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(54) Title: SUPER-2 COMPLEXES WITH ANTIBODY TO AUGMENT IL-2 THERAPY

(57) Abstract: Disclosed herein are cytokine compositions with enhanced biological activity and methods for their use. These com-
positions encompass interleukin-2 (IL-2) muteins in combination with antibodies capable of binding these muteins. The disclosed
methods encompass administering a combination of IL-2 mutein with antibody to treat neoplastic diseases, autoimmune diseases, in-
fectious diseases or for expanding a hematopoietic cell population.



SUPER-2 COMPLEXES WITH ANTIBODY TO AUGMENT IL-2 THERAPY

BACKGROUND

[0001] Interleukin 2 (IL-2) is a pluripotent cytokine produced primarily by activated CD4⁺ T cells and plays a crucial role in producing a normal immune response. IL-2 promotes proliferation and expansion of activated T lymphocytes, potentiates B cell growth, and activates monocytes and natural killer cells. It was by virtue of these activities that IL-2 was tested and is used as an approved treatment of cancer (aldesleukin, Proleukin®).

[0002] In eukaryotic cells human IL-2 is synthesized as a precursor polypeptide of 153 amino acids, from which 20 amino acids are removed to generate mature secreted IL-2 (Taniguchi 1983). Recombinant human IL-2 has been produced in *E. coli* (Rosenberg 1984), in insect cells (Smith 1985) and in mammalian COS cells (Taniguchi 1983).

[0003] IL-2 works by interacting with three different receptors: the interleukin 2 receptor alpha (IL-2R α ; CD25), the interleukin 2 receptor beta (IL-2R β ; CD122), and the interleukin 2 receptor gamma (IL-2R γ ; CD132; common gamma chain). The first receptor to be identified was the IL-2R α , which is a 55 kD polypeptide (p55) that appears upon T cell activation and was originally called Tac (for T activation) antigen. The IL-2R α binds IL-2 with a K_d of approximately 10^{-8} M, and is also known as the “low affinity” IL-2 receptor. Binding of IL-2 to cells expressing only the IL-2R α does not lead to any detectable biologic response.

[0004] The IL-2R β is a member of the type I cytokine receptor family characterized by the two cysteine/WSXWS motif. The IL-2R β is expressed coordinately with the IL-2R γ . The IL-2R γ , a 64 kD polypeptide, is also known as the common γ chain because it is shared among a number of cytokine receptors, including the receptor for interleukin-4 and interleukin-7. The IL-2R $\beta\gamma$ is the same signaling receptor complex that can bind to IL-15.

[0005] The low affinity IL-2 receptor is less sensitive to IL-2 than the high affinity IL-2 receptor. Thus IL-2 efficacy requires the administration of relatively high doses. Unfortunately, these doses can cause vascular leak syndrome (VLS). Upon antigen receptor-mediated T cell activation, the IL-2R α is rapidly expressed. Once the IL-2R α binds IL-2, it

then sequentially engages the IL-2R β and the IL-2R γ (Figure 1). IL-2 binding by the IL-2R $\alpha\beta\gamma$ complex results in signal transduction through a Jak/STAT signaling pathway and IL-2 mediated growth stimulation.

[0001] IL-2 efficacy requires the administration of relatively high doses (as discussed herein). Unfortunately, these doses can cause vascular leak syndrome (VLS), resulting in the accumulation of intravascular fluid in the lungs and liver and ultimately pulmonary edema and liver cell damage. Lower dose IL-2 regimens have been tested to avoid VLS, but the therapeutic results are suboptimal. Accordingly, the use of IL-2 as an antineoplastic agent has been limited by the serious toxicities that accompany the doses necessary for a tumor response.

[0002] The major side effect of IL-2 therapy is VLS, which leads to the accumulation of intravascular fluid in the lungs and liver resulting in pulmonary edema and liver damage as described above. Until recently it was believed that VLS was caused by the release of proinflammatory cytokines from IL-2 activated NK cells. However, a recent report points to the direct binding of IL-2 to lung endothelial cells, as a purported cause of VLS. (Krieg *et al.*, PNAS USA 107(26):11906-11911 (2010). In principle, an IL-2 variant with high affinity for IL-2R β , whose activity was not dependent on CD25 expression could have improved clinical utility and reduced toxicity.

[0006] IL-2 receptors (IL-2Rs) are either trimers composed of α (CD25), β (CD122), and γ (CD132, common γ chain), that is, $\alpha\beta\gamma$ IL-2Rs or dimers of $\beta\gamma$, $\beta\gamma$ IL-2Rs. Both forms are signaling competent, but the trimeric form possesses 10-100 fold higher affinity for IL-2 than the dimeric form.

[0007] A recent report points to the direct binding of IL-2 to lung endothelial cells as a potential cause of VLS. Krieg *et al.* (2010) PNAS USA 107(26):11906-11911. Complexes of IL-2 and a specific anti-IL-2 antibody were found to reduce VLS, while an anti-tumor response was still observed. This anti-IL-2 antibody purportedly interferes with the interaction of IL-2 with IL-2 receptor α (CD25).

[0008] IL-2 variants that preferentially bind β (CD122) have been developed to improve the clinical utility and reduce the toxicity associated with wild type IL-2, such as VLS (US Pat. App. No. 13/997,503, incorporated by reference in its entirety). Surprisingly, it is now disclosed herein that the efficacy of these IL-2 variants (muteins) is enhanced by interaction with anti-IL-2 antibodies and the formation of IL-2 mutein/antibody complexes.

[0009] Such findings not only support the use of the disclosed complexes in directly treating cancers, such as metastatic melanoma and metastatic renal cell carcinoma, but also treating immunodeficiency, such as HIV or human SCID patients; treating infectious disease, such as tuberculosis; its use as an adjuvant in “cancer vaccine” strategies; and for immune system stimulation indications, such as enhancing standard vaccination protocols (*e.g.*, elderly).

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1A and 1B** illustrate expansion of lymphocyte subsets after stimulation with IL-2 superkine or IL-2 superkine/mAb complexes. (A) Sorted carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled Thyl.1⁺ memory phenotype (MP) CD8⁺ cells were transferred to wild-type (WT) recipients, followed by daily injections of PBS, IL-2, H9 (IL-2 superkine), F42A (IL-2 containing the F42A mutation), or F42A-H9 alone; IL-2, H9, F42A, or F42A-H9, each complexed with anti-IL-2 monoclonal antibody (mAb) MAB602; or IL-2, H9, F42A, F42A-H9, each complexed with anti-IL-2 mAb5344 for 5 days. On day 6, spleen cells were analyzed for CFSE profiles of donor Thyl.1⁺ CD8⁺ cells (left column) and host CD25⁺ CD4⁺ regulatory T cells (right column). (B) Total cell numbers of host CD122^{high} MP CD8⁺ T cells, CD25⁺ CD4⁺ regulatory T cells, and CD3⁺CD122^{high} NK1.1⁺ NK cells in spleen of animals treated as in A. Numbers in histograms represent the percentage of divided (CFSE^{low}) donor cells.

[0011] **Figure 2A and 2B** illustrate affinity of anti-human IL-2 monoclonal antibodies MAB602 and 5344 to IL-2, IL-2 superkine (H9), and F42A as measure by enzyme-linked immunosorbent assay (ELISA). (A) Anti-human IL-2 MAB602 binds IL-2 and IL-2 superkine H9 with the same affinity (EC₅₀) of about 3nM but shows reduced affinity towards IL-2 carrying the F42A mutation (F42A). (B) Anti-human IL-2 mAb 5344 binds IL-2 and F42A with similar affinity (EC₅₀ of about 7.5nM), while binding of mAb 5344 to H9 is decreased by 4.5 fold compared to IL-2.

BRIEF SUMMARY OF THE INVENTION

[0012] IL-2 exerts a wide spectrum of effects on the immune system, and it plays crucial roles in regulating both immune activation and homeostasis. As an immune system stimulator, IL-2 has found use in the treatment of cancer and chronic viral infections. The

stimulatory affects of IL-2 can also cause havoc, mediating autoimmunity and transplant rejection. Because of its instrumental role in immune regulation and disease, the identification of molecules and methods that reduce the toxicities associated with IL-2 therapy while maintaining or improving its efficacy remains an active area of research.

[0013] In one embodiment, a composition is provided. The composition comprises an IL-2 mutein and an antibody, wherein the IL-2 mutein binds IL-2R β with higher affinity than wild-type IL-2 and wherein the antibody specifically binds IL-2 or an IL-2 mutein. In an embodiment, the IL-2 mutein comprises one or more amino acid substitutions at positions 24, 65, 74, 80, 81, 85, 86, 89, 92, and/or 93, wherein the amino acid numbering is in accordance with wild-type human IL-2. In an embodiment, the composition further comprises a substitution at amino acid position 42. In an embodiment, the antibody competes for binding with one or more of the following antibodies: S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12. In an embodiment, the antibody is a human antibody or humanized antibody. In an embodiment, the antibody is S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12. In an embodiment the antibody binds with higher affinity to the IL-2 mutein than IL-2. In an embodiment, the antibody comprises an Fc region and wherein the Fc region is an Fc mutein.

[0014] In another embodiment, a pharmaceutical composition is provided. The composition comprises the IL-2 mutein and an antibody as described herein.

[0015] In another embodiment, a method for treating an autoimmune disorder in a subject is provided. The method comprises the administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein.

[0016] In another embodiment, a method for treating an immune cell deficiency in a subject is provided. The method comprises administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein.

[0017] In another embodiment, a method for treating cancer in a subject is provided. The method comprises the administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein. In an embodiment, the IL-2 mutein is administered to the subject prior to the administration of the antibody, or wherein the IL-2 mutein and the antibody are administered simultaneously, or wherein the antibody is administered prior to the IL-2 mutein. In an embodiment, the IL-2 mutein and the antibody are combined with a second therapeutic agent. In an embodiment, the second therapeutic agent is wild-type IL-2.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0018] Stimulation of the immune system with IL-2 is an attractive treatment option for a number of maladies, including certain metastatic cancers. However, administration of IL-2 can lead to severe toxic effects including pulmonary edema. Pulmonary edema is thought to result from binding of IL-2 to lung endothelial cells, which express low to intermediate levels of the high affinity $\alpha\beta\gamma$ interleukin-2 receptor (IL-2R).

[0019] The use of IL-2 as an antineoplastic agent has been limited by the serious toxicities that accompany the doses necessary for a tumor response. The major side effect of IL-2 therapy is vascular leak syndrome (VLS), which leads to the accumulation of intravascular fluid in the lungs and liver resulting in pulmonary edema and liver damage. Until recently it was believed that VLS was caused by the release of proinflammatory cytokines from IL-2 activated NK cells. However, a recent report points to the direct binding of IL-2 to lung endothelial cells, as a purported cause of VLS. (Krieg *et al.*, PNAS USA 107(26)11906-11911 (2010). In principle, an IL-2 variant with high affinity for IL-2R $\beta\gamma$, whose activity was not dependent on CD25 expression could have improved clinical utility and reduced toxicity.

[0020] One IL-2 mutein of clinical interest is BAY 50-4798, which differs from wild-type IL-2 by the substitution of arginine for asparagine at position 88 (R88N) (Steppan *et al.* (2006) *J. Interferon and Cytokine Res.*, 26(3): 171-. This modification allegedly results in an IL-2 mutein with relatively reduced binding to the IL-2R $\beta\gamma$, and thought to possess lower toxicity relative to wild type IL-2. However, a clinical study found that patients receiving BAY 50-4798 experienced a similar degree of IL-2 mediated VLS.

[0021] For these reasons, it is clear that IL-2 muteins that exhibit unique properties are needed. Potential uses of such muteins include treating cancer (as a direct and/or adjunct therapy) and immunodeficiency (e.g., HIV and tuberculosis). Other potential uses of IL-2 are derived from its immunostimulatory activity, and include direct treatment of cancer, treating immunodeficiency, such as HIV or human SCID patients; treating infectious disease, such as tuberculosis; its use as an adjuvant in "cancer vaccine" strategies; and for immune system stimulation indications, such as enhancing standard vaccination protocols (e.g., elderly). For example, IL-2 muteins that exhibit reduced VLS would be advantageous.

[0022] IL-2R can exist as a trimer of subunits termed α (CD25), β (CD122), and the common gamma chain (γ , γ_c , CD132) or as a dimer of $\beta\gamma$. β and γ are crucial for signal transduction, whereas α is not essential for IL-2 signaling but rather confers high-affinity binding of IL-2 to its receptor, thereby increasing receptor affinities by about 10- to 100-fold.

[0023] High-affinity $\alpha\beta\gamma$ IL-2Rs are typically found on CD4⁺ T regulatory cells (Tregs) as well as recently-activated T cells. Low-affinity $\beta\gamma$ IL-2Rs are present at a low level on naïve CD8 cells but are prominent on antigen-experienced (memory) and memory-phenotype (MP) CD8⁺ T cells as well as natural killer (NK) cells. Both MP CD8⁺ T cells and NK cells express relatively high levels of β and readily respond to IL-2 injections *in vivo*.

[0024] Most cells, for example, resting T cells are less sensitive to IL-2 since they only express the low affinity IL-2R composed of $\beta\gamma$. Thus stimulation of low affinity IL-2 expressing cells needs high doses of IL-2. However, upon antigen receptor-mediated T cell activation, α is rapidly expressed. Once α binds IL-2, it then sequentially engages β and γ . The formed trimer complex results in signal transduction through a Jak/STAT signaling pathway and IL-2 mediated growth stimulation.

[0025] Accordingly, the combination treatment of an IL-2 mutein that preferentially bind β (CD122) and an anti-IL-2 antibody form IL-2 mutein/antibody complexes are extremely beneficial for the treatment of disease.

[0026] In order for the present disclosure to be more readily understood, certain terms and phrases are defined below as well as throughout the specification. The definitions provided herein are non-limiting and should be read in view of what one of skill in the art would know at the time of invention.

Definitions

[0027] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*see generally*, Sambrook *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (1989) Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference), which are provided throughout this document. The nomenclature used herein and the laboratory procedures of analytical and synthetic organic chemistry described below are those well known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0028] As used herein, "IL-2" means wild-type IL-2, whether native or recombinant. Mature human IL-2 occurs as a 133 amino acid sequence (less the signal peptide, consisting of an additional 20 N-terminal amino acids), as described in Fujita, *et. al.*, PNAS USA, 80, 7437-7441 (1983). The amino acid sequence of human IL-2 (SEQ ID NO: 1) is found in Genbank under accession locator NP_000577.2. The amino acid sequence of mature human IL-2 is depicted in SEQ ID NO: 2. The murine (*Mus musculus*) IL-2 amino acid sequence is found in Genbank under accession locator (SEQ ID NO: 3). The amino acid sequence of mature murine IL-2 is depicted in SEQ ID NO: 4.

SEQ ID NO: 1

MYRMQLLSIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTR
MLTFKFYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLEL
KGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT

SEQ ID NO: 2

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE
FLNRWITFCQSIISTLT

SEQ ID NO: 3

MYSMQLASCVTLTLVLLVNSAPTSSSTSSSTAEEAQQQQQQQQQQQHLEQLLMDLQ
ELLSRMENYRNKLPRMLTFKFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQ
LEDAENFISNIRVTVVKLKGS DNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ

SEQ ID NO: 4

APTSSSTSSSTAEEAQQQQQQQQQQQHLEQLLMDLQELLSRMENYRNKLPRMLTF
KFYLPKQATELKDLQCLEDELGPLRHVLDLTQSKSFQLEDAENFISNIRVTVVKLKGS
DNTFECQFDDESATVVDFLRRWIAFCQSIISTSPQ

[0029] As used herein, “IL-2 mutein” or “mutant IL-2” or “IL-2 mutant” means a polypeptide wherein specific substitutions to the interleukin-2 protein have been made. As used in reference to administrative modalities and treatments, the term “IL-2 mutein” means 1, 2, 3, 4, or 5 or more IL-2 muteins. For example, treatment using an IL-2 mutein may refer to treatment with a single IL-2 mutein, or a combination of multiple IL-2 muteins.

[0030] Figure 3, for example, discloses twelve IL-2 muteins and their corresponding relative binding affinity for the IL-2R β . The IL-2 muteins can also be characterized by amino acid insertions, deletions, substitutions and modifications at one or more sites in or at the other residues of the native IL-2 polypeptide chain. In accordance with this disclosure any such insertions, deletions, substitutions and modifications result in an IL-2 mutein that retains the IL-2R β binding activity. Exemplary muteins can include substitutions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids.

[0031] Muteins also include conservative modifications and substitutions at other positions of IL-2 (*e.g.*, those that have a minimal effect on the secondary or tertiary structure of the mutein). Such conservative substitutions include those described by Dayhoff in *The Atlas of Protein Sequence and Structure* 5 (1978), and by Argos in *EMBO J.*, 8:779-785 (1989). For example, amino acids belonging to one of the following groups represent conservative changes: Group I: ala, pro, gly, gln, asn, ser, thr; Group II: cys, ser, tyr, thr; Group III: val, ile, leu, met, ala, phe; Group IV: lys, arg, his; Group V: phe, tyr, trp, his; and Group VI: asp, glu.

[0032] As used herein, the term “anti-IL-2 antibody” as used in reference to administrative modalities and treatments, means 1, 2, 3, 4, or 5 or more anti-IL-2 antibodies. For example, treatment using an IL-2 mutein may refer to treatment with a single anti-IL-2 antibody, or a combination of multiple anti-IL-2 antibodies.

[0033] “Numbered in accordance with IL-2” means identifying a chosen amino acid with reference to the position at which that amino acid normally occurs in the mature sequence of wild type IL-2, for example R81 refers to the eighty-first amino acid, arginine, that occurs in SEQ ID NO: 2.

[0034] The term “complex” refers to the binding and non-binding association between an anti-IL-2 antibody and an IL-2 mutein. In certain embodiments, the complex may be formed by ionic or covalent bonds. In certain embodiments, the complex may be formed by non-

ionic and non-covalent bonding. In specific embodiments, the non-ionic and non-covalent bonding includes van der Waals forces or hydrophobic forces.

[0035] The term "identity," as used herein in reference to polypeptide or DNA sequences, refers to the subunit sequence identity between two molecules. When a subunit position in both of the molecules is occupied by the same monomeric subunit (*e.g.*, the same amino acid residue or nucleotide), then the molecules are identical at that position. The similarity between two amino acid or two nucleotide sequences is a direct function of the number of identical positions. In general, the sequences are aligned so that the highest order match is obtained. If necessary, identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux et al., *Nucleic Acids Res.* 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul et al., *J. Molecular Biol.* 215:403, 1990). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof.

[0036] The term "polypeptide," "protein" or "peptide" refer to any chain of amino acid residues, regardless of its length or post-translational modification (*e.g.*, glycosylation or phosphorylation).

[0037] By "protein variant" or "variant protein" or "variant polypeptide" herein is meant a protein that differs from a wild-type protein by virtue of at least one amino acid modification. The parent polypeptide may be a naturally occurring or wild-type (WT) polypeptide, or may be a modified version of a WT polypeptide. Variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, *e.g.* from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent.

[0038] By "parent polypeptide", "parent protein", "precursor polypeptide", or "precursor protein" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. A parent polypeptide may be a wild-type polypeptide, or a variant or engineered version of a wild-type polypeptide. Parent polypeptide may refer to the

polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it.

[0039] By "wild type" or "WT" or "native" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[0040] In the event the mutant IL-2 polypeptides of the disclosure are "substantially pure," they can be at least about 60% by weight (dry weight) the polypeptide of interest, for example, a polypeptide containing the mutant IL-2 amino acid sequence. For example, the polypeptide can be at least about 75%, about 80%, about 85%, about 90%, about 95% or about 99%, by weight, the polypeptide of interest. Purity can be measured by any appropriate standard method, for example, column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0041] As used herein, we may use the terms "cancer" (or "cancerous"), "hyperproliferative," and "neoplastic" to refer to cells having the capacity for autonomous growth (*e.g.*, an abnormal state or condition characterized by rapidly proliferating cell growth). Hyperproliferative and neoplastic disease states may be categorized as pathologic (*e.g.*, characterizing or constituting a disease state), or they may be categorized as non-pathologic (*e.g.*, as a deviation from normal but not associated with a disease state). The terms are meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair. The terms "cancer" or "neoplasm" are used to refer to malignancies of the various organ systems, including those affecting the lung, breast, thyroid, lymph glands and lymphoid tissue, gastrointestinal organs, and the genitourinary tract, as well as to adenocarcinomas which are generally considered to include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

[0042] The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system

carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

[0043] As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, *e.g.*, arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias (*e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia). Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) Crit Rev. in Oncol./Hematol. 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Sternberg disease.

IL-2 Muteins

[0044] In various embodiments, the present disclosure provides IL-2 polypeptides, which may be, but are not necessarily, substantially purified and that can function as an agonist of wild-type IL-2; carrying out one or more of the biological activities of IL-2 (*e.g.*, stimulation of cellular proliferation)). IL-2 has been characterized as a T cell growth factor that induces proliferation of antigen-activated T cells and stimulation of NK cells.

[0045] Also, described are IL-2 polypeptides that can function as an antagonist of wild-type IL-2; that is, preventing the biological activity of IL-2.

[0046] An exemplary mutant IL-2 polypeptide includes an amino acid sequence that is at least about 80% identical to SEQ ID NO:2 which binds the IL-2R β with an affinity that is greater than the affinity with which the polypeptide represented by SEQ ID NO: 2 binds the IL-2R β . For example, a mutant IL-2 polypeptide can have at least one mutation (*e.g.*, a deletion, addition, or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,

19, 20, or more amino acid residues) relative to a wild-type IL-2, and that binds the IL-2R β with higher affinity than a wild-type IL-2.

[0047] An exemplary mutant IL-2 polypeptide can also include an amino acid sequence that is at least about 80% identical to SEQ ID NO: 2 and that binds to an IL-2 receptor γ (IL-2R γ) with an affinity that is less than the affinity with which the polypeptide represented by SEQ ID NO: 2 binds the IL-2R γ . For example, a mutant IL-2 polypeptide can have at least one mutation (*e.g.*, a deletion, addition, or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acid residues) relative to a wild-type IL-2, and that binds the IL-2R γ with lower affinity than a wild-type IL-2.

[0048] Exemplary mutant IL-2 polypeptides can be at least about 50%, at least about 65%, at least about 70%, at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, or at least about 99% identical to wild-type IL-2. The mutation can consist of a change in the number or content of amino acid residues. For example, the mutant IL-2 can have a greater or a lesser number of amino acid residues than wild-type IL-2. Alternatively, or in addition, an exemplary mutant polypeptide can contain a substitution of one or more amino acid residues that are present in the wild-type IL-2. In various embodiments, the mutant IL-2 polypeptide can differ from wild-type IL-2 by the addition, deletion, or substitution of a single amino acid residue, for example, a substitution of the residue at position 69. Similarly, exemplary mutant polypeptides can differ from wild-type by a substitution of two amino acid residues, for example, the residues at positions 24, 65, 74, 80, 81, 85, 86, 89, 92, and 93 of SEQ ID NO:2.

[0049] By way of illustration, a polypeptide that includes an amino acid sequence that is at least 95% identical to a reference amino acid sequence of SEQ ID NO:2 is a polypeptide that includes a sequence that is identical to the reference sequence except for the inclusion of up to five alterations of the reference amino acid of SEQ ID NO: 2. For example, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence can occur at the amino (N--) or carboxy (C--) terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0050] The substituted amino acid residue(s) can be, but are not necessarily, conservative substitutions, which typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. These mutations can be at amino acid residues that contact the IL-2R β and/or the IL-2R γ .

[0051] More specifically, a mutation (whether conservative or non-conservative, by way of addition(s) or deletion(s)) can be made at one or more of positions. For example, the mutation can be: I24V, P65H, Q74R, Q74 H, Q74N, Q74S, L80F, L80V, R81I, R81T, R81D, L85V, I86V, I89V, I92F, V93I. The sequences of exemplary IL-2 muteins are as follows: 5-1 SEQ ID NO: 5; 5-2 SEQ ID NO: 6; 6-6 SEQ ID NO: 7; A2 SEQ ID NO: 8; B1 SEQ ID NO: 9; B11 SEQ ID NO: 10; C5 SEQ ID NO: 11; D10 SEQ ID NO: 12; E10 SEQ ID NO: 13; G8 SEQ ID NO: 14; H4 SEQ ID NO: 15; and H9 SEQ ID NO: 16.

SEQ ID NO: 5

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKGYMPKKATELKHLQ
CLEEELKPLEEVLNLARSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE
FLNRWITFCQSIISTLT

SEQ ID NO: 6

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKGYMPKKATELKHLQ
CLEEELKPLEEVLNLARSKNFHLRPRDVISNINVIVLELKGSETTFMCEYADETATIVE
FLNRWITFCQSIISTLT

SEQ ID NO: 7

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKGYMPKKATELKHLQ
CLEEELKPLEEVLNLARSKNFHLIPRDVISNINVIVLELKGSETTFMCEYADETATIVEF
LNRWITFCQSIISTLT

SEQ ID NO: 8

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKGYMPKKATELKHLQ
CLEEELKPLEEVLNLARHSKNFHLTPRDVVSNINVFILELKGSETTFMCEYADETATIV
EFLNRWITFCQSIISTLT

SEQ ID NO: 9

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKPLEEVLNLANNSKNFHFDPRDVVSNVNVFVLEELKGSETTFMCEYADETATI
VEFLNRWITFCQSIISTLT

SEQ ID NO: 10

APTSSSTKKTQLQLEHLLLDLQMVLNGINNYKNPKLTRMLTFKFYMPKKATELKHL
QCLEEELKPLEEVLNLASSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATI
VEFLNRWITFCQSIISTLT

SEQ ID NO: 11

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKHLEEVLNLANNSKNFHVTPRDVVSNINVFVLEELKGSETTFMCEYADETATI
VEFLNRWITFCQSIISTLT

SEQ ID NO: 12

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKPLEEVLNLAHSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATIV
EFLNRWITFCQSIISTLT

SEQ ID NO: 13

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKPLEEVLNLASSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATIV
EFLNRWITFCQSIISTLT

SEQ ID NO: 14

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKPLEEVLNLANNSKNFHFDPRDVVSNINVFVLEELKGSETTFMCEYADETATIV
EFLNRWITFCQSIISTLT

SEQ ID NO: 15

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
CLEEELKPLEEVLNLASSKNFHLTPRDVISNINVFVLEELKGSETTFMCEYADETATIVE
FLNRWITFCQSIISTLT

SEQ ID NO: 16

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ
 CLEELKPLEEVNLNAQSKNFHFDPRDVVSNINVFVLELKGSETTFMCEYADETATIV
 EFLNRWITFCQSIISTLT

[0052] With respect to affinity, this there are disclosed herein exemplary mutant IL-2 polypeptides that bind the IL-2R β with an affinity that is higher than the wild type IL-2 polypeptide by at least about 2%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, or at least about 40% higher affinity or more. The wild-type IL-2 polypeptide binds the IL-2R β with a K_d of about 280 nM. The binding affinity of exemplary disclosed mutant IL-2 polypeptides can also be expressed as 1.2, 1.4, 1.5, 2, 5, 10, 15, 20, 25, 50, 100, 200, 250 or more fold higher affinity for the IL-2R β than wild-type IL-2.

[0053] For example, the substitution Q74N refers to a variant polypeptide, in this instance wherein the glutamine at position 74 is replaced with asparagine. Also, a substitution can be made simply at position 74 within the parent polypeptide, for example 74N would refer to the substitution of the amino acid at position 74 with asparagine, irrespective of the amino acid occurring at position 74 of the parent polypeptide.

[0054] With respect to affinity, this there are disclosed herein exemplary mutant IL-2 polypeptides that bind the IL-2R β with an affinity that is higher than the wild type IL-2 polypeptide by at least about 2%, at least about 5%, at least about 10%, at least about 20%, at least about 30%, or at least about 40% higher affinity or more. The wild-type IL-2 polypeptide binds the IL-2R β with a K_d of about 280 nM. The binding affinity of exemplary disclosed mutant IL-2 polypeptides can also be expressed as 1.2, 1.4, 1.5, 2, 5, 10, 15, 20, 25, 50, 100, 200, 250 or more fold higher affinity for the IL-2R β than wild-type IL-2.

[0055] Alternatively, or in addition, an exemplary mutant IL-2 polypeptide can have increased potency in a T cell proliferation assay relative to wild-type IL-2. The ability of a mutant IL-2 polypeptide to bind the IL-2R β can be assessed by numerous assays, including the cell binding and proliferation assays described herein.

[0056] Exemplary mutant IL-2 polypeptides can have the ability to exhibit a decreased dissociation rate from the IL-2R β receptor subunit, such that signaling from the receptor/ligand complex persists for a longer time period following transient exposure to the mutant IL-2 polypeptide, as compared to a wild-type IL-2.

[0057] Also provided in the instant disclosure are mutant polypeptides that disrupt the association of the IL-2R β with the IL-2R γ such that this interaction is reduced by about 2%,

about 5%, about 10%, about 15%, about 20%, about 50%, about 75%, about 90%, about 95% or more relative to wild-type IL-2.

[0058] Exemplary mutant IL-2 polypeptides possessing both properties of increased affinity for the IL-2R β and disruption of the IL-2R β with the IL-2R γ interaction are also disclosed.

[0059] As described further below, an exemplary class of mutant IL-2 polypeptides is provided in the instant disclosure with increased binding affinity for the IL-2R β and/or decreased binding affinity to the IL-2R γ using yeast surface display relative to wild-type IL-2.

[0060] In various embodiments, polypeptides used in the practice of the instant invention are synthetic, or are produced by expression of a recombinant nucleic acid molecule. In the event the polypeptide is a chimera (e.g., a fusion protein containing at least a mutant IL-2 polypeptide and a heterologous polypeptide), it can be encoded by a hybrid nucleic acid molecule containing one sequence that encodes all or part of the mutant IL-2, and a second sequence that encodes all or part of the heterologous polypeptide. For example, the mutant IL-2 polypeptide may be fused to a hexa-histidine tag to facilitate purification of bacterially expressed protein, or to a hemagglutinin tag to facilitate purification of protein expressed in eukaryotic cells.

[0061] Methods for constructing a DNA sequence encoding the IL-2 muteins and expressing those sequences in a suitably transformed host include, but are not limited to, using a PCR-assisted mutagenesis technique. Mutations that consist of deletions or additions of amino acid residues to an IL-2 polypeptide can also be made with standard recombinant techniques. In the event of a deletion or addition, the nucleic acid molecule encoding IL-2 is optionally digested with an appropriate restriction endonuclease. The resulting fragment can either be expressed directly or manipulated further by, for example, ligating it to a second fragment. The ligation may be facilitated if the two ends of the nucleic acid molecules contain complementary nucleotides that overlap one another, but blunt-ended fragments can also be ligated. PCR-generated nucleic acids can also be used to generate various mutant sequences.

[0062] The complete amino acid sequence can be used to construct a back-translated gene. A DNA oligomer containing a nucleotide sequence coding for IL-2 mutein can be synthesized. For example, several small oligonucleotides coding for portions of the desired

polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[0063] In addition to generating mutant polypeptides via expression of nucleic acid molecules that have been altered by recombinant molecular biological techniques, mutant polypeptides can be chemically synthesized. Chemically synthesized polypeptides are routinely generated by those of skill in the art.

[0064] Once assembled (by synthesis, site-directed mutagenesis or another method), the DNA sequences encoding an IL-2 mutein will be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the IL-2 mutein in the desired transformed host. Proper assembly can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host. As is well known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[0065] The DNA sequence encoding the IL-2 mutein, whether prepared by site directed mutagenesis, chemical synthesis or other methods, can also include DNA sequences that encode a signal sequence. Such signal sequence, if present, should be one recognized by the cell chosen for expression of the IL-2 mutein. It can be prokaryotic, eukaryotic or a combination of the two. It can also be the signal sequence of native IL-2. The inclusion of a signal sequence depends on whether it is desired to secrete the IL-2 mutein from the recombinant cells in which it is made. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be encoded and most preferably that the wild-type IL-2 signal sequence be used.

[0066] As noted above, exemplary mutant IL-2 polypeptides can be prepared as fusion or chimeric polypeptides that include a mutant IL-2 polypeptide and a heterologous polypeptide (i.e., a polypeptide that is not IL-2 or a mutant thereof) (see, e.g., U.S. Pat. No. 6,451,308). Exemplary heterologous polypeptides can increase the circulating half-life of the chimeric polypeptide *in vivo*, and may, therefore, further enhance the properties of the mutant IL-2 polypeptides. In various embodiments, the polypeptide that increases the circulating half-life may be a serum albumin, such as human serum albumin, or the Fc region of the IgG subclass of antibodies that lacks the IgG heavy chain variable region. Exemplary Fc regions

can include a mutation that inhibits complement fixation and Fc receptor binding, or it may be lytic, i.e., able to bind complement or to lyse cells via another mechanism, such as antibody-dependent complement lysis (ADCC; USSN 08/355,502 filed Dec. 12, 1994).

[0067] The "Fc region" can be a naturally occurring or synthetic polypeptide that is homologous to the IgG C-terminal domain produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50 kDa. The mutant IL-2 polypeptides can include the entire Fc region, or a smaller portion that retains the ability to extend the circulating half-life of a chimeric polypeptide of which it is a part. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule. That is, they can contain mutations that may or may not affect the function of the polypeptides; as described further below, native activity is not necessary or desired in all cases.

[0068] The Fc region can be "lytic" or "non-lytic," but is typically non-lytic. A non-lytic Fc region typically lacks a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site of murine IgG Fc includes the Leu residue at position 235 of IgG Fc. Thus, the Fc receptor binding site can be destroyed by mutating or deleting Leu 235. For example, substitution of Glu for Leu 235 inhibits the ability of the Fc region to bind the high affinity Fc receptor. The murine C'1q binding site can be functionally destroyed by mutating or deleting the Glu 318, Lys 320, and Lys 322 residues of IgG. For example, substitution of Ala residues for Glu 318, Lys 320, and Lys 322 renders IgG1 Fc unable to direct antibody-dependent complement lysis. In contrast, a lytic IgG Fc region has a high affinity Fc receptor binding site and a C'1q binding site. The high affinity Fc receptor binding site includes the Leu residue at position 235 of IgG Fc, and the C'1q binding site includes the Glu 318, Lys 320, and Lys 322 residues of IgG1. Lytic IgG Fc has wild-type residues or conservative amino acid substitutions at these sites. Lytic IgG Fc can target cells for antibody dependent cellular cytotoxicity or complement directed cytotoxicity (CDC). Appropriate mutations for human IgG are also known (see, e.g., Morrison et al., *The Immunologist* 2:119-124, 1994; and Brekke et al., *The Immunologist* 2: 125, 1994).

[0069] In other embodiments, the chimeric polypeptide can include the mutant IL-2 polypeptide and a polypeptide that functions as an antigenic tag, such as a FLAG sequence. FLAG sequences are recognized by biotinylated, highly specific, anti-FLAG antibodies, as described herein (see also Blonar et al., *Science* 256:1014, 1992; LeClair et al., *Proc. Natl. Acad. Sci. USA* 89:8145, 1992). In some embodiments, the chimeric polypeptide further comprises a C-terminal c-myc epitope tag.

[0070] In other embodiments, the chimeric polypeptide includes the mutant IL-2 polypeptide and a heterologous polypeptide that functions to enhance expression or direct cellular localization of the mutant IL-2 polypeptide, such as the Aga2p agglutinin subunit (see, e.g., Boder and Wittrup, *Nature Biotechnol.* 15:553-7, 1997).

[0071] In other embodiments, a chimeric polypeptide including a mutant IL-2 and an antibody or antigen-binding portion thereof can be generated. The antibody or antigen-binding component of the chimeric protein can serve as a targeting moiety. For example, it can be used to localize the chimeric protein to a particular subset of cells or target molecule. Methods of generating cytokine-antibody chimeric polypeptides are described, for example, in U.S. Pat. No. 6,617,135.

[0072] In some embodiments the mutant IL-2 polypeptide, either alone or as a part of a chimeric polypeptide, such as those described above, can be obtained by expression of a nucleic acid molecule. Just as mutant IL-2 polypeptides can be described in terms of their identity with wild-type IL-2 polypeptides, the nucleic acid molecules encoding them will necessarily have a certain identity with those that encode wild-type IL-2. For example, the nucleic acid molecule encoding a mutant IL-2 polypeptide can be at least 50%, at least 65%, preferably at least 75%, more preferably at least 85%, and most preferably at least 95% (e.g., 99%) identical to the nucleic acid encoding wild-type IL-2 (e.g., SEQ ID NO:2). The nucleic acid sequence encoding mature IL-2 and its signal sequence are found in SEQ ID NO: 17.

[0073] The nucleic acid molecules provided can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the nucleic acid molecules can be double-stranded or single-stranded (i.e., either a sense or an antisense strand).

[0074] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence (e.g., the coding sequence of IL-2) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by performance of the polymerase chain reaction (PCR). In the

event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by *in vitro* transcription.

[0075] Exemplary isolated nucleic acid molecules of the present disclosure can include fragments not found as such in the natural state. Thus, this disclosure encompasses recombinant molecules, such as those in which a nucleic acid sequence (for example, a sequence encoding a mutant IL-2) is incorporated into a vector (e.g., a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location).

[0076] As described above, the mutant IL-2 polypeptide of the invention may exist as a part of a chimeric polypeptide. In addition to, or in place of, the heterologous polypeptides described above, a nucleic acid molecule of the invention can contain sequences encoding a "marker" or "reporter." Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r , $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, of additional sequences that can serve the function of a marker or reporter.

[0077] The nucleic acid molecules of the invention can be obtained by introducing a mutation into IL-2-encoding DNA obtained from any biological cell, such as the cell of a mammal. Thus, the nucleic acids of the invention (and the polypeptides they encode) can be those of a mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, baboon, dog, or cat. In one embodiment, the nucleic acid molecules will be those of a human.

Antibodies

[0078] The present invention provides anti-IL-2 antibodies, generally therapeutic and/or diagnostic antibodies as described herein. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. The invention provides antibody structures that contain a set of 6 CDRs as defined herein (including small numbers of amino acid changes as described below).

[0079] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE. It should be understood that therapeutic antibodies can also comprise hybrids of isotypes and/or subclasses.

[0080] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[0081] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[0082] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those

residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below.

[0083] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g. Kabat *et al.*, supra (1991)).

[0084] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope. For example, the commercially available antibodies S4B6, JES6-5, MAB602, MAB5344, and JES6-1A12 bind to specific epitopes on IL-2.

[0085] The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide; in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide.

[0086] Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. Conformational and nonconformational epitopes may be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0087] An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Antibodies that recognize the same epitope can be verified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, for example "binning." In the present invention, non-limiting examples of antibodies that bind IL-2 include S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12.

[0088] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat *et al.* collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[0089] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By “immunoglobulin (Ig) domain” herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, “CH” domains in the context of IgG are as follows: “CH1” refers to positions 118-220 according to the EU index as in Kabat. “CH2” refers to positions 237-340 according to the EU index as in Kabat, and “CH3” refers to positions 341-447 according to the EU index as in Kabat.

[0090] Another type of Ig domain of the heavy chain is the hinge region. By “hinge” or “hinge region” or “antibody hinge region” or “immunoglobulin hinge region” herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the “lower hinge” generally referring to positions 226 or 230.

[0091] Of particular interest in the present invention are the Fc regions. By “Fc” or “Fc region” or “Fc domain” as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains C γ 2 and C γ 3 (C γ 2 and C γ 3) and the lower hinge region between C γ 1 (C γ 1) and C γ 2 (C γ 2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually

defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0092] In some embodiments, the antibodies are full length. By “full length antibody” herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[0093] Alternatively, the antibodies can be a variety of structures, including, but not limited to, antibody fragments, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as “antibody mimetics”), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as “antibody conjugates”), and fragments of each, respectively. Structures that still rely

[0094] In one embodiment, the antibody is an antibody fragment. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward *et al.*, 1989, Nature 341:544-546, entirely incorporated by reference) which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, 1988, Science 242:423-426, Huston *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883, entirely incorporated by reference), (viii) bispecific single chain Fv (WO 03/11161, hereby incorporated by reference) and (ix) “diabodies” or “triabodies”, multivalent or multispecific fragments constructed by gene fusion (Tomlinson *et al.*, 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger *et al.*, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448, all entirely incorporated by reference).

[0095] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. That is, in the present invention, the CDR sets can be used with framework and constant regions other than those specifically described by sequence herein.

[0096] In general, both “chimeric antibodies” and “humanized antibodies” refer to antibodies that combine regions from more than one species. For example, “chimeric antibodies” traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. “Humanized antibodies” generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, *e.g.*, WO 92/11018, Jones, 1986, *Nature* 321:522-525, Verhoeyen *et al.*, 1988, *Science* 239:1534-1536, all entirely incorporated by reference. “Backmutation” of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque *et al.*, 2004, *Biotechnol. Prog.* 20:639-654, entirely incorporated by reference. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, *Humanization of Monoclonal Antibodies*, *Molecular Biology of B Cells*, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*, 1988, *Nature* 332:323-329; Verhoeyen *et al.*, 1988, *Science*, 239:1534-1536; Queen *et al.*, 1989, *Proc Natl Acad Sci, USA* 86:10029-33; He *et al.*, 1998, *J. Immunol.* 160: 1029-1035; Carter *et al.*, 1992, *Proc Natl Acad Sci USA* 89:4285-9, Presta *et al.*, 1997, *Cancer Res.* 57(20):4593-9; Gorman *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:4181-4185; O'Connor *et al.*, 1998, *Protein Eng* 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:969-973, entirely incorporated by reference. In one embodiment, the parent antibody has been affinity matured,

as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu *et al.*, 1999, J. Mol. Biol. 294:151-162; Baca *et al.*, 1997, J. Biol. Chem. 272(16):10678-10684; Rosok *et al.*, 1996, J. Biol. Chem. 271(37): 22611-22618; Rader *et al.*, 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss *et al.*, 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan *et al.*, 2002, J. Immunol. 169:1119-1125; De Pascalis *et al.*, 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[0097] In one embodiment, the antibodies of the invention can be multispecific antibodies, and notably bispecific antibodies, also sometimes referred to as “diabodies”. These are antibodies that bind to two (or more) different antigens, or different epitopes on the same antigen. Diabodies can be manufactured in a variety of ways known in the art (Holliger and Winter, 1993, Current Opinion Biotechnol. 4:446-449, entirely incorporated by reference), *e.g.*, prepared chemically or from hybrid hybridomas.

[0098] In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu *et al.*, 1996, Cancer Res. 56:3055-3061, entirely incorporated by reference. In some cases, the scFv can be joined to the Fc region, and may include some or the entire hinge region.

[0099] The antibodies of the present invention are generally isolated or recombinant. “Isolated,” when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An “isolated antibody,” refers to an antibody which is substantially free of other antibodies having different antigenic specificities. For instance, an isolated antibody that specifically binds to IL-2 is substantially free of antibodies that specifically bind antigens other than IL-2.

[00100] An isolated antibody that specifically binds to an epitope, isoform or variant of human IL-2 or cynomolgus IL-2 may, however, have cross-reactivity to other related

antigens, for instance from other species, such as IL-2 species homologs. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[00101] Isolated monoclonal antibodies, having different specificities, can be combined in a well defined composition. Thus for example the S4B6, JES6-5, MAB602, MAB5344, and JES6-1A12 antibodies can be combined in a single formulation, if desired.

[00102] The anti-IL-2 antibodies of the present invention specifically bind IL-2 ligands (e.g. the human and cynomolgus IL-2 proteins of SEQ ID NOs:1-4. "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00103] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

[00104] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KA or Ka for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.

[00105] The present invention further provides variant antibodies. That is, there are a number of modifications that can be made to the antibodies of the invention, including, but not limited to, amino acid modifications in the CDRs (affinity maturation), amino acid modifications in the Fc region, glycosylation variants, covalent modifications of other types, etc.

[00106] By "variant" herein is meant a polypeptide sequence that differs from that of a parent polypeptide by virtue of at least one amino acid modification. Amino acid

modifications can include substitutions, insertions and deletions, with the former being preferred in many cases.

[00107] In general, variants can include any number of modifications, as long as the function of the protein is still present, as described herein. That is, in the case of amino acid variants generated with the CDRs of either S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12, for example, the antibody should still specifically bind to both human and cynomolgus IL-2. Similarly, if amino acid variants are generated with the Fc region, for example, the variant antibodies should maintain the required receptor binding functions for the particular application or indication of the antibody.

[00108] However, in general, from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions are generally utilized as often the goal is to alter function with a minimal number of modifications. In some cases, there are from 1 to 5 modifications, with from 1-2, 1-3 and 1-4 also finding use in many embodiments.

[00109] It should be noted that the number of amino acid modifications may be within functional domains: for example, it may be desirable to have from 1-5 modifications in the Fc region of wild-type or engineered proteins, as well as from 1 to 5 modifications in the Fv region, for example. A variant polypeptide sequence will preferably possess at least about 80%, 85%, 90%, 95% or up to 98 or 99% identity to the parent sequences. It should be noted that depending on the size of the sequence, the percent identity will depend on the number of amino acids.

[00110] By “amino acid substitution” or “substitution” herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. By “amino acid insertion” or “insertion” as used herein is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. By “amino acid deletion” or “deletion” as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence.

[00111] By “parent polypeptide”, “parent protein”, “precursor polypeptide”, or “precursor protein” as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by “parent Fc polypeptide” as used herein is meant an Fc polypeptide that is

modified to generate a variant, and by “parent antibody” as used herein is meant an antibody that is modified to generate a variant antibody.

[00112] By “wild type” or “WT” or “native” herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00113] By “variant Fc region” herein is meant an Fc sequence that differs from that of a wild-type Fc sequence by virtue of at least one amino acid modification. Fc variant may refer to the Fc polypeptide itself, compositions comprising the Fc variant polypeptide, or the amino acid sequence.

[00114] In some embodiments, one or more amino acid modifications are made in one or more of the CDRs of the antibody (*e.g.*, S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12). In general, only 1 or 2 or 3 amino acids are substituted in any single CDR, and generally no more than from 4, 5, 6, 7, 8, 9 or 10 changes are made within a set of CDRs. However, it should be appreciated that any combination of no substitutions, 1, 2 or 3 substitutions in any CDR can be independently and optionally combined with any other substitution.

[00115] In some cases, amino acid modifications in the CDRs are referred to as “affinity maturation”. An “affinity matured” antibody is one having one or more alteration(s) in one or more CDRs which results in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some cases, although rare, it may be desirable to decrease the affinity of an antibody to its antigen, but this is generally not preferred.

[00116] Affinity maturation can be done to increase the binding affinity of the antibody for the antigen by at least about 10% to 50-100-150% or more, or from 1 to 5 fold as compared to the “parent” antibody. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by known procedures. See, for example, Marks et al., 1992, *Biotechnology* 10:779-783 that describes affinity maturation by variable heavy chain (VH) and variable light chain (VL) domain shuffling. Random mutagenesis of CDR and/or framework residues is described in: Barbas, et al. 1994, *Proc. Nat. Acad. Sci, USA* 91:3809-3813; Shier et al., 1995, *Gene*

169:147-155; Yelton et al., 1995, J. Immunol. 155:1994-2004; Jackson et al., 1995, J. Immunol. 154(7):3310-9; and Hawkins et al, 1992, J. Mol. Biol. 226:889-896, for example.

[00117] Alternatively, amino acid modifications can be made in one or more of the CDRs of the antibodies of the invention that are “silent”, e.g. that do not significantly alter the affinity of the antibody for the antigen. These can be made for a number of reasons, including optimizing expression (as can be done for the nucleic acids encoding the antibodies of the invention).

[00118] Thus, included within the definition of the CDRs and antibodies of the invention are variant CDRs and antibodies; that is, the antibodies of the invention can include amino acid modifications in one or more of the CDRs of S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12. In addition, as outlined below, amino acid modifications can also independently and optionally made in any region outside the CDRs, including framework and constant regions.

[00119] In some embodiments, the anti-IL-2 antibodies of the invention are composed of a variant Fc domain. As is known in the art, the Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. These Fc receptors include, but are not limited to, (in humans) FcγRI (CD64) including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158, correlated to antibody-dependent cell cytotoxicity (ADCC)) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2), FcRn (the neonatal receptor), C1q (complement protein involved in complement dependent cytotoxicity (CDC)) and FcRn (the neonatal receptor involved in serum half-life). Suitable modifications can be made at one or more positions as is generally outlined, for example in US Patent Application 11/841,654 and references cited therein, US 2004/013210, US 2005/0054832, US 2006/0024298, US 2006/0121032, US 2006/0235208, US 2007/0148170, USSN 12/341,769, US Patent No. 6,737,056, US Patent No. 7,670,600, US Patent No. 6,086,875 all of which are expressly incorporated by reference in their entirety, and in particular for specific amino acid substitutions that increase binding to Fc receptors.

[00120] In addition to the modifications outlined above, other modifications can be made. For example, the molecules may be stabilized by the incorporation of disulphide

bridges linking the VH and VL domains (Reiter et al., 1996, Nature Biotech. 14:1239-1245, entirely incorporated by reference). In addition, there are a variety of covalent modifications of antibodies that can be made as outlined below.

[00121] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[00122] The antibodies of the invention are cross reactive with human and cynomolgus IL-2 and are thus species cross-reactive antibodies. A “species cross-reactive antibody” is an antibody that has a binding affinity for an antigen from a first mammalian species that is nearly the same as the binding affinity for a homologue of that antigen from a second mammalian species. Species cross-reactivity can be expressed, for example, as a ratio of the KD of an antibody for an antigen of the first mammalian species over the KD of the same antibody for the homologue of that antigen from a second mammalian species wherein the ratio is 1.1, 1.2, 1.3, 1.4, 1.5, 2, 5, 10, 15, up to 20. Alternatively or additionally, an antibody is “species cross reactive” when it shows therapeutic or diagnostic efficacy when administered to the second species. Thus, in the present case, the antibodies of the invention are cross reactive with cynomolgus IL-2, show preclinical efficacy when administered to cynomolgus primates and thus are considered cross reactive.

[00123] In some embodiments, antibodies that compete with the antibodies of the invention (for example, with S4B6, JES6-5, MAB602, MAB5344, and/or JES6-1A12) for binding to human IL-2 and/or cynomolgus IL-2 are provided. Competition for binding to IL-2 or a portion of IL-2 by two or more anti-IL-2 antibodies may be determined by any suitable technique, as is known in the art.

[00124] In some embodiments, the anti-IL-2 antibody of the present invention specifically binds to one or more residues or regions in IL-2 but also does not cross-react with other proteins with homology to IL-2.

[00125] Typically, a lack of cross-reactivity means less than about 5% relative competitive inhibition between the molecules when assessed by ELISA and/or FACS analysis using sufficient amounts of the molecules under suitable assay conditions.

[00126] The disclosed anti-IL-2 antibodies may also inhibit cell growth, specifically when complexed (*i.e.*, combined with) an IL-2 mutein. "Inhibits growth" includes any measurable decrease in the cell growth when contacted with a an anti-IL-2 antibody-IL-2 mutein complex, as compared to the growth of the same cells not in contact with such a complex, for instance an inhibition of growth of a cell culture by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

[00127] The antibodies to cytokines or lymphokines of the present invention can be prepared by any suitable method known in the art. For example, cytokines or lymphokines of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. The term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technology.

[00128] Hybridoma techniques include those known in the art and taught in Harlow and Lane, *supra*; Hammerling et al., *Monoclonal Antibodies and T-Cell Hybridomas*, 563-681, 1981, said references incorporated by reference in their entireties. Fab and F(ab').sub.2 fragments can be produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab').sub.2 fragments).

[00129] Alternatively, antibodies to cytokines or lymphokines can be produced through the application of recombinant DNA and phage display technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182: 41-50, 1995; Ames et al., *J. Immunol. Methods* 184: 177-186,

1995; Kettleborough et al., Eur. J. Immunol. 24: 952-958, 1994; Persic et al., Gene 187: 9-18, 1997; Burton et al., Advances in Immunology 57: 191-280, 1994; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727 and 5,733,743, each incorporated herein by reference in their entirety and for all purposes.

[00130] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce antibody fragments including the Fc region of the antibody can be employed using methods known in the art. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax et al., BioTechniques 12: 864-869, 1992; and Sawai et al., AJRI 34: 26-34, 1995; and Better et al., Science 240: 1041-1043, 1988.

[00131] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498, each incorporated herein by reference in their entirety and for all purposes; Huston et al., Methods in Enzymology, 203: 46-88, 1991; Shu, L. et al., PNAS 90: 7995-7999, 1993; and Skerra et al., Science 240: 1038-1040, 1988. For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, Science 229: 1202, 1985; Oi et al., BioTechniques 4: 214, 1986; Gillies et al., J. Immunol. Methods, 125: 191-202, 1989; and U.S. Pat. No. 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; and U.S. Pat. Nos. 5,530,101 and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., Molecular Immunology, 28: 489-498, 1991; Studnicka et al., Protein Engineering 7: 805-814, 1994; Roguska et al., PNAS 91: 969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described above. See also, U.S. Pat. Nos. 4,444,887; 4,716,111; 5,545,806; and 5,814,318; and WO 98/46645; WO 98/50433;

WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each incorporated herein by reference in their entirety and for all purposes.

[00132] Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a cytokine or lymphokine of the present invention. The antibodies can be specific for antigens other than cytokines or lymphokines of the present invention. For example, antibodies can be used to target the cytokines or lymphokines of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention can also be used in in vitro immunoassays and purification methods using methods known in the art. See *e.g.*, Harbor et al., *supra*, and WO 93/21232; EP 0 439 095; Naramura et al., *Immunol. Lett.* 39: 91-99, 1994; U.S. Pat. No. 5,474,981, incorporated herein by reference in its entirety and for all purposes; Gillies et al., *PNAS* 89: 1428-1432, 1992; Fell et al., *J. Immunol.* 146: 2446-2452, 1991.

[00133] The present invention further includes compositions comprising the cytokines or lymphokines of the present invention fused or conjugated to antibody domains of an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention can comprise the hinge region, CH₁ domain, CH₂ domain, and CH₃ domain or any combination of whole domains or portions thereof. The cytokines or lymphokines of the present invention can be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. The polypeptides can also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the cytokine antibody complex of the present invention to antibody portions are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388; and WO 91/06570, each incorporated herein by reference in their entirety and for all purposes; Ashkenazi et al., *PNAS*, 88: 10535-10539, 1991; Zheng et al., *J. Immunol.*, 154: 5590-5600, 1995; and Vil et al., *PNAS*, 89: 11337-11341, 1992.

[00134] The invention further relates to antibodies which act as agonists of the cytokines or lymphokines of the present invention. For example, the present invention

includes antibodies which activate the receptor for cytokines or lymphokines. These antibodies can act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies can be specified as agonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. See *e.g.*, WO 96/40281; U.S. Pat. No. 5,811,097, each incorporated herein by reference in their entirety and for all purposes; Deng et al., *Blood* 92: 1981-1988, 1998; Chen, et al., *Cancer Res.*, 58: 3668-3678, 1998; Harrop et al., *J. Immunol.* 161: 1786-1794, 1998; Zhu et al., *Cancer Res.*, 58: 3209-3214, 1998; Yoon, et al., *J. Immunol.*, 160: 3170-3179, 1998; Prat et al., *J. Cell. Sci.*, 111: 237-247, 1998; Pitard et al., *J. Immunol. Methods*, 205: 177-190, 1997; Liautard et al., *Cytokine*, 9: 233-241, 1997; Carlson et al., *J. Biol. Chem.*, 272: 11295-11301, 1997; Taryman et al., *Neuron*, 14: 755-762, 1995; Muller et al., *Structure*, 6: 1153-1167, 1998; Bartunek et al., *Cytokine*, 8: 14-20, 1996. As discussed above, antibodies to cytokines or lymphokines on metastatic cells can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides as embodiments of the invention using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan et al., *FASEB J.* 7: 437-444, 1989 and Nissinoff, *J. Immunol.* 147: 2429-2438, 1991). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide as embodiments of the invention to ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide as embodiments of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Methods of Treatment

[00135] In some embodiments, mutant IL-2 polypeptides, and/or nucleic acids expressing them, can be administered in combination with an anti-IL-2 antibody to a subject to treat a disorder associated with abnormal apoptosis or a differentiative process (*e.g.*, cellular proliferative disorders or cellular differentiative disorders, such as cancer, by, for example, producing an active or passive immunity). In the treatment of such diseases, the disclosed IL-2 muteins may possess advantageous properties, such as reduced vascular leak syndrome. More specifically, in the treatment of such diseases, the combination of IL-2

muteins and anti-IL-2 antibodies may possess advantageous properties such as increased efficacy, decreased toxicity, unexpected additive effects, and unexpected synergistic effects wherein the combination of the mutein and the antibody produce more than a simple additive effect. Furthermore, in specific embodiments wherein IL-2 mutein treatment has been ineffective at treating abnormal apoptosis and proliferative/differentiative disorders, for example, the combination of the IL-2 mutein and the anti-IL-2 antibody is effective.

[00136] Examples of cellular proliferative and/or differentiative disorders include cancer (*e.g.*, carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, *e.g.*, leukemias). A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver. The compositions of the present invention (*e.g.*, mutant IL-2 polypeptides and/or the nucleic acid molecules that encode them) can also be administered to a patient who has a viral infection (*e.g.*, AIDS or an influenza).

[00137] The mutant IL-2 polypeptides can be used in combination with anti-IL-2 antibodies to treat patients who have, who are suspected of having, or who may be at high risk for developing any type of cancer, including renal carcinoma or melanoma, or any viral disease. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues.

[00138] Additional examples of proliferative disorders include hematopoietic neoplastic disorders.

[00139] Other examples of proliferative and/or differentiative disorders include skin disorders. The skin disorder may involve the aberrant activity of a cell or a group of cells or layers in the dermal, epidermal, or hypodermal layer, or an abnormality in the dermal-epidermal junction. For example, the skin disorder may involve aberrant activity of keratinocytes (*e.g.*, hyperproliferative basal and immediately suprabasal keratinocytes), melanocytes, Langerhans cells, Merkel cells, immune cell, and other cells found in one or more of the epidermal layers, *e.g.*, the stratum basale (stratum germinativum), stratum spinosum, stratum granulosum, stratum lucidum or stratum corneum. In other embodiments, the disorder may involve aberrant activity of a dermal cell, for example, a dermal endothelial, fibroblast, immune cell (*e.g.*, mast cell or macrophage) found in a dermal layer, for example, the papillary layer or the reticular layer.

[00140] Examples of skin disorders include psoriasis, psoriatic arthritis, dermatitis (eczema), for example, exfoliative dermatitis or atopic dermatitis, pityriasis rubra pilaris, pityriasis rosacea, parapsoriasis, pityriasis lichenoides, lichen planus, lichen nitidus, ichthyosiform dermatosis, keratodermas, dermatosis, alopecia areata, pyoderma gangrenosum, vitiligo, pemphigoid (*e.g.*, ocular cicatricial pemphigoid or bullous pemphigoid), urticaria, prokeratosis, rheumatoid arthritis that involves hyperproliferation and inflammation of epithelial-related cells lining the joint capsule; dermatitises such as seborrheic dermatitis and solar dermatitis; keratoses such as seborrheic keratosis, senile keratosis, actinic keratosis, photo-induced keratosis, and keratosis follicularis; acne vulgaris; keloids and prophylaxis against keloid formation; nevi; warts including verruca, condyloma or condyloma acuminatum, and human papilloma viral (HPV) infections such as venereal warts; leukoplakia; lichen planus; and keratitis. The skin disorder can be dermatitis, *e.g.*, atopic dermatitis or allergic dermatitis, or psoriasis.

[00141] Patients amenable to treatment may also have psoriasis. The term "psoriasis" is intended to have its medical meaning, namely, a disease which afflicts primarily the skin and produces raised, thickened, scaling, nonscarring lesions. The lesions are usually sharply demarcated erythematous papules covered with overlapping shiny scales. The scales are typically silvery or slightly opalescent. Involvement of the nails frequently occurs resulting in pitting, separation of the nail, thickening and discoloration. Psoriasis is sometimes associated with arthritis, and it may be crippling. Hyperproliferation of keratinocytes is a key feature of psoriatic epidermal hyperplasia along with epidermal inflammation and reduced differentiation of keratinocytes. Multiple mechanisms have been invoked to explain the keratinocyte hyperproliferation that characterizes psoriasis. Disordered cellular immunity has also been implicated in the pathogenesis of psoriasis. Examples of psoriatic disorders include chronic stationary psoriasis, psoriasis vulgaris, eruptive (gluttate) psoriasis, psoriatic erythroderma, generalized pustular psoriasis (Von Zumbusch), annular pustular psoriasis, and localized pustular psoriasis.

[00142] Alternatively, or in addition to methods of direct administration to patients, in some embodiments, mutant IL-2 polypeptides can be used in combination with anti-IL-2 antibodies in *ex vivo* methods. For example, cells (*e.g.*, peripheral blood lymphocytes or purified populations of lymphocytes isolated from a patient and placed or maintained in culture) can be cultured *in vitro* in culture medium and the contacting step can be affected by adding the IL-2 mutant and anti-IL-2 antibody to the culture medium. The culture step can

include further steps in which the cells are stimulated or treated with other agents, *e.g.*, to stimulate proliferation, or to expand a population of cells that is reactive to an antigen of interest (*e.g.*, a cancer antigen or a viral antigen). The cells are then administered to the patient after they have been treated.

[00143] In certain embodiments, the IL-2 mutein and the anti-IL-2 antibody are administered separately but at the same time to a patient in need thereof. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using the same administrative modality (*e.g.*, both are injected intravenously into a patient in need thereof) at the same time but separately. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using different administrative modalities (*e.g.*, one is injected intravenously and the other is injected subcutaneously into a patient in need thereof) at the same time but separately.

[00144] In certain embodiments, the IL-2 mutein and the anti-IL-2 antibody are administered separately and at different times to a patient in need thereof. In specific embodiments, the IL-2 mutein polypeptide is administered before the anti-IL-2 antibody. In specific embodiments, the IL-2 mutein polypeptide is administered 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, or any combination thereof, apart such that the IL-2 mutein polypeptide is administered first and is followed by a separate administration of the anti-IL-2 antibody. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using the same administrative modality (*e.g.*, both are injected intravenously into a patient in need thereof) at different times. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using different administrative modalities (*e.g.*, one is injected intravenously and the other is injected subcutaneously into a patient in need thereof) at different times.

[00145] In certain embodiments, the IL-2 mutein polypeptide is administered after the anti-IL-2 antibody. In specific embodiments, the IL-2 mutein polypeptide is administered 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13

hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 1 month, or any combination thereof, apart such that the anti-IL-2 antibody is administered first and is followed by a separate administration of the IL-2 mutein polypeptide. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using the same administrative modality (*e.g.*, both are injected intravenously into a patient in need thereof) at different times. In specific embodiments, the IL-2 mutein and anti-IL-2 antibody are administered using different administrative modalities (*e.g.*, one is injected intravenously and the other is injected subcutaneously into a patient in need thereof) at different times.

[00146] In certain embodiments the anti-IL-2 antibody and IL-2 mutein is combined prior to administration. In a specific embodiment, the anti-IL-2 antibody and IL-2 mutein are combined and allowed to incubate for a sufficient time for the anti-IL-2 antibody and IL-2 mutein to form a complex. In specific embodiments, the anti-IL-2 antibody and IL-2 mutein are allowed to incubate for 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 11 minutes, 12 minutes, 13 minutes, 14 minutes, 15 minutes, 16 minutes, 17 minutes, 18 minutes, 19 minutes, 20 minutes, 21 minutes, 22 minutes, 23 minutes, 24 minutes, 25 minutes, 26 minutes, 27 minutes, 28 minutes, 29 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 1 day, 2 days, 3 days, 4 days, or 5 or more days or any combination thereof prior to administration.

[00147] In certain embodiments, the anti-IL-2 antibody that is administered in combination with the IL-2 mutein is two anti-IL-2 antibodies. In certain embodiments, the anti-IL-2 antibody that is administered in combination with the IL-2 mutein is three anti-IL-2 antibodies. In certain embodiments, the anti-IL-2 antibody that is administered in combination with the IL-2 mutein is four anti-IL-2 antibodies. In certain embodiments, the anti-IL-2 antibody that is administered in combination with the IL-2 mutein is five or more anti-IL-2 antibodies.

[00148] In certain embodiments, the IL-2 mutein that is administered in combination with the anti-IL-2 antibody is two IL-2 muteins. In certain embodiments, the IL-2 mutein that is administered in combination with the anti-IL-2 antibody is three IL-2 muteins. In certain embodiments, the IL-2 mutein that is administered in combination with the anti-IL-2

antibody is four IL-2 muteins. In certain embodiments, the IL-2 mutein that is administered in combination with the anti-IL-2 antibody is five or more IL-2 muteins.

Pharmaceutical Compositions and Methods of Administration

[00149] In some embodiments, mutant IL-2 polypeptides and nucleic acids can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include the polypeptide or nucleic acid molecule and a pharmaceutically acceptable carrier.

[00150] A pharmaceutical composition is formulated to be compatible with its intended route of administration. In one embodiment, the mutant IL-2 polypeptides and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) are administered orally. In one embodiment, the mutant IL-2 polypeptides and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) are administered through a parenteral route. Examples of parenteral routes of administration include, for example, intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as mono- and/or di-basic sodium phosphate, hydrochloric acid or sodium hydroxide (*e.g.*, to a pH of about 7.2-7.8, *e.g.*, 7.5). The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00151] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™, (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved

against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.*, sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, 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, aluminum monostearate and gelatin.

[00152] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00153] Oral compositions, if used, generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes™; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00154] In certain embodiments wherein the administration by inhalation, the mutant IL-2 polypeptides, or the nucleic acids encoding them, and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) may be delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798. In other embodiments, inhalation is performed using pressurized, non-aerosol administration. In specific embodiments, the anti-IL-2 antibody and/or anti-IL-2 antibody complex are administered intranasally by inhalation wherein the inhalation is performed using a pressurized, non-aerosol device. In specific embodiments, the anti-IL-2 antibody and/or anti-IL-2 antibody complex are administered intranasally by inhalation wherein the inhalation is performed using an aerosol device.

[00155] Systemic administration of the mutant IL-2 polypeptides or nucleic acids and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00156] In some embodiments, compounds (mutant IL-2 polypeptides or nucleic acids, anti-IL-2 antibodies, or complexes of the IL-2 polypeptides and anti-IL-2 antibodies) can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00157] In some embodiments, compounds (mutant IL-2 polypeptides or nucleic acids, anti-IL-2 antibodies, or complexes of the IL-2 polypeptides and anti-IL-2 antibodies) can also be administered by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey et al. (*Nature* 418:6893, 2002), Xia et al. (*Nature Biotechnol.* 20: 1006-1010, 2002), or Putnam (*Am. J. Health Syst. Pharm.* 53: 151-160, 1996, erratum at *Am. J. Health Syst. Pharm.* 53:325, 1996).

[00158] In one embodiment, the mutant IL-2 polypeptides or nucleic acids and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) are

prepared with carriers that will protect the mutant IL-2 polypeptides and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) against rapid elimination from the body, 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, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[00159] Dosage, toxicity and therapeutic efficacy of such mutant IL-2 polypeptides or nucleic acids and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00160] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*e.g.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00161] As defined herein, a therapeutically effective amount of mutant IL-2 polypeptides and/or anti-IL-2 antibodies (including complexes of the IL-2 polypeptides and anti-IL-2 antibodies) (*e.g.*, an effective dosage) depends on the polypeptide and antibody selected. For instance, single dose amounts of the mutant IL-2 polypeptide are in the range of approximately 0.001 to 0.1 mg/kg of patient body weight can be administered; in some embodiments, about 0.005, 0.01, 0.05 mg/kg may be administered. In some embodiments, 600,000 IU/kg is administered (IU can be determined by a lymphocyte proliferation bioassay and is expressed in International Units (IU) as established by the World Health Organization 1st International Standard for Interleukin-2 (human)). The dosage may be similar to, but is expected to be less than, that prescribed for PROLEUKIN®. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the mutant IL-2 polypeptides of the invention can include a single treatment or, can include a series of treatments. In one embodiment, the compositions are administered every 8 hours for five days, followed by a rest period of 2 to 14 days, *e.g.*, 9 days, followed by an additional five days of administration every 8 hours.

[00162] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

EXAMPLE

Mice

[00163] Wild-type (C57BL/6) and Thy1.1-congenic mice on a C57BL/6 background (both from Charles River) were maintained under specific pathogen-free conditions and used at 3-6 months of age. Experiments were performed in accordance with the Swiss Federal Veterinary Office guidelines and approved by the Cantonal Veterinary Office.

Flow cytometry

[00164] Cell suspensions of spleen were prepared and stained for analysis by flow cytometry using phosphate-buffered saline (PBS) containing 4% FCS and 2.5 mM EDTA.

Fluorochrome-conjugated monoclonal antibodies (mAbs; from BD Biosciences unless otherwise stated) were used against: CD3 (145-2C11, eBioscience), CD4 (RM4-5, Caltag Laboratories), CD8a (53-6.7), CD25 (PC61), CD44 (IM7, eBioscience), NK1.1 (PK136), and Thy1.1 (HIS51, eBioscience). At least 100'000 viable cells were acquired on a BD FACSCanto II flow cytometer and analyzed using FlowJo software (TriStar Inc.).

IL-2/anti-IL-2 mAb complexes

[00165] IL-2/anti-IL-2 mAb complexes, recombinant human IL-2 (rhIL-2), F42A, H9, or F42A-H9 were premixed at a 2:1 molar ratio with anti-human IL-2 mAb using 15'000 international units (IU) of rhIL-2 or IL-2 muteins. RhIL-2 and anti-hIL-2 mAbs (clones MAB602 or 5344) were obtained from R&D Systems.

Adoptive transfer to T cells

[00166] T cell subsets were obtained by negative T cell enrichment (StemCell Technologies). Where indicated, purified cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular Probes). $2-3 \times 10^6$ CD8⁺ T cells from Thy1.1-congenic WT mice enriched for CD44^{high} memory-phenotype (MP) cells were injected intravenously (i.v.) to Thy1.2-congenic WT mice. Starting on the day of adoptive cell transfer, age- and gender-matched mice received daily intraperitoneal (i.p.) injections of either PBS, 15'000 IU human IL-2 (IL-2), or 15'000 IU IL-2 muteins H9, F42A, or F42A-H9 for 5 days. The other two groups received 15'000 IU human IL-2, H9, F42A, or F42A-H9 complexed with either the anti-human IL-2 monoclonal antibody MAB602 or 5344 as indicated. 6 days after adoptive cell transfer, spleens were removed and analyzed by flow cytometry.

Enzyme-linked immunosorbent assay (ELISA) and radio-labeled immunosorbent assay (RIA)

[00167] ELISA was set up by coating 96 well plates (Nunc) with carrier-free recombinant human IL-2 (BD Biosciences) or IL-2 muteins at 5µg/ml in PBS overnight at 4°C. Wells were blocked using 0.5% BSA in PBS, anti-human IL-2 mAbs were added in serial dilutions and incubated for 2 hours at room temperature, followed by incubation with biotinylated anti-mouse IgG (eBioscience) and streptavidin-HRP (R&D Systems). The ELISA was developed using TMB substrate (BioRad) and the reaction stopped with H₂SO₄. The optical density at 490nm was measured using a plate reader (BioRad). A

radioimmunoassay (RIA) was set up as described above with the modification of detecting plate bound IL-2 or IL-2 muteins by radio-labelled anti-human IL-2 monoclonal antibodies for 45 min. at 4°C. Anti-human IL-2 monoclonal antibodies were iodinated using the iodination reagent method. Supernatants were collected, wells were washed twice with PBS, and radioactivity in the bound wells and supernatants (unbound fraction) was determined using a gamma counter (PerkinElmer, Schwerzenbach, Switzerland).

Results

[00168] The efficacy of IL-2 variants (muteins) is enhanced by interaction with anti-IL-2 antibodies and the formation of IL-2 mutein/antibody complexes. As demonstrated in **Figure 1**, IL-2 variant expansion of MPCD8⁺ T cells, regulatory T cells, and NK cells is enhanced when complexed with IL-2 antibodies.

[00169] The affinity of anti-human IL-2 monoclonal antibodies MAB602 and 5344 to IL-2, IL-2 superkine (H9), and F42A as measure by enzyme-linked immunosorbent assay (ELISA) is demonstrated in **Figure 2**. Anti-human IL-2 MAB602 binds IL-2 and IL-2 superkine H9 with the same affinity (EC₅₀) of about 3nM but shows reduced affinity towards IL-2 carrying the F42A mutation (F42A) (**Fig. 2A**). Anti-human IL-2 mAb 5344 binds IL-2 and F42A with similar affinity (EC₅₀ of about 7.5nM), while binding of mAb 5344 to H9 is decreased by 4.5 fold compared to IL-2 (**Fig. 2B**). The EC₅₀-values obtained by ELISA are summarized in **Table 1**. Similar results were obtained using radio-labeled immunosorbent assay (RIA) as summarized in **Table 2**.

Table 1. EC₅₀ values obtained by ELISA.

ELISA	IL-2 EC ₅₀ (nM)	H9 EC ₅₀ (nM)	F42A EC ₅₀ (nM)
MAB602	2	3.5	<1000
5344	7.5	32	7.6

Table 2. EC₅₀ values obtained by radio-labeled immunosorbent assay (RIA).

RIA	IL-2 EC ₅₀ (nM)	H9 EC ₅₀ (nM)	F42A EC ₅₀ (nM)
MAB602	1.8	2.5	3342
5344	8.3	29.8	6.3

WE CLAIM:

1. A composition comprising an IL-2 mutein and an anti-IL-2 antibody, wherein the IL-2 mutein binds IL-2R β with higher affinity than wild-type IL-2 and wherein the antibody specifically binds IL-2 or an IL-2 mutein.
2. The composition of claim 1, wherein the IL-2 mutein comprises one or more amino acid substitutions at positions 24, 65, 74, 80, 81, 85, 86, 89, 92, and/or 93 of SEQ ID NO.: 1 or 2, wherein the amino acid numbering is in accordance with wild-type human IL-2.
3. The composition of claim 2 further comprising a substitution at amino acid position 42.
4. The composition of claim 1, wherein the antibody competes for binding with one or more of the following antibodies: S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12.
5. The composition of claim 4, wherein the antibody is a human antibody or humanized antibody.
6. The composition of claim 1, wherein the antibody is S4B6, JES6-5, MAB602, MAB5344, or JES6-1A12.
7. The composition of claim 1, wherein the antibody binds with higher affinity to the IL-2 mutein than IL-2.
8. The composition of claims 1, 4, 5, 6, and 7, wherein the antibody comprises an Fc region and wherein the Fc region is an Fc mutein.
9. A pharmaceutical composition comprising the IL-2 mutein and the antibody of any of claims 1-8.
10. A method for treating an autoimmune disorder in a subject, the method comprising the administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein.
11. A method for treating an immune cell deficiency in a subject, the method comprising the administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein.

12. A method for treating cancer in a subject, the method comprising the administration of an IL-2 mutein and an antibody that specifically binds IL-2 or an IL-2 mutein.
13. The methods of claims 10-12, wherein the IL-2 mutein is administered to the subject prior to the administration of the antibody, or wherein the IL-2 mutein and the antibody are administered simultaneously, or wherein the antibody is administered prior to the IL-2 mutein.
14. The methods of claims 10-12, wherein the IL-2 mutein and the antibody are combined with a second therapeutic agent.
15. The method of claim 14, wherein the second therapeutic agent is wild-type IL-2.

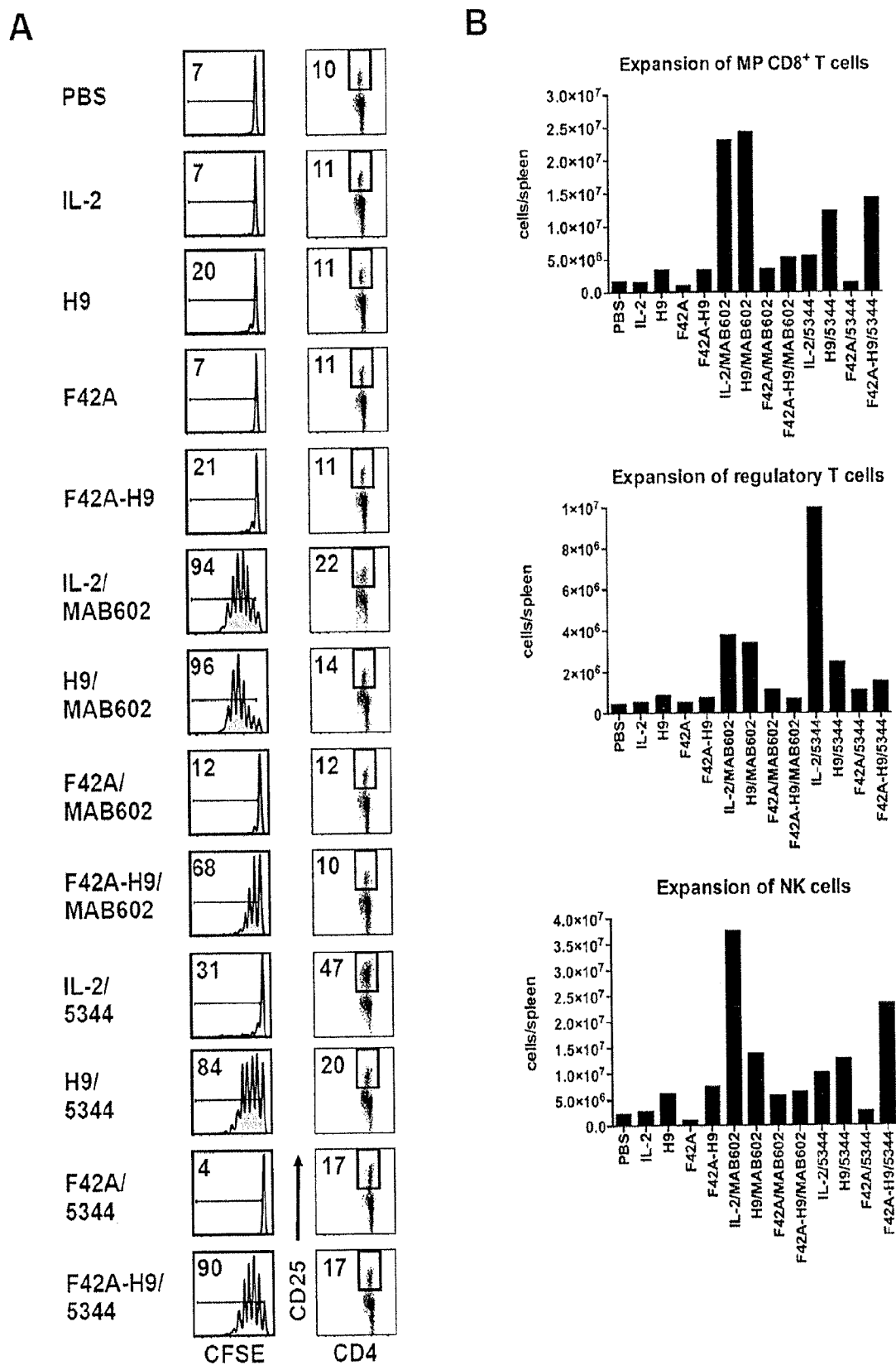


FIGURE 1

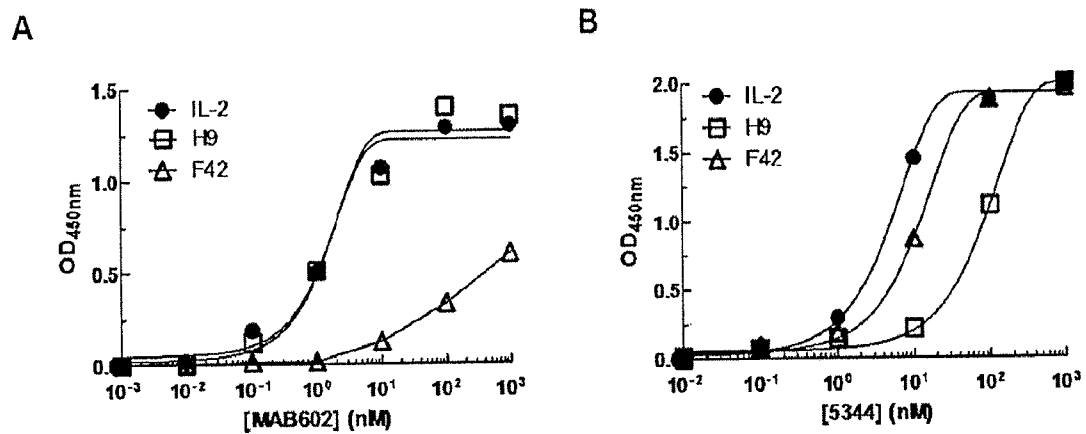


FIGURE 2

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395, A61K 45/00 (2014.01)

USPC - 424/145.1, 424/85.2, 530/351, 530/388.23

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 424/145.1, 424/85.2, 530/351, 530/388.23Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/133.1, 424/141.1, 530/388.1Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB), Google/Scholar: Interleukin 2, IL-2, variant, mutation, substitution, F42A, BAY50-4798, R88N, MAB602, MAB5344, S4B6, JES6-5, JES6-IA12, affinity, interleukin 2 receptor beta, IL-2Rb, CD122, IL-2 immunocomplex
GenCore 6.4.1: SEQ ID NO: 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Tomala, et al. In Vivo Expansion of Activated Naive CD8+ T Cells and NK Cells Driven by Complexes of IL-2 and Anti-IL-2 Monoclonal Antibody As Novel Approach of Cancer Immunotherapy. The Journal of Immunology 2009, 183(8):4904-4912; Abstract, pg 4905, col 1, 1st para	1-7 and 10-12
Y	Levin, et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature 25 March 2012, 484(7395):529-33; Abstract, pg 8 [according to the posted document], Fig 1c and its legend; pg 11 [according to the posted Supplementary Materials document], Supplementary Fig 10 and its legend	1-7 and 10-12
A	NCBI. Direct Submission NP_000577.2. Interleukin-2 precursor [Homo sapiens]. 04 November 2012. [Retrieved from the Internet 10 March 2014: < http://www.ncbi.nlm.nih.gov/protein/28178861?sat=17&satkey=19158726 >]	2, 3
A	WO 2012/088446 A1 (Garcia, et al.) 28 June 2012 (28.06.2012) Abstract, claims 1-13; para [0021]-[0026], Fig 3-7, 10, and 11	1-7 and 10-12
A	Letourneau, et al. IL-2/anti-IL-2 antibody complexes show strong biological activity by avoiding interaction with IL-2 receptor alpha subunit CD25. Proc Natl Acad Sci USA 2010, 107(5):2171-2176; Abstract	1-7 and 10-12
A	Steppan, et al. Reduced secondary cytokine induction by BAY 50-4798, a high-affinity receptor-specific interleukin-2 analog. J Interferon Cytokine Res. 2006, 26(3):171-8; Abstract	1-7 and 10-12

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 March 2014 (10.03.2014)

Date of mailing of the international search report

31 MAR 2014

Name and mailing address of the ISA/US

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Lee, et al. Interleukin-2/anti-interleukin-2 monoclonal antibody immune complex suppresses collagen-induced arthritis in mice by fortifying interleukin-2/STAT5 signalling pathways. Immunology ePub 20 November 2012, 137(4):305-16; Abstract	1-7 and 10-12
A	Polhill, et al. IL-2/IL-2Ab Complexes Induce Regulatory T Cell Expansion and Protect against Proteinuric CKD. Am Soc Nephrol. August 2012, 23(8):1303-1308; Abstract	1-7 and 10-12
A	Boyman, et al. Selectively expanding subsets of T cells in mice by injection of interleukin-2/ antibody complexes: implications for transplantation tolerance. Transplant Proc. May 2012, 44(4):1032-4; Abstract	1-7 and 10-12

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8, 9, 13-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.