-6 of less than about 50 picomolar, or a rate of dissociation (Kd) from IL-6 of less than or equal to 10^8 S⁻¹.

75. The method or use of any one of claims 15-71, wherein the antibody or antibody fragment thereof, specifically binds to the same linear or conformational epitope(s) and/or competes for binding to the same linear or conformational epitope(s) on an intact human IL-6
polypeptide or fragment thereof as an anti-IL-6 antibody comprising the polypeptides of
SEQ ID NO: 702 and SEQ ID NO: 704 or the polypeptides of SEQ ID NO: 2 and SEQ ID
NO: 3.

76. The method or use of claim 75, wherein said binding to the same linear or conformational
epitope(s) and/or competition for binding to the same linear or conformational epitope(s)
on an intact human IL-6 polypeptide or fragment thereof is ascertained by epitopic
mapping using overlapping linear peptide fragments which span the full length of the
native human IL-6 polypeptide and includes at least one residues comprised in IL-6
fragments selected from those respectively encompassing amino acid residues 37-51,
amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45
and/or amino acid residues 58-72 of SEQ ID NO: 1.

77. The method or use of any one of claims 15-76, wherein the antibody or antibody fragment
thereof, is aglycosylated.

78. The method or use of any one of claims 15-76, wherein the antibody or antibody fragment
thereof, contains an Fc region that has been modified to alter effector function, half-life,
proteolysis, and/or glycosylation.

79. The method or use of any one of claims 15-76, wherein the antibody or antibody fragment
thereof, is a human, humanized, single chain, or chimeric antibody.

80. The method or use of any one of claims 15-76, wherein the antibody or antibody fragment
thereof, further comprises a human Fc.

81. The method or use of claim 80, wherein said human Fc is derived from IgGl, IgG2, IgG3,
IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgGlO, IgGl 1, IgG12, IgG13, IgG14, IgG15, IgGl 6,
IgGl 7, IgGl 8, or IgGl 9.

82. The method or use of any one of claims 1-81, wherein said anemia is anemia associated
with chemotherapy, anemia associated with radiotherapy, and drug-induced immune
hemolytic anemia (DIIHA).

83. The method or use of claim 3 or 8, wherein said chemotherapy comprises administration of
at least one of the following chemotherapy agents Alemtuzumab (Campath®),
Asparaginase (Elspar®), Bleomycin (Blenoxane®), Busulfan (Mylan®), Busulfex®,
Capecitabine (Xeloda®), Carboplatin (Paraplatin®), Cisplatin (PLATINOL®),
Cyclophosphamide (Cytoxan®), Cytarabine (Cytosar-U®), Daunorubicin (Cerubidine®),
Docetaxel (Taxotere®), Doxorubicin (Adriamycin®), Epirubicin (Ellence®), Etoposide
(VePesid®), Fluorouracil (5-FU®), Gemcitabine (Gemzar®), Gemtuzumab ozogamicin
(Mylotarg®), Hydroxyurea (Hydrea®), Idarubicin (Idamycin®), Interleukin 2
(Proleukin®), Irinotecan (Camptosar®), Lomustine (CeeNU®), Mechlorethamine (Mustargen®), Melphanal (Alkeran®), Methotrexate (Rheumatrex®), Mitomycin (Mutamycin®), Mitoxantrone (Novantrone®), Oxaliplatin (Eloxatin®), Paclitaxel (Taxol®), Pemetrexed (Alimta®), Pentostatin (Nipent®), Procarbazine (Matulane®), Thiotepa (Thioplex®), Topotecan (Hycamtin®), Trastuzumab (Herceptin®), Tretinoin (Vesanoid®), Vinblastine (Velban®), or Vincristine (Oncovin®).

84. The method or use of any one of claims 1-83, wherein the patient has elevated C-reactive protein ("CRP").
85. The method or use of any one of claims 1-83, wherein the patient has elevated IL-6 serum level.
86. The method or use of any one of claims 1-83, wherein the patient has elevated IL-6 level in the joints.
87. The method or use of any one of claims 1-86, wherein said IL-antagonist inhibits at least one activity associated with IL-6.
88. The method or use of claim 87, wherein at least one of the at least one activity associated with IL-6 is an in vitro activity comprising stimulation of proliferation of T1 165 cells; binding of IL-6 to IL-6R; activation (dimerization) of the gpl30 signal-transducing glycoprotein; formation of IL-6/IL-6R/gpl30 multimers; stimulation of haptoglobin production by HepG2 cells modified to express human IL-6 receptor; or any combination thereof.
89. The method or use of any one of claims 1-88, wherein prior to administration of the IL-6 antagonist, optionally an antibody or antibody fragment thereof, the subject has exhibited or is at risk for developing at least one of the following symptoms: elevated serum C-reactive protein ("CRP"); elevated erythrocyte sedimentation rate; or a combination thereof.
90. The method or use of any one of claims 15-89, wherein said antibody has a half-life of at least about 30 days.
91. The method or use of any one of claims 1-90, wherein the patient has cancer or is being treated for cancer.
92. The method or use of claim 91, wherein the cancer is selected from the group consisting of Acanthoma, Acinic cell carcinoma, Acoustic neuroma, Acral lentiginous melanoma, Acrospiroma, Acute eosinophilic leukemia, Acute lymphoblastic leukemia, Acute megakaryoblastic leukemia, Acute monocytic leukemia, Acute myeloblastic leukemia with maturation, Acute myeloid dendritic cell leukemia, Acute myeloid leukemia, Acute

93. The method or use of claim 92, wherein the cancer is comprising Colorectal Cancer, Non-Small Cell Lung Cancer, Cholangiocarcinoma, Mesothelioma, Castleman's disease, Renal Cell Carcinoma, and any combination thereof.

94. The method or use of claim 92, wherein the patient has a cancer selected from head and neck cancer, esophageal cancer, throat cancer, lung cancer, gastrointestinal cancers such as stomach cancer, colorectal cancer, pancreatic cancer, as well as hematological cancers such as multiple myeloma, leukemia, and lymphoma.

95. The method or use of any one of claims 1-94, wherein prior to administration of the IL-6 antagonist, optionally an antibody or antibody fragment, the subject has exhibited or is at risk for developing at least one of the following symptoms: decreased serum albumin; elevated serum C-reactive protein ("CRP"); fatigue; fever; anorexia (loss of appetite); weight loss; cachexia; weakness; decreased Glasgow Prognostic Score ("GPS"); elevated serum D-dimer; abnormal coagulation profile; and any combination thereof.
96. The method or use of claim 95, wherein said symptom is a side-effect of another therapeutic agent administered to the subject prior to, concurrent with, or subsequent to administration of the antibody or antibody fragment.

97. The method or use of any one of claims 1-96, wherein the IL-6 antagonist, optionally an antibody or antibody fragment, is administered in a therapeutically effective amount for prevention or treatment of at least one symptom associated with anemia, optionally, anemia associated with chemotherapy, anemia associated with radiotherapy, and drug-induced immune hemolytic anemia (DIIHA).

98. The method or use of any one of claims 1-96, wherein the therapeutically effective amount is between about 0.1 and 20 mg/kg of body weight of recipient subject.

99. The method or use of any one of claims 1-98, further comprising monitoring the subject to assess said symptom subsequent to administration of the IL-6 antagonist, optionally an antibody or antibody fragment.

100. The method or use of claim 99, wherein said symptom is exhibited prior to antibody or antibody fragment administration.

101. The method or use of claim 100, wherein said symptom is improved or restored to a normal condition within about 1-5 weeks of antibody administration.

102. The method or use of claim 101, wherein said symptom thereafter remains improved for an entire period intervening two consecutive antibody administrations.

103. The method or use of any one of claims 1-102, wherein said patient suffers from a disease or disorder selected from the group consisting of general fatigue, exercise-induced fatigue, cancer-related fatigue, inflammatory disease-related fatigue, chronic fatigue syndrome, cancer-related cachexia, cardiac-related cachexia, respiratory-related cachexia, renal-related cachexia, age-related cachexia, rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic juvenile idiopathic arthritis, psoriasis, psoriatic arthropathy, ankylosing spondylitis, inflammatory bowel disease (IBD), polymyalgia rheumatica, giant cell arteritis, autoimmune vasculitis, graft versus host disease (GVHD), Sjogren's syndrome, adult onset Still's disease, rheumatoid arthritis, systemic juvenile idiopathic arthritis, osteoarthritis, osteoporosis, Paget's disease of bone, osteoarthritis, multiple myeloma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, prostate cancer, leukemia, renal cell cancer, multicentric Castleman's disease, ovarian cancer, drug resistance in cancer chemotherapy, cancer chemotherapy toxicity, ischemic heart disease, atherosclerosis, obesity, diabetes, asthma, multiple sclerosis, Alzheimer's disease, cerebrovascular disease, fever, acute phase response, allergies, anemia, anemia of inflammation (anemia of chronic
disease), hypertension, depression, depression associated with a chronic illness, thrombosis, thrombocytosis, acute heart failure, metabolic syndrome, miscarriage, obesity, chronic prostatitis, glomerulonephritis, pelvic inflammatory disease, reperfusion injury, transplant rejection, graft versus host disease (GVHD), avian influenza, smallpox, pandemic influenza, adult respiratory distress syndrome (ARDS), severe acute respiratory syndrome (SARS), sepsis, and systemic inflammatory response syndrome (SIRS).

104. The method or use of any one of claims 1-103, wherein the anemia is severe anemia

105. The method or use of any one of claims 1-103, wherein the patient treated has at least one symptom of anemia, optionally wherein the patient exhibits:
   (a) hematocrit levels below about 42-52% for men or about 36-48% for women;
   (b) serum ferritin levels below about 30-400 ng/mL for men or about 13-150 ng/mL for women; serum iron levels below about 60-170 µg/dL;
   (c) reticulocyte count below about 0.5%—1.5%;
   (d) white blood cell (WBC) count of below about 5,000-1 000/mL;
   (e) red blood cell (RBC) count of below about 4.5-5.5x10^6/mL for men and below about 4.0-5.0x10^6/mL for women;
   (f) platelet count below about 1.4-4.0x10^5/mL; or
   (g) total iron binding capacity (TIBC) below about 250-370 µg/dL.

106. The method or use of any one of claims 1-105, wherein the patient treated has at least one symptom of anemia, optionally wherein the patient exhibits fatigue, lack of energy, dizziness, headaches, diminished sex drive, rapid heartbeat, inability to concentrate, paleness, or shortness of breath.

107. The method or use of any one of claims 1-106, wherein the patient has or is to receive autologous stem cell or bone marrow transplant.

108. The method or use of any one of claims 1-106, wherein said IL-antagonist, optionally an anti-IL-6 antibody or antibody fragment, is administered prior, concurrent or after administration of said chemotherapeutic or radiation.

109. The method or use of claim 108, wherein the chemotherapeutic is an EGFR inhibitor.

110. The method or use of claim 109, wherein said EGFR inhibitor is selected from the group consisting of Cetuximab (Erbitux), Erlotinib (Tarceva), Gefitinib (Iressa), Lapatinib (Tykerb), Panitumumab (Vectibix), Sunitinib or Sutent (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-lH-indol-3-ylidene)methyl]-2,4-dimethyl-lH-pyrrole-3-carboxamide), Gefitinib or N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-y1propoxy)quinazolin-4-amine, and Zalutumumab.
111. The method or use of any one of claims 1-110, wherein the patient has a cancer that has exhibited resistance to said chemotherapeutic or radiation after at least one round of chemotherapy or radiation.

112. The method or use of claim 111, wherein said chemotherapeutic or radiation reduces or prevents the treated cancer from invading or metastasizing to other sites in the body.

113. The method or use of claim 111, wherein said chemotherapeutic or radiation results in increased apoptosis of the treated cancer cells.

114. The method or use of claim 111, wherein the treated cancer is selected from advanced and non-advanced cancers including metastasized cancers such as metastatic and non-metastatic lung cancer, breast cancer, head and neck cancer, (HNSCC), pharyngeal cancer, pancreatic cancer, colorectal cancer, anal cancer, glioblastoma multiforme, epithelial cancers, renal cell carcinomas, acute or chronic myelogenous leukemia and other leukemias.

115. The method or use of any one of claims 1-114, wherein the results are used to facilitate design of an appropriate therapeutic regimen for anemia or a disease associated with anemia.

116. The method or use of any one of claims 1-115, wherein the IL-6 antagonist, anti-IL-6 antibody or antibody fragment is co-administered with another therapeutic agent selected from the group consisting of analgesics, antibiotics, anti-cachexia agents, anti-coagulants, anti-cytokine agents, antiemetic agents, anti-fatigue agent, anti-fever agent, anti-inflammatory agents, anti-nausea agents, antipyretics, antiviral agents, anti-weakness agent, chemotherapy agents, cytokine antagonist, cytokines, cytotoxic agents, gene therapy agents, growth factors, IL-6 antagonists, immunosuppressive agents, local anesthetic, statins, other therapeutic agents, or any combination thereof.

117. The method or use of claim 116, wherein said analgesic is acetaminophen, amitriptyline, benzocaine, carbamazepine, codeine, dyclonine hydrochloride (HQ), dihydromorphine, fentanyl patch, Flupirtine, fluriprofen, gabapentin, hydrocodone APAP, hydromorphone, ibuprofen, ketoprofen, lidocaine, morphine, an optiate and derivatives thereof, oxycodone, pentazocine, pethidine, phenacetin, pregabalin, propoeylphene, propoyl APA, salicylamide, tramadol, tramadol APAP, Ulcerase® (0.6% Phenol), or voltaren.

118. The method or use of claim 116, wherein said local anesthetic is amethocaine, articaine, benzocaine, bupivacaine, mepivacaine, cocaine, cinchocaine, chlorprocaine, cyclomethycaine, dibuacaine, dimethocaine, EMLA® (eutectic mixture of lidocaine and prilocaine), etidocaine, larocaine, levobupivacaine, lidocaine, lignocaine, procaine,
piperocaine, prilocaine, proparacaine, propoxycaine, ropivacaine, saxitoxin, tetracaine, tetrodotoxin, or trimecaine.

119. The method or use of claim 116, wherein the anti-cachexia agent is cannabis, dronabinol (Marinol®), nabilone (Cesamet), cannabidiol, cannabichromene, tetrahydrocannabinol, Sativex, megestrol acetate, or any combination thereof.

120. The method or use of claim 151, wherein the anti-coagulant is abciximab (ReoPro®), acenocoumarol, antithrombin III, argatroban, aspirin, bivalirudin (Angiomax®), desirudin (Revasc®/Iprivask®), dipyridamole, eptifibatide (Integrilin®), fondaparinux, heparin, hirudin, idraparinux, lepirudin (Refudan®), low molecular weight heparin, melagatran, phenindione, phenprocoumon, ticlopidine, tirofiban (Aggrastat®), warfarin, ximelagatran, ximelagatran (Exanta®/Exarta®), or any combination thereof.

121. The method or use of claim 116, wherein the anti-inflammatory agent is acetaminophen, azapropazone, diclofenac, diflunisal, etodolac, fenbufen, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, mefenamic, meloxicam, nabumetone, naproxen, phenylbutazone, piroxicam, a salicylate, sulindac, tenoxicam, tiaprofenic acid, or tolfenamic acid. In still further embodiment, the salicylate is acetylsalicylic acid, amoxiprin, benorylate, choline magnesium salicylate, ethenzamide, faislamine, methyl salicylate, magnesium salicylate, salicyl salicylate, or salicylamide.

122. The method or use of claim 116, wherein the anti-nausea agent or antiemetic agent is comprising 5-HT3 receptor antagonists, ajwain, alizapride, anticholinergics, antihistamines, aprepitant, benzodiazepines, cannabichromene, cannabidiol, cannabinoids, cannabis, casopitant, chlorpromazine, cyclizine, dexamethasone, dexamethasone, dimenhydrinate (Gravol®), diphenhydramine, dolasetron, domperidone, dopamine antagonists, doxylamine, dronabinol (Marinol®), droperidol, emetrol, ginger, granisetron, haloperidol, hydroxyzine, hyoscine, lorazepam, meclizine, metoclopramide, midazolam, muscimol, nabilone (Cesamet), nkl receptor antagonists, ondansetron, palonosetron, peppermint, Phenergan, prochlorperazine, Promacot, promethazine, Pentazine, propofol, sativex, tetrahydrocannabinol, trimethobenzamide, tropisetron, nandrolone, stilbestrol, thalidomide, lenalidomide, ghrelin agonists, myostatin antagonists, anti-myostatin antibodies, selective androgen receptor modulators, selective estrogen receptor modulators, angiotensin All antagonists, beta two adenergic receptor agonists, beta three adenergic receptor agonists, or any combination thereof.
123. The method or use of claim 116, wherein said antiviral agent is selected from the group consisting of abacavir, aciclovir, acyclovir, adefovir, amantadine, amprenavir, an antiretroviral fixed dose combination, an antiretroviral synergistic enhancer, arbidol, atazanavir, atripla, brivudine, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, famiclovir, fomiviren, fosamprenavir, foscarnet, fosfonet, fusion inhibitor, ganciclovir, gardasil, ibacitabine, idoxuridine, imiquimod, imunovir, indinavir, inosine, integrase inhibitor, interferon, interferon type I, interferon type II, interferon type III, lamivudine, lopinavir, loviride, maraviroc, MK-0518, moroxydine, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, penclofivir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine, or any combination thereof.

124. The method or use of claim 116, wherein the cytotoxic agent, chemotherapeutic agent, or immunosuppressive agent is comprising 1-dehydrotestosterone, 1-methylnitrosourea, 5-fluorouracil, 6-mercaptopurine, 6-mercaptopurine, 6-thioguanine, Abatacept, abraxane, acitretin, aclarubicin, Actinium-225 \(^{225}\text{Ac}\), actinomycin, Adalimumab, adenosine deaminase inhibitors, Afelimomab, Aflibercept, Afutuzumab, Alefacept, alitretinoin, alkyl sulfonates, alkylating agents, altretamine, alvocidib, aminolevulinic acid/methyl aminolevulinate, aminopterin, aminopterin, amrubicin, amsacrine, amsacrine, anagrelide, Anakinra, anthracenediones, anthracyclines, anthracyclines, anthracyclines, anthramycin (AMC); antimirototic agents, antibiotics, anti-CD20 antibodies, antifolates, Anti-lymphocyte globulin, Antimetabolites, Anti-thymocyte globulin, arsenic trioxide, Aselizumab, asparaginase, asparagine depleters, Astatine-21 I \(^{211}\text{At}\), Atlizumab, Atorolimumab, atrasentan, Avastin®, azacitidine, Azathioprine, azelastine, aziridines, Basiliximab, BAYX antibodies, Belatacept, Belimumab, belotecan, bendamustine, Bertilimumab, bexarotene, bisantrene, Bismuth-213 \(^{213}\text{Bi}\), Bismuth-212 \(^{212}\text{Bi}\), bleomycin, bleomycin, bleomycin, BLYS antibodies, bortezomib, busulfan, busulfan, Calcineurin inhibitors, calicheamicin, camptothecin, camptothecins, capcitabine, carboplatin (paraplatin), carboquone, carminomycin, carmofur, carmustine, carmustine (BSNU), CAT antibodies, CD11a antibodies, CD147/Basigin antibodies, CD154 antibodies, CD18 antibodies, CD20 antibodies, CD23 antibodies, CD3 antibodies, CD4 antibodies, CD40 antibodies, CD62L/L-selectin antibodies, CD80 antibodies, CDK inhibitors, Cedelizumab, celecoxib,
Certolizumab pegol, chlorambucil, chlorambucils, Cyclosporin, cis-dichlorodiamine platinum (II) (DDP) cisplatin, cladribine, Clenoliximab, clofarabine, colchicin, Complement component 5 antibodies, Copper-67 (67Cu), corticosteroids, CTLA-4 antibodies, CTLA-4 fusion proteins, Cyclophillin inhibitors, cyclophosphamides, cyclothophamide, cytarabine, cytarabine, cytochalasin B, cytotoxic ribonucleases, dacarbazine, Daclizumab, dactinomycin, dactinomycin (actinomycin D), daunorubicin, daunorubicin, daunorubicin (formerly daunomycin), decitabine, Deforolimus, demecolcine, detorubicin, dibromomannitol, diethylcarbamazine, dihydrofolate reductase inhibitors, dihydroxy anthracin dione, diptheria toxin, DNA polymerase inhibitors, docetaxel, Dorlimomab aritox, Dorlixizumab, doxorubicin (adriamycin), DXL625, Eculizumab, Efalizumab, efaproxiral, EGFR antagonists, elesclomol, elsamitrucin, Elsilimomab, emetine, endothelin receptor antagonists, epipodophyllotoxins, epirubicin, epothilones, Erbitux®, Erlizumab, estramustine, Etanercept, ethidium bromide, etogolucid, etoposide, etoposide phosphate, Everolimus, Faralimomab, farnesyltransferase inhibitors, FKBP inhibitors, floxuridine, fludarabine, fluorouracil, Fontolizumab, fotemustine, Galiximab, Gallium-67 (67Ga), Gantenerumab, Gavilimomab, gemcitabine, glucocorticoids, Golimumab, Gomiliximab, gramicidin D, Gusperimus, Herceptin®, hydrazines, hydroxyurea, hypomethylating agents, idarubicin, Idarubicine, ifosfamide, IL-1 antagonists, IL-1 receptor antagonists, IL-12, IL-12 antibodies, IL-12R antagonists, IL-13 antibodies, IL-2, IL-2 inhibitors, IL-2 receptor/CD25 antibodies, IL-6 antibodies, imatinib mesylate, Immunoglobulin E antibodies, IMP dehydrogenase inhibitors, Infliximab, Inolimomab, Integrin antibodies, Interferon antibodies, interferons, Interleukin 5 antibodies, Interleukin-6 receptor antibodies, interleukins, Iodine- 125 (125I), Iodine- 131 (131I), Ipilimumab, irinocteanc, ixabepilone, Keliximab, larotaxel, Lead-212 (212Pb), Lebrilizumab, Leflunomide, Lenalidomide, Lerdelimumab, leucovorine, LFA-1 antibodies, lidocaine, lipoxygenase inhibitors, lomustine (CCNU), lonidamine, lucanthone, Lumiliximab, Lutetium- 177 (177Lu), Macrolides, mannosulfan, Maslimomab, masoprocol, mechlorethamine, melphalan, Mepolizumab, mercaptopurine, Metelimumab, Methotrexate, microtubule assembly inhibitors, microtubule stability enhancers, mithramycin, mitobronitol, mitoguazone, mitomycin, mitomycin C, mitotane, mitoxantrone, Morolimumab, mTOR inhibitors, Muromonab-CD3, mustines, Mycophenolic acid, mytontane (0,P’-(DDD)), Natalizumab, nedaplatin, Nerelimomab, nimustine, nitrogen mustards, nitrosoureas, nordihydroguaiaretic acid, oblimersen, ocrelizumab, Ocrelimomab, Odulimomab, ofatumumab, olaparib, Omalizumab, ortataxel, Otelixizumab, oxaliplatin,
oxaliplatin, paclitaxel (taxol), Pascolizumab, PDGF antagonists, pegasparagse, pemetrexed, Pentostatin, Pertuzumab, Pexelizumab, phosphodiesterase inhibitors, phosphorus-32 (^{32}P), Pimecrolimus Abetimus, pirarubicin, pixaantrone, platins, plicamycin, poly ADP ribose polymerase inhibitors, pofhner sodium, porphyrin derivatives, prednimustine, procaine, procarbazine, procarbazine, propranolol, proteasome inhibitors, pseudomonas exotoxin, *Pseudomonas* toxin, purine synthesis inhibitors, puromycin, pyrimidine synthesis inhibitors, radionuclides, radiotherapy, raltitrexed, ranimustine, Reslizumab, retinoid X receptor agonists, retinoids, Rhenium-186 (^{186}Re), Rhenium-188 (^{188}Re), ribonucleotide reductase inhibitors, ricin, Rilonacept, Rituxan®, Rovelizumab, rubitecan, Ruplizumab, Samarium-153 (^{153}Sm), satraplatin, Scandium-47 (^{47}Sc), selective androgen receptor modulators, selective estrogen receptor modulators, seliciclib, semustine, sex hormone antagonists, Siplizumab, Sirolimus, steroid aromatase inhibitors, steroids, streptozocin, streptozotocin, Tacrolimus, talaporfain, Talizumab, taxanes, taxols, tegafur, Telimomab aritox, temoporfin, temozolomide, temsirolimus, Temsirolimus, Teneliximab, teniposide, Teplizumab, Teriflunomide, testolactone, tetracaine, Thalidomide, thioepa chlorambucil, thiopurines thioguanine, ThioTEPA, thymidylate synthase inhibitors, tiazofurin, tipifarnib, T-lymphocyte antibodies, TNF antagonists, TNF antibodies, TNF fusion proteins, TNF receptor fusion proteins, *TNF-alpha* inhibitors, Tocilizumab, topoisomerase inhibitors, topotecan, Toralizumab, trabectedin, Tremelimumab, treosulfan, tretinoin, triazines, triaziquone, triethylenemelamine, triplatin tetranitrate, trofosfamide, tumor antigen specific monoclonal antibodies, tyrosine kinase inhibitors, uramustine, Ustekinumab, valrubicin, Valrubicine, Vapaliximab, VEGF antagonists, Vepalimomab, verteporfin, vinblastine, vinca alkaloids, vincristine, vìnhesine, vinflunine, vinorelbine, Visilizumab, vorinostat, Yttrium-88 (^{88}Y), Yttrium-90 (^{90}Y), Zanolimumab, zileuton, Ziralimumab, Zolimomab aritox, zorubicin, Zotarolimus, or any combination thereof.

The method or use of claim 116, wherein the chemotherapy agent is selected from the group consisting of VEGF antagonists, EGFR antagonists, platins including cisplatin and carboplatin, taxols, irinotecan, 5-fluorouracil, gemcytabine, leucovorin, steroids, cyclophosphamide, melphalan, vinca alkaloids, vinblastine, vincristine, vindesine, vinorelbine, mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins, IL-12, IL-2, IL-12R antagonists, Toxin conjugated monoclonal antibodies, tumor antigen specific monoclonal
antibodies, Erbitux®, Avastin®, Pertuzumab, anti-CD20 antibodies, Rituxan®,
ocrelizumab, ofatumumab, DXL625, Herceptin®, ibandronate (Boniva),
Obatoclax, ABT-263, gossypol, gefitinib, epidermal growth factor receptor tyrosine kinase

126. The method or use of claim 116, wherein the cytokine antagonist is an antagonist of a
factor comprising tumor necrosis factor-alpha, interferon gamma, interleukin 1 alpha,
interleukin 1 beta, interleukin 6, or any combination thereof.

127. The method or use of claim 126, wherein the cytokine antagonist is an antagonist of TNF-
a, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-a, IFN-γ, BAFF,
CXCL13, IP-10, leukemia-inhibitory factor, or a combination thereof.

128. The method or use of claim 127, wherein said growth factor is VEGF, EPO, EGF, HRG,
Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof.

129. The method or use of claim 128, wherein the IL-6 antagonist comprises anti-IL-6
antibodies or antibody fragments thereof, antisense nucleic acids, polypeptides, small
molecules, or any combination thereof.

130. The method or use of claim 129, wherein the antisense nucleic acid comprises at least
approximately 10 nucleotides of a sequence encoding IL-6, IL-6 receptor alpha, gpl30,
p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, or SYK.

131. The method or use of claim 129, wherein the antisense nucleic acid comprises DNA, RNA,
peptide nucleic acid, locked nucleic acid, morpholino (phosphorodiamidate morpholino
oligo), glycerol nucleic acid, threose nucleic acid, or any combination thereof.

132. The method or use of claim 129, wherein the IL-6 antagonist comprises a fragment of a
polypeptide having a sequence selected from the group consisting IL-6, IL-6 receptor
alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, STAT3, or any combination
thereof.

133. The method or use of claim 129, wherein the IL-6 antagonist is coupled to a half-life
increasing moiety.

134. The method or use of claim 116, wherein the statin is comprising atorvastatin, cerivastatin,
fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or
any combination thereof.

135. The method or use of claim 116, wherein the other therapeutic agent is an antagonist of a
factor comprising tumor necrosis factor-alpha, Interferon gamma, Interleukin 1 alpha,
Interleukin 1 beta, Interleukin 6, proteolysis inducing factor, leukemia-inhibitory factor,
tamoxifen, BCL-2 antagonists, estrogen, bisphosphonates, teriparatide, strontium ranelate,
sodium alendronate (Fosamax), risedronate (Actonel), raloxifene, ibandronate (Boniva),
Obatoclax, ABT-263, gossypol, gefitinib, epidermal growth factor receptor tyrosine kinase
Sulfamethizole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Sulfonamides, Teicoplanin, Telithromycin, Tetracycline, Tetracyclines, Ticarcillin, Tinidazole, Tobramycin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Troleandomycin, Trovafloxacin, and Vancomycin. Active agents also include Aldosterone, Beclometasone, Betamethasone, Corticosteroids, Cortisol, Cortisone acetate, Deoxycorticosterone acetate, Dexamethasone, Fludrocortisone acetate, Glucocorticoids, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone, Steroids, and Triamcinolone, an agonist, antagonist, or modulator of a factor comprising TNF-alpha, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, WH-alpha, WN-gamma, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof.

136. The method or use of any one of claims 1-135, wherein the antibody or antibody fragment is directly or indirectly coupled to a detectable label, cytotoxic agent, therapeutic agent, or an immunosuppressive agent.

137. The method or use of claim 136, wherein the detectable label is comprising fluorescent dyes, bioluminescent materials, radioactive materials, chemiluminescent moieties, streptavidin, avidin, biotin, radioactive materials, enzymes, substrates, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, β-galactosidase, luciferase, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin, dansyl chloride, luminol, luciferin, aequorin, Iodine 125 (125I), Carbon 14 (14C), Sulfur 35 (35S), Tritium (3H), Phosphorus 32 (32P), or any combination thereof.

138. The method or use of any one of the foregoing claims, wherein said subject is receiving concomitant chemotherapy.

139. The method or use of any one of the foregoing claims, wherein said subject is receiving concomitant radiotherapy.

140. The method or use of any one of the foregoing claims, wherein said antibody is the Abl antibody.

141. The method or use of any one of the foregoing claims, wherein said composition may be administered intravenously for at least about 1 hour.

142. The method or use of any one of the foregoing claims, wherein said composition comprises at least about 25, 80, 100, 160, 200, 320, or 640 mg of the IL-6 antagonist.

143. The method or use of any one of the foregoing claims, wherein the effective amount is between about 0.1 and 100 mg/kg of body weight of the subject.

144. The method or use of any one of the foregoing claims, wherein said subject is administered at least 1, 2, 3, 4, or 5 doses.
145. The method or use of any one of the foregoing claims, wherein said composition is administered every 4 weeks.

146. The method or use of any one of the foregoing claims, wherein said subject is administered 80 mg every 4 weeks for a total of 2 doses.

147. The method or use of any one of the foregoing claims, wherein said subject is administered 160 mg every 4 weeks for a total of 2 doses.

148. The method or use of any one of the foregoing claims, wherein said subject is administered 320 mg every 4 weeks for a total of 2 doses.

149. The method or use of any one of the foregoing claims, wherein said subject is administered 640 mg every 4 weeks for a total of 2 doses.

150. The method or use of any one of claims 1-149, wherein said anemia is induced by chemoradiation (CRT) regimens used for the treatment of cancers of the head and neck.

151. The method or use of claim 150, wherein said method further comprises assessment of the status of the anemia or head and neck cancer.

152. The method or use of claim 151, wherein said assessment comprises imaging modality selected from the group consisting of CAT, PET, and MRI exams.

153. A method of identifying cancers that are potentially resistant to the effects of a chemotherapeutic or radiation by assaying for IL-6 using an antibody according to the invention in order to detect whether elevated IL-6 levels are present at the site of the treated cancer.

154. A method for the reduction of anemia in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy comprising administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6.

155. A method for the treating anemia in a subject with lung cancer receiving chemotherapy comprising administering an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Ab1.

156. Use of an antibody or antibody fragment according to the invention for preparing a composition for identifying cancers that are potentially resistant to the effects of a chemotherapeutic or radiation by assaying for IL-6 using in order to detect whether elevated IL-6 levels are present at the site of the treated cancer.

157. Use of a humanized antibody or antibody fragment that selectively binds IL-6 for preparing a composition for the reduction of anemia in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy.
158. Use of a humanized monoclonal antibody or antibody fragment that selectively binds IL-6, wherein said antibody is Ab 1 or a humanized variant or fragment thereof, for preparing a composition for treating anemia in a subject with lung cancer receiving or having received before or after administration of said composition, chemotherapy and/or radiation.

159. A composition for treating or preventing anemia comprising an effective amount of an IL-6 antagonist.

160. A composition for treating or preventing drug-induced immune hemolytic anemia (DIHA) comprising an effective amount of an IL-6 antagonist.

161. A composition for treating or preventing anemia associated with chemotherapy comprising an effective amount of an IL-6 antagonist.

162. A composition for treating or preventing anemia associated with radiotherapy comprising an effective amount of an IL-6 antagonist.

163. A composition for treating or preventing anemia associated with cancer an effective amount of an IL-6 antagonist.

164. The composition of any one of claims 159-163, wherein said composition further comprises an excipient, carrier, optionally a pharmaceutically acceptable carrier, or diluent.

165. The composition of any one of claims 159-163, wherein said IL-6 antagonists targets IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, SYK, STAT3, or any combination thereof.

166. The composition of any one of claims 159-163, wherein said IL-6 antagonist is an antibody, an antibody fragment, a peptide, a glycoalkoid, an antisense nucleic acid, a ribozyme, a retinoid, an avemir, a small molecule, or any combination thereof.

167. The composition of any one of claims 159-163, wherein said IL-6 antagonist is an anti-IL-6R, anti-gpl30, anti-p38 MAP kinase, anti-JAK1, anti-JAK2, anti-JAK3, anti-STAT3, or anti-SYK antibody or antibody fragment.

168. The composition of any one of claims 159-163, wherein said IL-6 antagonist is a small molecule comprising thalidomide, lenalidomide, or any combination thereof.

169. The composition of any one of claims 159-163, wherein said IL-6 antagonist is an anti-IL-6 antibody or antibody fragment.

170. The composition of claim 169, wherein said anti-IL-6 antibody or antibody fragment thereof, is Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an
antibody fragment thereof, to a subject in need thereof, wherein the antibody or antibody
fragment thereof, specifically binds to IL-6.

171. The composition of claim 170, wherein said antibody comprises at least one light chain
selected from the group consisting of an amino acid sequence with at least about 90%
sequence identity to an amino acid sequence of SEQ ID NO: 2, 20, 21, 37, 53, 69, 85, 101,
119, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378,
394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 647, 648, 649, 650, 651, 655,
660, 666, 667, 671, 675, 679, 683, 687, 693, 699, 702, 706, or 709.

172. The composition of claim 170, wherein said antibody comprises at least one light chain
selected from the group consisting of nucleic acid sequences with at least about 90%
sequence identity to a nucleic acid sequence of SEQ ID NO: 10, 29, 45, 61, 77, 93, 109,
418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 662, 669, 673, 677, 681, 685, 689,
698, 701, 705, 720, 721, 722, or 723, wherein said nucleic acid sequence encodes said light
chain.

173. The composition of claim 170, wherein said antibody comprises at least one heavy chain
selected from the group consisting of an amino acid sequence with at least about 90%
sequence identity to an amino acid sequence of SEQ ID NO: 3, 18, 19, 22, 38, 54, 70, 86,
102, 117, 118, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347,
363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, 652, 653, 654, 655,

174. The composition of claim 170, wherein said antibody comprises at least one heavy chain
selected from the group consisting of nucleic acid sequences with at least about 90%
sequence identity to a nucleic acid sequence of SEQ ID NO: 11, 30, 46, 62, 78, 94, 110,
131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403,
419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579, 663, 670, 674, 678, 682, 686, 690,
700, 703, 707, 724, or 725, wherein said nucleic acid sequence encodes said heavy chain.

175. The composition of claim 170, wherein said antibody comprises at least two CDR
sequences selected from the group consisting of an amino acid sequence with at least about
90% sequence identity to an amino acid sequence of SEQ ID NO: 4, 7, 23, 26, 39, 42, 55,
58, 71, 74, 87, 90, 103, 106, 124, 127, 140, 143, 156, 159, 172, 175, 188, 191, 204, 207,
220, 223, 236, 239, 252, 255, 268, 271, 284, 287, 300, 303, 316, 319, 332, 335, 348, 351,
364, 367, 380, 383, 396, 399, 412, 415, 428, 431, 444, 447, 460, 463, 476, 479, 492, 495,
508, 511, 524, 527, 540, 543, 556, 559, 572, 575, 710, 711, 712, 716, 5, 8, 24, 27, 40, 43,

178. The composition of claim 170, wherein the antibody or antibody fragment thereof comprises a light chain CDR1 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 4, 23, 39, 55, 71, 74, 87, 103, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 710, 711, or 712.

179. The composition of claim 170, wherein the antibody or antibody fragment thereof comprises a light chain CDR2 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 5, 24, 40, 56, 72, 88, 104, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, 713, 714, 715, or 718.

180. The composition of claim 179, wherein the antibody or antibody fragment thereof comprises a light chain CDR3 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 6, 25, 41, 57, 73, 89, 105, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, or 574.

181. The composition of any one of claims 179-181, wherein said antibody or antibody fragment thereof comprises three light chain CDR polypeptides.


183. The composition of any one of claims 159-181 wherein the antibody or antibody fragment thereof comprises a heavy chain CDR1 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 7, 26, 42, 58, 74, 90, 106, 127, 143, 159, 175, 191,
184. The composition of any one of claims 159-181 wherein the antibody or antibody fragment thereof comprises a heavy chain CDR2 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 8, 27, 43, 59, 75, 91, 107, 120, 121, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, 659, 717, or 718.

185. The composition of any one of claims 159-181 wherein the antibody or antibody fragment thereof comprises a heavy chain CDR3 polypeptide sequence selected from the group consisting of an amino acid sequence with at least about 90% sequence identity to an amino acid sequence of SEQ ID NO: 9, 28, 44, 60, 76, 92, 108, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, or 577.

186. The composition of any one of claims 181-185, wherein said antibody or antibody fragment thereof comprises three heavy chain CDR polypeptides.

187. The composition of any one of claims 181-185, wherein the light chain of said antibody or antibody fragment thereof is selected from the amino acid sequences of light chains listed in TABLE 4.

188. The composition of any one of claims 181-185, wherein at least one heavy chain of said antibody or antibody fragment thereof is selected from the amino acid sequences of heavy chains listed in TABLE 4.

189. The composition of any one of claims 181-185, wherein at least one CDR of said antibody or antibody fragment thereof is selected from the amino acid sequences of CDRs listed in TABLE 4.

190. The composition of any one of claims 1-189, wherein said light chain comprises at least about 95% sequence identity said amino acid sequence.

191. The composition of any one of claims 1-181, wherein said heavy chain comprises at least about 95% sequence identity said amino acid sequence.

192. The composition of any one of claims 1-18, wherein said CDR sequence comprises at least about 95% sequence identity said amino acid sequence.

193. The composition of any one of claims 159-192, wherein said 11-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, which comprises at least two of the CDRs contained in the V_H polypeptide sequences comprising: SEQ ID NO: 3, 18, 19, 22, 38, 54,

194. The composition of any one of claims 159-193, wherein said IL-6 antagonist is an antibody is an Ab<sub>1</sub> antibody or antibody fragment thereof.

195. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises a light chain comprising the amino acid sequence of SEQ ID NO: 2, 20, 647, 648, 649, 650, 651, 660, 666, 699, 702, 706, or 709.

196. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises a humanized light chain comprising the amino acid sequence of SEQ ID NO: 648, 649, and 650.

197. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises at least one light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 4, 5, 6, 710, 711, 712, 713, 714, and 715.

198. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises at least one humanized light chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 710, 711, 712, 713, 714, and 715.

199. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 3, 18, 19, 652, 653, 654, 655, 656, 657, 658, 661, 664, 665, 704, 708.
200. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-II-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises a humanized heavy chain comprising the amino acid sequence of SEQ ID NO: 653, 654, and 655.

201. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-II-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises at least one heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 7, 9, 74, 716, 8, 120, 659, 717, and 718.

202. The method or use of any one of claims 1-10, or composition of any one of Claims 159-193, wherein the IL-6 antagonist is an anti-II-6 antibody or antibody fragment thereof, wherein said antibody or antibody fragment thereof comprises at least one humanized heavy chain CDR comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 74, 716, 717, and 718.

203. The composition of any one of claims 159-163, wherein the IL-6 antagonist is an anti-II-6 antibody or antibody fragment containing a light chain polypeptide that comprises at least one Ab 1 light chain CDR polypeptide comprising

   (a) a light chain CDR1 having at least 72.7% sequence identity to SEQ ID NO: 4;
   (b) a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5;
   (c) a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6;
   (d) a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4;
   (e) a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or
   (f) a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6;

and wherein the heavy chain polypeptide comprises at least one Ab 1 heavy chain CDR polypeptide comprising

   (g) a heavy chain CDR1 having at least 80% sequence identity to SEQ ID NO: 7;
   (h) a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120;
   (i) a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9;
   (j) a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7;
   (k) a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or
   (l) a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9.
204. The composition of any one of claims 159-203, wherein the wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment containing a light chain polypeptide that comprises at least one Ab light chain CDR polypeptide comprising
   (a) a light chain CDR1 having at least 81.8% sequence identity to SEQ ID NO: 4;
   (b) a light chain CDR2 having at least 71.4% sequence identity to SEQ ID NO: 5; or
   (c) a light chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 6;
and wherein the heavy chain polypeptide comprises at least one Ab heavy chain CDR polypeptide comprising
   (d) a heavy chain CDR1 having at least 60% sequence identity to SEQ ID NO: 7;
   (e) a heavy chain CDR2 having at least 87.5% sequence identity to SEQ ID NO: 120; or
   (f) a heavy chain CDR3 having at least 83.3% sequence identity to SEQ ID NO: 9.

205. The composition of either of claims 203 or 204, wherein the Ab antibody or antibody fragment comprises at least two of said light chain CDR polypeptides and at least two of said heavy chain CDR polypeptides.

206. The composition of any one of claims 159-205, wherein the wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment comprising an Ab antibody or antibody fragment that comprises
   (a) two or more Ab light chain CDR polypeptides comprising
   (b) a light chain CDR1 having at least 72.7% sequence identity to SEQ ID NO: 4;
   (c) a light chain CDR2 having at least 85.7% sequence identity to SEQ ID NO: 5; or
   (d) a light chain CDR3 having at least about 90% sequence identity to SEQ ID NO: 6;
and two or more Ab heavy chain CDR polypeptide comprising
   (e) a heavy chain CDR1 having at least 80% sequence identity (identical to at least 4 out of 5 residues) to SEQ ID NO: 7;
   (f) a heavy chain CDR2 having at least about 90% sequence identity to SEQ ID NO: 120; or
   (g) a heavy chain CDR3 having at least 33.3% sequence identity to SEQ ID NO: 9; wherein the Ab antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.

207. The composition of any one of claims 159-206, wherein the wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment comprising an Ab antibody or antibody fragment comprises
(a) two or more Ab 1 light chain CDR polypeptides comprising
(b) a light chain CDR1 having at least 90.9% sequence identity to SEQ ID NO: 4;
(c) a light chain CDR2 having at least 100% sequence identity to SEQ ID NO: 5; or
(d) a light chain CDR3 having at least 66.6% sequence identity to SEQ ID NO: 6;
and two or more Ab 1 heavy chain CDR polypeptide comprising
(e) a heavy chain CDR1 having at least 100% sequence identity to SEQ ID NO: 7;
(f) a heavy chain CDR2 having at least 56.2% sequence identity to SEQ ID NO: 120; or
(g) a heavy chain CDR3 having at least 50% sequence identity to SEQ ID NO: 9;
wherein the Ab 1 antibody or antibody fragment specifically binds to IL-6 and
antagonizes at least one activity associated with IL-6.

208. The composition of either of claim 206 or 207, wherein said Ab 1 antibody or antibody
fragment comprises said light chain CDR1, said light chain CDR3, said heavy chain
CDR2, and said heavy chain CDR3.

209. The composition of any one of claims 159-208, wherein the wherein the IL-6 antagonist is
an anti-IL-6 antibody or antibody fragment that is administered to the subject in the form
of at least one nucleic acids that encode the antibody or antibody fragment thereof.

210. The composition of claim 209, wherein the light chain of said antibody or antibody
fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ
ID NOs: 10, 29, 45, 61, 77, 93, 109, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290,
306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578,
662, 669, 673, 677, 681, 685, 689, 698, 701, 705, 720, 721, 722, or 723.

211. The composition of claim 209, wherein the heavy chain of said antibody or antibody
fragment thereof is encoded by at least one of the following nucleic acid sequences of SEQ
ID NOs: 11, 30, 46, 62, 78, 94, 110, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291,
307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, 579,
663, 670, 674, 678, 682, 686, 690, 700, 703, 707, 724, or 725.

212. The composition of claim 209, wherein at least one of the CDRs of said antibody or
antibody fragment thereof is encoded by at least one of the following nucleic acid
sequences of SEQ ID NOs: 12, 15, 31, 34, 47, 50, 63, 66, 79, 82, 95, 98, 111, 114, 132,
135, 148, 151, 164, 167, 180, 183, 196, 199, 212, 215, 228, 231, 244, 247, 260, 263, 276,
423, 436, 439, 452, 455, 468, 471, 484, 487, 500, 503, 516, 519, 532, 535, 548, 551, 564,
567, 580, 583, 694, 13, 16, 32, 35, 48, 51, 64, 67, 80, 83, 96, 99, 112, 115, 133, 136, 149,
213. The composition of claim 209, wherein at least one of the nucleic acids comprise the heavy and light chain polynucleotide sequences of SEQ ID NO: 723 and SEQ ID NO: 700; SEQ ID NO: 701 and SEQ ID NO: 703; SEQ ID NO: 705 and SEQ ID NO: 707; SEQ ID NO: 720 and SEQ ID NO: 724; and SEQ ID NO: 10 and SEQ ID NO: 11.

214. The composition of any one of claims 159-213, wherein said IL-6 antagonist is an anti-IL-6 antibody or antibody fragment that comprises the humanized variable light sequence of amino acid sequence of SEQ ID NO: 20.

215. The composition of any one of claims 159-214, wherein said wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment that comprises the humanized variable heavy sequence of amino acid sequence of SEQ ID NO: 19.

216. The composition of any one of claims 159-215, wherein said wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment that comprises at least one light chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 4, 5, or 6.

217. The composition of any one of claims 159-216, wherein said wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment containing at least one heavy chain CDRs as set forth in the amino acid sequence of SEQ ID NOs: 7, 120, or 9.

218. The composition of any one of claims 159-217, wherein said wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment containing which is an asialated, humanized anti-IL-6 monoclonal antibody with a half-life of -30 days comprising the humanized variable light and heavy sequences as set forth in SEQ ID NO: 20 and 19 or 709 and 657.

219. The composition of any one of claims 159-218, wherein wherein the IL-6 antagonist is an anti-IL-6 antibody or antibody fragment that is expressed from a recombinant cell.

220. The composition of claim 220, wherein the cell is selected from a mammalian, yeast, bacterial, and insect cell.

221. The composition of claim 221, wherein the cell is a yeast cell.

222. The composition of claim 222, wherein the cell is a diploidal yeast cell.
223. The composition of claim 222, wherein the yeast cell is a *Pichia* yeast.

224. The composition of any one of claims 159-223, wherein said antibody is asialated.

225. The composition of any one of claims 159-223, wherein said antibody is humanized.

226. The composition of any one of claims 159-223, wherein said antibody or antibody fragment comprises a Fab, Fab', F(ab')2, Fv, scFv, IgNAR, SMIP, camelbody, or nanobody.

227. The composition of any one of claims 159-226, wherein the antibody or antibody fragment thereof, has an *in vivo* half-life of at least about 30 days in a healthy human subject.

228. The composition of any one of claims 159-226, wherein the antibody or antibody fragment thereof, has a binding affinity (Kd) for IL-6 of less than about 50 picomolar, or a rate of dissociation (K_off) from IL-6 of less than or equal to 10^(-4) S^{-1}.

229. The composition of any one of claims 159-226, wherein the antibody or antibody fragment thereof, specifically binds to the same linear or conformational epitope(s) and/or competes for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof as an anti-IL-6 antibody comprising the polypeptides of SEQ ID NO: 702 and SEQ ID NO: 704 or the polypeptides of SEQ ID NO: 2 and SEQ ID NO: 3.

230. The composition of claim 229, wherein said binding to the same linear or conformational epitope(s) and/or competition for binding to the same linear or conformational epitope(s) on an intact human IL-6 polypeptide or fragment thereof is ascertained by epitopic mapping using overlapping linear peptide fragments which span the full length of the native human IL-6 polypeptide and includes at least one residues comprised in IL-6 fragments selected from those respectively encompassing amino acid residues 37-51, amino acid residues 70-84, amino acid residues 169-183, amino acid residues 31-45 and/or amino acid residues 58-72 of SEQ ID NO: 1.

231. The composition of any one of claims 159-230, wherein the antibody or antibody fragment thereof, is aglycosylated.

232. The composition of any one of claims 159-230, wherein the antibody or antibody fragment thereof, contains an Fc region that has been modified to alter effector function, half-life, proteolysis, and/or glycosylation.

233. The composition of any one of claims 159-230, wherein the antibody or antibody fragment thereof, is a human, humanized, single chain, or chimeric antibody.

234. The composition of any one of claims 159-230, wherein the antibody or antibody fragment thereof, further comprises a human Fc.
235. The composition of claim 234, wherein said human Fc is derived from IgG1, IgG2, IgG3, IgG4, IgG5, IgG6, IgG7, IgG8, IgG9, IgG10, IgG11, IgG12, IgG13, IgG14, IgG15, IgG16, IgG7, IgG8, or IgG9.

236. The composition of claim 161, wherein said chemotherapy comprises administration of at least one of the following chemotherapy agents Alemtuzumab (Campath®), Asparaginase (Elspar®), Bleomycin (Blenoxane®), Busulfan (Myleran®, Busulfex®), Capecitabine (Xeloda®), Carboplatin (Paraplatin®), Cisplatin (PLATINOL®), Cyclophosphamide (Cytoxan®), Cytarabine (Cytosar-U®), Daunorubicin (Cerubidine®), Docetaxel (Taxotere®), Doxorubicin (Adriamycin®), Epirubicin (Ellence®), Etoposide (VePesid®), Fluorouracil (5-FU®), Gemcitabine (Gemzar®), Gemtuzumab ozogamicin (Mylotarg®), Hydroxyurea (Hydrea®), Idarubicin (Idamycin®), Interleukin 2 (Proleukin®), Irinotecan (Camptosar®), Lomustine (CeeNU®), Methotrexate (Pvheumatrex®), Mitomycin (Mutamycin®), Mitoxantrone (Novantrone®), Oxaliplatin (Eloxatin®), Paclitaxel (Taxol®), Pemetrexed (Alimta®), Pentostatin (Nipent®), Procarbazine (Matulane®), Thiopeta (Thioplex®), Topotecan (Hycamtin®), Trastuzumab (Herceptin®), Tretinoin (Vesanoid®), Vinblastine (Velban®), or Vincristine (Oncovin®).

237. The composition of any one of claims 159-236, wherein the patient has elevated C-reactive protein ("CRP").

238. The composition of any one of claims 159-236, wherein the patient has elevated IL-6 serum level.

239. The composition of any one of claims 159-236, wherein the patient has elevated IL-6 level in the joints.

240. The composition of any one of claims 159-236, wherein said IL-antagonist inhibits with at least one activity associated with IL-6.

241. The composition of claim 240, wherein at least one of the at least one activity associated with IL-6 is an in vitro activity comprising stimulation of proliferation of T165 cells; binding of IL-6 to IL-6R; activation (dimerization) of the gpl30 signal-transducing glycoprotein; formation of IL-6/IL-6R/gpl30 multimers; stimulation of haptoglobin production by HepG2 cells modified to express human IL-6 receptor; or any combination thereof.

242. The composition of any one of claims 159-241, wherein prior to administration of the antibody or antibody fragment thereof, the subject has exhibited or is at risk for developing...
at least one of the following symptoms: elevated serum C-reactive protein ("CRP"); elevated erythrocyte sedimentation rate; or a combination thereof.

243. The composition of any one of claims 159-242, wherein said antibody has a half-life of at least about 30 days.

244. The composition of any one of claims 159-243, wherein the symptom of anemia are selected from the group consisting of:
(a) hematocrit levels below about 42-52% for men or about 36-48% for women;
(b) serum ferritin levels below about 30-400 ng/mL for men or about 13-150 ng/mL for women; serum iron levels below about 60-170 µg/dL;
(c) reticulocyte count below about 0.5%—1.5%;
(d) white blood cell (WBC) count of below about 5,000-10,000/mL;
(e) red blood cell (RBC) count of below about 4.5-5.5×10⁹/mL for men and below about 4.0-5.0×10⁹/mL for women;
(f) platelet count below about 1.4-4.0×10⁹/mL;
(g) total iron binding capacity (TIBC) below about 250-370 µg/dL;
(h) fatigue;
(i) lack of energy;
(j) dizziness;
(k) headaches;
(l) diminished sex drive;
(m) rapid heartbeat;
(n) inability to concentrate;
(o) paleness; or
(p) shortness of breath.

245. The composition of any one of claims 159-244, wherein the patient has cancer or is being treated for cancer.

246. The composition of claim 245, wherein the cancer is selected from the group consisting of Acanthoma, Acinic cell carcinoma, Acoustic neuroma, Acral lentiginous melanoma, Acrospiroma, Acute eosinophilic leukemia, Acute lymphoblastic leukemia, Acute megakaryoblastic leukemia, Acute monocytic leukemia, Acute myeloblastic leukemia with maturation, Acute myeloid dendritic cell leukemia, Acute myeloid leukemia, Acute promyelocytic leukemia, Adamantinoma, Adenocarcinoma, Adenoid cystic carcinoma, Adenoma, Adenomatoid odontogenic tumor, Adrenocortical carcinoma, Adult T-cell leukemia, Aggressive NK-cell leukemia, AIDS-Related Cancers, AIDS-related lymphoma,

247. The composition of claim 246, wherein the cancer is comprising Colorectal Cancer, Non-Small Cell Lung Cancer, Cholangiocarcinoma, Mesothelioma, Castleman's disease, Renal Cell Carcinoma, and any combination thereof.

248. The composition of claim 246, wherein the patient has a cancer selected from head and neck cancer, esophageal cancer, throat cancer, lung cancer, gastrointestinal cancers such as stomach cancer, colorectal cancer, pancreatic cancer, as well as hematological cancers such as multiple myeloma, leukemia, and lymphoma.

249. The composition of any one of claims 159-248, wherein prior to administration of the antibody or antibody fragment the subject has exhibited or is at risk for developing at least one of the following symptoms: decreased serum albumin; elevated serum C-reactive protein ("CRP"); fatigue; fever; anorexia (loss of appetite); weight loss; cachexia; weakness; decreased Glasgow Prognostic Score ("GPS"); elevated serum D-dimer; abnormal coagulation profile; and any combination thereof.

250. The composition of claim 249, wherein said symptom is a side-effect of another therapeutic agent administered to the subject prior to, concurrent with, or subsequent to administration of the antibody or antibody fragment.
251. The composition of any one of claims 159-250, wherein the antibody or antibody fragment
is administered in a therapeutically effective amount for prevention or treatment of at least
one symptom associated with oral mucositis.
252. The composition of any one of claims 159-250, wherein the therapeutically effective
amount is between about 0.1 and 20 mg/kg of body weight of recipient subject
253. The composition of any one of claims 159-252, further comprising monitoring the subject
to assess said symptom subsequent to administration of the antibody.
254. The composition of claim 253, wherein said symptom is exhibited prior to antibody or
antibody fragment administration.
255. The composition of claim 254, wherein said symptom is improved or restored to a normal
condition within about 1-5 weeks of antibody administration.
256. The composition of claim 255, wherein said symptom thereafter remains improved for an
entire period intervening two consecutive antibody administrations.
257. The composition of any one of claims 159-256, wherein said patient suffers from a disease
or disorder selected from the group consisting of: general fatigue, exercise-induced fatigue,
cancer-related fatigue, inflammatory disease-related fatigue, chronic fatigue syndrome,
cancer-related cachexia, cardiac-related cachexia, respiratory-related cachexia, renal-
related cachexia, age-related cachexia, rheumatoid arthritis, systemic lupus erythematosis
(SLE), systemic juvenile idiopathic arthritis, psoriasis, psoriatic arthropathy, ankylosing
spondylitis, inflammatory bowel disease (IBD), polymyalgia rheumatica, giant cell
arteritis, autoimmune vasculitis, graft versus host disease (GVHD), Sjogren's syndrome,
adult onset Still's disease, rheumatoid arthritis, systemic juvenile idiopathic arthritis,
osteoarthritis, osteoporosis, Paget's disease of bone, osteoarthritis, multiple myeloma,
Hodgkin's lymphoma, non-Hodgkin's lymphoma, prostate cancer, leukemia, renal cell
cancer, multicentric Castleman's disease, ovarian cancer, drug resistance in cancer
chemotherapy, cancer chemotherapy toxicity, ischemic heart disease, atherosclerosis,
obesity, diabetes, asthma, multiple sclerosis, Alzheimer's disease, cerebrovascular disease,
fever, acute phase response, allergies, anemia, anemia of inflammation (anemia of chronic
disease), hypertension, depression, depression associated with a chronic illness,
thrombosis, thrombocytosis, acute heart failure, metabolic syndrome, miscarriage, obesity,
chronic prostatitis, glomerulonephritis, pelvic inflammatory disease, reperfusion injury,
transplant rejection, graft versus host disease (GVHD), avian influenza, smallpox,
pandemic influenza, adult respiratory distress syndrome (ARDS), severe acute respiratory
syndrome (SARS), sepsis, and systemic inflammatory response syndrome (SIRS).
258. The composition of claim 161, or method or use of any of claims 1-10, wherein said anemia is associated with the administration of Cisplatin (PLATINOL®).

259. The composition of claim 161, or method or use of any of claims 1-10, wherein said anemia is associated with the combination of radiation therapy and Cisplatin (PLATINOL®).

260. The composition of any one of claims 159-259, wherein said composition further comprises an iron supplement or a medication that stimulate the formation of red blood cells, optionally, Epoetin alfa (Epogen®, Procrit®) or Darbepoetin alfa (Aranesp®).

261. The composition of any one of claims 159-259, wherein the patient has or is to receive autologous stem cell or bone marrow transplant or hematopoietic stem cell transplant (HSCT).

262. The composition of any one of claims 159-261, wherein said IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment, is administered prior, concurrent or after administration of said chemotherapeutic or radiation.

263. The composition of claim 262, wherein the chemotherapeutic is an EGFR inhibitor.

264. The composition of claim 263, wherein said EGFR inhibitor is selected from the group consisting of Cetuximab (Erbitux), Erlotinib (Tarceva), Gefitinib (Iressa), Lapatinib (Tykerb), Panitumumab (Vectibox), Sunitinib or Sutent (N-(2-diethylaminoethyl)-5-[(Z)-5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide), Gefitinib or N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-y1propoxy)quinazolin-4-amine, and Zalutumumab.

265. The composition of any one of claims 159-264, wherein the patient has a cancer that has exhibited resistance to said chemotherapeutic or radiation after at least one round of chemotherapy or radiation.

266. The composition of claim 265, wherein said chemotherapeutic or radiation reduces or prevents the treated cancer from invading or metastasizing to other sites in the body.

267. The composition of claim 265, wherein said chemotherapeutic or radiation results in increased apoptosis of the treated cancer cells.

268. The composition of claim 265, wherein the treated cancer is selected from advanced and non-advanced cancers including metastasized cancers such as metastatic and non-metastatic lung cancer, breast cancer, head and neck cancer, (HNSCC), pharyngeal cancer, pancreatic cancer, colorectal cancer, anal cancer, glioblastoma multiforme, epithelial cancers, renal cell carcinomas, acute or chronic myelogenous leukemia and other leukemias.
269. The composition of any one of claims 159-268, wherein the results are used to facilitate design of an appropriate therapeutic regimen for mucositis or a disease associated with mucositis.

270. The composition of any one of claims 159-269, wherein said composition further comprises another therapeutic agent selected from the group consisting of analgesics, antibiotics, anti-cachexia agents, anti-coagulants, anti-cytokine agents, anti-emetic agents, anti-fatigue agent, anti-fever agent, anti-inflammatory agents, anti-nausea agents, antipyretics, antiviral agents, anti-weakeness agent, chemotherapy agents, cytokine antagonist, cytokines, cytoxic agents, gene therapy agents, growth factors, IL-6 antagonists, immunosuppressive agents, local anesthetic, statins, other therapeutic agents, or any combination thereof.

271. The composition of claim 270, wherein said analgesic is acetaminophen, amitriptyline, benzocaine, carbamazepine, codeine, dyclonine hydrochloride (HQ), dihydromorphine, fentanyl patch, Flupirtine, fluriprofen, gabapentin, hydrocodone APAP, hydromorphone, ibuprofen, ketoprofen, lidocaine, morphine, an opiate and derivatives thereof, oxycodone, pentazocine, pethidine, phenacetin, pregabalin, propoxyphene, propoyl APA, salicylamide, tramadol, tramadol APAP, Ulcerease® (0.6% Phenol), or voltaren.

272. The composition of claim 270, wherein said local anesthetic is amethocaine, articaine, benzocaine, bupivacaine, mepivacaine, cocaine, cinchocaine, chlorprocaine, cyclomethycaine, dibucaine, dimethocaine, EMLA® (eutectic mixture of lidocaine and prilocaine), etidocaine, lanocaine, levobupivacaine, lidocaine, lignocaine, procaine, piperocaine, prilocaine, propacaine, propoxycaine, ropivacaine, saxitoxin, tetracaine, tetrodotoxin, or trimecaine.

273. The composition of claim 270, wherein the anti-cachexia agent is cannabis, dronabinol (Marinol®), nabilone (Cesamet), cannabidiol, cannabichromene, tetrahydrocannabinol, Sativex, megestrol acetate, or any combination thereof.

274. The composition of claim 270, wherein the anti-coagulant is abciximab (ReoPro®), acenocoumarol, antithrombin III, argatroban, aspirin, bivalirudin (Angiomax®), clopidogrel, dabigatran, dabigatran etexilate (Pradaxa®/Pradax®), desirudin (Revasc®/Iprivask®), dipyridamole, eptifibatide (Integrilin®), fondaparinux, heparin, hirudin, idraparinux, lepirudin (Refludan®), low molecular weight heparin, melagatran, phenindione, phenprocoumon, ticlopidine, tirofiban (Aggrastat®), warfarin, ximelagatran, ximelagatran (Exanta®/Exarta®), or any combination thereof.
275. The composition of claim 270, wherein the anti-inflammatory agent is acetaminophen, azapropazone, diclofenac, diflunisal, etodolac, fenbufen, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, mefenamic, meloxicam, nabumetone, naproxen, phenylbutazone, piroxicam, a salicylate, sulindac, tenoxicam, tiaprofenic acid, or tolfenamic acid. In still further embodiment, the salicylate is acetylsalicylic acid, amoxiprin, benorylate, choline magnesium salicylate, ethenzamide, fainosilamine, methyl salicylate, magnesium salicylate, salicycyl salicylate, or salicylamide.

276. The composition of claim 270, wherein the anti-nausea agent or antiemetic agent is comprising 5-HT3 receptor antagonists, ajwain, alizapride, anticholinergics, antihistamines, aprepitant, benzodiazepines, cannabichromene, cannabidiol, cannabinoids, cannabis, casopitant, chlorpromazine, cyclizine, dexamethasone, dexamethasone, dimenhydrinate (Gravol®), diphenhydramine, dolasetron, domperidone, dopamine antagonists, doxylamine, dronabinol (Marinol®), droperidol, emetrol, ginger, granisetron, haloperidol, hydroxyzine, hyoscine, lorazepam, metoclopramide, midazolam, muscimol, nabilone (Cesamet), NK1 receptor antagonists, ondansetron, palonosetron, peppermint, Phenergan, prochlorperazine, Promact, promethazine, Pentazine, propofol, sativex, tetrahydrocannabinol, trimethobenzamide, tropisetron, nalbione (Cesamet), thalidomide, lenalidomide, ghrelin agonists, anti-myostatin antibodies, selective androgen receptor modulators, selective estrogen receptor modulators, angiotensin II antagonists, beta two adrenergic receptor agonists, beta three adrenergic receptor agonists, or any combination thereof.

277. The composition of claim 270, wherein said antiviral agent is selected from the group consisting of abacavir, aciclovir, acyclovir, adefovir, amantadine, amprenavir, an antiretroviral fixed dose combination, an antiretroviral synergistic enhancer, arbidol, atazanavir, atipla, brivudine, cidofovir, combivir, darunavir, delavirdine, didanosine, docosanol, edoxudine, efavirenz, emtricitabine, enfuvirtide, entecavir, entry inhibitors, famciclovir, famiviren, fosamprenavir, foscarad, fosfonet, fusion inhibitor, ganciclovir, gardasil, ibacitabine, idoxuridine, imiquimod, imunovir, indinavir, inosine, integrase inhibitor, interferon, interferon type I, interferon type II, interferon type III, lamivudine, lopinavir, loviride, maraviroc, MK-0518, moroxydine, nelfinavir, nevirapine, nexavir, nucleosome analogues, oseltamivir, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, reverse transcriptase inhibitor, ribavirin, rimantadine, rivotavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir,
tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, zidovudine, or any combination thereof.

278. The composition of claim 270, wherein the cytotoxic agent, chemotherapeutic agent, or immunosuppressive agent is comprising 1-dehydrottestosterone, 1-methylnitrosourea, 5-fluorouracil, 6-mercaptopurine, 6-mercaptopurine, 6-thioguanine, Abatacept, abraxane, acitretin, aclarubicin, Actinium-225 (225Ac), actinomycin, Adalimumab, adenosine deaminase inhibitors, Afelimomab, Afibercept, Afutuzumab, Alefacept, altretamine, alkyl sulfonates, alkylation agents, altretamine, alvocidib, aminolevulinic acid/methyl aminolevulinate, aminopterin, aminopterin, amrubicin, amsacrine, amsacrine, anagrelide, Anakinra, anthracenediones, anthracyclines, anthracyclines, anthracyclines, amyramycin (AMC); antimitotic agents, antibiotics, anti-CD20 antibodies, antifolates, Anti-lymphocyte globulin, Antimetabolites, Anti-thymocyte globulin, arsenic trioxide, Aselizumab, asparaginase, asparagine depleters, Astatine-211, (211At), Atlizumab, Atorolimumab, atrasentan, Avastin®, azacitidine, Azathioprine, azelastine, azidothymidine, Basiliximab, BAYX antibodies, Belatacept, Belimumab, belotecan, bendamustine, Bertilimumab, bexarotene, bisantrene, Bismuth-213 (213Bi), Bismuth-212 (212Bi), bleomycin, bleomycin, bleomycin, BLYS antibodies, bortezomib, busulfan, busulfan, Calcineurin inhibitors, calicheamicin, camptothecin, camptothecins, carboxatin, carboxatin, copper, carminomycin, camphor, carmustine, carmustine (BSNU), CAT antibodies, CD11a antibodies, CD147/Basigin antibodies, CD154 antibodies, CD18 antibodies, CD20 antibodies, CD23 antibodies, CD3 antibodies, CD4 antibodies, CD40 antibodies, CD62L/L-selectin antibodies, CD80 antibodies, CDK inhibitors, Cedelizumab, celecoxib, Certolizumab pegol, chlorambucil, chlorambucils, Ciclosporin, cis-dichlorodiamine platinum (II) (DDP) cisplatin, cladribine, Clinoliximab, clofarabine, colchicin, Complement component 5 antibodies, Copper-67 (67Cu), corticosteroids, CTLA-4 antibodies, CTLA-4 fusion proteins, Cyclophilin inhibitors, cyclophosphamides, cyclophosphamide, cytarabine, cytarabine, cytarabine, cytarabine, cytarabine, cytarabine, cytochalasin B, cytotoxic ribonucleases, dacarbazine, Daclizumab, dactinomycin, dactinomycin (actinomycin D), daunorubicin, daunorubicin, daunorubicin (formerly daunomycin), decitabine, Deforolimus, demecolcine, detorubicin, dibromomannitol, diethylcarbamazine, dihydrofolate reductase inhibitors, dihydroxy anthracin dione, diphtheria toxin, DNA polymerase inhibitors, docetaxel, Dorlimomab aritox, Dorlixizumab, doxorubicin (adriamycin), DXL625, Eculizumab, Efalizumab, efaproxiral, EGFR antagonists, elesclomol, elasmatrinuc, Elsilimomab, emetine, endothelin receptor antagonists, epipodophyllotoxins, epirubicin, epothilones,
Erbitux®, Erlizumab, estramustine, Etanercept, ethidium bromide, etogluclid, etoposide, etoposide phosphate, Everolimus, Faralimomab, farnesyltransferase inhibitors, FKBP inhibitors, floxuridine, fludarabine, fluouracil, Fontolizumab, fotemustine, Galiximab, Gallium-67 (67Ga), Gantenerumab, Gavilimomab, gemcitabine, glucocorticoids, Golimumab, Gomiliximab, glucocorticoids, Immunoglobulin E antibodies, IMP dehydrogenase inhibitors, Infliximab, Inolimomab, Integrin antibodies, Interferon antibodies, interferons, Interleukin-5 antibodies, Interleukin-6 receptor antibodies, interleukins, Iodine-125 (125I), Iodine-131 (131I), Ipilimumab, irinotecan, ixabepilone, Keliximab, larotaxel, Lead-212 (212Pb), Lebrilizumab, Leflunomide, Lenalidomide, Lerdelimumab, leucovorine, LFA-1 antibodies, lidocaine, lipoxygenase inhibitors, lomustine (CCNU), lonidamine, lucanthone, Lumiliximab, Lutetium-177 (177Lu), Macrolides, mannosulfan, Maslimomab, masoprocol, mechlorethamine, melphalan, Mepolizumab, mercaptopurine, Metelimumab, Methotrexate, mitobronitol, mitoguazone, nitrosoureas, nordihydroguaiaretic acid, oblimersen, ocrelizumab, Ocrelizumab, Odulimomab, ofatumumab, olaparib, Omalizumab, ortataxel, Otelixizumab, oxaliplatin, oxaliplatin (taxol), Pascolizumab, PDGF antagonists, pegaspargase, pemtrexed, Pentostatin, Pertuzumab, Pexelizumab, phosphodiesterase inhibitors, Phosphorus-32 (32P), Pimecrolimus Abetimus, pirarubicin, pixantrone, platins, plicamycin, poly ADP ribose polymerase inhibitors, porphyrin derivatives, prednimustine, procaine, procarbazine, procarbazine, propranolol, proteasome inhibitors, Pseudomonas exotoxin, Pseudomonas toxin, purine synthesis inhibitors, puromycin, pyrimidine synthesis inhibitors, radionuclides, radiotherapy, raltitrexed, ranimustine, Reslizumab, retinoid X receptor agonists, retinoids, Rhenium-186 (186Re), Rhenium-188 (188Re), ribonucleotide reductase inhibitors, ricin, Rilonacept, Rituxan®, Rovelizumab, rubitecan, Ruplizumab, Samarium-153 (153Sm), satraplatin, Scandium-47 (47Sc), selective androgen receptor modulators, selective estrogen receptor modulators, seliciclib, semustine, sex hormone antagonists, Siplizumab, Sirolimus, steroid aromatase inhibitors,
steroids, streptozocin, streptozotocin, Tacrolimus, talaporfin, Talizumab, taxanes, taxols, tegafur, Telimomab aritox, temoporfin, temozolomide, temsirolimus, Temozolomide, Teneliximab, teniposide, Teplizumab, thioepa chlorambucil, thiopurines thioguanine, ThioTEPA, thymidylate synthase inhibitors, tiazofurin, tipifarnib, T-lymphocyte antibodies, TNF antagonists, TNF antibodies, TNF fusion proteins, TNF receptor fusion proteins, TNF-alpha inhibitors, Tocilizumab, topoisomerase inhibitors, topotecan, Toralizumab, trabectedin, Tremelimumab, treosulfan, tretonin, triazenes, triaziquone, triethylenemelamine, triplatin tetranitrate, trofosfamide, tumor antigen specific monoclonal antibodies, tyrosine kinase inhibitors, uramustine, Ustekinumab, valrubicin, Valrubicine, Vapaliximab, VEGF antagonists, Vepalimomab, verteporfin, vinblastine, vinca alkaloids, vincristine, vindesine, vinflunine, vinorelbine, Visilizumab, vorinostat, Yttrium-88 (88Y), Yttrium-90 (90Y), Zanolimumab, zileuton, Ziralimumab, Zolimomab aritox, zorubicin, Zotarolimus, or any combination thereof.

279. The composition of claim 270, wherein the chemotherapy agent is selected from the group consisting of VEGF antagonists, EGFR antagonists, platins including cisplatin and carboplatin, taxols, irinotecan, 5-fluorouracil, gemcytabine, leucovorine, steroids, cyclophosphamide, melphalan, vinca alkaloids, vinblastine, vincristine, vindesine, vinorelbine, mustines, tyrosine kinase inhibitors, radiotherapy, sex hormone antagonists, selective androgen receptor modulators, selective estrogen receptor modulators, PDGF antagonists, TNF antagonists, IL-1 antagonists, interleukins, IL-12, IL-2, IL-12R antagonists, Toxin conjugated monoclonal antibodies, tumor antigen specific monoclonal antibodies, Erbitux®, Avastin®, Pertuzumab, anti-CD20 antibodies, Rituxan®, ocrelizumab, ofatumumab, DXL625, Herceptin®, or any combination thereof.

280. The composition of claim 270, wherein the cytokine antagonist is an antagonist of a factor comprising tumor necrosis factor-alpha, interferon gamma, interleukin 1 alpha, interleukin 1 beta, interleukin 6, or any combination thereof.

281. The composition of claim 280, wherein the cytokine antagonist is an antagonist of TNF-a, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-a, IFN-γ, BAFF, CXCL13, IP-10, leukemia-inhibitory factor, or a combination thereof.

282. The composition of claim 281, wherein said growth factor is VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof.

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283. The composition of claim 281, wherein the IL-6 antagonist comprises anti-IL-6 antibodies or antibody fragments thereof, antisense nucleic acids, polypeptides, small molecules, or any combination thereof.

284. The composition of claim 283, wherein the antisense nucleic acid comprises at least approximately 10 nucleotides of a sequence encoding IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, or SYK.

285. The composition of claim 284, wherein the antisense nucleic acid comprises DNA, RNA, peptide nucleic acid, locked nucleic acid, morpholino (phosphorodiamidate morpholino oligo), glycerol nucleic acid, threose nucleic acid, or any combination thereof.

286. The composition of claim 284, wherein the IL-6 antagonist polypeptide comprises a fragment of a polypeptide having a sequence selected from the group consisting of IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, and SYK.

287. The composition of claim 286, wherein the IL-6 antagonist comprises a soluble IL-6, IL-6 receptor alpha, gpl30, p38 MAP kinase, JAK1, JAK2, JAK3, STAT3, SYK, or any combination thereof.

288. The composition of claim 270, wherein the statin is comprising atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin, or any combination thereof.

289. The composition of claim 270, wherein the other therapeutic agent is an antagonist of a factor comprising tumor necrosis factor-alpha, Interferon gamma, Interleukin 1 alpha, Interleukin 1 beta, Interleukin 6, proteolysis inducing factor, leukemia-inhibitory factor, tamoxifen, BCL-2 antagonists, estrogen, bisphosphonates, teriparatide, strontium ranelate, sodium alendronate (Fosamax), risedronate (Actonel), raloxifene, ibandronate (Boniva), Obatoclax, ABT-263, gossypol, gefitinib, epidermal growth factor receptor tyrosine kinase inhibitors, erlotinib, epidermal growth factor receptor inhibitors, psoralens, trioxysalen, methoxsalen, bergapten, retinoids, etretinate, acitretin, infliximab (Remicade®), adalimumab, infliximab, etanercept, Zenapax®, Cyclosporine, Methotrexate, granulocyte-colony stimulating factor, filgrastim, lenograstim, Neupogen, Neulasta, 2-Arylpropionic acids, Aceclofenac, Acemetacin, Acetylsalicylic acid (Aspirin), Alclofenac, Alminoprobe, Anoxiprin, Ampyrene, Arylalkanoic acids, Azapropazone, Benorylate/Benorilate, Benoxaprofen, Bromfenac, Carprofen, Celecoxib, Choline magnesium salicylate, Clofezone, COX-2 inhibitors, Dexibuprofen, Dextroproben, Diclofenac, Diflunisal, Droxican, Ethanazaide, Etoheolac, Etoricoxib, Faislamine, fenamic acids, Fenbufen, Fenoprofen, Flufenamic acid, Flunoxaprofen, Flurbiprofen, Ibuprofen, Ibuproam,
Indometacin, Indoprofen, Kebuzone, Ketoprofen, Ketorolac, Lornoxicam,洛索洛芬，
Lumiracoxib, Magnesium salicylate, Meclofenamic acid, Mefenamic acid, Meloxicam,
Metamizole, Methyl salicylate, Mofebutazone, Nabumetone, Naproxen, N-Arylanthranilic acids, Oxametacin, Oxpazoline, Oxicams, Oxyphenbutazone, Parecoxib, Phenazone,
Phenylbutazone, Phenylbutazone, Piroxicam, Piroprofen, profens, Proglumetacin,
Pyrazolidine derivatives, Rofecoxib, Salicyl salicylate, Salicylamide, Salicylates,
Sulfinpyrazone, Sulindac, Suprofen, Tenoxicam, Tiaprofenic acid, Tolmetin, and Valdecoxib.

Antibiotics include Amikacin, Aminoglycosides, Amoxicillin, Ampicillin, Ansamycins, Arsenic, Azithromycin, Azlocillin, Aztreonam, Bacitracin, Carbacephem, Carbapenems, Carbencillin, Cefaclor, Cefadroxil, Cefalexin, Ceftazidime, Cefamandole, Cefazolin, Cefdinir, Cefditoren, Cefepime, Ceftobiprole, Ceftriaxone, Cefuroxime, Cefuroxime, Cephalosporins, Chloramphenicol, Cilastatin, Ciprofloxacin, Clarithromycin, Clindamycin, Cloxacillin, Colistin, Cotrimoxazole, Dalfopristin, Demeclocycline, Dicloxacillin, Dirithromycin, Doripenem, Doxycycline, Enoxacin, Erythromycin, Ertapenem, Erythromycin, Ethambutol, Flucloxacillin, Fosfomycin, Furazolidone, Fusidic acid, Gatifloxacin, Geldanamycin, Gentamicin, Glycopeptides, Herbimycin, Imipenem, Isoniazid, Kanamycin, Levofloxacins, Lincomycin, Linezolid, Loracarbef, Macrolides, Mafenide, Meropenem, Meticillin, Metronidazole, Mezlocillin, Minocycline, Monobactams, Moxifloxacin, Mupirocin, Nafcillin, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Oxytetracycline, Paromomycin, Penicillin, Penicillins, Piperacillin, Platensimycin, Polymyxin B, Polypeptides, Prontosil, Pyrazinamide, Quinolones, Quinupristin, Rifampicin, Rifampin, Roxithromycin, Spectinomycin, Streptomycin, Sulfacetamide, Sulfamethizole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Sulfonamides, Teicoplanin, Telithromycin, Tetracycline, Tetracyclines, Ticarcillin, Tinidazole, Tobramycin, Trimethoprim, Trimethoprim-Sulfamethoxazole, Troleandomycin, Trovafloxacin, and Vancomycin. Active agents also include Aldosterone, Beclometasone, Betamethasone, Corticosteroids, Cortisol, Cortisone acetate, Deoxytocicosterone acetate, Dexamethasone, Fluorocorticoids acetate, Glucocorticoids, Hydrocortisone, Methylprednisolone, Prednisolone, Prednisone, Steroids, and Triamcinolone, an agonist, antagonist, or modulator of a factor comprising TNF-alpha, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, WH-alpha, WN-gamma, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, or any combination thereof.
290. The composition of any one of claims 159-289, wherein the IL-6 antagonist, optionally an anti-IL-6 antibody or antibody fragment, is directly or indirectly coupled to a detectable label, cytotoxic agent, therapeutic agent, an immunosuppressive agent, or a half-life increasing moiety.

291. The composition of claim 290, wherein the detectable label is comprising fluorescent dyes, bioluminescent materials, radioactive materials, chemiluminescent moieties, streptavidin, avidin, biotin, radioactive materials, enzymes, substrates, horseradish peroxidase, acetylcholinesterase, alkaline phosphatase, β-galactosidase, luciferase, rhodamine, fluorescein, fluorescein isothiocyanate, umbelliferone, dichlorotriazinylamine, phycoerythrin, dansyl chloride, luminol, luciferin, aequorin, Iodine 125 ([125I]), Carbon 14 ([14C]), Sulfur 35 ([35S]), Tritium ([3H]), Phosphorus 32 ([32P]), or any combination thereof.

292. The composition of any one of claims 159-291, wherein said subject is receiving concomitant chemotherapy.

293. The composition of any one of claims 159-291, wherein said subject is receiving concomitant radiotherapy. The composition of any one of claims 151-291, wherein said antibody is the Ab 1 antibody.

294. The composition of any one of the foregoing claims, wherein said composition may be administered intravenously for at least about 1 hour.

295. The composition of any one of claims 159-295, wherein the effective amount is between about 0.1 and 100 mg/kg of body weight of the subject.

296. The composition of any one of claims 159-295, wherein said composition comprises at least about 25, 80, 100, 160, 200, 320, or 640 mg of IL-6 antagonist.

297. The composition of any one of claims 159-295, wherein said composition is formulated for intravenous administration.

298. The composition of any one of claims 159-295, wherein said composition comprises an excipient selected from the group consisting of histidine, sorbitol, and polysorbate 80.

299. The composition of any one of claims 159-295, wherein said composition is administered every 4 weeks.

300. The composition of any one of claims 159-295, wherein said composition is administered 80 mg every 4 weeks for a total of 2 doses.

301. The composition of any one of claims 159-295, wherein said composition is administered 160 mg every 4 weeks for a total of 2 doses.

302. The composition of any one of claims 159-295, wherein said composition is administered 320 mg every 4 weeks for a total of 2 doses.
303. A composition for the reduction of oral mucositis in subjects with head and neck cancer receiving concomitant chemotherapy and radiotherapy comprising an effective amount of a humanized monoclonal antibody that selectively binds IL-6.

304. A composition for the treating oral mucositis in a subject with head and neck cancer receiving concomitant chemotherapy comprising an effective amount of a humanized monoclonal antibody that selectively binds IL-6, wherein said antibody is Abl.

305. A composition comprising a humanized monoclonal antibody or fragment thereof that selectively binds IL-6 for treating oral mucositis induced by chemoradiation (CRT) regimens used for the treatment of cancers of the head and neck.

306. A composition for treatment or prevention of oral mucositis comprising a humanized monoclonal antibody that selectively binds IL-6 and saline solution.

307. The composition of any one of claims 303-306, wherein said oral mucositis is induced by chemoradiation (CRT) regimens used for the treatment of cancers of the head and neck.

308. A method of treating rheumatoid arthritis by subcutaneously administering a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab1 or an antibody that competes with Ab1 for binding to IL-6 to a patient in need thereof.

309. Use of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Abl or an antibody that competes with Abl for binding to IL-6, for preparing a subcutaneously administrable composition for treating rheumatoid arthritis in a patient in need thereof.

310. The method or use of claim 308 or 309, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that is formulated for subcutaneous administration.

311. The method or use of claim 310, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

312. The method or use of claim 310, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 to 280 mM sorbitol or sorbitol in combination with sucrose, and 0.015% (w/w) Polysorbate 80, said formulation having a nitrogen headspace in the shipping vials.
313. The method or use of any one of claims 308-312, wherein the concentration of said anti-IL-6 antibody or antibody fragment is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/mL or at least about 10-100 mg/mL.

314. A method of treating rheumatoid arthritis by intravenously administering a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab1 or an antibody that competes with Ab1 for binding to IL-6 to a patient in need thereof.

315. Use of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab1 or an antibody that competes with Ab1 for binding to IL-6, for preparing an intravenously administrable composition for treating rheumatoid arthritis in a patient in need thereof.

316. The method or use of claim 314 or 315, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that is formulated for intravenous administration.

317. The method or use of claim 316, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, anti-IL-6 antibody or antibody fragment, 25 mM Histidine base, Phosphoric acid q.s. to pH 6, and 250 mM sorbitol.

318. The method or use of claim 317, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, 12.5 mM Histidine base, 12.5 mM Histidine HC1 (or 25 mM Histidine base and Hydrochloric acid q.s. to pH 6), 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

319. The method or use of claim 317, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HC1 to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

320. The method or use of any one of claims 309-319, wherein the concentration of said anti-IL-6 antibody or antibody fragment is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/mL or at least about 10-100 mg/mL.

321. The method or use of any one of claims 309-320, wherein the administered dosage of said anti-IL-6 antibody or antibody fragment is at least about 50 or 100 mg.

322. The method or use of any one of claims 309-320, wherein the administered dosage of said anti-IL-6 antibody or antibody fragment is about 80 mg, about 160 mg, or about 320 mg.
323. The method or use of any one of claims 309-322, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 709.

324. The method or use of any one of claims 309-323, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising a polypeptide encoded by a polynucleotide that has at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 723.

325. The method or use of any one of claims 309-324, wherein said antibody or antibody fragment comprises a heavy chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 657.

326. The method or use of any one of claims 309-324, wherein said antibody or antibody fragment comprises a heavy chain polypeptide comprising a polypeptide encoded by a polynucleotide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 700.

327. The method or use of any one of claims 309-326, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 709, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 723, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723; and a heavy chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 657, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 700, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 700, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence
of the reverse complement of SEQ ID NO: 700; wherein the Abl antibody or antibody fragment specifically binds to IL-6 and antagonizes at least one activity associated with IL-6.

328. The method or use of any one of claims 309-327, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 90% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

329. The method or use of any one of claims 309-327, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 95% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

330. The method or use of any one of claims 309-327, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 98% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

331. The method or use of any one of claims 309-327, wherein said anti-IL-6 antibody comprises the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

332. The method or use of any one of claims 309-331, wherein said anti-IL-6 antibody further comprises the constant light chain sequence contained in SEQ ID NO: 586.

333. The method or use of any one of claims 309-332, wherein said anti-IL-6 antibody comprises the constant heavy chain sequence contained in SEQ ID NO: 588.

334. The method or use any one of claims 309-333, wherein said dosage is administered at least twice.

335. The method or use of claim 334, wherein the patient receives at least a first dosage and a second dosage, and said second dosage is about eight weeks subsequent to said first dosage.

336. The method or use of claim 335, wherein the patient is administered said dosage every 8 weeks or 2 months.

337. The method or use of any one of claims 309-336, wherein said patient has previously received or is concurrently receiving methotrexate.

338. The method or use of claim 337, wherein the dosage of said methotrexate is at least 10 mg/week.

339. The method or use of claim 338, wherein said patient continues to receive methotrexate for at least 8 weeks after administration of said anti-IL-6 antibody or antibody fragment.

340. The method or use any one of claims 337-339, wherein the dosage of said patient exhibits methotrexate resistance at the time of administration of said anti-IL-6 antibody or antibody fragment.
341. The method or use of any one of claims 309-3340, wherein at least one anti-inflammatory agent, analgesic agent, or disease-modifying antirheumatic drug (DMARD) is administered to said patient.

342. The method or use of claim 341, wherein said anti-inflammatory agent is selected from the group consisting of steroids, Cortisone, Glucocorticoids, prednisone, prednisolone, Hydrocortisone (Cortisol), Cortisone acetate, Methylprednisolone, Dexamethasone, Betamethasone, Triamcinolone, Beclometasone, and Fludrocortisone acetate, non-steroidal anti-inflammatory drug (NSAIDs), ibuprofen, naproxen, meloxicam, etodolac, nabumetone, sulindac, tolemtinin, choline magnesium salicylate, diclofenac, diflunisal, indomethicin, Ketoprofen, Oxaprozin, piroxicam, and nimesulide, Salicylates, Aspirin (acetylsalicylic acid), Diflunisal, Salsalate, p-amino phenol derivatives, Paracetamol, Phenacetin, Propionic acid derivatives, Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Acetic acid derivatives, Indomethacin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone, Enolic acid (Oxicam) derivatives, Piroxicam, Meloxicam, Tenoxicam, Droxicam, Loroxicam, Isolexicam, Fenamic acid derivatives (Fenamates), Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid, Selective COX-2 inhibitors (Coxibs), Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, Sulphonanilides, Nimesulide, and Licofelone.

343. The method or use of claim 341, wherein said analgesic agent is selected from the group consisting of NSAIDs, COX-2 inhibitors, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, acetaminophen, opiates, Dextropropoxyphene, Codeine, Tramadol, Anileridine, Pethidine, Hydrocodone, Morphine, Oxycodone, Methadone, Diacetylmorphine, Hydromorphone, Oxymorphone, Levorphanol, Buprenorphine, Fentanyl, Sufentanil, Etorphine, Carfentanil, dihydromorphine, dihydrocodeine, Thebaine, Papaverine, diproqualone, Flupirtine, Tricyclic antidepressants, and lidocaine.

344. The method or use of claim 341, wherein said DMARD is selected from the group consisting of mycophenolate mofetil (CellCept), calcineurin inhibitors, cyclosporine, sirolimus, everolimus, oral retinoids, azathioprine, tumoric acid esters, D-penicillamine, cyclophosphamide, immunoadsorption column, ProSORB(r) column, a gold salt, auranofin, sodium aurothiomalate (Myocrisin), hydroxychloroquine, chloroquine, leflunomide, methotrexate (MTX), minocycline, sulfasalazine (SSZ), tumor necrosis factor alpha (TNFa) blockers, etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira),
certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers, e.g.,
anakinra (Kineret), monoclonal antibodies against B cells, rituximab (Rituxan)), T cell
costimulation blockers, abatacept (Orencia), Interleukin 6 (IL-6) blockers, tocilizumab,
RoActemra, and Actemra.

345. The method or use of claim 343 or 344, wherein said DMARD is not an antibody.

346. The method or use of any one of claims 309-345, wherein the efficacy of said
administration is determined by detecting at least one of the following: (i) improved DAS-
28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR
scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one
or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy
is measured relative to said patient's baseline prior to administration of said antibody or
antibody fragment, relative untreated patients, relative to patients receiving a placebo or
control formulation, or relative to age/gender norms.

347. The method or use of any one of claims 309-346, wherein said administration results in an
improvement in disease as manifested by at least one of the following: (i) improved DAS-
28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR
scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one
or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy
is measured relative to said patient's baseline prior to administration of said antibody or
antibody fragment, relative untreated patients, relative to patients receiving a placebo or
control formulation, or relative to age/gender norms.

348. The method or use of any one of claims 309-347, wherein said administration results in a
prolonged improvement in disease (observed at least 4, 6, 8, 10, 12, 14 or 16 weeks after
antibody administration) as manifested by at least one of the following: (i) improved DAS-
28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR
scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one
or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy
is measured relative to said patient's baseline prior to administration of said antibody or
antibody fragment, relative untreated patients, relative to patients receiving a placebo or
control formulation, or relative to age/gender norms.

349. The method or use of any one of claims 309-348, which improves at least one of the
following: (i) improved DAS-28 scores, (ii) improved EULAR scores, (iii) improved
LDAS scores (iv) improved ACR scores, (v) an increase in serum albumin, (vi) a decrease
in CRP, (vii) improvement in one or more SF-36 domain scores, (viii) an improvement in
SF-6D score, wherein said efficacy is measured relative to said patient's baseline prior to administration of said antibody or antibody fragment, relative untreated patients, relative to patients receiving a placebo or control formulation, or relative to age/gender norms.

350. The method or use of any one of claims 346-349, wherein said improvement in SF-6D score is at least equal to the Minimum Important Difference (MID) relative to the patient's SF-6D prior to said administration.

351. The method or use of any one of claims 346-350, wherein said improvement in SF-6D score is at least twice the MID relative to the patient's SF-6D prior to said administration.

352. The method or use of any one of claims 346-351, wherein said improvement in SF-6D score is at least three times the MID relative to the patient's SF-6D prior to said administration.

353. The method or use of any one of claims 346-352, wherein said improvement in SF-36 comprises an improvement in the physical functioning domain score, said improvement being at least equal to the minimum clinically important difference (MCID), at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

354. The method or use of any one of claims 346-353, wherein said improvement in SF-36 comprises an improvement in the role physical domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

355. The method or use of any one of claims 346-354, wherein said improvement in SF-36 comprises an improvement in the bodily pain domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

356. The method or use of any one of claims 346-355, wherein said improvement in SF-36 comprises an improvement in the general health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

357. The method or use of any one of claims 346-356, wherein said improvement in SF-36 comprises an improvement in the role emotional domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4
times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

358. The method or use of any one of claims 346-357, wherein said improvement in SF-36 comprises an improvement in the vitality domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

359. The method or use of any one of claims 346-358, wherein said improvement in SF-36 comprises an improvement in the social functioning domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

360. The method or use of any one of claims 346-359, wherein said improvement in SF-36 comprises an improvement in the mental health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

361. A composition for treating rheumatoid arthritis comprising a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab 1 or an antibody that competes with Ab 1 for binding to IL-6 to a patient in need thereof that is formulated for subcutaneous administration.

362. The composition of claim 361, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

363. The composition of claim 361, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition that comprises, or alternatively consists of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HCl to make final pH 6, 250 to 280 mM sorbitol or sorbitol in combination with sucrose, and 0.015% (w/w) Polysorbate 80, said formulation having a nitrogen headspace in the shipping vials.

364. The composition of any one of claims 361-363, wherein the concentration of said anti-IL-6 antibody or antibody fragment is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/mL or at least about 10-100 mg/mL.
365. A composition for treating rheumatoid arthritis comprising a therapeutically effective dosage of an anti-IL-6 antibody or antibody fragment having the same epitopic specificity as Ab1 or an antibody that competes with Ab1 for binding to IL-6 to a patient in need thereof that is formulated for intravenous administration.

366. The composition of claim 365, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, anti-IL-6 antibody or antibody fragment, 25 mM Histidine base, Phosphoric acid q.s. to pH 6, and 250 mM sorbitol.

367. The composition of claim 365, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, 12.5 mM Histidine base, 12.5 mM Histidine HC1 (or 25 mM Histidine base and Hydrochloric acid q.s. to pH 6), 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

368. The composition of claim 365, wherein said anti-IL-6 antibody or antibody fragment is contained in a composition comprising, or alternatively consisting of, said anti-IL-6 antibody or antibody fragment, about 5 mM Histidine base, about 5 mM Histidine HC1 to make final pH 6, 250 mM sorbitol, and 0.015% (w/w) Polysorbate 80.

369. The composition of any one of claims 365-367, wherein the concentration of said anti-IL-6 antibody or antibody fragment is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/mL or at least about 10-100 mg/mL.

370. The composition of any one of claims 365-369, which comprises at least about 50 or 100 mg of said anti-IL-6 antibody or antibody fragment.

371. The composition of any one of claims 365-370, which comprises at least about 80 mg, about 160 mg, or about 320 mg of said anti-IL-6 antibody or antibody fragment.

372. The composition of any one of claims 361-371, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 709.

373. The composition of any one of claims 361-372, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising a polypeptide encoded by a polynucleotide that has at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 723.
374. The composition of any one of claims 361-373, wherein said antibody or antibody fragment comprises a heavy chain polypeptide comprising a polypeptide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 657.

375. The composition of any one of claims 361-374, wherein said antibody or antibody fragment comprises a heavy chain polypeptide comprising a polypeptide encoded by a polynucleotide having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96%, at least 97% identity, at least 98%, at least 99% identity, or 100% identity to SEQ ID NO: 700.

376. The composition of any one of claims 361-375, wherein said antibody or antibody fragment comprises a light chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 709, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 723, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 723; and a heavy chain polypeptide comprising: a polypeptide having at least 75% identity to SEQ ID NO: 657, a polypeptide encoded by a polynucleotide that has at least 75% identity to the polynucleotide of SEQ ID NO: 700, a polypeptide encoded by a polynucleotide that hybridizes under medium stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 700, or a polypeptide encoded by a polynucleotide that hybridizes under high stringency conditions to a polynucleotide having the sequence of the reverse complement of SEQ ID NO: 700; wherein the Abl antibody or antibody fragment specifically binds to IL-6 and antagonizes one or more activity associated with IL-6.

377. The composition of any one of claims 361-376, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 90% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

378. The composition of any one of claims 361-377, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 95% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.
The composition of any one of claims 361-378, wherein said anti-IL-6 antibody comprises variable heavy and light chain sequences which are at least 98% identical to the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

The composition of any one of claims 361-379, wherein said anti-IL-6 antibody comprises the variable heavy and light sequences contained in SEQ ID NO: 19 and 20.

The composition of any one of claims 361-380, wherein said anti-IL-6 antibody further comprises the constant light chain sequence contained in SEQ ID NO: 586.

The composition of any one of claims 361-381, wherein said anti-IL-6 antibody comprises the constant heavy chain sequence contained in SEQ ID NO: 588.

The composition of any one of claims 361-382, further comprising methotrexate.

The composition of any one of claims 361-383, further comprising at least one anti-inflammatory agent, analgesic agent, or disease-modifying antirheumatic drug (DMARD).

The composition of claim 384, wherein said anti-inflammatory agent is selected from the group consisting of steroids, Cortisone, Glucocorticoids, prednisone, prednisolone, Hydrocortisone (Cortisol), Cortisone acetate, Methylprednisolone, Dexamethasone, Betamethasone, Triamcinolone, Beclometasone, and Fludrocortisone acetate, non-steroidal anti-inflammatory drug (NSAIDs), ibuprofen, naproxen, meloxicam, etodolac, nabumetone, sulindac, tolemtin, choline magnesium salicylate, diclofenac, diflusinal, indomethin, Ketoprofen, Oxaprozin, piroxicam, and nimesulide, Salicylates, Aspirin (acetylsalicylic acid), Diflunisal, Salsalate, p-amino phenol derivatives, Paracetamol, phenacetin, Propionic acid derivatives, Ibuprofen, Naproxen, Fenoprofen, Ketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen, Acetic acid derivatives, Indomethacin, Sulindac, Etodolac, Keterolac, Diclofenac, Nabumetone, Enolic acid (Oxicam) derivatives, Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lornoxicam, Ioxicam, Fenamic acid derivatives (Fenamates), Mefenamic acid, Meclomenamic acid, Flufenamic acid, Tolfenamic acid, Selective COX-2 inhibitors (Coxibs), Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, Sulphonanilides, Nimesulide, and Licofelone.

The composition of claim 384, wherein said analgesic agent is selected from the group consisting of NSAIDs, COX-2 inhibitors, Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib, acetaminophen, opiates, Dextropropoxyphene, Codeine, Tramadol, Anileridine, Pethidine, Hydrocodone, Morphine, Oxycodone, Methadone, Diacyl morphine, Hydromorphone, Oxymorphone, Levorphanol, Buprenorphine, Fentanyl, Sufentanyl, Etorphine, Carfentanil, dihydromorphine,
dihydrocodeine, Thebaine, Papaverine, diroqualalone, Flupirtine, Tricyclic antidepressants, and lidocaine.

387. The composition of claim 384, wherein said DMARD is selected from the group consisting of mycophenolate mofetil (CellCept), calcineurin inhibitors, cyclosporine, sirolimus, everolimus, oral retinoids, azathioprine, tumeric acid esters, D-penicillamine, cyclophosphamide, immunoadsorption column, Prosorba(r) column, a gold salt, auranofin, sodium aurothiomalate (Myocrisin), hydroxychloroquine, chloroquine, leflunomide, methotrexate (MTX), minocycline, sulfasalazine (SSZ), tumor necrosis factor alpha (TNFa) blockers, etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers, e.g., anakinra (Kineret), monoclonal antibodies against B cells, rituximab (Rituxan)), T cell costimulation blockers, abatacept (Orencia), Interleukin 6 (IL-6) blockers, tocilizumab, RoActemra, and Actemra.

388. The composition of claim 387, wherein said DMARD is not an antibody.

389. The composition of any one of claims 361-388, wherein administration of said composition to a patient in need thereof results in an improvement in at least one of the following: (i) improved DAS-28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy is measured relative to said patient's baseline prior to administration of said antibody or antibody fragment, relative untreated patients, relative to patients receiving a placebo or control formulation, or relative to age/gender norms.

390. The composition of any one of claims 361-389, wherein administration of said composition to a patient in need thereof results in a prolonged improvement in disease (observed at least 4, 6, 8, 10, 12, 14 or 16 weeks after antibody administration) as manifested by at least one of the following: (i) improved DAS-28 scores, (ii) improved EULAR scores, (iii) improved LDAS scores (iv) improved ACR scores, (v) an increase in serum albumin, (vi) a decrease in CRP, (vii) improvement in one or more SF-36 domain scores, (viii) an improvement in SF-6D score, wherein said efficacy is measured relative to said patient's baseline prior to administration of said antibody or antibody fragment, relative untreated patients, relative to patients receiving a placebo or control formulation, or relative to age/gender norms.

391. A composition according to claim 390, wherein said improvement in SF-6D score is at least equal to the Minimum Important Difference (MID) relative to the patient's SF-6D prior to said administration.
392. A composition according to claim 390 or 391, wherein said improvement in SF-6D score is
at least twice the MID relative to the patient's SF-6D prior to said administration.
393. A composition according to claim 390 or 391, wherein said improvement in SF-6D score is
at least three times the MID relative to the patient's SF-6D prior to said administration.
394. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the physical functioning domain score, said improvement being at least equal to the minimum clinically important difference (MCID), at least 2
times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the
MCID, or at least 6 times the MCID for that domain score.
395. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the role physical domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4
times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain
score.
396. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the bodily pain domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4
times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain
score.
397. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the general health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4
times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain
score.
398. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the role emotional domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4
times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain
score.
399. A composition according to claim 390 or 391, wherein said improvement in SF-36
comprises an improvement in the vitality domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times
the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.
400. A composition according to claim 390 or 391, wherein said improvement in SF-36 comprises an improvement in the social functioning domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

401. A composition according to claim 390 or 391, wherein said improvement in SF-36 comprises an improvement in the mental health domain score, said improvement being at least equal to the MCID, at least 2 times the MCID, at least 3 times the MCID, at least 4 times the MCID, at least 5 times the MCID, or at least 6 times the MCID for that domain score.

402. A method for treating rheumatoid arthritis comprising administering a composition comprising at least about 10 mg/mL of an anti-IL-6 antibody to a patient in need thereof.

403. Use of an anti-IL-6 antibody for preparing a pharmaceutical composition comprising at least about 10 mg/mL of said anti-IL-6 antibody for use in treating rheumatoid arthritis to a patient in need thereof.

404. The method or use of claim 402 or 403, wherein said composition comprising at least about 20, 30, 40, 50, 60, 70, 80, or 100 mg/mL of an anti-IL-6 antibody.

405. The method or use of claim 404, wherein said composition comprises at least about 10-100 mg/mL of an anti-IL-6 antibody.

406. The method or use of claim 404, wherein said composition is administered subcutaneously and comprises at least about 100 mg/mL of an anti-IL-6 antibody.

407. The method or use of claim 405, wherein said composition is administered intravenously and comprises at least about 10, 20, 30, or 40 mg/mL, or 10-40 mg/mL of an anti-IL-6 antibody.

408. The method or use of any one of claims 402-407, wherein said anti-IL-6 antibody or antibody fragment thereof, is Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab1O, Ab1, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody or antibody fragment thereof, specifically binds to IL-6.

409. A composition for treating rheumatoid arthritis comprising at least about 10 mg/mL of an anti-IL-6 antibody to a patient in need thereof.

410. The composition of claim 409, wherein said composition comprising at least about 20, 30, 40, 50, 60, 70, 80, or 100 mg/mL of an anti-IL-6 antibody.
411. The composition of claim 409, wherein said composition comprises at least about 10-100 mg/mL of an anti-IL-6 antibody.
412. The composition of claim 409, wherein said composition is formulated for subcutaneous administration and comprises at least about 100 mg/mL of an anti-IL-6 antibody.
413. The composition of claim 409, wherein said composition is formulated for intravenous administration and comprises at least about 10, 20, 30, or 40 mg/mL, or 10-40 mg/mL of an anti-IL-6 antibody.
414. The method or use of any one of claims 409-413, wherein said anti-IL-6 antibody or antibody fragment thereof, is Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36 antibody, or an antibody fragment thereof, to a subject in need thereof, wherein the antibody or antibody fragment thereof, specifically binds to IL-6.
415. The method or use of claim 414, wherein said anti-IL-6 antibody or antibody fragment thereof, contains one, two, three, four, five or six of the CDR's of an anti-IL-6 antibody or antibody fragment thereof, is Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36.
416. The method or use of claim 415, wherein said anti-IL-6 antibody or antibody fragment thereof, contains 2 or 3 of the heavy chain CDRs in an anti-IL-6 antibody or antibody fragment thereof, selected from Abl, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36.
417. The method or use of claim 415, wherein said anti-IL-6 antibody or antibody fragment thereof, contains 2 or 3 of the light chain CDRs in an anti-IL-6 antibody or antibody fragment thereof, selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36.
418. The method or use of claim 416, wherein said anti-IL-6 antibody or antibody fragment thereof, further contains 2 or all 3 of the light chain CDRs in an anti-IL-6 antibody or antibody fragment thereof, selected from Ab1, Ab2, Ab3, Ab4, Ab5, Ab6, Ab7, Ab8, Ab9, Ab10, Ab11, Ab12, Ab13, Ab14, Ab15, Ab16, Ab17, Ab18, Ab19, Ab20, Ab21, Ab22, Ab23, Ab24, Ab25, Ab26, Ab27, Ab28, Ab29, Ab30, Ab31, Ab32, Ab33, Ab34, Ab35, or Ab36.
**FIGURE 2 – PREFERRED ANTI-IL-6 ANTIBODY HUMANIZATION**

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**FIGURE 3 – PREFERRED ANTI-IL-6 ANTIBODY HUMANIZATION**

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**FIGURE 4A – Alignment of Ab1 light chains**

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FIGURE 4B – Alignment of Ab1 light chains (continued)

FR4

**kappa constant light chain**

| SEQ ID NO:2  | FGGGTEVVVKR T VAAPSVFIFPPSDEQLKSGTASVVCLLNN |
| SEQ ID NO:20 | FGGGTEVVVKR |
| SEQ ID NO:647| FGGGTEVVVKR |
| SEQ ID NO:651| FGGGTKVEIKR |
| SEQ ID NO:660| FGGGTKVEIKR T VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN |
| SEQ ID NO:666| FGGGTKVEIKR T VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN |
| SEQ ID NO:699| FGGGTKVEIKR T |
| SEQ ID NO:702| FGGGTKVEIKR T VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN |
| SEQ ID NO:706| FGGGTKVEIKR T VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN |
| SEQ ID NO:709| FGGGTKVEIKR |

**kappa constant light chain (continued)**

| SEQ ID NO:2  | SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:20 | SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:647| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:651| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:660| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:666| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:699| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:702| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
| SEQ ID NO:706| SQESVTEQDSKDSYSLSSTLTLSKADYEHKVYACEVTHQGLSSPVTSFNRGEC |
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### FIGURE 5A – Alignment of Ab1 heavy chains

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<td>WQGTLTVTSS</td>
</tr>
<tr>
<td>656</td>
<td>IYYG-SDEATAYATWAIG</td>
<td>RFTISRDNSKNTLYQMNLSRAEDTAVYYCAR</td>
<td>DDSSDWDAKFNL</td>
<td>WQGTLTVTSS</td>
</tr>
<tr>
<td>657</td>
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<td>DDSSDWDAKFNL</td>
<td>WQGTLTVTSS</td>
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<td>658</td>
<td>IYYG-SDEATAYATWAIG</td>
<td>RFTISKTST--TVDLMKMTSLTAADTATYFCAR</td>
<td>DDSSDWDAKFNL</td>
<td>WQGTLTVTSS</td>
</tr>
<tr>
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<td>IYYG-SDEATAYATWAIG</td>
<td>RFTISKTST--TVDLMKMTSLTAADTATYFCAR</td>
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<td>WQGTLTVTSS</td>
</tr>
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<td>RFTISRDNSKNTLYQMNLSRAEDTAVYYCAR</td>
<td>DDSSDWDAKFNL</td>
<td>WQGTLTVTSS</td>
</tr>
<tr>
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<td>RFTISRDNSKNTLYQMNLSRAEDTAVYYCAR</td>
<td>DDSSDWDAKFNL</td>
<td>WQGTLTVTSS</td>
</tr>
</tbody>
</table>
FIGURE 5B – Alignment of Ab1 heavy chains, continued

gamma-1 constant heavy chain polypeptide

SEQ ID NO: 3  ASTKGPSVFPLAPSSKSTSGTAALGCLVK
SEQ ID NO: 658  ASTKGPSVFPLAPSSKSTSGTAALGCLVK

SEQ ID NO: 664  ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPFETVSVNLAGLTSVHFPAVLQSSGLYSLSSVTVSSS
SEQ ID NO: 665  ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPFETVSVNLAGLTSVHFPAVLQSSGLYSLSSVTVSSS
SEQ ID NO: 704  ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPFETVSVNLAGLTSVHFPAVLQSSGLYSLSSVTVSSS
SEQ ID NO: 705  ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPFETVSVNLAGLTSVHFPAVLQSSGLYSLSSVTVSSS
SEQ ID NO: 708  ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPFETVSVNLAGLTSVHFPAVLQSSGLYSLSSVTVSSS

gamma-1 constant heavy chain polypeptide, continued

SEQ ID NO: 664  LTGQTYICNVMHNPSNTKVDKVEPKSDKHTCPCPEAPEELGGPSVFLLFPKPKDPDTLMISRTPSVTCVVDVS
SEQ ID NO: 665  LTGQTYICNVMHNPSNTKVDKVEPKSDKHTCPCPEAPEELGGPSVFLLFPKPKDPDTLMISRTPSVTCVVDVS
SEQ ID NO: 704  LTGQTYICNVMHNPSNTKVDKVEPKSDKHTCPCPEAPEELGGPSVFLLFPKPKDPDTLMISRTPSVTCVVDVS
SEQ ID NO: 708  LTGQTYICNVMHNPSNTKVDKVEPKSDKHTCPCPEAPEELGGPSVFLLFPKPKDPDTLMISRTPSVTCVVDVS

gamma-1 constant heavy chain polypeptide, continued

SEQ ID NO: 664  HEDPQVFENWVVGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQ
SEQ ID NO: 665  HEDPQVFENWVVGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQ
SEQ ID NO: 708  HEDPQVFENWVVGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQ

gamma-1 constant heavy chain polypeptide, continued

SEQ ID NO: 664  PREPQVFYTLPSDELTKNQVSLSLCLVKGYFPSIDAEWESNSQPENYKTPFPLDSDGSFFLYSKLTVKSRW
SEQ ID NO: 665  PREPQVFYTLPSDELTKNQVSLSLCLVKGYFPSIDAEWESNSQPENYKTPFPLDSDGSFFLYSKLTVKSRW
SEQ ID NO: 704  PREPQVFYTLPSDELTKNQVSLSLCLVKGYFPSIDAEWESNSQPENYKTPFPLDSDGSFFLYSKLTVKSRW
SEQ ID NO: 708  PREPQVFYTLPSDELTKNQVSLSLCLVKGYFPSIDAEWESNSQPENYKTPFPLDSDGSFFLYSKLTVKSRW

gamma-1 constant heavy chain polypeptide, continued

SEQ ID NO: 664  QGNNVFSCVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 665  QGNNVFSCVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 704  QGNNVFSCVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 708  QGNNVFSCVMHEALHNHYTQKSLSLSPGK
\[ \text{ID}_{50} = 0.09273 \text{ mg/kg} \]
\[ \chi^2 = 0.9701 \]

Log Dose [Ab1]

A2M 24h (ng/ml)

Control

0.3 mg/kg [Ab1]

0.1 mg/kg [Ab1]

1 mg/kg [Ab1]

3 mg/kg [Ab1]

FIG. 6
FIG. 13

Neutrophil Count (x10^9/l)

Time (Weeks)

FIG. 14A

Median Serum CRP Concentration (µg/ml)

Time (h)

- Placebo i.v. (n=14)
- Ab1 10mg i.v. (n=6)
- Ab1 1mg i.v. (n=6)
- Ab1 30mg i.v. (n=5)
- Ab1 3mg i.v. (n=6)
- Ab1 1000mg i.v. (n=5)
**FIG. 14B**

- □ Placebo i.v. (n=14)
- ◇ Ab1 10mg i.v. (n=6)
- △ Ab1 1mg i.v. (n=6)
- ○ Ab1 30mg i.v. (n=5)
- ◀ Ab1 3mg i.v. (n=6)
- ■ Ab1 100mg i.v. (n=5)

**FIG. 15A**

- □ Ab1 80mg (n=29)
- △ Ab1 160mg (n=32)
- ◀ Ab1 320mg (n=32)
- ○ Placebo (n=31)
Fig. 15B: Graph showing the median CRP concentration over time for different treatment groups.

Fig. 16: Graph showing the mean CRP plasma concentration over time for a placebo group.
FIG. 18B

Mean Change from Baseline Hemoglobin (g/dl)

- □ Ab1 80 mg (n=29)
- ▼ Ab1 320 mg (n=32)
- △ Ab1 160 mg (n=32)
- ○ Placebo (n=31)

Time (Weeks)

FIG. 18C

Hemoglobin Concentration (g/l)

- □ Ab1 320 mg (n=11)
- ▼ Ab1 80 mg (n=10)
- △ Ab1 160 mg (n=9)
- ○ Placebo (n=8)

Time (Weeks)
Figure 20C

- Ab1 320mg (n=7)
- Ab1 80mg (n=10)
- Ab1 160mg (n=8)
- Placebo (n=10)

Albumin Concentration (g/l) vs Time (Weeks)
*p<0.05 versus placebo using Wilcoxon Rank Sum Test. SC=subcutaneous; IV=intravenous

FIG. 21A
Day 10

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Isotype Control</th>
<th>Anti-IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Ulceration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulceration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIG. 22

Day 10

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Days Ulcerated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 Gy</td>
<td>2</td>
<td>0.0134</td>
</tr>
<tr>
<td>30 Gy</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>35 Gy</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>40 Gy</td>
<td>8</td>
<td>0.0237</td>
</tr>
<tr>
<td>45 Gy</td>
<td>8</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

FIG. 23

SUBSTITUTE SHEET (RULE 26)
Changes from Baseline to Week 12 in SF-36 Domain Scores Compared with Age/Gender Matched Norms

* p<0.05 for ALD-518 dose versus placebo at week 12; ** p<0.05 for ALD-518 dose versus placebo at week 12 and maintained to week 16.
*p<0.05 for ALD-518 dose versus placebo at week 12; †p<0.05 for ALD-518 dose versus placebo at week 12 and maintained to week 16;

Changes from Baseline to Week 12 in SF-36 Domain Scores Compared with Age/Gender Matched Norms

FIG. 28C

FIG. 28D
Changes from Baseline to Week 12 and 16 in SF-36 Domain Scores Compared with Age/Gender Matched Norms

**FIG. 29A**

**FIG. 29B**
WHO oral mucositis grade versus cumulative IMRT (Gy): ALD518 160 mg intravenous at week 0 and week 4

FIG. 30