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(54) Title: TARGETING IL-7 SIGNALING AS A THERAPY FOR MULTIPLE SCLEROSIS AND OTHER IL-7 SIGNALING DEPENDENT DISORDERS

(57) Abstract: Compositions directed to interleukin 7 and interleukin 7 receptors are provided. Methods of preventing and treating diseases involving signaling events via the interleukin-7 receptor include autoimmune diseases, transplantation rejection and other disorders which include a T lymphocyte response.



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**TARGETING IL-7 SIGNALING AS A THERAPY FOR MULTIPLE SCLEROSIS
AND OTHER IL-7 SIGNALING DEPENDENT DISORDERS**

STATEMENT AS TO FEDERALLY FUNDED RESEARCH

[001] This invention was made with United States government support under grant number AI040114 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

RELATED APPLICATIONS

[002] This application claims priority under 35 USC § 119 to U.S. Provisional Patent Application No. 61/146,591 filed January 22, 2009, the disclosure of which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

[003] Embodiments of the invention relate to compositions and methods utilizing IL-7 receptor agents that can modulate IL-7 receptor mediated signaling.

BACKGROUND

[004] Multiple sclerosis (MS) is the most common cause of chronic neurological deficits in young adults; with women being afflicted 2-3 times more frequently than men. Both human studies and animal models (EAE and others) provide strong evidence that MS has an autoimmune component resulting in severe demyelination and axonal injury. Recent genetic studies suggest that this disease is inheritable and that several immune related genes are risk factors for MS.

SUMMARY

[005] This Summary is provided to present a summary of the invention to briefly indicate the nature and substance of the invention. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims.

[006] Compositions which modulate interleukin 7 receptor signaling are useful in treating diseases involving interleukin 7 signaling, such as for example, autoimmune diseases.

The compositions comprise an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or peptides, organic molecules or inorganic molecules.

[007] Using a disease model which lacks interleukin 7 receptor (IL-7R) in all tissues except thymus, it was shown that interleukin-7 (IL-7) was required for the induction of experimental autoimmune encephalomyelitis (EAE). The animal disease model and experimental data support IL-7R as protective: 1) EAE was induced and WT mice showed typical EAE progression with disease onset occurring about 2 weeks into the study. Conversely, mice lacking interleukin 7 receptor alpha chain (IL-7R α) signaling outside the thymus showed significant protection against the disease phenotype over 40 days. 2) Mice injected with IL-7R α blocking antibody were also significantly protected when compared to controls. When antibody is delivered prior to disease induction the animals are protected. When antibody delivered after the disease is induced the animals improve, therefore, IL-7R α modulating agents are therapeutic, as well as protective.

[008] In a preferred embodiment, a method of modulating interleukin-7 (IL-7) receptor or interleukin-7 receptor α chain mediated signaling *in vivo* or *in vitro*, comprising contacting a IL-7R α (CD127) or molecule comprising a IL-7R α (CD127) or functional domains thereof, with an agent which specifically targets or binds to interleukin 7 receptor alpha chain molecules.

[009] In another preferred embodiment, the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.

[0010] In another preferred embodiment, the agent modulates interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, mediated cell signaling.

[0011] In another preferred embodiment, a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, comprises: receptor molecules, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.

[0012] In another preferred embodiment, the agent comprises: an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.

[0013] In another preferred embodiment, the agent targets or specifically binds to the interleukin 7 receptor (IL-7R), a molecule comprising an interleukin-7 receptor alpha (IL-

7R α) chain (CD127), or functional domains thereof, and inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0014] In another preferred embodiment, the agent inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0015] In another preferred embodiment, the agent protects or treats a patient at risk of developing or suffering from an immune system related disease or disorder, comprising: autoimmune diseases or disorders, inflammatory diseases or disorders, Graft versus Host Disease (GVHD), neuroinflammatory diseases or disorders.

[0016] In another preferred embodiment, the agent protects or treats a patient suffering from or at risk of developing cancer, bacterial, viral, fungal, or parasitic organisms.

[0017] In another preferred embodiment, a method of modulating cells of a patient's immune system, comprising: administering to a patient an effective amount of an agent which modulates expression, function or activity of interleukin-7 receptors, variants, isoforms, mutants, alleles or fragments thereof. Preferably, the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.

[0018] In another preferred embodiment, the agent modulates interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, mediated cell signaling.

[0019] In another preferred embodiment, a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, comprises: receptor molecules, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof. Preferably, the agent targets or specifically binds to the interleukin 7 receptor (IL-7R), a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, and inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0020] In another preferred embodiment, the cells of a patient's immune system comprise: T lymphocytes, B lymphocytes, natural killer cells, mononuclear cells, hematopoietic or non-hematopoietic cells.

[0021] In another preferred embodiment, the agent protects or treats a patient at risk of developing or suffering from an immune system related disease or disorder, comprising:

autoimmune diseases or disorders, inflammatory diseases or disorders, Graft versus Host Disease (GVHD), neuroinflammatory diseases or disorders.

[0022] In another preferred embodiment, the agent protects or treats a patient suffering from or at risk of developing cancer, bacterial, viral, fungal, or parasitic organisms.

[0023] In yet another preferred embodiment, a method of treating a patient suffering from an autoimmune disease or disorder comprising: administering to a patient a composition comprising a therapeutically effective amount of an agent that modulates IL-7R α mediated signaling. Preferably, the agent inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0024] In another preferred embodiment, a pharmaceutical composition comprising an agent for modulating expression, function, signaling or activity of an interleukin-7 receptor (IL-7R), variants, isoforms, mutants, alleles or fragments thereof. Preferably, the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.

[0025] In another preferred embodiment, the agent targets or specifically binds to the interleukin 7 receptor (IL-7R) or molecules comprising an IL-7R α chain or functional domains thereof; and inhibiting IL-7R α chain expression, function, activity or signaling through activation of the molecules comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0026] In another preferred embodiment, a fusion protein comprising at least one molecule which inhibits interleukin 7 receptor alpha chain (IL-7R α) mediated signaling. Preferably, the at least one molecule inhibiting interleukin 7 receptor alpha chain mediated signaling comprises an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.

[0027] In another preferred embodiment, a vector expressing a molecule which modulates interleukin-7 receptor alpha chain mediated signaling.

[0028] In another preferred embodiment, an isolated cell comprising a vector expressing a molecule which modulates interleukin-7 receptor alpha chain mediated signaling.

[0029] In another preferred embodiment, a method of identifying candidate therapeutic agents comprising: contacting an interleukin 7 receptor alpha chain containing molecule or functional domains thereof, or a cell expressing the molecule, with a candidate therapeutic agent; measuring signaling mediated by the IL-7R α chain as compared to a baseline control.

Preferably, the candidate therapeutic agent modulates IL-7R α signaling by at least about 20% as compared to a baseline control.

[0030] Other aspects are described *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Figures 1A and 1B are graphs showing MOG-induced EAE in IL7R α Tg^{IL7R^{-/-}} and WT animals. Figure 1A: EAE was induced with the MOG₃₅₋₅₅ peptide in IL7R α Tg^{IL7R^{-/-}} mice ($\circ$, n=7) and C57B/6 wild type controls ($\blacksquare$, n=8). Clinical symptoms were scored daily for 40 days and the results are expressed as the mean $\pm$ SEM. The two curves are significantly different when the entire data set is analyzed (p=0.0061, Mann Whitney t test). Significance is further enhanced when analysis starts from disease onset (p<0.0001). Figure 1B: Spleen cells were isolated from either WT or IL7R α Tg^{IL7R^{-/-}} mice 40 days post EAE induction. Cells were analyzed by flow cytometry using antibodies against CD127, TCR β , B220, or NK1.1. CD127 is the marker for IL-7R α . Results shown are the percentages of positive staining relative to total leukocyte population.

[0032] Figures 2A and 2B are graphs showing the clinical scores of MOG-induced EAE in WT animals that received IL-7R α neutralizing antibody either before disease onset or at peak disease. EAE was induced in C57B/6 mice using the MOG₃₅₋₅₅ peptide. Antibody treatments were administered at 20 μ g/g body weight via i.p. injections every other day, for 20 days (shaded regions). Clinical symptoms were scored daily for 50 days and the results are expressed as the mean $\pm$ SEM. Figure 2A: IL-7R α neutralizing antibody ($\blacksquare$, n=12) or IgG isotype control ($\circ$, n=14) was administered prior to disease onset, starting from day 7. The two curves are significantly different when analyzed from the appearance of clinical symptoms to 4 days post treatment (p=0.0038, Mann Whitney t test) but this significance does not hold for the duration of the study (p=0.2180). Figure 2B: IL-7R α neutralizing antibody ($\blacksquare$, n=13) or IgG isotype control ($\circ$, n=12) was administered after the disease had peaked, starting from day 19. The difference between the curves is statistically significant for the entire study (p=0.0004, Mann Whitney t test).

[0033] Figure 3 is a schematic representation showing a protocol used in EAE induction *in vivo*.

[0034] Figure 4 is a graph showing that IL7R α Tg^{IL7R^{-/-}} mice are less susceptible to EAE.

[0035] Figure 5 is a graph showing IL-7 neutralization *in vitro*. IL-7 and IL-7R α neutralize T cell proliferation with equal capacities *in vitro*.

[0036] Figure 6 is a schematic illustration showing the immunization protocols and neutralizing antibody treatment. Antibody treatments: 20µg/g (400 µg injections) every other day, for a total of 10 injections.

[0037] Figures 7A and 7B are graphs showing antibody treatment in vivo at the peak of EAE. Figure 7A: IL-7Rα neutralizing antibody lead to significant recovery, which held even after cessation of treatment. Figure 7B: IL-7 neutralizing antibody did not improve recovery.

[0038] Figure 8 is a schematic representation showing the effects of EAE in the chimeric mice.

[0039] Figure 9 is a schematic representation showing the results of EAE in chimeric mice after wild type or Tg BMT were injected into Rag^{-/-} mice.

DETAILED DESCRIPTION

[0040] Several aspects of the invention are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the invention. One having ordinary skill in the relevant art, however, will readily recognize that the invention can be practiced without one or more of the specific details or with other methods. In other instances, well-known structures or operations are not shown in detail to avoid obscuring the invention. The present invention is not limited by the illustrated ordering of acts or events, as some acts may occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the present invention.

[0041] All genes, gene names, and gene products disclosed herein are intended to correspond to homologs from any species for which the compositions and methods disclosed herein are applicable. Thus, the terms include, but are not limited to genes and gene products from humans and mice. It is understood that when a gene or gene product from a particular species is disclosed, this disclosure is intended to be exemplary only, and is not to be interpreted as a limitation unless the context in which it appears clearly indicates. Thus, for example, for the genes disclosed herein, which in some embodiments relate to mammalian nucleic acid and amino acid sequences are intended to encompass homologous and/or orthologous genes and gene products from other animals including, but not limited to other mammals, fish, amphibians, reptiles, and birds. In preferred embodiments, the genes or nucleic acid sequences are human.

[0042] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the relevant art and will not be interpreted in an idealized or overly formal sense unless expressly so defined herein.

Definitions

[0043] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0044] Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0045] Immune conditions, diseases, disorders and reactions or responses to be treated according to the methods and compositions of the invention means a disease in which the immune system contributes to pathogenesis, for example T cells. These reactions include, but are not limited to, autoimmune conditions, disorders or diseases and persistent and progressive immune reactions to infectious non self antigens from bacterial, viral (e.g., HCV), fungal, or parasitic organisms which invade and persist within mammals and humans. Such conditions and disorders include allergies and/or asthma. The allergies and asthma may be due to sensitization with foreign or non-self antigens as pollen, animal dander and food proteins. The source of the provoking foreign antigen can be plant, fungal, mold, or other environmental contaminant.

[0046] "Cells of the immune system" or "immune cells", is meant to include any cells of the immune system that may be assayed, including, but not limited to, B lymphocytes, also called B cells, T lymphocytes, also called T cells, natural killer (NK) cells, natural killer T (NK) cells, lymphokine-activated killer (LAK) cells, monocytes, macrophages, neutrophils, granulocytes, mast cells, platelets, Langerhans cells, stem cells, dendritic cells, peripheral blood mononuclear cells, tumor-infiltrating (TIL) cells, gene modified immune cells

including hybridomas, drug modified immune cells, and derivatives, precursors or progenitors of the above cell types.

[0047] "Immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response selective for the antigen. These cells include, but are not limited to, T cells (T lymphocytes), B cells (B lymphocytes), monocytes, macrophages, natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates.

[0048] "Immune related molecules" refers to any molecule identified in any immune cell, whether in a resting ("non-stimulated") or activated state, and includes any receptor, ligand, cell surface molecules, nucleic acid molecules, polypeptides, variants and fragments thereof.

[0049] "T cells" or "T lymphocytes" are a subset of lymphocytes originating in the thymus and having heterodimeric receptors associated with proteins of the CD3 complex (e.g., a rearranged T cell receptor, the heterodimeric protein on the T cell surfaces responsible for antigen/MHC specificity of the cells). T cell responses may be detected by assays for their effects on other cells (e.g., target cell killing, activation of other immune cells, such as B-cells) or for the cytokines they produce.

[0050] The phrase "T cell response" means an immunological response involving T cells. The T cells that are "activated" divide to produce antigen specific memory T cells or antigen specific cytotoxic T cells. The cytotoxic T cells bind to and destroy cells recognized as containing the antigen. The memory T cells are activated by the antigen and thus provide a response to an antigen already encountered. This overall response to the antigen is the antigen specific T cell response, e.g. tumor specific.

[0051] "CD," "cluster of differentiation" or "common determinant" as used herein refers to cell surface molecules recognized by antibodies. Expression of some CDs (e.g., CD4, CD8, CD25, CD127) is specific for cells of a particular lineage or maturational pathway, and the expression of others varies according to the state of activation, position, or differentiation of the same cells. Preferably, in some embodiments, the CD determinants are human when the isolated cells are to be administered to a human or a human immune response is being studied.

[0052] As used herein, the term "CD127" refers to the "interleukin-7 receptor," present on a cell surface. However, in some preferred embodiments the molecule is soluble CD127. The IL-7 receptor alpha chain is described in the literature. See, e.g., Goodwin *et al.* (1990) *Cell* 60:941-951; GenBank Accession Nos. NP 032398 and NP002176. IL-7R is also referred to in the literature as CD127. The term CD127 ligand refers to a compound that

binds to the IL-7 receptor. CD127⁺ refers to cells which stain intensely or brightly when treated with a labeled antibody directed toward CD127. Generally, the cells are distinguished according to their CD127 expression levels based upon a readily discernible differences in staining intensity as is known to one of ordinary skill in the art.

[0053] "Inhibitors," "activators," and "modulators" of expression, signaling, function or activity are used to refer to inhibiting, activating, or modulating IL-7R expression, function or activity, signaling, and these modulators are identified using *in vitro* and/or *in vivo* assays for expression or activity. The term "modulator" includes inhibitors and activators. A modulator can be an antibody or a soluble ligand which binds a protein of interest, small molecule and the like. Inhibitors are agents that, e.g., inhibit expression of a polypeptide or polynucleotide of the invention or bind to, partially or totally block stimulation or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide of the invention, e.g., antagonists. Inhibitors or modulators are compared to control samples without the inhibitor or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control sample is about 80%, optionally 50% or 25 to 1%, or less. Activation is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control sample is 110%, optionally 150%, optionally 200-500%, or 1000-3000%, or higher.

[0054] An "anti-X antibody" or "X antibody" according to the invention is an antibody which can specifically bind to X. For instance, the anti-CD127 antibody or CD127 antibody is capable of binding CD127. The antibodies for use according to the invention include, but are not limited to, recombinant antibodies, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, human monoclonal antibodies, humanized or primatized monoclonal antibodies, and antibody fragments. The antigenic target, for example, IL-7R α , can be from any species. Thus, an antibody, for example, anti-IL-7R α can be mouse anti-human IL-7R α , goat anti-human IL-7R α ; goat anti-mouse IL-7R α ; rat anti-human IL-7R α ; mouse anti-rat IL-7R α and the like. The combinations of antibody generated in a certain species against an antigen target, e.g. IL-7R α , from another species, or in some instances the same species (for example, in autoimmune or inflammatory response) are limitless and all species are embodied in this invention. The term antibody is used in the broadest sense and includes fully assembled antibodies, monoclonal antibodies (including human, humanized or chimeric antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and

antibody fragments that can bind antigen (e.g., Fab', F'(ab)₂, Fv, single chain antibodies, diabodies), comprising complementarity determining regions (CDRs) of the foregoing as long as they exhibit the desired biological activity.

[0055] The phrase "specifically (or selectively) binds" or "is specific for" or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. For example, specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. "Specifically modulates" as used herein refers to modulating the activity, functions, etc, of the IL-7R α chain, or functional domains or peptide sequences, as opposed to any other molecule. "Targeting" or "targets" is another term for specificity. Thus "specificity" or "targeting" also refers to the specific functions e.g. signaling of IL-7R alpha chain containing molecules, activity, specific binding of a molecule to the IL-7R, etc.

[0056] As used herein, the term "variant", when used in the context of a peptide or polypeptide, means a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity; and, when used in the context of an oligonucleotide, means an oligonucleotide that differs in nucleotide sequence by the insertion, deletion, or substitution of nucleotides. A particular nucleic acid sequence also implicitly encompasses "splice variants" and "allelic variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant or allelic variant of that nucleic acid. "Splice variants," are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. Thus, when referring to, for example, IL-7, or IL-7 receptor the terms include all variants encompassed by this definition.

[0057] As used herein, the term "safe and effective amount" or "therapeutic amount" refers to the quantity of a component which is sufficient to yield a desired therapeutic

response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. By "therapeutically effective amount" is meant an amount of a compound of the present invention effective to yield the desired therapeutic response. The specific safe and effective amount or therapeutically effective amount will vary with such factors as the particular condition being treated, the physical condition of the patient, the type of mammal or animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

[0058] As used herein, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression, *in vivo* amounts of a gene. This includes any amounts *in vivo*, functions and the like as compared to normal controls. The term includes, for example, increased, enhanced, increased, agonized, promoted, decreased, reduced, suppressed blocked, or antagonized. Modulation can increase activity or amounts more than 1-fold, 2-fold, 3-fold, 5-fold, 10-fold, 100-fold, etc., over baseline values. Modulation can also decrease its activity or amounts below baseline values.

Interleukin-7 and Interleukin-7 Receptor (CD127)

[0059] Interleukin-7 (IL-7) was originally described as a B cell growth factor secreted by bone marrow stromal cells. Subsequently, IL-7 was found to promote the growth of pro-T cells as well, produced by cortical epithelial cells in the thymus. It is required for normal murine T and B cell development as well as human T cell development. IL-7 acts as a modulator of low affinity peptide-induced proliferation, a central feature of homeostatic regulation of T cell populations in humans. Although not absolutely required for B cell development in humans, IL-7 still plays an important role in human B cell development. IL-7 provides a survival signal to early B lymphoid precursors. IL-7 signals through the IL-7 receptor (IL-7R), a heterodimer receptor composed of two subunits, the gamma common (γ_c) chain (CD132) and the IL-7R α chain (CD127). The γ_c chain is shared by other cytokine receptors including IL-2R, IL-4R, IL-9R, IL-15R, and IL-21R, while the IL-7R α chain is unique to the IL-7 receptor, whose expression varies with different stages of lymphoid development. IL-7R α chain is expressed from the early pro-B cell stage through the pre-B cell stage. IL-7 promotes the formation of a functional pre-B cell receptor (pre-BCR) in pro-B cells and the transition to pre-B cells. Down-regulation of IL-7 signaling in pre-B cells serves as a trigger for initiating apoptosis during negative selection of B cells with unproductive Ig rearrangements. Finally, IL-7R α expression ceases very late in the late pre-B

cell stage. When IL-7 engages the IL-7R on pro-B cells, IL-7R tyrosine phosphorylation and PI turnover occurs, resulting in clonal proliferation. Because the IL-7R itself has no intrinsic kinase activity, IL-7-induced phosphorylation occurs as a result of recruiting intracellular kinases, including the JAK/STAT system as well as activation of PI-3 kinase and src family kinases.

[0060] The IL-7R α chain is believed to be involved in 30% of all Multiple sclerosis (MS) cases. The mutation within this gene, that enhances the risk for MS, is linked to an SNP (rs897932), which resides within the transmembrane domain of IL-7R α . This mutation results in the alternative splicing of exon 6. Without wishing to be bound by theory, the alternative splicing of exon 6 possibly results in the production of a secreted IL7R α .

[0061] In order to identify the underlying role of IL-7R α signaling in MS, a myelin oligodendrocyte glycoprotein (MOG)-induced model of EAE in IL-7R α mutants was used, which are detailed in the Examples section which follows. The mice express the IL-7R α gene exclusively in the thymus and not in the periphery. This was accomplished by crossing IL-7R $^{-/-}$ mice with transgenic mice expressing wild type IL-7R α driven by the thymic proximal *lck* promoter. The resulting progeny of this cross was referred to as IL-7R α Tg^{IL7R $^{-/-}$} . These genetic manipulations allowed for sufficient T cell development, yet prevent IL-7 cytokine signaling once mature cells leave the thymus. Although both naïve and memory T cells require IL-7 signaling for homeostatic survival in the periphery, the naïve cells can still last an abbreviated 2-4 weeks in its absence. This seems to be a sufficient length of time to produce normal CD4/CD8 distributions in the periphery of IL-7R α Tg^{IL7R $^{-/-}$} mice. B cell development, however, was still compromised due to the lack of IL-7R signaling in the bone marrow.

[0062] In preferred embodiments, molecules that bind or interact with IL-7R and/or IL-7R α and inhibit IL-7R α mediated signaling are used in the treatment of disorders or diseases associated with IL-7R α mediated signaling diseases or disorders, such as for example autoimmune diseases or disorders. Examples of autoimmune diseases or disorders comprise: Addison's Disease, Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's Disease), ankylosing spondylitis, nephritis, aplastic anemia, arthritis, asthma; atopic allergy, autoimmune lymphoproliferative syndrome (ALPS), Behcet's Disease, cardiomyopathy, colitis, Crohn's Disease, diabetes, alveolitis, gastritis, Goodpasture's Disease, Grave's Disease, Guillian-Barre Syndrome, Hashimoto's Thyroiditis, hepatitis, inflammatory demyelinating polyneuropathy, irritable bowel syndrome, Lou Gehrig's Disease, lupus, multiple sclerosis, myasthenia gravis,

polyglandular autoimmune syndromes, rheumatoid arthritis, sarcoidosis, scleritis, scleroderma, Sjögren's Syndrome, Systemic Lupus Erythematosus (SLE), or ulcerative colitis.

[0063] Activated T-lymphocytes express high numbers of IL-7 receptors, and proliferation of the cells is driven by this receptor. T-cell activation is a process that occurs and leads to clinical symptoms and tissue damage in patients with autoimmune disorders or a transplant rejection (e.g., a graft rejection, such as an allograft rejection). Inhibition of the proliferation and signal transduction of these cells by candidate therapeutic agents, antibodies, small molecules, enzymes, peptides, proteins, nucleic acid molecules, IL-7R mutants, IL-7R α mutants, and the like, can decrease or eliminate symptoms of these diseases.

[0064] In another preferred embodiment, a method of modulating interleukin-7 (IL-7) receptor or interleukin-7 receptor α chain mediated signaling *in vivo* or *in vitro*, comprising contacting a IL-7R α (CD127) or molecule comprising a IL-7R α (CD127) or functional domains thereof, with an agent which specifically targets or binds to interleukin 7 receptor alpha chain molecules.

[0065] In another preferred embodiment, the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof. In another preferred embodiment, the agent modulates interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, mediated cell signaling.

[0066] *Hybrid Molecules:* In a preferred embodiment, one or more IL-7R α nucleic acids, proteins or peptides can be linked or fused to another moiety. For example, a targeting sequence, such as, for example, an aptamer, antibody sequence; a therapeutic effector molecule, e.g. cytokines, antibiotics, toxins, radiolabels; signal leader peptide; intracellular targeting moiety; a receptor, receptor binding domain, signaling peptide, intramembrane molecule; intracellular localization signal, and the like. The IL-7R α molecules, peptides or functional domains thereof, can be genetically fused, or linked via linker molecules. It is understood that the term "IL-7R α " as used throughout the specification and claims also encompasses any IL-7R α chain, or IL-7R α mutant or IL-7R α containing molecule, mutant IL-7R α containing molecule or any molecule comprising IL-7R α chains, IL-7R α peptides, or functional domains thereof, or nucleic acid sequences, variants, derivatives, allelic variants, splice variants, species variants, is inclusive of all family members, isoforms, orthologs, precursors, mutants, alleles, fragments, species, sense and antisense polynucleotide strands, etc. of IL-7R α . Thus the terms "a hybrid IL-7R α " or "chimeric IL-7R α molecule" encompasses all of the IL-7R α species, mutants, or any permutations, etc.

[0067] In one embodiment, IL-7R α fusion molecules can be fused or linked to a therapeutically effective domain which can be a modulatory or cytolytic moiety having a significant serum half-life ($t_{1/2}$) beyond that of either antibody or modulatory/cytolytic moiety alone.

[0068] Accordingly, embodiments of the invention are further directed to chimeric fusion molecules comprising single or multivalent therapeutically active domains which can be modulatory and/or cytolytic; an antigen-binding domain; compositions of single-chain and multivalent modulatory and cytolytic antigen-binding fusion proteins and the like. Other moieties include integrin motifs and NGR motifs.

[0069] Furthermore, the chimeric or hybrid IL-7R α molecules to be used in the present invention, as described herein, may comprise at least one further domain, *inter alia*, domains which provide for purification means, like, e.g. histidine stretches. The further domain(s) may be linked by covalent or non-covalent bonds.

[0070] The linkage can be based on genetic fusion according to the methods known in the art and described herein or can be performed by, e.g., chemical cross-linking as described in, e.g., WO 94/04686. The additional domain present in the construct may be linked by a flexible linker, such as a polypeptide linker to one of the binding site domains; the polypeptide linker can comprise plural, hydrophilic or peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of one of the domains and the N-terminal end of the other of the domains when the polypeptide assumes a conformation suitable for binding when disposed in aqueous solution.

[0071] In another preferred embodiment, the molecule comprises a label for detecting the fusion molecule *in vivo* and to monitor the effects of the chimeric molecule during therapy.

[0072] "Detectable moiety" or a "label" refers to a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P , ^{35}S , fluorescent dyes, electron-dense reagents, enzymes (e.g., as commonly used in an ELISA), biotin-streptavidin, dioxigenin, haptens and proteins for which antisera or monoclonal antibodies are available, or nucleic acid molecules with a sequence complementary to a target. The detectable moiety often generates a measurable signal, such as a radioactive, chromogenic, or fluorescent signal, that can be used to quantify the amount of bound detectable moiety in a sample. Quantitation of the signal is achieved by, e.g., scintillation counting, densitometry, or flow cytometry.

[0073] In other embodiments, the molecules can be in any stereoisomeric form, for example, enantiomers, diastereomers, tautomers and the like. The fusion molecule or parts thereof includes all variants, mutations, alleles, substitutes, fragments and analogs thereof.

[0074] In another preferred embodiment, a moiety which can be used in embodiments of the invention can be determined based on the desired treatment. For example, if the disease is cancer, various anticancer agents can be used. If a disease relates to transplantation or autoimmunity, immune suppressor molecules can be used. If the disease is an infection, antibiotics, antiviral moieties, anti-inflammatory moieties can be used. The invention contemplates the use of one or more moieties with different properties can be used, for example, an anti-angiogenic moiety and a toxin. The various moieties and combinations are only limited by the imagination of the user. As illustrative examples, the moieties can include one or more molecules' comprising: endostatin, angiogenin, angiostatin, chemokines, angioarrestin, angiostatin (plasminogen fragment), basement-membrane collagen-derived anti-angiogenic factors (tumstatin, canstatin, or arrestin), anti-angiogenic antithrombin III, signal transduction inhibitors, cartilage-derived inhibitor (CDI), CD59 complement fragment, fibronectin fragment, gro-beta, heparinases, heparin hexasaccharide fragment, human chorionic gonadotropin (hCG), interferon alpha/beta/gamma, interferon inducible protein (IP-10), interleukin-12, kringle 5 (plasminogen fragment), metalloproteinase inhibitors (TIMPs), 2-methoxyestradiol, placental ribonuclease inhibitor, plasminogen activator inhibitor, platelet factor-4 (PF4), prolactin 16 kD fragment, proliferin-related protein (PRP), various retinoids, tetrahydrocortisol-S, thrombospondin-1 (TSP-1), transforming growth factor-beta (TGF- β), vasculostatin, vasostatin (calreticulin fragment) and the like. These molecules include all forms, variants, mutations, alleles, substitutes, fragments and analogs thereof.

[0075] In another preferred embodiment, the targeting domain comprises antibody, aptamer, a ligand for a receptor (e.g. VEGF), diabodies, peptides, lipopolysaccharides, integrins and the like.

[0076] In another preferred embodiment, the IL-7R α comprises a receptor moiety such as for example, a hormone binding site, a growth hormone binding site, a growth factor binding site, a cytokine or monokine binding site etc.

[0077] In another preferred embodiment, the chimeric fusion molecules comprise an antigen binding domain specific for other tumor antigens. The antigen binding domain can be an antibody or aptamer, receptor, ligand etc.

[0078] In one preferred embodiment, the invention provides for antibody fusion molecules comprising: F_c region, C_H1, C_H2 and/or C_H3, Fab, Fab', F(ab')₂, single chain Fv

(S_CFv) and Fv fragments, as well as any portion of an antibody having specificity toward a desired target epitope or epitopes. Also preferred are antibodies or antibody fragments or to single chain, two-chain, and multi-chain proteins and glycoproteins belonging to the classes of polyclonal, monoclonal, chimeric, bispecific and hetero immunoglobulins (monoclonal antibodies being preferred); it also includes synthetic and genetically engineered variants of these immunoglobulins.

[0079] In another preferred embodiment, the antigen binding domain is an aptamer. In the preferred embodiment, the chimeric molecule comprises an aptamer fused to the IL-7R α molecule, variants, mutants and fragments thereof. The aptamer can be specific for any one or more tumor antigens.

[0080] As used herein, the term "aptamer" or "selected nucleic acid binding species" shall include non-modified or chemically modified RNA or DNA. The method of selection may be by, but is not limited to, affinity chromatography and the method of amplification by reverse transcription (RT) or polymerase chain reaction (PCR).

[0081] In another embodiment, IL-7R α or functional domains thereof, are associated with a molecule of interest (e.g. peptide, antigen, enzyme, etc) either by binding, as a fusion moiety, or linked via a chemical moiety to modulate the molecule of interest's half-life, retention in an intracellular body, degradation, altering of intracellular or surface expression of the molecule of interest, etc.

[0082] In another preferred embodiment, the molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, comprises: receptor molecules, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof. For example, the IL-7R α , or any functional domain thereof (for example, the signaling domain or peptide sequence involved in IL-7R α mediated signaling) can be a hybrid molecule which is linked, fused, encoded for etc, with one or more molecules such as for example, a receptor, ligand, label, synthetic or natural molecule etc. In another preferred embodiment, the IL-7R α molecule is a stand alone molecule. All of these molecules can comprise one or more modifications, substitutions, analogs, derivatives etc. (see, below). The hybrid molecule can be fused, linked etc to an IL-7 molecule, mutants, or fragments thereof. Other molecules include, for example, receptors, receptor domains, immunoglobulins, peptides, organic molecules, inorganic molecules, fluorescent or radioactive labels, polypeptides, peptides, oligonucleotides, polynucleotides, aptamers, synthetic or natural molecules, therapeutic agents, enzymes, or combinations thereof. Any

desired molecule can be used to provide a molecule comprising an IL-7R α chain, peptides or functional domains thereof, limited only by the imagination of the user.

[0083] In another preferred embodiment, the agent targets or specifically binds to the interleukin 7 receptor (IL-7R), a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, and inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0084] In another preferred embodiment, the agent inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

[0085] In a preferred embodiment, a molecule that binds to or targets IL-7R or inhibits IL-7R α mediated signaling comprises an antibody which specifically binds to IL-7R, variants, mutants, derivatives and fragments thereof.

[0086] A mutant IL-7R α polypeptide, either alone or as part of a chimeric polypeptide, can be encoded by a substantially pure nucleic acid molecule, including a molecule of genomic DNA, cDNA, or synthetic DNA. The nucleic acid molecule encoding a mutant IL-7R α polypeptide will be at least 65%, at least 75%, at least 85%, or at least 95% (e.g., 99%) identical to the nucleic acid encoding wild-type IL-7R α . For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

[0087] In another preferred embodiment, a molecule that binds to IL-7R or inhibits IL-7R mediated signaling comprises an antibody which specifically binds IL-7R, the IL-7R α chain or combinations thereof.

[0088] In another preferred embodiment, the antibody is specific for human IL-7R, preferably human IL-7R α chain, or human interleukin-7R (IL-7R) variants, or fragments thereof.

[0089] In another preferred embodiment, the anti-IL-7R α antibody is specific for membrane bound and/or soluble IL-7R α molecules.

[0090] In another preferred embodiment, the anti-IL-7 antibody is specific for membrane bound and/or soluble IL-7 molecules. IL-7 has been characterized functionally as a T cell growth factor that stimulates the proliferation and differentiation of B cells, T cells, natural killer (NK) cells, and lymphocyte-activated killer (LAK) cells *in vitro*.

[0091] In another preferred embodiment, the antibody specific for IL-7R or IL-7R α is human or humanized antibody. Preferably, the target is mammalian, preferably, the antibody specifically binds to a human IL-7R α molecule or a human interleukin IL-7R molecule.

[0092] In another preferred embodiment, a modulator of interleukin-7R mediated signaling or molecules comprising an IL-7R α chain or functional peptides or domains thereof, through the interleukin-7 receptor alpha chain comprises a mutant interleukin-7 molecule which may bind to the receptor and receptor mediated signaling does not occur. For example, structural modeling led to the prediction that the carboxy terminus of IL-7 is within a hydrophobic moment that is directed to a solvent interface. This indicates that the carboxy terminus is involved in protein-protein interactions (Cosenza *et al.*, *Protein Sci.* 9:916-926, 2000; see also Cosenza *et al.*, *J. Biol. Chem.* 272:32995-33000, 1997). Accordingly, IL-7 molecules containing one or more mutations within the carboxy terminal region are within the scope of the invention (particular IL-7 mutants include those having an addition, deletion, or substitution at amino acids in the carboxy region of the human IL-7 sequence or at analogous positions in IL-7 molecules of other species). As described herein, other residues can be mutated as well, and the invention encompasses mutant IL-7 polypeptides in which a single residue is changed (e.g., deleted or replaced with another residue), a pair of residues are changed (e.g., double mutants), or more than a pair of residues are changed (e.g., the mutant can be a triple mutant). The mutations can be of amino acid residues that are contiguous with one another, or the mutant residues may be separated by one or more wild type residues. In addition, the mutations can be in either the amino terminal, or carboxy terminal ends of the molecule, inclusive of amino acids in between the two terminals.

[0093] *Interleukin-7R α mediated signaling:* To determine whether any given IL-7R α chain, or IL-7R α mutant or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof, has a biological activity that differs from wild type IL-7R α ; whether mutant IL-7 binds to but does not result in signaling mediated by the IL-7 receptor; whether a IL-7R α , mutant IL-7R α chain or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof, expressed on a cell either fails to mediate a signal; whether the signal mediated by the IL-7R α , mutant IL-7R α or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof, is weak compared to a wild type IL-7R α , the signal mediated by IL-7R α is strong compared to wild type IL-7R α ; one can assess the ability of the mutants to perform as the wild type molecules would in the same circumstances. For example, IL-7R α mediated signaling, as (or others known in the art) can be assessed to determine whether a particular

molecule is an IL-7R antagonist or agonist and, whether such molecules are candidate therapeutic agents for treating diseases or disorders associated with IL-7 and IL-7R α mediated signaling, other T-cell mediated processes (including, but not limited to, autoimmune disorders, cancer, bone marrow immune cell development), as well as transplant rejection, such as an allograft rejection). Following IL-7R crosslinking, rapid activation of several kinases occurs, including members of the Janus and src families and P13-kinase (accordingly, one can assay kinase activation as a means of determining whether a molecule binds to and modulates the IL-7R; for example, inhibition of activation indicates a useful mutant and one that can be assessed further in cell-based assays in cell culture or *in vivo* as an inhibitor of IL-7R mediated signaling). A number of transcription factors are subsequently activated, including STATs, c-myc, NFAT and AP-1 (assays designed to evaluate these transcription factors can be used to assess any given interaction between candidate therapeutic agents and IL-7R α . For example, a candidate agent which modulates IL-7R α mediated signaling can indicate a molecule that agonizes or antagonizes an IL-7R). Jak1 and P13 kinase are complexed to the intracytoplasmic domains of the IL-7R α subunit, whereas Jak3 is complexed to the γc component, similar to IL-2R and IL-4R. Phosphorylation of both Jak1 and Jak3 initiate proliferation in activated T cells. The specific binding of IL-7 to the IL-7R α subunit initiates heterodimerization with γc and phosphorylation of the Jak kinases. The Tyr residues in the cytoplasmic tail of the receptor thus provide docking sites for proteins with phosphotyrosine-binding SH2 domains, which in turn are also Jak substrates.

[0094] Signal transducers and activators of transcription, STAT3 and STAT5, have been shown to undergo phosphorylation upon ligand engagement of the IL-7R (Foxwell *et al.*, *Eur. J. Immunol.* 25:3041-3046, 1995). In human peripheral blood T lymphoblasts, IL-2 and IL-7 were shown to be potentially equivalent in their ability to induce tyrosine phosphorylation of both STAT5 isoforms, STAT5a and STAT5b. The isoforms of STAT5 were shown to bind to related but distinct docking sites on the IL-7R α chain. IL-7 induces STAT5a/STAT5b heterodimerization, and STAT3 seems to be associated constitutively with each STAT5 isoform. STAT1 is also activated upon stimulation of precursor B cells by IL-7.

[0095] IL-7R engagement also activates the src family kinases p59^{fyn} and p53^{lyn} in pre-B cells and in myeloid cell lines. In contrast to p53/p56^{lyn}, p59^{fyn} is associated constitutively with IL-7R in these cells. In mature human T cells, p56^{lck} was activated by IL-7 and IL-7R was distinctly shown to be physically associated with both p59^{fyn} and p56^{lck}. Signaling through p59^{fyn} is unlikely to mediate all of the responses generated by IL-7 (Hofmeister *et al.*, *Cytokine Growth Factor Rev.* 10: 14-60, 1999; Venkitaraman and Cowling, *Eur. J. Immunol.*

24:2168-2174, 1994). Thus, an IL-7R α or functional domains thereof, can have a mutation in any one or more of the residues which are involved in IL-7R α mediated signaling pathways.

[0096] The invention is not limited to wild type IL-7R α but includes without limitation, allelic variants, species variants, splicing variants, mutants, fragments, and the like.

[0097] In a preferred embodiment, IL-7R α peptides comprise at least five consecutive amino acid residues with the understanding that they are "active" peptides. "Active" includes one or more functions of IL-7R α which includes known functions as described herein but also any other function that is innate to the IL-7R and IL-7R α molecule or including one which may be altered based on any manipulation by the end user.

[0098] In another preferred embodiment, IL-7R α includes the peptide itself, chemical equivalents thereto, isomers thereof (e.g., isomers, stereoisomers, retro isomers, retro-inverso isomers, all-[D] isomers, all-[L] isomers, or mixed [L] and [D] isomers thereof), conservative substitutions therein, precursor forms thereof, endoproteolytically-processed forms thereof, such as cleavage of single amino acids from N or C terminals or immunologically active metabolites of the peptides of the invention, pharmaceutically-acceptable salts and esters thereof, and other forms resulting from post-translational modification. Also included is any parent sequence, up to and including 10, 9, 8, 7, 6, 5 and 4 amino acids in length (cyclized, or linear, or branched from the core parent sequence), for which the specified sequence is a subsequence. A person skilled in the art would appreciate that where the peptide can be a monomer, dimer, a trimer, etc. The use of the peptides of the present invention include use of peptides wherein the active fragment or fragments are complexed to one or more binding partners. Modified peptides which retain the activity of the peptides of the invention are encompassed within the scope of the present invention.

[0099] In another preferred embodiment, an IL-7R α , IL-7R α peptides, or functional domains thereof, comprises at least one non-native amino acid residue or a non-amino acid molecule. A "non-native" amino acid residue comprises any change to an amino acid which is encoded by the IL-7R nucleic acid sequence. Thus, a non-native amino acid residue or non-amino acid molecule comprises, without limitation: a chemical equivalent, analog, synthetic molecule, derivative, variant, substitution, peptide nucleic acid, a linker molecule, inorganic molecule etc.

[00100] The mutations can be introduced at the nucleic acid level or at the amino acid level. With respect to particular nucleic acid sequences, because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine.

Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. If mutations at the nucleic acid level are introduced to encode a particular amino acid, then one or more nucleic acids are altered. For example proline is encoded by CCC, CCA, CCG, CCU; thus, one base change, e.g. CCC (proline) to GCC gives rise to alanine. Thus by way of example every natural or non-natural nucleic acid sequence herein which encodes a natural or non-natural polypeptide also describes every possible silent variation of the natural or non-natural nucleic acid. One of skill will recognize that each codon in a natural or non-natural nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule or a different molecule. Accordingly, each silent variation of a natural and non-natural nucleic acid which encodes a natural and non-natural polypeptide is implicit in each described sequence.

[00101] As to amino acid sequences, individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single natural and non-natural amino acid or a small percentage of natural and non-natural amino acids in the encoded sequence, the alteration results in the deletion of an amino acid, addition of an amino acid, or substitution of a natural and non-natural amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar natural amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the methods and compositions described herein.

[00102] A "non-natural amino acid" refers to an amino acid that is not one of the 20 common amino acids or pyrrolysine or selenocysteine. Other terms that may be used synonymously with the term "non-natural amino acid" is "non-naturally encoded amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and variously hyphenated and non-hyphenated versions thereof. The term "non-natural amino acid" includes, but is not limited to, amino acids which occur naturally by modification of a naturally encoded amino acid (including but not limited to, the 20 common amino acids or pyrrolysine and selenocysteine) but are not themselves incorporated, without user manipulation, into a growing polypeptide chain by the translation complex. Examples of naturally-occurring amino acids that are not naturally-encoded include, but are not limited to, N-acetylglucosaminy-L-serine, N-acetylglucosaminy-L-threonine, and O-phosphotyrosine.

Additionally, the term "non-natural amino acid" includes, but is not limited to, amino acids which do not occur naturally and may be obtained synthetically or may be obtained by modification of non-natural amino acids.

[00103] In some cases, the non-natural amino acid substitution(s) or incorporation(s) will be combined with other additions, substitutions, or deletions within the polypeptide to affect other chemical, physical, pharmacologic and/or biological traits. In some cases, the other additions, substitutions or deletions may increase the stability (including but not limited to, resistance to proteolytic degradation) of the polypeptide or increase affinity of the polypeptide for its appropriate receptor, ligand and/or binding proteins. In some cases, the other additions, substitutions or deletions may increase the solubility of the polypeptide. In some embodiments sites are selected for substitution with a naturally encoded or non-natural amino acid in addition to another site for incorporation of a non-natural amino acid for the purpose of increasing the polypeptide solubility following expression in recombinant host cells. In some embodiments, the polypeptides comprise another addition, substitution, or deletion that modulates affinity for the associated ligand, binding proteins, and/or receptor, modulates (including but not limited to, increases or decreases) receptor dimerization, stabilizes receptor dimers, modulates circulating half-life, modulates release or bio-availability, facilitates purification, or improves or alters a particular route of administration. Similarly, the non-natural amino acid polypeptide can comprise chemical or enzyme cleavage sequences, protease cleavage sequences, reactive groups, antibody-binding domains (including but not limited to, FLAG or poly-His) or other affinity based sequences (including but not limited to, FLAG, poly-His, GST, etc.) or linked molecules (including but not limited to, biotin) that improve detection (including but not limited to, GFP), purification, transport thru tissues or cell membranes, prodrug release or activation, size reduction, or other traits of the polypeptide.

[00104] The methods and compositions described herein include incorporation of one or more non-natural amino acids into a polypeptide. One or more non-natural amino acids may be incorporated at one or more particular positions which does not disrupt activity of the polypeptide. This can be achieved by making "conservative" substitutions, including but not limited to, substituting hydrophobic amino acids with non-natural or natural hydrophobic amino acids, bulky amino acids with non-natural or natural bulky amino acids, hydrophilic amino acids with non-natural or natural hydrophilic amino acids) and/or inserting the non-natural amino acid in a location that is not required for activity.

[00105] A variety of biochemical and structural approaches can be employed to select the desired sites for substitution with a non-natural amino acid within the polypeptide. Any position of the polypeptide chain is suitable for selection to incorporate a non-natural amino acid, and selection may be based on rational design or by random selection for any or no particular desired purpose. Selection of desired sites may be based on producing a non-natural amino acid polypeptide (which may be further modified or remain unmodified) having any desired property or activity, including but not limited to agonists, super-agonists, partial agonists, inverse agonists, antagonists, receptor binding modulators, receptor activity modulators, modulators of binding to binder partners, binding partner activity modulators, binding partner conformation modulators, dimer or multimer formation, no change to activity or property compared to the native molecule, or manipulating any physical or chemical property of the polypeptide such as solubility, aggregation, or stability. For example, locations in the polypeptide required for biological activity of a polypeptide can be identified using methods including, but not limited to, point mutation analysis, alanine scanning or homolog scanning methods. Residues other than those identified as critical to biological activity by methods including, but not limited to, alanine or homolog scanning mutagenesis may be good candidates for substitution with a non-natural amino acid depending on the desired activity sought for the polypeptide. Alternatively, the sites identified as critical to biological activity may also be good candidates for substitution with a non-natural amino acid, again depending on the desired activity sought for the polypeptide. Another alternative would be to make serial substitutions in each position on the polypeptide chain with a non-natural amino acid and observe the effect on the activities of the polypeptide. Any means, technique, or method for selecting a position for substitution with a non-natural amino acid into any polypeptide is suitable for use in the methods, techniques and compositions described herein.

[00106] The structure and activity of naturally-occurring mutants of a polypeptide that contain deletions can also be examined to determine regions of the protein that are likely to be tolerant of substitution with a non-natural amino acid. Once residues that are likely to be intolerant to substitution with non-natural amino acids have been eliminated, the impact of proposed substitutions at each of the remaining positions can be examined using methods including, but not limited to, the three-dimensional structure of the relevant polypeptide, and any associated ligands or binding proteins. X-ray crystallographic and NMR structures of many polypeptides are available in the Protein Data Bank (PDB, www.rcsb.org), a centralized database containing three-dimensional structural data of large molecules of

proteins and nucleic acids, one can be used to identify amino acid positions that can be substituted with non-natural amino acids. In addition, models may be made investigating the secondary and tertiary structure of polypeptides, if three-dimensional structural data is not available. Thus, the identity of amino acid positions that can be substituted with non-natural amino acids can be readily obtained. Exemplary sites of incorporation of a non-natural amino acid include, but are not limited to, those that are excluded from potential receptor binding regions, or regions for binding to binding proteins or ligands may be fully or partially solvent exposed, have minimal or no hydrogen-bonding interactions with nearby residues, may be minimally exposed to nearby reactive residues, and/or may be in regions that are highly flexible as predicted by the three-dimensional crystal structure of a particular polypeptide with its associated receptor, ligand or binding proteins.

[00107] A wide variety of non-natural amino acids can be substituted for, or incorporated into, a given position in a polypeptide. By way of example, a particular non-natural amino acid may be selected for incorporation based on an examination of the three dimensional crystal structure of a polypeptide with its associated ligand, receptor and/or binding proteins, a preference for conservative substitutions

[00108] As further used herein, a "chemical equivalent" of a peptide of the invention is a molecule which possesses the same desired activity, e.g. immunological activity, as peptides described herein, and exhibits a trivial chemical different, or a molecule which is converted, under mild conditions, into a peptide of the invention (e.g., esters, ethers, reduction products, and complexes of the peptides of the invention).

[00109] Additionally, as used herein, "conservative substitutions" are those amino acid substitutions which are functionally equivalent to the substituted amino acid residue, either because they have similar polarity or steric arrangement, or because they belong to the same class as the substituted residue (e.g., hydrophobic, acidic, or basic). The term "conservative substitutions", as defined herein, includes substitutions having an inconsequential effect on the ability of the peptide of the invention to enhance innate immunity. Examples of conservative substitutions include the substitution of a polar (hydrophilic) residue for another (e.g., arginine/lysine, glutamine/asparagine, or threonine/serine); the substitution of a non-polar (hydrophobic) residue (e.g. isoleucine, leucine, methionine, phenylalanine, tyrosine) for another, the substitution of an acidic residue (e.g., aspartic acid or glutamic acid) for another; or the substitution of a basic residue (e.g., arginine, histidine, lysine or ornithine) for another.

[00110] The term "analogue", as used herein, includes any peptide having an amino acid sequence substantially identical to a sequence described herein, in which at least one residue

has been conservatively substituted with a functionally-similar residue. An "analogue" includes functional variants and obvious chemical equivalents of an amino acid sequence of an IL-7R α , IL-7R α peptides, or functional domains thereof. As further used herein, the term "functional variant" refers to the activity of a peptide that demonstrates an ability to signal; interacting with one or more molecules; activating a transcription pathway, etc. An "analogue" further includes any pharmaceutically-acceptable salt of an analogue as described herein.

[00111] A "derivative", as used herein, refers to a peptide of the invention having one or more amino acids chemically derivatized by reaction of a functional side group. Exemplary derivatized molecules include, without limitation, peptide molecules in which free amino groups have been derivatized to form salts or amides, by adding acetyl groups, amine hydrochlorides, carbobenzoxy groups, chloroacetyl groups, formyl groups, p-toluene sulfonyl groups, or t-butyloxycarbonyl groups. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. Furthermore, free carboxyl groups may be derivatized to form salts, esters (e.g., methyl and ethyl esters), or hydrazides. Thus, a "derivative" further includes any pharmaceutically-acceptable salt of a derivative as described herein.

[00112] In one embodiment of the present invention, the isolated peptide of the invention has a modified C-terminus and/or a modified N-terminus. For example, the isolated peptide may have an amidated C-terminus. For example, the amino terminus can be acetylated (Ac) or the carboxy terminus can be amidated (NH₂). However, in one embodiment of the invention, the peptides of the invention are preferably not acetylated if such a modification would result in loss of desired immunological activity. Amino terminus modifications include methylating (i.e., --NHCH₃ or --NH(CH₃)₂), acetylating, adding a carbobenzoxy group, or blocking the amino terminus with any blocking group containing a carboxylate functionality defined by RCOO--, where R is selected from the group consisting of naphthyl, acridinyl, steroidyl, and similar groups. Carboxy terminus modifications include replacing the free acid with a carboxamide group or forming a cyclic lactam at the carboxy terminus to introduce structural constraints.

[00113] In one embodiment backbone substitutions can be made, such as NH to NCH₃. The isolated peptide may also be a modification (e.g., a point mutation, such as an insertion or a deletion, or a truncation). By way of example, the peptide may comprise an amino acid sequence comprising a modified residue by at least one point insertion of a D amino acid as long as desired IL-7R α activity is retained. In particular, proline analogs in which the ring size of the proline residue is changed from 5 members to 4, 6, or 7 members can be employed.

Cyclic groups can be saturated or unsaturated, and if unsaturated, can be aromatic or non-aromatic.

[00114] In another preferred embodiment, the naturally occurring side chains of the 20 genetically encoded amino acids (or D amino acids) are replaced with other side chains with similar properties, for instance with groups such as alkyl, lower alkyl, cyclic 4-, 5-, 6-, to 7-membered alkyl amide, amide lower alkyl amide di(lower alkyl), lower alkoxy, hydroxy, carboxy and the lower ester derivatives thereof, and with 4-, 5-, 6-, to 7-membered heterocyclic.

[00115] Such substitutions can include but are not necessarily limited to: (1) non-standard positively charged amino acids, like: ornithine, Nlys; N-(4-aminobutyl)-glycine which has the lysine side chain attached to the "N-terminus" and compounds with aminopropyl or aminoethyl groups attached to the amino group of glycine. (2), Non-naturally occurring amino acids with no net charge and side-chains similar to arginine, such as, Cit; citrulline and Hci; citrulline with one more methylene group; (3) non-standard non-naturally occurring amino acids with OH (e.g., like serine), such as, hSer; homoserine (one more methylene group, Hyp; hydroxyproline, Val(β OH); hydroxyvaline, Pen; penicillamin, (Val(β SH)); (4) proline derivatives, such as, D-Pro, such as, 3,4-dehydropyrrolidine, Pyr; pyroglutamine (proline with C=O in ring), Proline with fluorine substitutions on the ring, 1,3-thiazolidine-4-carboxylic acid (proline with S in ring); (5) Histidine derivative, such as, Thi; beta-(2-thienyl)-alanine; or (6) alkyl derivatives, such as, Abu; 2-aminobutyric acid (ethyl group on C α), Nva; norvaline (propyl group on C α), Nle; norleucine (butyl group on C α), Hol; homoleucine (propyl group on C α), Aib, alpha-aminoisobutyric acid (valine without methylene group). A person skilled in the art would appreciate that those substitutions that retain the activity of the parent peptide/sequence.

[00116] In another alternative embodiment, the C-terminal carboxyl group or a C-terminal ester can be induced to cyclize by internal displacement of the --OH or the ester (--OR) of the carboxyl group or ester respectively with the N-terminal amino group to form a cyclic peptide. For example, after synthesis and cleavage to give the peptide acid, the free acid is converted to an activated ester by an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride (CH₂Cl₂), dimethyl formamide (DMF) mixtures. The cyclic peptide is then formed by internal displacement of the activated ester with the N-terminal amine. Internal cyclization as opposed to polymerization can be enhanced by use of very dilute solutions. Such methods are well known in the art.

[00117] The peptides of the invention can be cyclized, or a desamino or descarboxy residue at the termini of the peptide can be incorporated, so that there is no terminal amino or carboxyl group, to decrease susceptibility to proteases or to restrict the conformation of the peptide. C-terminal functional groups of the compounds of the present invention include amide, amide lower alkyl, amide di(lower alkyl), lower alkoxy, hydroxy, and carboxy, and the lower ester derivatives thereof, and the pharmaceutically acceptable salts thereof.

[00118] The peptides of the invention can be cyclized by adding an N and/or C terminal cysteine and cyclizing the peptide through disulfide linkages or other side chain interactions.

[00119] A desamino or descarboxy residue at the termini of the peptide can be incorporated, so that there is no terminal amino or carboxyl group, to decrease susceptibility to proteases or to restrict the conformation of the peptide.

[00120] Mutant polypeptides, e.g. IL-7R, IL-7R α , molecules comprising IL-7R α or functional domains thereof, which can be assessed in one or more of the assays described above, encompass substantially pure polypeptides having an amino acid sequence that is identical to a wild type sequence. The polypeptides can include amino acid residues (naturally occurring, synthetic, analogs, derivatives, or modified (e.g., glycosylated or phosphorylated residues) that are linked by a peptide bond.

[00121] In addition to containing one or more mutations, a polypeptide of the invention can be substantially pure (i.e., separated from one or more of the components that naturally accompany the polypeptide). Typically, a polypeptide is substantially pure when it is at least 60%, by weight, free from naturally occurring organic molecules. Alternatively, the preparation can be at least 75%, at least 90%, or at least 99%, by weight, of mutant polypeptide. A substantially pure mutant polypeptide can be obtained, for example, by expression of a recombinant nucleic acid encoding a mutant polypeptide, e.g. IL-7 or IL-7R α , or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, including column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. Polypeptides that are derived from eukaryotic organisms but synthesized in *E. coli*, or other prokaryotes, and polypeptides that are chemically synthesized will be substantially free from their naturally associated components.

[00122] A wild type IL-7R α can be a polypeptide that is identical to the naturally-occurring IL-7R α polypeptide. A mutant polypeptide can be a polypeptide or portion thereof having at least one mutation relative to the wild-type molecule. A mutant IL-7R α or functional domains thereof, that is biologically active (functional domain or functional peptides) generally modifies at least 40%, more preferably at least 70%, and most preferably

at least 90% of the activity of the wild-type IL-7R α molecule (for example, a molecule may modulate an IL-7R α receptor signaling by about 40%, 50%, 60%, 70%, or more). The ability of a mutant IL-7R α polypeptide to modify wild-type IL-7R α activity can be assayed by numerous methods, including the cell proliferation and phosphorylation assays.

[00123] Mutations in polypeptides can be effected in many following ways, for example: deletion of one or more of the amino acids, addition of one or more amino acids, or substitution of one or more of the amino acids. In the event the mutation is a substitution, the substitution can be a conservative or non-conservative substitution. Non-conservative substitutions occur when one amino acid residue in a polypeptide sequence is replaced by another amino acid that has a different physical property (e.g., a different size, charge, or polarity) as the amino acid being replaced. For example, substitution of a non-aromatic amino acid in the place of an aromatic amino acid (e.g., substitution of an alanine in the place of tryptophan) is an example of a non-conservative substitution. Alternately, the substitution can be a conservative amino acid substitution. A conservative substitution can be the replacement of one amino acid in a polypeptide sequence by another amino acid, wherein the replacement amino acid has similar physical properties (e.g., size, charge, and polarity) as the amino acid being replaced. For example, replacing one aromatic amino acid with another aromatic amino acid can be a conservative substitution. Some typical examples of conservative amino acid substitutions include substitutions with the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. The substitution can be, for example, a non-aromatic amino acid substitution. The substitution can also be at the corresponding position of a polypeptide from another species (for example, a domesticated animal such as a cow, pig, sheep, rabbit, goat, dog or cat).

[00124] In some embodiments, the agents described herein can effectively compete with wild type IL-7 for binding, targeting, modulating activity or signaling etc., to the IL-7 receptor alpha chain. The polypeptides described herein can include a heterologous (i.e., non-IL-7) sequence (i.e., a polypeptide can be a chimeric polypeptide).

[00125] *Polynucleotides:* A mutant polypeptide, whether alone or as a part of a chimeric polypeptide, can be encoded by a nucleic acid molecule, and substantially pure nucleic acid molecules that encode the mutant polypeptides described herein are within the scope of the invention. The nucleic acid can be a molecule of genomic DNA, cDNA, synthetic DNA, or RNA. The nucleic acid molecule encoding, for example, IL-7R α , a mutant IL-7R or mutant IL-7R α polypeptide will be at least 65%, at least 75%, at least 85%, or at least 95% (e.g.,

99%) identical to the nucleic acid encoding wild-type molecules. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 10 nucleotides.

[00126] In a preferred embodiment, an expression vector comprises a nucleic acid molecule with a sequence encoding polypeptides described above, including antibodies specific for IL-7R or IL-7R α . The vector can be capable of directing expression of an IL-7R α polypeptide in, for example, a cell that has been transduced with the vector. These vectors can be viral vectors, such as retroviral, adenoviral, or adenoviral-associated vectors, as well as plasmids or cosmids. Prokaryotic or eukaryotic cells that contain and express DNA encoding any of the molecules comprising IL-7R α , mutant IL-7R α , IL-7R α domains, antibodies, etc, are also features of the invention. The method of transduction, the choice of expression vector, and the host cell may vary. The precise components of the expression system are not critical. It matters only that the components are compatible with one another, a determination that is well within the abilities of skilled artisans. Furthermore, for guidance in selecting an expression system, skilled artisans may consult Ausubel *et al.*, Current Protocols in Molecular Biology (1993, John Wiley and Sons, New York, N.Y.) and Pouwels *et al.*, Cloning Vectors: A Laboratory Manual (1987). The vector can also have a sequence that encodes a detectable marker, such as β -galactosidase, α -glucuronidase (GUS), luciferase, horseradish peroxidase (HRP), alkaline phosphatase, acetylcholinesterase, or chloramphenicol acetyl transferase. Fluorescent reporter genes include, but are not limited to, green fluorescent protein (GFP), red fluorescent protein (RFP), cyan fluorescent protein (CFP), and blue fluorescent protein (BFP). The detectable marker can also be an epitope tag, such as a myc, FLAG, or HA tag.

[00127] The vector may comprise any regulatory sequence allowing proper expression of the coding nucleic acid in a selected host cell, e.g., a promoter, terminator, polyA, origin of replication, integration region (e.g., homologous region), intron, UTR sequences, marker gene, etc.

[00128] In another preferred embodiment, an expression vector comprising a nucleic acid molecule as defined above is provided, including a signal peptide, operably linked to regulatory elements allowing expression of said nucleic acid in a mammalian host or host cell. Preferred regulatory elements include a promoter, which may be selected, without limitation, from viral, cellular and synthetic promoters, including constitutive, tissue-specific or regulated promoters, in particular from the group consisting of the CMV promoter, E1Fa promoter and metallothionein promoter. Further regulatory elements that may be contained

within the vectors of this invention include, without limitation, a Bcl-2 gene, UTR sequences and MAR sequences.

[00129] The above nucleic acids and vectors may be used for instance to produce recombinant mammalian IL-7R α polypeptides in various competent host or host cells, as well as for gene therapy purposes.

[00130] The vector may be a plasmid, virus, phage, cosmid, episome, etc. Preferred vectors are viral vectors (e.g., recombinant adenoviruses) and plasmids, which can be produced based on commercially available backbones, such as pBR, pcDNA, pUC, pET, pVITRO, etc. The vector typically comprises regulatory elements or sequences to control or mediate expression of a polypeptide. The regulatory sequences may be chosen from promoters, enhancers, silencers, tissue-specific signals, peptide signals, introns, terminators, polyA sequences, GC regions, etc., or a combination thereof. Such regulatory elements or sequences may be derived from mammalian, fungal, plant, bacterial, yeast, bacteriophage or viral genes, or from artificial sources. Useful promoters for prokaryote expression (such as *E. coli*) include T7 RNA polymerase promoter (pT7), TAC promoter (pTAC), Trp promoter, Lac promoter, Tre promoter, PhoA promoter for example. Suitable promoters for expression in mammalian cells include viral promoters (e.g., CMV, LTR, RSV, SV40, TK, pCAG, etc.), domestic gene promoters (e.g., E1f α , chicken Pactine, Ubiquitine, INSM1, etc.), hybrid promoters (e.g., actine/globin, etc.), etc. A vector may comprise more than one promoter. The promoters may be inducible or regulated. For instance, the use of inducible or regulated promoters allows a better control of production by dissociating the culture from production phases. Inducible or regulated promoters may be found in the literature, such as the Tetracycline system, the Geneswitch system, the Ecdysone system, the Oestradiol system, the RU486 system, the Cumate system, the metallothionein promoter etc. Other systems are based on electric currents or microwaves, such as focalized ultrasound system, AIR induced expression system and the like. These systems can be used to control expression of a polypeptide according to the invention.

[00131] The polypeptides may be co-expressed with other factors. The cDNAs coding for each may be both placed downstream of the same promoter, but separated by an IRES sequence, or each of them downstream of its own promoter.

[00132] The vector may further comprise an origin of replication and/or a marker gene, which may be selected from conventional sequences. An amplification selection marker such as the DHFR gene can be inserted in the backbone of the vector. The vector may further

comprise various combinations of these different elements which may be organized in different ways.

[00133] *Cells:* In a preferred embodiment, a cell comprises any of the polypeptides described herein, any of the nucleic acid molecules described herein, or any of the expression vectors described herein (for example, a T cell or a B cell, in culture or *in vivo*).

[00134] The present invention also provides recombinant host cells comprising a nucleic acid or a vector as described above. The host cell may be selected from any eukaryotic and prokaryotic cells, typically from a mammalian cell (in particular a human, rodent, canine cell), a bacterial cell (in particular *E. coli*, *Bacillus brevis*, *Bacillus subtilis*), a yeast cell, a plant cell and an insect cell. These host cells may be adapted to serum-free media. Production may also be accomplished in a transgenic animal or plant.

[00135] Preferred recombinant host cells are selected from mammalian cells, in particular human cells as well as derivatives or mutants thereof, including bone marrow cells, stem cells, immune cells and the like. "Cells of the immune system" or "immune cells", is meant to include any cells of the immune system that may be assayed, including, but not limited to, B lymphocytes, also called B cells, T lymphocytes, also called T cells, natural killer (NK) cells, natural killer T (NK) cells, lymphokine-activated killer (LAK) cells, monocytes, macrophages, neutrophils, granulocytes, mast cells, platelets, Langerhan's cells, stem cells, dendritic cells, peripheral blood mononuclear cells, tumor-infiltrating (TIL) cells, gene modified immune cells including hybridomas, drug modified immune cells, antigen presenting cells and derivatives, precursors or progenitors of the above cell types. The cells also comprise hematopoietic and non-hematopoietic cells.

[00136] Other examples of suitable host cells include Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells, Human Embryonic Kidney (HEK-293) cells, human epidermal keratinocytes (HEK), human stromal or epithelial cells, PERC6, etc. In such mammalian cells, polypeptides may be produced as a secreted protein using functional signal peptide sequences.

Antibodies:

[00137] Also within the scope of the invention are antibodies (e.g., polyclonal or monoclonal antibodies) that specifically bind any of the polypeptides described herein. These antibodies can be made by methods known to those in the art of molecular biology and cellular biochemistry, and they can be used to detect the polypeptides of the invention in diagnostic or therapeutic contexts.

[00138] In one embodiment, the composition comprises an anti-IL-7R α or anti IL-7R antibody. As described above, the antibody can be generated in any species and can be against IL-7R α or IL-7R from any species. Both monoclonal and polyclonal antibodies are contemplated and comprise embodiments of the invention. The antibody further be bispecific and be targeted to one or more IL-7R or IL-7R α from different species or to mutants, derivatives or fragments of these molecules.

[00139] *Polyclonal Antibodies:* Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. Alternatively, antigen may be injected directly into the animal's lymph node (see Kilpatrick *et al.*, *Hybridoma*, 16:381-389, 1997). An improved antibody response may be obtained by conjugating the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride or other agents known in the art.

[00140] *Monoclonal Antibodies:* Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods.

[00141] In the hybridoma method, a mouse or other appropriate host animal, such as rats, hamster or macaque monkey, is immunized as herein described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

[00142] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00143] Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a

medium. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Exemplary murine myeloma lines include those derived from MOP-21 and M.C.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA.

[00144] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by BIAcore or Scatchard analysis (Munson *et al.*, *Anal. Biochem.*, 107:220 (1980)).

[00145] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal. The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00146] *Recombinant Production of Antibodies:* The amino acid sequence of an immunoglobulin of interest may be determined by direct protein sequencing, and suitable encoding nucleotide sequences can be designed according to a universal codon table.

[00147] Alternatively, DNA encoding the monoclonal antibodies may be isolated and sequenced from the hybridoma cells using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). Sequence determination will generally require isolation of at least a portion of the gene or cDNA of interest. Usually this requires cloning the DNA or, preferably, mRNA (i.e., cDNA) encoding the monoclonal antibodies. Cloning is carried out using standard techniques (see, e.g., Sambrook *et al.* (1989) *Molecular*

Cloning: A Laboratory Guide, Vols 1-3, Cold Spring Harbor Press, which is incorporated herein by reference). For example, a cDNA library may be constructed by reverse transcription of polyA⁺ mRNA, preferably membrane-associated mRNA, and the library screened using probes specific for human immunoglobulin polypeptide gene sequences. In a preferred embodiment, however, the polymerase chain reaction (PCR) is used to amplify cDNAs (or portions of full-length cDNAs) encoding an immunoglobulin gene segment of interest (e.g., a light chain variable segment). The amplified sequences can be readily cloned into any suitable vector, e.g., expression vectors, minigene vectors, or phage display vectors. It will be appreciated that the particular method of cloning used is not critical, so long as it is possible to determine the sequence of some portion of the immunoglobulin polypeptide of interest.

[00148] As used herein, an "isolated" nucleic acid molecule or "isolated" nucleic acid sequence is a nucleic acid molecule that is either (1) identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the nucleic acid or (2) cloned, amplified, tagged, or otherwise distinguished from background nucleic acids such that the sequence of the nucleic acid of interest can be determined. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

[00149] One source for RNA used for cloning and sequencing is a hybridoma produced by obtaining a B cell from the transgenic mouse and fusing the B cell to an immortal cell. An advantage of using hybridomas is that they can be easily screened, and a hybridoma that produces a human monoclonal antibody of interest selected. Alternatively, RNA can be isolated from B cells (or whole spleen) of the immunized animal. When sources other than hybridomas are used, it may be desirable to screen for sequences encoding immunoglobulins or immunoglobulin polypeptides with specific binding characteristics. One method for such screening is the use of phage display technology. Phage display is described in e.g., Dower *et al.*, WO 91/17271, McCafferty *et al.*, WO 92/01047, and Caton and Koprowski, *Proc. Natl. Acad. Sci. USA*, 87:6450-6454 (1990), each of which is incorporated herein by reference. In one embodiment using phage display technology, cDNA from an immunized transgenic mouse (e.g., total spleen cDNA) is isolated, the polymerase chain reaction is used to amplify a cDNA sequences that encode a portion of an immunoglobulin polypeptide, e.g., CDR regions, and the amplified sequences are inserted into a phage vector. cDNAs encoding

peptides of interest, e.g., variable region peptides with desired binding characteristics, are identified by standard techniques such as panning.

[00150] The sequence of the amplified or cloned nucleic acid is then determined. Typically the sequence encoding an entire variable region of the immunoglobulin polypeptide is determined, however, it will sometimes be adequate to sequence only a portion of a variable region, for example, the CDR-encoding portion. Typically the portion sequenced will be at least 30 bases in length, more often bases coding for at least about one-third or at least about one-half of the length of the variable region will be sequenced.

[00151] Sequencing can be carried out on clones isolated from a cDNA library, or, when PCR is used, after subcloning the amplified sequence or by direct PCR sequencing of the amplified segment. Sequencing is carried out using standard techniques (see, e.g., Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Guide*, Vols 1-3, Cold Spring Harbor Press, and Sanger, F. *et al.* (1977) *Proc. Natl. Acad. Sci. USA* 74: 5463-5467, which is incorporated herein by reference). By comparing the sequence of the cloned nucleic acid with published sequences of human immunoglobulin genes and cDNAs, one of skill will readily be able to determine, depending on the region sequenced, (i) the germline segment usage of the hybridoma immunoglobulin polypeptide (including the isotype of the heavy chain) and (ii) the sequence of the heavy and light chain variable regions, including sequences resulting from N-region addition and the process of somatic mutation. One source of immunoglobulin gene sequence information is the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md.

[00152] Once isolated, the DNA may be operably linked to expression control sequences or placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to direct the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies is well known in the art.

[00153] Expression control sequences refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[00154] Nucleic acid is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates

in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, operably linked means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00155] Cell, cell line, and cell culture are often used interchangeably and all such designations herein include progeny. Transformants and transformed cells include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

[00156] The invention also provides isolated nucleic acids encoding specific binding agents or antibodies of the invention, optionally operably linked to control sequences recognized by a host cell, vectors and host cells comprising the nucleic acids, and recombinant techniques for the production of the specific binding agents or antibodies, which may comprise culturing the host cell so that the nucleic acid is expressed and, optionally, recovering the specific binding agent or antibody from the host cell culture or culture medium.

[00157] Many vectors are known in the art. Vector components may include one or more of the following: a signal sequence (that may, for example, direct secretion of the specific binding agent or antibody), an origin of replication, one or more selective marker genes (that may, for example, confer antibiotic or other drug resistance, complement auxotrophic deficiencies, or supply critical nutrients not available in the media), an enhancer element, a promoter, and a transcription termination sequence, all of which are well known in the art.

[00158] Suitable host cells include prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis*, *Pseudomonas*, and *Streptomyces*. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for specific binding agent-encoding vectors. *Saccharomyces cerevisiae*, or common baker's

yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Pichia*, e.g. *P. pastoris*, *Schizosaccharomyces pombe*; *Kluyveromyces*, *Yarrowia*; *Candida*; *Trichoderma reesia*; *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

[00159] Suitable host cells for the expression of glycosylated specific binding agent or antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection of such cells are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV.

[00160] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become routine procedure. Examples of useful mammalian host cell lines are Chinese hamster ovary cells, including CHOK1 cells (ATCC CCL61), DXB-11, DG-44, and Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77: 4216 (1980)); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, [Graham *et al.*, *J. Gen Virol.* 36: 59 (1977)]; baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human hepatoma cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383: 44-68 (1982)); MRC 5 cells or FS4 cells.

[00161] Host cells are transformed or transfected with the above-described nucleic acids or vectors for specific binding agent or antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In addition, novel vectors and transfected cell lines with multiple copies of transcription units separated by a selective

marker are particularly useful and preferred for the expression of specific binding agents or antibodies.

[00162] The host cells used to produce the specific binding agent or antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58: 44 (1979), Barnes *et al.*, *Anal. Biochem.* 102: 255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO90103430; WO 87/00195; or U.S. Pat. Re. No. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GentamycinTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan. The expression vectors, pDC323 and pDC324 as described in U.S. Patent Application No. 20030082735, containing the appropriate respective light chain and heavy chain pair were transfected into the CS9 host cell line.

[00163] Upon culturing the host cells, the specific binding agent or antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the specific binding agent or antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration.

[00164] The specific binding agent or antibody composition can be purified using, for example, hydroxyl apatite chromatography, cation or anion exchange chromatography, or preferably affinity chromatography, using the antigen of interest or protein A or protein G as an affinity ligand. Protein A can be used to purify specific binding agents or antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62: 1-13 (1983)). Protein G is recommended for all mouse isotypes and for human $\gamma 3$ (Guss *et al.*, *EMBO J.* 5: 1567-1575 (1986)). The matrix to which the affinity ligand is attached is

most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the specific binding agent or antibody comprises a C_H3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as ethanol precipitation, Reverse Phase HPLC, chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also possible depending on the specific binding agent or antibody to be recovered.

[00165] *Chimeric and Humanized Antibodies*: Since chimeric or humanized antibodies are less immunogenic in humans than the parental mouse monoclonal antibodies, they can be used for the treatment of humans with far less risk of anaphylaxis. Thus, these antibodies are preferred in therapeutic applications that involve *in vivo* administration to a human.

[00166] Chimeric monoclonal antibodies, in which the variable Ig domains of a rodent monoclonal antibody are fused to human constant Ig domains, can be generated using standard procedures known in the art (See Morrison, S. L., *et al.* (1984) Chimeric Human Antibody Molecules; Mouse Antigen Binding Domains with Human Constant Region Domains, *Proc. Natl. Acad. Sci. USA* 81, 6841-6855; and, Boulianne, G. L., *et al.*, *Nature* 312, 643-646. (1984)). Although some chimeric monoclonal antibodies have proved less immunogenic in humans, the rodent variable Ig domains can still lead to a significant human anti-rodent response.

[00167] Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting the non-human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as humanizing through "CDR grafting"), or, alternatively, (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering"). These methods are disclosed in, e.g., Jones *et al.*, *Nature* 321:522 525 (1986); Morrison *et al.*, *Proc. Natl. Acad. Sci.*, U.S.A., 81:6851 6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65 92 (1988); Verhoeyer *et al.*, *Science* 239:1534 1536 (1988); Padlan, *Molec. Immunol.* 28:489 498 (1991); Padlan, *Molec. Immunol.* 31(3):169 217 (1994); and Kettleborough, C. A. *et al.*, *Protein Eng.* 4(7):773 83 (1991) each of which is incorporated herein by reference.

[00168] In particular, a rodent antibody on repeated *in vivo* administration in man either alone or as a conjugate will bring about an immune response in the recipient against the rodent antibody; the so-called HAMA response (Human Anti Mouse Antibody). The HAMA

response may limit the effectiveness of the pharmaceutical if repeated dosing is required.

The immunogenicity of the antibody may be reduced by chemical modification of the antibody with a hydrophilic polymer such as polyethylene glycol or by using the methods of genetic engineering to make the antibody binding structure more human like.

[00169] CDR grafting involves introducing one or more of the six CDRs from the mouse heavy and light chain variable Ig domains into the appropriate framework regions of a human variable Ig domain. This technique (Riechmann, L., *et al.*, *Nature* 332, 323 (1988)), utilizes the conserved framework regions (FR1-FR4) as a scaffold to support the CDR loops which are the primary contacts with antigen. A significant disadvantage of CDR grafting, however, is that it can result in a humanized antibody that has a substantially lower binding affinity than the original mouse antibody, because amino acids of the framework regions can contribute to antigen binding, and because amino acids of the CDR loops can influence the association of the two variable Ig domains. To maintain the affinity of the humanized monoclonal antibody, the CDR grafting technique can be improved by choosing human framework regions that most closely resemble the framework regions of the original mouse antibody, and by site-directed mutagenesis of single amino acids within the framework or CDRs aided by computer modeling of the antigen binding site (e.g., Co, M. S., *et al.* (1994), *J. Immunol.* 152, 2968-2976).

[00170] One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region which disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRs of the parent non-human antibody. Such humanized antibodies may be further derivatized to facilitate uptake and clearance, e.g., via Ashwell receptors (See, e.g., U.S. Pat. Nos. 5,530,101 and 5,585,089).

[00171] A number of humanizations of mouse monoclonal antibodies by rational design have been reported (See, for example, 20020091240 published Jul. 11, 2002, WO 92/11018 and U.S. Pat. No., 5,693,762, U.S. Pat. No. 5,766,866).

[00172] Human engineering of antibodies has also been described in, e.g., Studnicka *et al.* U.S. Pat. No. 5,766,886; Studnicka *et al.* *Protein Engineering* 7: 805-814 (1994).

[00173] *Production of Antibody Variants:* Amino acid sequence variants of the desired specific binding agent or antibody may be prepared by introducing appropriate nucleotide changes into the encoding DNA, or by peptide synthesis. Such variants include, for example, deletions and/or insertions and/or substitutions of residues within the amino acid sequences of the specific binding agents or antibodies. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the specific binding agent or humanized or variant antibody, such as changing the number or position of glycosylation sites.

[00174] Nucleic acid molecules encoding amino acid sequence variants of the specific binding agent or antibody are prepared by a variety of methods known in the art. Such methods include oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the specific binding agent or antibody.

[00175] A useful method for identification of certain residues or regions of the specific binding agent or antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis," as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed variants are screened for the desired activity.

[00176] Ordinarily, amino acid sequence variants of the specific binding agent or antibody will have an amino acid sequence having at least 60% amino acid sequence identity with the original specific binding agent or antibody (murine or humanized) amino acid sequences of either the heavy or the light chain, or at least 65%, or at least 70%, or at least 75% or at least 80% identity, more preferably at least 85% identity, even more preferably at least 90% identity, and most preferably at least 95% identity, including for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100%. Identity or homology with respect to this sequence is defined

herein as the percentage of amino acid residues in the candidate sequence that are identical with the original sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the specific binding agent or antibody sequence shall be construed as affecting sequence identity or homology. Thus, sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences, or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff *et al.*, in Atlas of protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

[00177] *Insertions:* Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intra-sequence insertions of single or multiple amino acid residues. Examples of terminal insertions include a specific binding agent or antibody with an N-terminal methionyl residue or the specific binding agent or antibody (including antibody fragment) fused to an epitope tag or a salvage receptor epitope. Other insertional variants of the specific binding agent or antibody molecule include the fusion to a polypeptide which increases the serum half-life of the specific binding agent or antibody, e.g. at the N-terminus or C-terminus.

[00178] Examples of epitope tags include the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.* 8: 2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Mol. Cell. Biol.* 5(12): 3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering* 3(6): 547-553 (1990)]. Other exemplary tags are a poly-histidine sequence, generally around six histidine residues, that permits isolation of a compound so labeled using nickel chelation. Other labels and tags, such as the FLAG™ tag (Eastman Kodak, Rochester, N.Y.) are well known and routinely used in the art.

[00179] The term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

[00180] *Substitutions*: Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the specific binding agent or antibody molecule removed and a different residue inserted in its place. Substitutional mutagenesis within any of the hypervariable or CDR regions or framework regions is contemplated. Exemplary residue substitutions comprise: Ala (A) val; leu; ile val Arg (R) lys; gln; asn lys Asn (N) gln; his; asp, lys; gln arg Asp (D) glu; asn glu Cys (C) ser; ala ser Gln (Q) asn; glu asn Glu (E) asp; gln asp Gly (G) ala His (H) asn; gln; lys; arg Ile (I) leu; val; met; ala; leu phe; norleucine Leu (L) norleucine; ile; val; ile met; ala; phe Lys (K) arg; gln; asn arg Met (M) leu; phe; ile leu Phe (F) leu; val; ile; ala; tyr Pro (P) ala Ser (S) thr Thr (T) ser ser Trp (W) tyr; phe tyr Tyr (Y) trp; phe; thr; ser phe Val (V) ile; leu; met; phe; leu ala; norleucine

[00181] Substantial modifications in the biological properties of the specific binding agent or antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties: (1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr; (3) acidic: asp, glu; (4) basic: asn, gin, his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic: trp, tyr, phe.

[00182] Conservative substitutions involve replacing an amino acid with another member of its class. Non-conservative substitutions involve replacing a member of one of these classes with a member of another class.

[00183] Any cysteine residue not involved in maintaining the proper conformation of the specific binding agent or humanized or variant antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the specific binding agent or antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[00184] *Affinity Maturation*: Affinity maturation involves preparing and screening specific binding agent or antibody variants that have mutations (deletions, insertions or substitutions) within the CDRs of a parent specific binding agent or antibody and selecting

variants that have improved biological properties such as binding affinity relative to the parent specific binding agent or antibody. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino substitutions at each site. The specific binding agent or antibody variants thus generated may be displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity).

[00185] Alanine scanning mutagenesis can be performed to identify hypervariable region residues that contribute significantly to antigen binding. Alternatively, or in addition, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the specific binding agent or antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and specific binding agents or antibodies with superior properties in one or more relevant assays may be selected for further development.

[00186] Techniques utilizing gene shuffling and directed evolution may also be used to prepare and screen specific binding agent or antibody variants for desired activity. For example, Jermutus *et al.*, *Proc Nat'l Acad Sci USA*. 2001 Jan. 2; 98(1):75-80 reports that tailored *in vitro* selection strategies based on ribosome display were combined with *in vitro* diversification by DNA shuffling to evolve either the off-rate or thermodynamic stability of single-chain Fv antibody fragments (scFvs); Fermer *et al.*, *Tumor Biol*. 2004 Jan.-Apr.; 25(1-2):7-13 reports that use of phage display in combination with DNA shuffling raised affinity by almost three orders of magnitude.

[00187] *Altered Glycosylation:* Specific binding agent or antibody variants can also be produced that have a modified glycosylation pattern relative to the parent specific binding agent or antibody, for example, deleting one or more carbohydrate moieties found in the specific binding agent or antibody, and/or adding one or more glycosylation sites that are not present in the specific binding agent or antibody.

[00188] Glycosylation of polypeptides including antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. The presence

of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. Thus, N-linked glycosylation sites may be added to a specific binding agent or antibody by altering the amino acid sequence such that it contains one or more of these tripeptide sequences. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. O-linked glycosylation sites may be added to a specific binding agent or antibody by inserting or substituting one or more serine or threonine residues to the sequence of the original specific binding agent or antibody.

[00189] *Other Modifications:* Cysteine residue(s) may be removed or introduced in the Fc region, thereby eliminating or increasing interchain disulfide bond formation in this region. The homodimeric specific binding agent or antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176: 1191-1195 (1992) and Shopes, B. *J. Immunol.* 148: 2918-2922 (1992). Homodimeric specific binding agents or antibodies may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.*, *Cancer Research* 53: 2560-2565 (1993). Alternatively, a specific binding agent or antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design* 3: 219-230 (1989).

[00190] It has been shown that sequences within the CDR can cause an antibody to bind to MHC Class II and trigger an unwanted helper T-cell response. A conservative substitution can allow the specific binding agent or antibody to retain binding activity yet reduce its ability to trigger an unwanted T-cell response.

[00191] It is also contemplated that one or more of the N-terminal 20 amino acids of the heavy or light chain are removed.

[00192] Modifications to increase serum half-life also may be desirable, for example, by incorporation of or addition of a salvage receptor binding epitope (e.g., by mutation of the appropriate region or by incorporating the epitope into a peptide tag that is then fused to the specific binding agent or antibody at either end or in the middle, e.g., by DNA or peptide synthesis) (see, e.g., WO96/32478) or adding molecules such as PEG or other water soluble polymers, including polysaccharide polymers.

[00193] The salvage receptor binding epitope preferably constitutes a region wherein any one or more amino acid residues from one or two loops of a Fc domain are transferred to an

analogous position of the specific binding agent or antibody or fragment. Even more preferably, three or more residues from one or two loops of the Fe domain are transferred. Still more preferred, the epitope is taken from the C_H2 domain of the Fc region (e.g., of an IgG) and transferred to the C_H1, C_H3, or V_H region, or more than one such region, of the specific binding agent or antibody. Alternatively, the epitope is taken from the C_H2 domain of the Fc region and transferred to the C_L region or V_L region, or both, of the specific binding agent or antibody fragment. See also International applications WO 97/34631 and WO 96/32478 which describe Fc variants and their interaction with the salvage receptor.

[00194] Other sites of the constant region have been identified that are responsible for complement dependent cytotoxicity (CDC), such as the C1q binding site and/or the antibody-dependent cellular cytotoxicity (ADCC) [see, e.g., *Mol. Immunol.* 29 (5): 633-9 (1992); Shields *et al.*, *J. Biol. Chem.*, 276(9):6591-6604 (2001), incorporated by reference herein in its entirety]. Mutation of residues within Fc receptor binding sites can result in altered (i.e. increased or decreased) effector function, such as altered ADCC or CDC activity, or altered half-life. As described above, potential mutations include insertion, deletion or substitution of one or more residues, including substitution with alanine, a conservative substitution, a non-conservative substitution, or replacement with a corresponding amino acid residue at the same position from a different subclass (e.g. replacing an IgG1 residue with a corresponding IgG2 residue at that position).

[00195] *Human Antibodies:* Human antibodies to can also be produced using transgenic animals that have no endogenous immunoglobulin production and are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Pat. No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chains, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

[00196] Using a transgenic animal described above, for example, an immune response can be produced to a selected antigenic molecule, and antibody producing cells can be removed from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein. WO 96/33735 discloses that monoclonal antibodies against IL-8, derived from immune cells of transgenic mice immunized with IL-8, blocked IL-8 induced functions of neutrophils. Human monoclonal antibodies with specificity for the antigen used to immunize transgenic animals are also disclosed in WO 96/34096 and U.S. patent application no. 20030194404; and U.S. patent application no. 20030031667). See also Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993); and U.S. Pat. No. 5,591,669, U.S. Pat. No. 5,589,369, U.S. Pat. No. 5,545,807; and U.S. Patent Application No. 20020199213. U.S. Patent Application No. and 20030092125 describes methods for biasing the immune response of an animal to the desired epitope. Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275).

[00197] *Production by Phage Display Techniques:* The development of technologies for making repertoires of recombinant human antibody genes, and the display of the encoded antibody fragments on the surface of filamentous bacteriophage, has provided another means for making human antibodies directly. The antibodies produced by phage technology are produced as antigen binding fragments-usually Fv or Fab fragments-in bacteria and thus lack effector functions. Effector functions can be introduced by one of two strategies: The fragments can be engineered either into complete antibodies for expression in mammalian cells, or into bispecific antibody fragments with a second binding site capable of triggering an effector function.

[00198] Typically, the Fd fragment (V_H - C_H1) and light chain (V_L - C_L) of antibodies are separately cloned by PCR and recombined randomly in combinatorial phage display libraries, which can then be selected for binding to a particular antigen. The antibody fragments are expressed on the phage surface, and selection of Fv or Fab (and therefore the phage containing the DNA encoding the antibody fragment) by antigen binding is accomplished through several rounds of antigen binding and re-amplification, a procedure termed panning. Antibody fragments specific for the antigen are enriched and finally isolated.

[00199] In 1994, an approach for the humanization of antibodies, called "guided selection", was described. Guided selection utilizes the power of the phage display technique for the humanization of mouse monoclonal antibody (See Jespers, L. S., *et al.*, *BioTechnology* 12, 899-903 (1994)). For this, the Fd fragment of the mouse monoclonal antibody can be displayed in combination with a human light chain library, and the resulting hybrid Fab library may then be selected with antigen. The mouse Fd fragment thereby provides a template to guide the selection. Subsequently, the selected human light chains are combined with a human Fd fragment library. Selection of the resulting library yields entirely human Fab.

[00200] A variety of procedures have been described for deriving human antibodies from phage-display libraries (See, for example, Hoogenboom *et al.*, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991); U.S. Pat. Nos. 5,565,332 and 5,573,905; Clackson, T., and Wells, J. A., *TIBTECH* 12, 173-184 (1994)). In particular, *in vitro* selection and evolution of antibodies derived from phage display libraries has become a powerful tool (See Burton, D. R., and Barbas III, C. F., *Adv. Immunol.* 57, 191-280 (1994); and, Winter, G., *et al.*, *Annu. Rev. Immunol.* 12, 433-455 (1994); U.S. patent application no. 20020004215 and WO92/01047; U.S. patent application no. 20030190317 published Oct. 9, 2003 and U.S. Pat. No. 6,054,287; U.S. Pat. No. 5,877,293.

[00201] Watkins, "Screening of Phage-Expressed Antibody Libraries by Capture Lift," *Methods in Molecular Biology, Antibody Phage Display: Methods and Protocols* 178: 187-193, and U.S. patent application no. 200120030044772 published Mar. 6, 2003 describes methods for screening phage-expressed antibody libraries or other binding molecules by capture lift, a method involving immobilization of the candidate binding molecules on a solid support.

[00202] *Other Covalent Modifications:* Covalent modifications of the agent or antibody are also included within the scope of this invention. They may be made by chemical synthesis or by enzymatic or chemical cleavage of the agent or antibody, if applicable. Other types of covalent modifications can be introduced into the specific binding agent or antibody by reacting targeted amino acid residues with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[00203] Cysteinyll residues most commonly are reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyll residues also are derivatized by reaction with bromotrifluoroacetone, α -bromo- β -(5-imidazolyl)propionic acid, chloroacetyl phosphate, N-

alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[00204] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0.

[00205] Lysinyl and amino-terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α -amino-containing residues include imidoesters such as methyl picolinimide, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

[00206] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[00207] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylmidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay.

[00208] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N-CN-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl)carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[00209] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. These residues are deamidated under neutral or basic conditions. The deamidated form of these residues falls within the scope of this invention.

[00210] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[00211] Another type of covalent modification involves chemically or enzymatically coupling glycosides to the specific binding agent or antibody. These procedures are advantageous in that they do not require production of the specific binding agent or antibody in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO87/05330, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

[00212] Removal of any carbohydrate moieties present on the specific binding agent or antibody may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the specific binding agent or antibody to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the specific binding agent or antibody intact. Chemical deglycosylation is described by Hakimuddin, *et al. Arch. Biochem. Biophys.* 259: 52 (1987) and by Edge *et al. Anal. Biochem.*, 118: 131 (1981). Enzymatic cleavage of carbohydrate moieties on a specific binding agent or antibody can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al. Meth. Enzymol.* 138: 350 (1987).

[00213] Another type of covalent modification of the specific binding agent or antibody comprises linking the specific binding agent or antibody to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyethylated polyols, polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol, polyoxyalkylenes, or polysaccharide polymers such as dextran. Such methods are known in the art, see, e.g. U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192, 4,179,337, 4,766,106, 4,179,337, 4,495,285, 4,609,546 or EP 315 456.

Treatments of Disease or Disorders

[00214] In a preferred embodiment, the compositions of the invention are administered to patients with autoimmune, immune cell disorders, such as those mediated by or involve, for example, T cells.

[00215] In another preferred embodiment, the agent or composition protects or treats a patient at risk of developing or suffering from an immune system related disease or disorder, comprising: autoimmune diseases or disorders, inflammatory diseases or disorders, Graft versus Host Disease (GVHD), neuroinflammatory diseases or disorders.

[00216] In another preferred embodiment, the agent or composition, protects or treats a patient suffering from or at risk of developing cancer, bacterial, viral, fungal, or parasitic organisms. In a preferred embodiment, a method of treating a viral infection in a patient comprises administering to the patient, a therapeutically effective amount of : (i) an agent which modulates IL-7R α signaling.

[00217] In another preferred embodiment, an IL-7R α specific agent is administered to a patient to prevent or treat cancer. Cancer refers to all types of cancer or neoplasm or malignant tumors found in mammals, including, but not limited to: leukemias, lymphomas, melanomas, carcinomas and sarcomas. Examples of cancers are cancer of the brain, breast, pancreas, cervix, colon, head and neck, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and Medulloblastoma. As used herein, the terms "cancer," "neoplasm," and "tumor," are used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and *in vitro* cultures and cell lines derived from cancer cells. When referring to a type of cancer that normally manifests as a solid tumor, a "clinically detectable" tumor is one that is detectable on the basis of tumor mass; e.g., by procedures such as CAT scan, MR imaging, X-ray, ultrasound or palpation, and/or which is detectable because of the expression of one or more cancer-specific antigens in a sample obtainable from a patient.

[00218] In a preferred embodiment, a modulator of IL-7R α chain mediated signaling and/or IL-7R mediated signaling is administered to a patient with an autoimmune disease or disorder. The compositions of the invention, such as for example, modulators of interleukin-7 receptor alpha chain mediated signaling, modulators of interleukin-7R mediated signaling

are administered to a patient diagnosed or at risk of developing an autoimmune disease. See, for example, Figures 2A, 2B and Table 1 in the examples which follow.

[00219] A patient with an autoimmune disease may be diagnosed as known to one of ordinary skill in the art. Such patients may be identified symptomatically and/or by obtaining a sample from a patient and isolating autoreactive T cells and comparing the level of autoreactive T cells in a patient to a control (see, U.S. Patent Application Publication No. 20060105336). For instance, type 1 diabetes may be identified by age of on-set and dependence on insulin injections to maintain glucose homeostasis.

[00220] The response of a patient with an autoimmune disease to treatment may be monitored by determining the severity of their symptoms or by determining the frequency of autoreactive T cells in a sample from a patient with an autoimmune disease. The severity of symptoms of the autoimmune disease may correlate with the number of autoreactive T cells (see, U.S. Patent Application Publication No. 20060105336). In addition, an increase in the number of autoreactive T cells in the sample may be used as an indication to apply treatments intended to minimize the severity of the symptoms and/or treat the disease before the symptoms appear.

[00221] Autoimmunity and Autoimmune Disorders and Diseases. Autoimmunity is defined as persistent and progressive immune reactions to non infectious self antigens, as distinct from infectious non self antigens from bacterial, viral, fungal, or parasitic organisms which invade and persist within mammals and humans. Autoimmune conditions include scleroderma, Grave's disease, Crohn's disease, Sjorgen's disease, multiple sclerosis, Hashimoto's disease, psoriasis, myasathenia gravis, Autoimmune Polyendocrinopathy syndromes, Type I diabetes mellitus (T1DM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, and thyroiditis, as well as in the generalized autoimmune diseases typified by human Lupus. "Autoantigen" or "self-antigen" as used herein refers to an antigen or epitope which is native to the mammal and which is immunogenic in said mammal disease.

[00222] Also, or in the alternative, methods are provided for treating a human at risk for having an autoimmune disorder. The methods include administering to the human a composition including any of the compositions described herein, any of the nucleic acids described herein, or any of the expression vectors described herein. The amount of the composition administered will be sufficient to inhibit the symptoms of the autoimmune disorder in the patient. Moreover, these compositions can be administered together with (before, during, or after) other therapeutic regimens (such as physical therapy, as for an arthritic condition, or extracorporeal photophoresis (ECP), such as in cases of GVHD). A

human having or at risk for developing an autoimmune disorder can be diagnosed as having or at risk for developing (1) a rheumatic disease, such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome or Behcet's disease; (2) type I (insulin dependent) or type II diabetes mellitus; (3) an autoimmune disease of the thyroid, such as Hashimoto's thyroiditis or Graves' Disease; (4) an autoimmune disease of the central nervous system, such as multiple sclerosis, myasthenia gravis, or encephalomyelitis; (5) a variety of pemphigus, such as pemphigus vulgaris, pemphigus vegetans, pemphigus foliaceus, Senear-Usher syndrome, or Brazilian pemphigus; (6) psoriasis (e.g., psoriasis vulgaris) or atopic dermatitis; or (7) inflammatory bowel disease (e.g., ulcerative colitis or Crohn's Disease). The IL-7, IL-7R, IL-7R α polypeptides, nucleic acids, agents, and vectors described herein can be used to treat other autoimmune disorders including, but not limited to, endogenous uveitis, nephrotic syndrome, primary biliary cirrhosis, lichen planus, pyoderma gangrenosum, alopecia areata, a Bullous disorder, chronic viral active hepatitis, autoimmune chronic active hepatitis, and acquired immune deficiency syndrome (AIDS).

[00223] Methods are also provided for treating a human having or at risk for developing an autoimmune disorder resulting from a transplant rejection, including an allograft (including xenograft) or autograft rejection, and including rejections of tissue, organ, or cell transplants. The disorder can be, for example, graft-versus-host-disease (GVHD), including acute or chronic GVHD, or aplastic anemia. The methods include administering to the human a composition including any of the polypeptides described herein (including an IL-7 polypeptide, small molecule, antibodies etc), any of the nucleic acids described herein, or any of the expression vectors described herein. The amount of the composition administered will be sufficient to inhibit the symptoms of the transplant rejection in the human. For example, the human can reject a transplanted organ (such as a heart, liver, or kidney), a tissue graft (such as a skin graft), or a cell transplant (such as a bone marrow transplant). The treatment methods can improve or prevent any symptoms of a transplant rejection, including but not limited to symptoms associated with GVHD (acute or chronic GVHD). Moreover, these compositions can be administered together with (before, during, or after) other therapeutic regimens, such as extracorporeal photophoresis (ECP), as in cases of GVHD.

[00224] In therapeutic applications, the compositions can be administered with a physiologically-acceptable carrier, such as physiological saline by any standard route including intraperitoneally, intramuscularly, subcutaneously, or intravenously. It is expected that the intravenous route will be preferred. It is well known in the medical arts that dosages

for any one patient depend on many factors, including the general health, sex, weight, body surface area, and age of the patient, as well as the particular compound to be administered, the time and route of administration, and other drugs being administered concurrently.

Dosages, for example, for the polypeptides, of the invention will vary, but a preferred dosage for intravenous administration is approximately 0.01 mg to 100 mg/ml blood volume.

Determination of correct dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology.

[00225] In addition, or in the alternative, methods are provided for inhibiting the proliferation of a cell that expresses an IL-7 receptor (for example, a lymphoid or a myeloid cell). The methods include providing a cell that expresses an IL-7 receptor and exposing the cell to a composition (for example, a pharmaceutical composition) having any of the molecules described herein, any of the nucleic acid molecules described herein, or any of the expression vectors described herein, wherein the amount of the composition to which the cell is exposed is sufficient to inhibit the proliferation of the cell. In addition, or in the alternative, methods are provided for diagnosing a patient as having a disease or condition that could be treated with any of the polypeptides described herein, any of the nucleic acids described herein, or any of the expression vectors described herein. The methods include determining whether a biological sample obtained from the patient contains cells that are bound by a polypeptide comprising IL-7, the occurrence of binding indicating that the cells can be bound by any of the polypeptides described herein *in vivo* and thereby inhibited from proliferating in response to wild-type IL-7 *in vivo*.

[00226] The DNA molecules described can be contained within a vector that is capable of directing expression of a modulator of an IL-7 polypeptide or IL-7R α in, for example, a cell that has been transduced with the vector. These vectors can be viral vectors, such as retroviral, adenoviral, or adenoviral-associated vectors, as well as plasmids or cosmids. Prokaryotic or eukaryotic cells that contain and express DNA encoding any of the polypeptides are also features of the invention. The method of transduction, the choice of expression vector, and the host cell may vary. The precise components of the expression system are not critical. It matters only that the components are compatible with one another, a determination that is well within the abilities of skilled artisans. Furthermore, for guidance in selecting an expression system, skilled artisans may consult Ausubel *et al.*, Current Protocols in Molecular Biology (1993, John Wiley and Sons, New York, N.Y.) and Pouwels *et al.*, Cloning Vectors: A Laboratory Manual (1987).

Gene Therapy

[00227] Delivery of a therapeutic specific binding agent polypeptide or antibody to appropriate cells can be effected via gene therapy *ex vivo*, *in situ*, or *in vivo* by use of any suitable approach known in the art. For example, for *in vivo* therapy, a nucleic acid encoding the desired specific binding agent or antibody, either alone or in conjunction with a vector, liposome, or precipitate may be injected directly into the subject, and in some embodiments, may be injected at the site where the expression of the specific binding agent or antibody compound is desired. For *ex vivo* treatment, the subject's cells are removed, the nucleic acid is introduced into these cells, and the modified cells are returned to the subject either directly or, for example, encapsulated within porous membranes which are implanted into the patient. See, e.g. U.S. Pat. Nos. 4,892,538 and 5,283,187.

[00228] There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, chemical treatments, DEAE-dextran, and calcium phosphate precipitation. Other *in vivo* nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, adeno-associated virus or retrovirus) and lipid-based systems. The nucleic acid and transfection agent are optionally associated with a microparticle. Exemplary transfection agents include calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, quaternary ammonium amphiphile DOTMA ((dioleoyloxypropyl)trimethylammonium bromide, commercialized as Lipofectin by GIBCO-BRL)) (Felgner *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 84, 7413-7417; Malone *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86 6077-6081); lipophilic glutamate diesters with pendent trimethylammonium heads (Ito *et al.* (1990) *Biochem. Biophys. Acta* 1023, 124-132); the metabolizable parent lipids such as the cationic lipid dioctadecylamido glycylspermine (DOGS, Transfectam, Promega) and dipalmitoylphosphatidyl ethanolamylspermine (DPPES) (J. P. Behr (1986) *Tetrahedron Lett.* 27, 5861-5864; J. P. Behr *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86, 6982-6986); metabolizable quaternary ammonium salts (DOTB, N-(1-[2,3-dioleoyloxy]propyl)-N,N,N-trimethylammonium methylsulfate (DOTAP) (Boehringer Mannheim), polyethyleneimine (PEI), dioleoyl esters, ChoTB, ChoSC, DOSC) (Leventis *et al.* (1990) *Biochim. Inter.* 22, 235-241); 3beta[N--(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), dioleoylphosphatidyl ethanolamine (DOPE)/3beta[N--(N',N'-dimethylaminoethane)-

carbamoyl]cholesterolDC-Chol in one to one mixtures (Gao *et al.*, (1991) *Biochim. Biophys. Acta* 1065, 8-14), spermine, spermidine, lipopolyamines (Behr *et al.*, *Bioconjugate Chem*, 1994, 5: 382-389), lipophilic polylysines (LPLL) (Zhou *et al.*, (1991) *Biochim. Biophys. Acta* 939, 8-18), [[(1,1,3,3-tetramethylbutyl)cre-soxy]ethoxy]ethyl]dimethylbenzylammonium hydroxide (DEBDA hydroxide) with excess phosphatidylcholine/cholesterol (Ballas *et al.*, (1988) *Biochim. Biophys. Acta* 939, 8-18), cetyltrimethylammonium bromide (CTAB)/DOPE mixtures (Pinnaduwa *et al.*, (1989) *Biochim. Biophys. Acta* 985, 33-37), lipophilic diester of glutamic acid (TMAG) with DOPE, CTAB, DEBDA, didodecylammonium bromide (DDAB), and stearylamine in admixture with phosphatidylethanolamine (Rose *et al.*, (1991) *Biotechniques* 10, 520-525), DDAB/DOPE (TransfectACE, GIBCO BRL), and oligogalactose bearing lipids. Exemplary transfection enhancer agents that increase the efficiency of transfer include, for example, DEAE-dextran, polybrene, lysosome-disruptive peptide (Ohmori N I *et al.*, *Biochem Biophys Res Commun* Jun. 27, 1997; 23 5(3):726-9), chondroitin-based proteoglycans, sulfated proteoglycans, polyethylenimine, polylysine (Pollard H *et al.* *J Biol Chem*, 1998 273 (13):7507-11), integrin-binding peptide CYGGRGDTP, linear dextran nonasaccharide, glycerol, cholesteryl groups tethered at the 3'-terminal internucleoside link of an oligonucleotide (Letsinger, R. L. 1989 *Proc Natl Acad Sci USA* 86: (17):6553-6), lysophosphatide, lysophosphatidylcholine, lysophosphatidylethanolamine, and 1-oleoyl lysophosphatidylcholine.

[00229] In some situations it may be desirable to deliver the nucleic acid with an agent that directs the nucleic acid-containing vector to target cells. Such "targeting" molecules include antibodies specific for a cell-surface membrane protein on the target cell, or a ligand for a receptor on the target cell. Where liposomes are employed, proteins which bind to a cell-surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake. Examples of such proteins include capsid proteins and fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. In other embodiments, receptor-mediated endocytosis can be used. Such methods are described, for example, in Wu *et al.*, 1987 or Wagner *et al.*, 1990. For review of the currently known gene marking and gene therapy protocols, see Anderson 1992. See also WO 93/25673 and the references cited therein. For additional reviews of gene therapy technology, see Friedmann, *Science*, 244: 1275-1281 (1989); Anderson, *Nature*, supplement to vol. 392, no 6679, pp. 25-30 (1998); and Miller, *Nature*, 357: 455-460 (1992).

Treatment

[00230] As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent described herein, or identified by a method described herein, to a patient, or application or administration of the therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease, or the predisposition toward disease.

[00231] As defined herein, a therapeutically effective amount of a compound (i.e., an effective dosage) means an amount sufficient to produce a therapeutically (e.g., clinically) desirable result. 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 can 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 compounds of the invention can include a single treatment or a series of treatments.

[00232] In a preferred embodiment, the compositions of the invention are administered to patients for the prevention and/or treatment of disorders associated with IL-7R α mediated signaling, such as autoimmune diseases, diabetes, graft rejection, etc.

[00233] In one embodiment, the compositions are administered to patients as antibodies or cells producing antibodies targeted to IL-7R α chain, or IL-7R α mutant or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof. Peptides can be administered to a patient, for example, in the form of a peptide in a pharmaceutical compositions, as a vector expressing the peptides, and the like.

[00234] In another embodiment, IL-7R α mediated signaling or expression of IL-7R α molecule can be disrupted, modulated, increased, decreased, silenced by antibodies specific to IL-7R α , siRNA, antisense oligonucleotides, small molecule inhibition of IL-7R, IL-7R α peptides and the like. Preferably, in the case of autoimmune diseases, IL-7R, IL-7R α expression IL-7R α chain, or IL-7R α mutant or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof, signaling is disrupted or modulated to decrease the levels to those levels found in normal individuals, e.g. healthy individuals.

[00235] The disruption of a desired target nucleic acid can be carried out in several ways known in the art. For example, siRNA. Enzymatic nucleic acid molecules (e.g., ribozymes) are nucleic acid molecules capable of catalyzing one or more of a variety of reactions,

including the ability to repeatedly cleave other separate nucleic acid molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be used, for example, to target virtually any RNA transcript (Zaug *et al.*, 324, *Nature* 429 1986; Cech, 260 *JAMA* 3030, 1988; and Jefferies *et al.*, 17 *Nucleic Acids Research* 1371, 1989).

[00236] Because of their sequence-specificity, trans-cleaving enzymatic nucleic acid molecules show promise as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Enzymatic nucleic acid molecules can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the mRNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited.

[00237] In general, enzymatic nucleic acids with RNA cleaving activity act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

[00238] Several approaches such as *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc. London, B* 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing a variety of reactions, such as cleavage and ligation of phosphodiester linkages and amide linkages, (Joyce, 1989, *Gene*, 82, 83-87; Beaudry *et al.*, 1992, *Science* 257, 635-641; Breaker *et al.*, 1994, *TIBTECH* 12, 268; Bartel *et al.*, 1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.*, 1995, *FASEB J.*, 9, 1183; Breaker, 1996, *Curr. Op. Biotech.*, 7, 442).

[00239] The development of ribozymes that are optimal for catalytic activity would contribute significantly to any strategy that employs RNA-cleaving ribozymes for the purpose of regulating gene expression. The hammerhead ribozyme, for example, functions with a catalytic rate (k_{cat}) of about 1 min^{-1} in the presence of saturating (10 mM) concentrations of Mg^{2+} cofactor. An artificial "RNA ligase" ribozyme has been shown to catalyze the corresponding self-modification reaction with a rate of about 100 min^{-1} . In addition, it is known that certain modified hammerhead ribozymes that have substrate

binding arms made of DNA catalyze RNA cleavage with multiple turn-over rates that approach 100 min^{-1} . Finally, replacement of a specific residue within the catalytic core of the hammerhead with certain nucleotide analogues gives modified ribozymes that show as much as a 10-fold improvement in catalytic rate. These findings demonstrate that ribozymes can promote chemical transformations with catalytic rates that are significantly greater than those displayed *in vitro* by most natural self-cleaving ribozymes. It is then possible that the structures of certain self-cleaving ribozymes may be optimized to give maximal catalytic activity, or that entirely new RNA motifs can be made that display significantly faster rates for RNA phosphodiester cleavage.

[00240] Intermolecular cleavage of an RNA substrate by an RNA catalyst that fits the "hammerhead" model was first shown in 1987 (Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600). The RNA catalyst was recovered and reacted with multiple RNA molecules, demonstrating that it was truly catalytic.

[00241] Catalytic RNAs designed based on the "hammerhead" motif have been used to cleave specific target sequences by making appropriate base changes in the catalytic RNA to maintain necessary base pairing with the target sequences (Haseloff and Gerlach, *Nature*, 334, 585 (1988); Walbot and Bruening, *Nature*, 334, 196 (1988); Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600; Koizumi, M., Iwai, S. and Ohtsuka, E. (1988) *FEBS Lett.*, 228: 228-230). This has allowed use of the catalytic RNA to cleave specific target sequences and indicates that catalytic RNAs designed according to the "hammerhead" model may possibly cleave specific substrate RNAs *in vivo*. (see Haseloff and Gerlach, *Nature*, 334, 585 (1988); Walbot and Bruening, *Nature*, 334, 196 (1988); Uhlenbeck, O. C. (1987) *Nature*, 328: 596-600).

[00242] RNA interference (RNAi) has become a powerful tool for blocking gene expression in mammals and mammalian cells. This approach requires the delivery of small interfering RNA (siRNA) either as RNA itself or as DNA, using an expression plasmid or virus and the coding sequence for small hairpin RNAs that are processed to siRNAs. This system enables efficient transport of the pre-siRNAs to the cytoplasm where they are active and permit the use of regulated and tissue specific promoters for gene expression.

[00243] *Methods of Formulation:* The compounds described herein can be incorporated into pharmaceutical compositions. Such compositions typically include the active ingredient and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with

pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

[00244] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous 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 hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00245] 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™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must 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. 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, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00246] 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.

[00247] Oral compositions 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.

[00248] For administration by inhalation, the compounds can 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. Compositions for inhalation can also include propellants, surfactants, and other additives, e.g., to improve dispersion, flow, and bioavailability.

[00249] Systemic administration 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.

[00250] Compounds comprising nucleic acids, mutants, fragments and variants thereof, 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.* (2002), *Nature*, 418(6893), 38-9 (hydrodynamic transfection); Xia *et al.* (2002), *Nature Biotechnol.*, 20(10), 1006-10 (viral-mediated delivery); or Putnam (1996), *Am. J. Health Syst. Pharm.*, 53(2), 151-160, *erratum at Am. J. Health Syst. Pharm.*, 53(3), 325 (1996). Compounds comprising nucleic acids can also be administered by method suitable for administration of DNA vaccines. These methods include gene guns, bio injectors, and skin patches as well as needle-free methods such as the micro-particle DNA vaccine technology disclosed in U.S. Pat. No. 6,194,389, and the mammalian transdermal needle-free vaccination with powder-form vaccine as disclosed in U.S. Pat. No. 6,168,587. Additionally, intranasal delivery is possible, as described in, inter alia, Hamajima *et al.* (1998), *Clin. Immunol. Immunopathol.*, 88(2), 205-10. Liposomes (e.g., as described in U.S. Pat. No. 6,472,375) and microencapsulation can also be used. Biodegradable targetable microparticle delivery systems can also be used (e.g., as described in U.S. Pat. No. 6,471,996).

[00251] In one embodiment, the compounds are prepared with carriers that will protect the active ingredient 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.

[00252] In some embodiments, the compounds (e.g., polypeptides) are modified to enhance delivery into cells, e.g., by the addition of an optimized or native TAT protein transduction domain (PTD), e.g., as described in Ho *et al.*, *Cancer Res.* 61(2):474-7 (2001). Where the compound is a polypeptide, the polypeptide can be a fusion protein comprising an active portion (e.g., an active fragment of Apoptin) and a TAT PTD fused in frame.

[00253] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00254] Therapeutic agents include, for example, proteins, nucleic acids, small molecules, peptides, antibodies, siRNAs, ribozymes, and antisense oligonucleotides. Dosage, toxicity and therapeutic efficacy of such 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.

[00255] 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 (i.e., 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.

Candidate Therapeutic Agents

[00256] Embodiments of the invention encompass methods of screening compounds to identify those agent that modulate or inhibit IL-7R α mediated signaling, as described above. Screening assays for drug candidates will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

[00257] The methods include administering the compound to a model of the condition, e.g., contacting a cell (*in vitro*) model with the compound, or administering the compound to an animal model of the condition. The model is then evaluated for an effect of the candidate compound on signaling events mediated via the IL-7 receptor.

[00258] The test compounds utilized in the assays and methods described herein can be, *inter alia*, nucleic acids, small molecules, organic or inorganic compounds, antibodies or antigen-binding fragments thereof, polynucleotides, peptides, or polypeptides. For example, polypeptide variants including truncation mutants, deletion mutants, and point mutants; nucleic acids including sense, antisense, aptamers, and small inhibitory RNAs (siRNAs)

including short hairpin RNAs (shRNAs) and ribozymes) can be used as test compounds in the methods described herein.

[00259] Candidate agents may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of e.g. bacterial, fungal and animal extracts are available or readily produced.

[00260] *Chemical Libraries:* Developments in combinatorial chemistry allow the rapid and economical synthesis of hundreds to thousands of discrete compounds. These compounds are typically arrayed in moderate-sized libraries of small molecules designed for efficient screening. Combinatorial methods can be used to generate unbiased libraries suitable for the identification of novel compounds. In addition, smaller, less diverse libraries can be generated that are descended from a single parent compound with a previously determined biological activity. In either case, the lack of efficient screening systems to specifically target therapeutically relevant biological molecules produced by combinatorial chemistry such as inhibitors of important enzymes hampers the optimal use of these resources.

[00261] A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks," such as reagents. For example, a linear combinatorial chemical library, such as a polypeptide library, is formed by combining a set of chemical building blocks (amino acids) in a large number of combinations, and potentially in every possible way, for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[00262] A "library" may comprise from 2 to 50,000,000 diverse member compounds. Preferably, a library comprises at least 48 diverse compounds, preferably 96 or more diverse compounds, more preferably 384 or more diverse compounds, more preferably, 10,000 or more diverse compounds, preferably more than 100,000 diverse members and most preferably more than 1,000,000 diverse member compounds. By "diverse" it is meant that greater than 50% of the compounds in a library have chemical structures that are not identical to any other member of the library. Preferably, greater than 75% of the compounds in a library have chemical structures that are not identical to any other member of the collection, more preferably greater than 90% and most preferably greater than about 99%.

[00263] The preparation of combinatorial chemical libraries is well known to those of skill in the art. For reviews, see Thompson *et al.*, Synthesis and application of small molecule libraries, *Chem Rev* 96:555-600, 1996; Kenan *et al.*, Exploring molecular diversity with combinatorial shape libraries, *Trends Biochem Sci* 19:57-64, 1994; Janda, Tagged versus untagged libraries: methods for the generation and screening of combinatorial chemical libraries, *Proc Natl Acad Sci USA* 91:10779-85, 1994; Lebl *et al.*, One-bead-one-structure combinatorial libraries, *Biopolymers* 37:177-98, 1995; Eichler *et al.*, Peptide, peptidomimetic, and organic synthetic combinatorial libraries, *Med Res Rev* 15:481-96, 1995; Chabala, Solid-phase combinatorial chemistry and novel tagging methods for identifying leads, *Curr Opin Biotechnol* 6:632-9, 1995; Dolle, Discovery of enzyme inhibitors through combinatorial chemistry, *Mol Divers* 2:223-36, 1997; Fauchere *et al.*, Peptide and nonpeptide lead discovery using robotically synthesized soluble libraries, *Can J. Physiol Pharmacol* 75:683-9, 1997; Eichler *et al.*, Generation and utilization of synthetic combinatorial libraries, *Mol Med Today* 1: 174-80, 1995; and Kay *et al.*, Identification of enzyme inhibitors from phage-displayed combinatorial peptide libraries, *Comb Chem High Throughput Screen* 4:535-43, 2001.

[00264] Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to, peptoids (PCT Publication No. WO 91/19735); encoded peptides (PCT Publication WO 93/20242); random bio-oligomers (PCT Publication No. WO 92/00091); benzodiazepines (U.S. Pat. No. 5,288,514); diversomers, such as hydantoins, benzodiazepines and dipeptides (Hobbs, *et al.*, *Proc. Nat. Acad. Sci. USA*, 90:6909-6913 (1993)); vinylogous polypeptides (Hagihara, *et al.*, *J. Amer. Chem. Soc.* 114:6568 (1992)); nonpeptidal peptidomimetics with β -D-glucose scaffolding (Hirschmann, *et al.*, *J. Amer. Chem. Soc.*, 114:9217-9218 (1992)); analogous organic syntheses of small compound libraries (Chen, *et al.*, *J. Amer. Chem. Soc.*, 116:2661 (1994)); oligocarbamates (Cho, *et al.*, *Science*, 261:1303 (1993)); and/or peptidyl phosphonates (Campbell, *et al.*, *J. Org. Chem.* 59:658 (1994)); nucleic acid libraries (see, Ausubel, Berger and Sambrook, all *supra*); peptide nucleic acid libraries (see, e.g., U.S. Pat. No. 5,539,083); antibody libraries (see, e.g., Vaughn, *et al.*, *Nature Biotechnology*, 14(3):309-314 (1996) and PCT/US96/10287); carbohydrate libraries (see, e.g., Liang, *et al.*, *Science*, 274:1520-1522 (1996) and U.S. Pat. No. 5,593,853); small organic molecule libraries (see, e.g., benzodiazepines, Baum C&E News, January 18, page 33 (1993); isoprenoids (U.S. Pat. No. 5,569,588); thiazolidinones and metathiazanones (U.S. Pat. No. 5,549,974); pyrrolidines (U.S.

Pat. Nos. 5,525,735 and 5,519,134); morpholino compounds (U.S. Pat. No. 5,506,337); benzodiazepines (U.S. Pat. No. 5,288,514); and the like.

[00265] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem. Tech, Louisville Ky., Symphony, Rainin, Woburn, Mass., 433A Applied Biosystems, Foster City, Calif., 9050 Plus, Millipore, Bedford, Mass.). In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, Mo., ChemStar, Ltd., Moscow, RU, 3D Pharmaceuticals, Exton, Pa., Martek Bio sciences, Columbia, Md., etc.).

[00266] *Small Molecules:* Small molecule test compounds can initially be members of an organic or inorganic chemical library. As used herein, "small molecules" refers to small organic or inorganic molecules of molecular weight below about 3,000 Daltons. The small molecules can be natural products or members of a combinatorial chemistry library. A set of diverse molecules should be used to cover a variety of functions such as charge, aromaticity, hydrogen bonding, flexibility, size, length of side chain, hydrophobicity, and rigidity. Combinatorial techniques suitable for synthesizing small molecules are known in the art, e.g., as exemplified by Obrecht and Villalgordo, Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries, Pergamon-Elsevier Science Limited (1998), and include those such as the "split and pool" or "parallel" synthesis techniques, solid-phase and solution-phase techniques, and encoding techniques (see, for example, Czarnik, *Curr. Opin. Chem. Bio.*, 1:60 (1997)). In addition, a number of small molecule libraries are commercially available.

[00267] The whole procedure can be fully automated. For example, sampling of sample materials may be accomplished with a plurality of steps, which include withdrawing a sample from a sample container and delivering at least a portion of the withdrawn sample to test platform. Sampling may also include additional steps, particularly and preferably, sample preparation steps. In one approach, only one sample is withdrawn into the auto-sampler probe at a time and only one sample resides in the probe at one time. In other embodiments, multiple samples may be drawn into the auto-sampler probe separated by solvents. In still other embodiments, multiple probes may be used in parallel for auto sampling.

[00268] In the general case, sampling can be effected manually, in a semi-automatic manner or in an automatic manner. A sample can be withdrawn from a sample container manually, for example, with a pipette or with a syringe-type manual probe, and then manually delivered to a loading port or an injection port of a characterization system. In a

semi-automatic protocol, some aspect of the protocol is effected automatically (e.g., delivery), but some other aspect requires manual intervention (e.g., withdrawal of samples from a process control line). Preferably, however, the sample(s) are withdrawn from a sample container and delivered to the characterization system, in a fully automated manner—for example, with an auto-sampler.

[00269] In one embodiment, auto-sampling may be done using a microprocessor controlling an automated system (e.g., a robot arm). Preferably, the microprocessor is user-programmable to accommodate libraries of samples having varying arrangements of samples (e.g., square arrays with "n-rows" by "n-columns," rectangular arrays with "n-rows" by "m-columns," round arrays, triangular arrays with "r-" by "r-" by "r-" equilateral sides, triangular arrays with "r-base" by "s-" by "s-" isosceles sides, etc., where n, m, r, and s are integers).

[00270] Automated sampling of sample materials optionally may be effected with an auto-sampler having a heated injection probe (tip). An example of one such auto sampler is disclosed in U.S. Pat. No. 6,175,409 B1 (incorporated by reference).

[00271] According to the present invention, one or more systems, methods or both are used to identify a plurality of sample materials. Though manual or semi-automated systems and methods are possible, preferably an automated system or method is employed. A variety of robotic or automatic systems are available for automatically or programmably providing predetermined motions for handling, contacting, dispensing, or otherwise manipulating materials in solid, fluid liquid or gas form according to a predetermined protocol. Such systems may be adapted or augmented to include a variety of hardware, software or both to assist the systems in determining mechanical properties of materials. Hardware and software for augmenting the robotic systems may include, but are not limited to, sensors, transducers, data acquisition and manipulation hardware, data acquisition and manipulation software and the like. Exemplary robotic systems are commercially available from CAVRO Scientific Instruments (e.g., Model NO. RSP9652) or BioDot (Microdrop Model 3000).

[00272] Generally, the automated system includes a suitable protocol design and execution software that can be programmed with information such as synthesis, composition, location information or other information related to a library of materials positioned with respect to a substrate. The protocol design and execution software is typically in communication with robot control software for controlling a robot or other automated apparatus or system. The protocol design and execution software is also in communication with data acquisition hardware/software for collecting data from response measuring hardware. Once the data is collected in the database, analytical software may be used to

analyze the data, and more specifically, to determine properties of the candidate drugs, or the data may be analyzed manually.

[00273] *Data and Analysis:* The practice of the present invention may also employ conventional biology methods, software and systems. Computer software products of the invention typically include computer readable medium having computer-executable instructions for performing the logic steps of the method of the invention. Suitable computer readable medium include floppy disk, CD-ROM/DVD/DVD-ROM, hard-disk drive, flash memory, ROM/RAM, magnetic tapes and etc. The computer executable instructions may be written in a suitable computer language or combination of several languages. Basic computational biology methods are described in, for example Setubal and Meidanis *et al.*, Introduction to Computational Biology Methods (PWS Publishing Company, Boston, 1997); Salzberg, Searles, Kasif, (Ed.), Computational Methods in Molecular Biology, (Elsevier, Amsterdam, 1998); Rashidi and Buehler, Bioinformatics Basics: Application in Biological Science and Medicine (CRC Press, London, 2000) and Ouelette and Bzevanis Bioinformatics: A Practical Guide for Analysis of Gene and Proteins (Wiley & Sons, Inc., 2nd ed., 2001). See U.S. Pat. No. 6,420,108.

[00274] The present invention may also make use of various computer program products and software for a variety of purposes, such as probe design, management of data, analysis, and instrument operation. See, U.S. Pat. Nos. 5,593,839, 5,795,716, 5,733,729, 5,974,164, 6,066,454, 6,090,555, 6,185,561, 6,188,783, 6,223,127, 6,229,911 and 6,308,170.

[00275] Additionally, the present invention relates to embodiments that include methods for providing genetic information over networks such as the Internet.

Diagnostics

[00276] In a preferred embodiment, the invention provides for diagnosis of patients at risk of developing a disorder associated with IL-7R α mediated signaling, for example, autoimmune diseases such as multiple sclerosis. In one embodiment, detection of a SNP (rs897932), which resides within the transmembrane domain of IL7R α is predictive of an individual presenting with a disorder or disease associated with IL-7R α mediated signaling events. Studies have shown that this mutation results in the alternative splicing of exon 6; possibly resulting in the production of a secreted IL-7R α .

[00277] In another preferred embodiment, detection of soluble IL-7R α in a patient at levels which are higher as compared to levels detected in a normal healthy individual are diagnostic of an individual at risk of developing or in the early stages of diseases associated

with IL-7R α mediated signaling events found in individuals with, for example, multiple sclerosis.

[00278] In a preferred embodiment, oligonucleotides can be used in a variety of diagnostic assays. For example, the sequences can be radiolabeled to identify hybridization, use of the primers in PCR, generation of peptides, aptamers and antibodies directed to the desired sequences, etc.

[00279] In one embodiment, a method of diagnosing patients with disorders related to IL-7R α mediated signaling events as described herein, comprises obtaining a biological sample from a patient; identifying IL-7R α mutants or a portion thereof; and comparing the sequences to wild type IL-7R α sequences.

[00280] In another preferred embodiment, the patient or individual is a mammal. This includes humans of any age. For example, an embryo, neonate, infant, child, teenager or adult.

[00281] In another preferred embodiment, an IL-7R α mutant peptide or nucleic acid is identified by an antibody or aptamer.

[00282] In some instances, such as when unusually small amounts of RNA are recovered and only small amounts of cDNA are generated therefrom, it is desirable or necessary to perform a PCR reaction on the first PCR reaction product. That is, if difficult to detect quantities of amplified DNA are produced by the first reaction, a second PCR can be performed to make multiple copies of DNA sequences of the first amplified DNA. A nested set of primers are used in the second PCR reaction. The nested set of primers hybridize to sequences downstream of the 5' primer and upstream of the 3' primer used in the first reaction.

[00283] The present invention includes oligonucleotide which are useful as primers for performing PCR methods to amplify mRNA or cDNA that encodes IL-7R α peptides and mutants thereof.

[00284] According to the invention, diagnostic kits can be assembled which is useful to practice methods of detecting the presence of mRNA or cDNA that encodes IL-7R α mutants in tissue samples. Such diagnostic kits comprise oligonucleotides which are useful as primers for performing PCR methods.

[00285] In another preferred embodiment, a kit comprises reagents for identifying and measuring the levels of soluble IL-7R α or membrane bound IL-7R α having at least one mutation using real-time PCR (RT-PCR). The kit can include one or more primers that specifically hybridize to different regions of the IL-7R α molecules.

[00286] Another method of determining whether a sample contains cells expressing IL-7R α mutants by Northern blot analysis of mRNA extracted from a tissue sample. The techniques for performing Northern blot analyses are well known by those having ordinary skill in the art and are described in Sambrook, J. *et al.*, Molecular Cloning: A Laboratory Manual, (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. mRNA extraction, electrophoretic separation of the mRNA, blotting, probe preparation and hybridization are all well known techniques that can be routinely performed using readily available starting material.

[00287] One having ordinary skill in the art, performing routine techniques, could design probes to identify mRNA encoding, for example, IL-7R α SNP mutants. The mRNA is extracted using poly dT columns and the material is separated by electrophoresis and, for example, transferred to nitrocellulose paper. Labeled probes made from an isolated specific fragment or fragments can be used to visualize the presence of a complementary fragment fixed to the paper.

[00288] According to the invention, diagnostic kits can be assembled which is useful to practice methods of detecting the presence of mRNA that encodes IL-7R α in tissue samples by Northern blot analysis. Such diagnostic kits comprise oligonucleotides which are useful as probes for hybridizing to the mRNA. The probes may be radiolabeled. It is preferred that diagnostic kits according to the present invention comprise a container comprising a size marker to be run as a standard on a gel. It is preferred that diagnostic kits according to the present invention comprise a container comprising a positive control which will hybridize to the probe.

[00289] Another method of detecting the presence of mRNA encoding IL-7R α mutant protein is by oligonucleotide hybridization technology. Oligonucleotide hybridization technology is well known to those having ordinary skill in the art. Briefly, detectable probes which contain a specific nucleotide sequence that will hybridize to nucleotide sequence of mRNA encoding IL-7R α mutant protein. RNA or cDNA made from RNA from a sample is fixed, usually to filter paper or the like. The probes are added and maintained under conditions that permit hybridization only if the probes fully complement the fixed genetic material. The conditions are sufficiently stringent to wash off probes in which only a portion of the probe hybridizes to the fixed material. Detection of the probe on the washed filter indicates complementary sequences. Hybridization conditions can be routinely optimized to minimize background signal by non-fully complementary hybridization.

[00290] The present invention includes labeled oligonucleotides which are useful as probes for performing oligonucleotide hybridization. That is, they are fully complementary with mRNA sequences but not genomic sequences. For example, the mRNA sequence includes portions encoded by different exons. The labeled probes of the present invention are labeled with radiolabeled nucleotides or are otherwise detectable by readily available nonradioactive detection systems.

[00291] Another aspect of the invention relates to methods of analyzing tissue samples which are fixed sections routinely prepared by surgical pathologists to characterize and evaluate cells. In some embodiments, the cells are from various organs, including bone marrow.

[00292] *In situ* hybridization technology is well known by those having ordinary skill in the art. Briefly, cells are fixed and detectable probes which contain a specific nucleotide sequence are added to the fixed cells. If the cells contain complementary nucleotide sequences, the probes, which can be detected, will hybridize to them.

[00293] For *in situ* hybridization according to the invention, it is preferred that the probes are detectable by fluorescence. A common procedure is to label probe with biotin-modified nucleotide and then detect with fluorescently-tagged avidin. Hence, the probe does not itself have to be labeled with fluorescent but can be subsequently detected with fluorescent marker.

[00294] Cells are fixed and the probes are added to the genetic material. Probes will hybridize to the complementary nucleic acid sequences present in the sample. Using a fluorescent microscope, the probes can be visualized by their fluorescent markers.

[00295] According to the invention, diagnostic kits can be assembled which is useful to practice *in situ* hybridization methods of the invention are fully complementary with mRNA sequences but not genomic sequences. For example, the mRNA sequence includes portions encoded by different exons. It is preferred that labeled probes of the *in situ* diagnostic kits according to the present invention are labeled with a fluorescent marker.

[00296] Immunohistochemistry techniques may be used to identify and essentially stain cells containing an IL-7R α chain, or IL-7R α mutant or IL-7R α containing molecule, or any molecule comprising IL-7R α chains, or functional domains thereof.

[00297] The following examples are offered by way of illustration, not by way of limitation. While specific examples have been provided, the above description is illustrative and not restrictive. Any one or more of the features of the previously described embodiments can be combined in any manner with one or more features of any other embodiments in the

present invention. Furthermore, many variations of the invention will become apparent to those skilled in the art upon review of the specification.

[00298] All publications and patent documents cited in this application are incorporated by reference in pertinent part for all purposes to the same extent as if each individual publication or patent document were so individually denoted. By their citation of various references in this document, Applicants do not admit any particular reference is "prior art" to their invention.

EXAMPLES

[00299] The following examples serve to illustrate the invention without limiting it thereby. It will be understood that variations and modifications can be made without departing from the spirit and scope of the invention.

Example 1: IL-7 receptor mediated signaling

[00300] *Methods*

[00301] *Mice:* All mice used in these studies are 2-4 months old and on a C57BL/6 background. Age-matched WT C57B/6 are used as controls. IL-7R α Tg^{IL7R-/-} mice were provided by Dr. Thomas Malek. Rag^{-/-} mice were purchased from Jackson Laboratories. Animals are housed in a 12h light/dark cycle in a virus/antigen free facility with controlled temperature and humidity and provided with water and food as needed.

[00302] *Induction of active EAE and assessment of functional recovery:* All mice used in this study are C57BL/6 females between 8 and 12 weeks of age at the time of the first immunization. Mice are immunized with MOG peptide 35-55, as previously described. Briefly, MOG peptide is synthesized by standard 9-fluorenyl-methoxycarbonyl chemistry and shown to be >95% pure as determined by reversed-phase-HPLC (Biosynthesis, Lewisville, TX). Mice are injected s.c. on days 0 and 7 with 150 μ g of peptide emulsified in complete Freund's adjuvant. In addition, on days 0 and 2 post immunization (i.p.), mice are given pertussis toxin (500 ng/mouse) by i.p. injection. Clinical signs of EAE are assessed daily using a standard scale of 0-6 as follows: 0, no clinical signs; 1, slight loss of tail tone; 1.5 moderate to severe loss of tail tone; 2, flaccid tail; 2.5 flaccid tail and difficulty in walking; 3, complete hindlimb paralysis; 3.5, complete hindlimb paralysis and partial forelimb paralysis; 4, total paralysis; 4.5 total paralysis and very poor health (incontinence, etc); 5, moribund; and 6, death. Mice are considered to have EAE when they achieve a score of 2 or more for at least 2 consecutive days.

[00303] *In Vitro Neutralization:* Splenocytes were isolated from C57B/6 and stimulated with anti-CD3 (clone 145-2C11) for 48hrs. Cells were plated at 2×10^4 cell/well in the presence of anti-IL7R α (A7R34, BioXcell), anti-IL7 (M25, BioXcell), or rat IgG (Sigma) at 10 μ g/mL. Cells were stimulated with IL-7 at 0.1-30 ng/mL overnight. Cells were pulsed with tritiated thymidine (1 μ Ci/well) for 4 hrs. A MicroBeta Liquid Scintillation Counter was used to assess levels of ^{3} H incorporation at 1min/well.

[00304] *In Vivo Neutralizing Antibody Paradigm:* Mice were i.p. injected at peak disease (~17-25 dpi) with anti-IL-7R α (A7R34, BioXcell), anti-IL7 (M25, BioXcell), or rat IgG (Sigma) at 20 μ g/g body weight. Injections were administered at 48-hour intervals, for a total of 10 injections.

[00305] *Flow Cytometry Analysis:* Following EAE induction and neutralizing antibody treatments, spinal cords (SC) were pooled from respective groups. Mononuclear cells were isolated via Percoll gradient centrifugation and stained for surface markers (CD4, CD8, CD45, B220, NK1.1, CD11b). Cells were analyzed using an LSRII Flow Cytometer.

[00306] *Mice Chimeras:* One day before irradiation mice are placed on antibiotic water and maintained on oral antibiotics until day 14 post-bone marrow transplant (BMT). On the evening prior to irradiation, food is removed from cages and mice fasted for approximately 18 hrs to minimize intestinal distress. One day prior to BMT, Rag $^{-/-}$ mice are placed into plexi-glass holders and irradiated at a sublethal dose of 300rad, approximately 35-40 rads/minute (GC40 or Cobalt-60 Irradiator). One day post irradiation, donor C57B/6 or IL-7R α Tg $^{IL7R^{-/-}}$ mice are sacrificed and BM single cell suspensions prepared. Recipient mice are injected i.v. with 5×10^5 donor cells.

[00307] *Relative Leukocyte Distribution in the Spinal Cord:* SC were pooled in each group and analyzed via flow cytometry (Table 1). The animals treated with anti-IL7R α antibody, which subsequently improved EAE clinical signs, showed a significant reduction in the number of infiltrating CD4 $^{+}$ and CD8 $^{+}$ T cells when compared to controls, along with an increase in the number of microglia cells. Conversely, the number of infiltrating CD4 $^{+}$ and CD8 $^{+}$ T cells actually increased in the animals treated with the anti-IL7 antibody.

[00308] *Results:*

[00309] *EAE in IL-7R α Tg $^{IL7R^{-/-}}$:* IL-7R α Tg $^{IL7R^{-/-}}$ mice express the IL-7R α gene exclusively in the thymus and not in the periphery. The genetic manipulation allows for sufficient T cell development, yet prevents IL-7R α signaling on mature T cells that have left the thymus, as well as any other cell type throughout the body that may signal through this receptor. The

fact that attenuation of the EAE phenotype following disease induction is observed in these animals evidences that IL-7R α signaling plays a role in disease progression.

[00310] *IL-7 Neutralization In Vitro:* The neutralizing capacity of two different monoclonal antibodies was compared (Figure 4). One antibody blocks the signaling pathway by binding to the receptor (anti-IL7R α) while the other blocks by binding to the ligand (anti-IL7). The *in vitro* assay shows that each neutralizing antibody is capable of blocking IL7-mediated splenocyte proliferation with comparable rates and magnitude.

[00311] *Neutralizing Antibody Treatment at EAE Peak Disease:* EAE animals were treated with neutralizing antibody against the receptor (anti-IL7R α) or the ligand (anti-IL7) and compared to IgG controls (Figures 8A, 8B). Although these two antibodies functioned similarly *in vitro*, they did not confer parallel results *in vivo*. The antibody blocking the receptor directly yielded significant recovery in the EAE phenotype. However, antibody blocking the ligand had no affect on disease outcome.

[00312] *EAE in Chimeric Mice:* Chimeric mice were generated by transplanting bone marrow (BM) from IL-7R α Tg^{IL7R α -/-} mice or CD45.1 congenic mice to Rag^{-/-} recipient mice, which lack mature T and B cells (Figure 9). Upon reconstitution of the immune compartment, EAE was induced. Surprisingly, the animals that received IL-7R α Tg^{IL7R α -/-} donor BM were not protected from disease. Since these animals are still capable of IL-7R α signaling in non-hematopoietic cell lineage, it evidences that this signaling pathway may be playing a role in EAE progression through more cell types than merely T cells alone.

[00313] EAE was induced in IL7R α Tg^{IL7R α -/-} mice and C57B/6 wild type controls using standard protocols (Figures 1A, 1B). WT mice showed typical EAE progression with disease onset occurring about 2 weeks into the study. Conversely, mice lacking IL-7R α signaling outside the thymus show significant protection against the disease phenotype over 40 days.

[00314] Figures 1A and 1 B show the results obtained from MOG-induced EAE in IL7R α Tg^{IL7R α -/-} and WT animals. EAE was induced with the MOG₃₅₋₅₅ peptide in IL7R α Tg^{IL7R α -/-} mice. Spleen cells were isolated from either WT or IL7R α Tg^{IL7R α -/-} mice 40 days post EAE induction. Results shown are the percentages of positive staining relative to total leukocyte population. The disease protection in IL7R α Tg^{IL7R α -/-} animals was not likely to result from their diminished B cell pool. To demonstrate this, standard protocols were used for MOG₃₅₋₅₅ to induce EAE in μ MT B cell deficient mice, which are on the same C57B/6 background as the IL7R α Tg^{IL7R α -/-} animals. It was found that there was no significant difference between μ MT and wild type (WT) mice in the overall severity of EAE (n=5 per group). Flow cytometry analysis performed on isolated spleen cells after EAE induction

confirmed that μ MT mice were lacking only the B cell population while the WT animals showed normal cell distributions.

[00315] The next set of experiments used a neutralizing antibody against IL-7R α to therapeutically mimic the genetic manipulations in IL7R α Tg^{IL7R^{-/-}} mice. Two treatment paradigms were conducted in the MOG-induced model using the rat anti-mouse IL-7R α neutralizing antibody and the isotype control. In both cases, diseased C57B/6 mice received a total of 10 antibody injections over a period of 20 days. The two studies differed in that the antibody treatments were started either before disease onset (early, days 7-25) or after disease had peaked (late, days 19-37). Mice injected with IL7R α blocking antibody in the early treatment regimen were significantly healthier than IgG controls when analyzed from the appearance of clinical symptoms to 4 days post treatment (Figure 2A). However, upon cessation of the antibody therapy, disease progression resumed in the treated mice. On the other hand, mice receiving the IL-7R α neutralizing antibody late in treatment during peak disease showed significant recovery over the entire duration of the study (Figure 2B). Furthermore, flow cytometry analysis of the spinal cords (50 dpi) using a panel of leukocyte markers revealed that there was a dramatic decrease in the relative levels of T cell infiltrates for animals receiving IL-7R α neutralizing antibody in the late treatment group (Table 1).

[00316] Table 1 shows the relative leukocyte distributions within the spinal cord of EAE-induce mice after IL-7R α neutralizing antibody treatments.

Table 1. Relative Leukocyte Distributions in the Spinal Cord (% of total CD45 ⁺ population)							
Time and Treatment Groups		T cells CD4 ⁺	T cells CD8 ⁺	B cells B220 ⁺	Microglia CD45 ^{lo} /CD11b ⁺	Macrophage CD45 ^{hi} /CD11b ⁺	NKs NK1.1 ⁺
Early (7-20 dpi)	IgG	7.8	5.3	5.7	24.2	35.4	2.5
	IL-7R α	9.1	6.5	8.8	30.2	32.2	4.1
Late (19-27dpi)	IgG	22.1	15.9	6.7	33	6.1	4.7
	IL-7R α	3.9	0.5	5.3	75.3	2.3	2.6

[00317] In Table 1, EAE-induced animals from early and late antibody treatment groups were sacrificed at 50dpi. Spinal cords were removed and stained with various immunologic markers of cell surface antigens (CD45, CD11b, CD4, CD8, B220, NK1.1) for flow cytometry analysis. The numbers in the table represent the percentage of cells staining positive for a given marker, gated on the CD45⁺ population (leukocytes). The almost

complete lack of T-cells in the spinal cord of mice treated with a neutralizing antibody to IL7R α was noted.

[00318] Figure 3 shows the methodology used in EAE induction in mice. Mice were injected with 350ng PTX (i.p.), 300 μ g MOG₃₅₋₅₅ in CFA (s.c.; complete Freund's adjuvant (1 mg *M. tuberculosis*/ injection) on days shown in the figure. Behavior assessed daily using the following clinical scores: 0 No clinical signs; 1 Slight loss of tail tone; 2 Flaccid tail; 3 Hindlimb paralysis; 4 Total paralysis; 5 Moribund; 6 Death.

[00319] Figure 4 shows that IL7R α Tg^{IL7R^{-/-}} mice are less susceptible to EAE as compared to wild type mice. EAE was induced with the MOG₃₅₋₅₅ peptide in IL7R α Tg^{IL7R^{-/-}} mice (■, n=7) and C57B/6 wild type controls (○, n=8). Clinical symptoms were scored daily for 40 days and the results are expressed as the mean $\pm$ SEM. The two curves are significantly different when the entire data set is analyzed (p=0.0061, Mann Whitney t test). Significance is further enhanced when analysis starts from disease onset (p<0.0001).

[00320] Figure 5 shows IL7 and IL7R α neutralize T cell proliferation with equal capacities *in vitro*. Splenocytes were stimulated with anti CD3 for 48 hrs. 2×10^6 cells stimulated with 0-30 ng/mL IL-7. Proliferation was measured via ³H incorporation in presence of 10 μ g/mL neutralizing antibodies.

[00321] Table 2: Spinal Cord Cell Profile

Relative Leukocyte Distributions in the Spinal Cord (% of total CD45+ population)						
Treatment at EAE Peak	T cells CD4+	T cells CD8+	B cells B220+	Microglia CD45 ^{lo} /CD11b+	Macrophage CD45 ^{hi} /CD11b+	NKs NK1.1+
IgG	22.1	15.9	6.7	33	6.1	4.7
IL-7R α	3.9	0.5	5.3	75.3	2.3	2.5

Relative Leukocyte Distributions in the Spinal Cord (% of total CD45+ population)						
Treatment at EAE Peak	T cells CD4+	T cells CD8+	B cells B220+	Microglia CD45 ^{lo} /CD11b+	Macrophage CD45 ^{hi} /CD11b+	NKs NK1.1+
IgG	4.8	6.6	15.7	42	34.8	3.5
IL-7	12.5	10.6	12.4	35.7	37.9	7.5

[00322] The results in Table 2 show that IL-7R α neutralizing antibody dramatically decreases CD4/CD8 T cell infiltration which was accompanied by an extensive increase in microglia cells. These effects were not seen with IL-7 neutralizing antibodies.

[00323] *Summary:* The results evidence that inhibition of IL-7R α signaling during peak disease is therapeutic and confirm the important role that this pathway has in EAE. Additionally, the successful outcome of these experiments advocates further development of this medicinal strategy.

[00324] All references cited herein, are incorporated herein by reference. Although the invention has been illustrated and described with respect to one or more implementations, equivalent alterations and modifications will occur to others skilled in the art upon the reading and understanding of this specification and the annexed drawings. In addition, while a particular feature of the invention may have been disclosed with respect to only one of several implementations, such feature may be combined with one or more other features of the other implementations as may be desired and advantageous for any given or particular application.

[00325] The Abstract will allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the following claims.

What is claimed:

1. A method of modulating interleukin-7 (IL-7) receptor or interleukin-7 receptor α chain mediated signaling *in vivo* or *in vitro*, comprising contacting a IL-7R α (CD127) or molecule comprising a IL-7R α (CD127) or functional domains thereof, with an agent which specifically targets or binds to interleukin 7 receptor alpha chain molecules; and, modulating interleukin-7 (IL-7) receptor or interleukin-7 receptor α chain mediated signaling.
2. The method of claim 1, wherein the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.
3. The method of claim 1, wherein the agent modulates interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, mediated cell signaling.
4. The method of claim 1, wherein a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, comprises: receptor molecules, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.
5. The method of claim 1, wherein the agent comprises: an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.
6. The method of claim 1, wherein the agent targets or specifically binds to the interleukin 7 receptor (IL-7R), a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, and inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

7. The method of claim 1, wherein the agent inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.
8. The method of claim 1, wherein the agent protects or treats a patient at risk of developing or suffering from an immune system related disease or disorder, comprising: autoimmune diseases or disorders, inflammatory diseases or disorders, Graft versus Host Disease (GVHD), neuroinflammatory diseases or disorders.
9. The method of claim 1, wherein the agent protects or treats a patient suffering from or at risk of developing cancer, bacterial, viral, fungal, or parasitic organisms.
10. A method of modulating cells of a patient's immune system, comprising:
administering to a patient an effective amount of an agent which modulates expression, function or activity of interleukin-7 receptors, variants, isoforms, mutants, alleles or fragments thereof; and,
modulating a patients immune system.
11. The method of claim 10, wherein the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.
12. The method of claim 10, wherein the agent modulates interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, mediated cell signaling.
13. The method of claim 10, wherein a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, comprises: receptor molecules, oligonucleotide, polynucleotide, peptide, polypeptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.
14. The method of claim 10, wherein the agent targets or specifically binds to the interleukin 7 receptor (IL-7R), a molecule comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127), or functional domains thereof, and inhibits IL-7R α chain expression,

function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

15. The method of claim 10, wherein the agent comprises: an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.

16. The method of claim 10, wherein the cells of a patient's immune system comprise: T lymphocytes, B lymphocytes, natural killer cells, or mononuclear cells.

17. The method of claim 10, wherein the agent protects or treats a patient at risk of developing or suffering from an immune system related disease or disorder, comprising: autoimmune diseases or disorders, inflammatory diseases or disorders, Graft versus Host Disease (GVHD), neuroinflammatory diseases or disorders.

18. The method of claim 10, wherein the agent protects or treats a patient suffering from or at risk of developing cancer, bacterial, viral, fungal, or parasitic organisms.

19. A method of treating a patient suffering from an autoimmune disease or disorder comprising: administering to a patient a composition comprising a therapeutically effective amount of an agent that modulates IL-7R α mediated signaling.

20. The method of claim 19, wherein the agent comprises at least one of: an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules or inorganic molecules.

21. The method of claim 19, wherein the agent inhibits IL-7R α chain expression, function, activity or signaling through activation of receptors comprising the IL-7R α chain by at least about 20% as compared to a normal control.

22. A pharmaceutical composition comprising an agent for modulating expression, function, signaling or activity of an interleukin-7 receptor (IL-7R), variants, isoforms, mutants, alleles or fragments thereof.

23. The pharmaceutical composition of claim 23, wherein the agent specifically modulates molecules comprising an interleukin-7 receptor alpha (IL-7R α) chain (CD127) or functional domains thereof.
24. The pharmaceutical composition of claim 23, wherein the agent targets or specifically binds to the interleukin 7 receptor (IL-7R) or molecules comprising an IL-7R α chain or functional domains thereof; and inhibiting IL-7R α chain expression, function, activity or signaling through activation of the molecules comprising the IL-7R α chain by at least about 20% as compared to a normal control.
25. The pharmaceutical composition of claim 23, wherein the agent comprises an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or fusion peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.
26. A fusion protein comprising at least one molecule which inhibits interleukin 7 receptor alpha chain (IL-7R α) mediated signaling.
27. The fusion protein of claim 26, wherein the at least one molecule inhibiting interleukin 7 receptor alpha chain mediated signaling comprises an antibody, ligand, aptamer, oligonucleotide, polynucleotide, peptide, protein, fusion proteins or peptides, natural or synthetic molecules, organic molecules, inorganic molecules or combinations thereof.
28. A vector expressing a molecule which modulates interleukin-7 receptor alpha chain mediated signaling.
29. An isolated cell comprising a vector expressing a molecule which modulates interleukin-7 receptor alpha chain mediated signaling.
30. A method of identifying candidate therapeutic agents comprising: contacting an interleukin 7 receptor alpha chain containing molecule or functional domains thereof, or a cell expressing the molecule, with a candidate therapeutic agent;
measuring signaling mediated by the IL-7R α chain as compared to a baseline control;
and,

identifying a candidate therapeutic agent.

31. The method of claim 30, wherein a candidate therapeutic agent modulates IL-7R α signaling by at least about 20% as compared to a baseline control.
32. The method of claim 30, wherein a candidate therapeutic agent inhibits IL-7R α mediated signaling by at least about 20% as compared to a baseline control.

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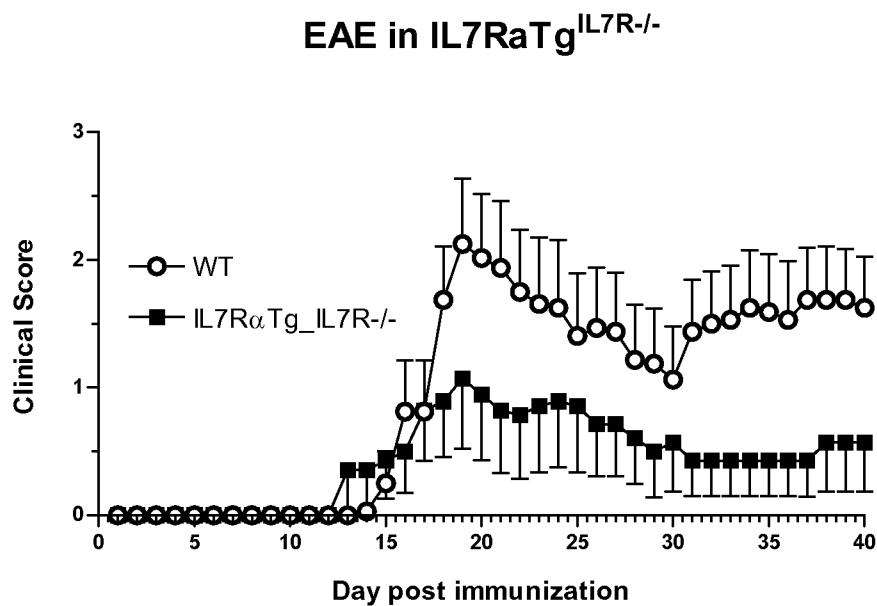


FIGURE 1A

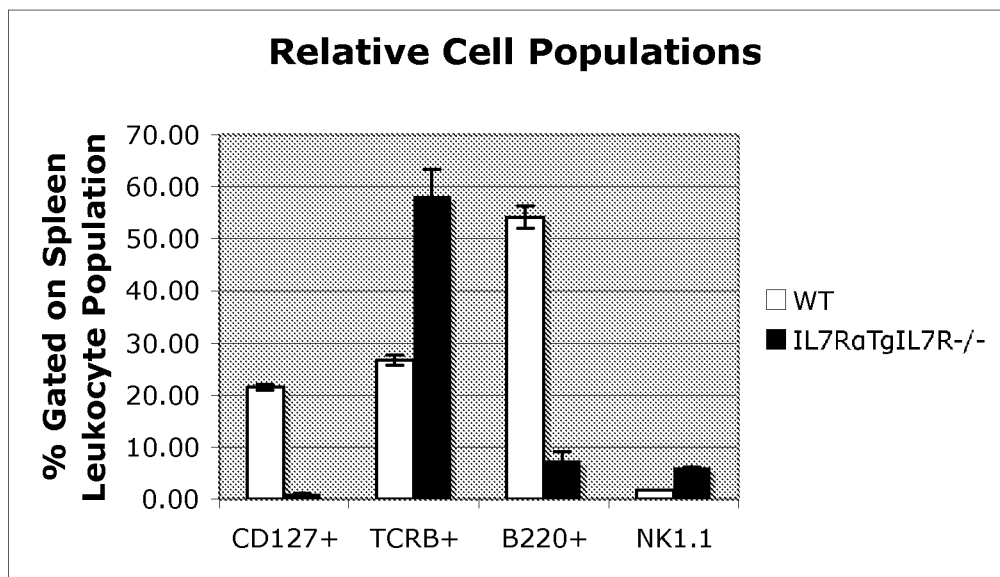


FIGURE 1B

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Ab Treatment Prior to EAE onset (Day 7-25)

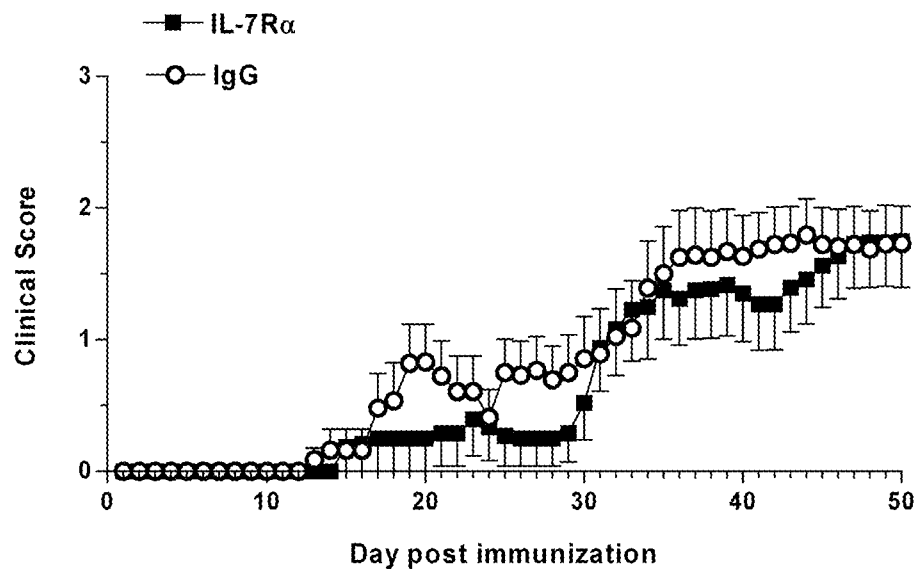


FIGURE 2A

Ab Treatment at EAE Peak (Day 19-37)

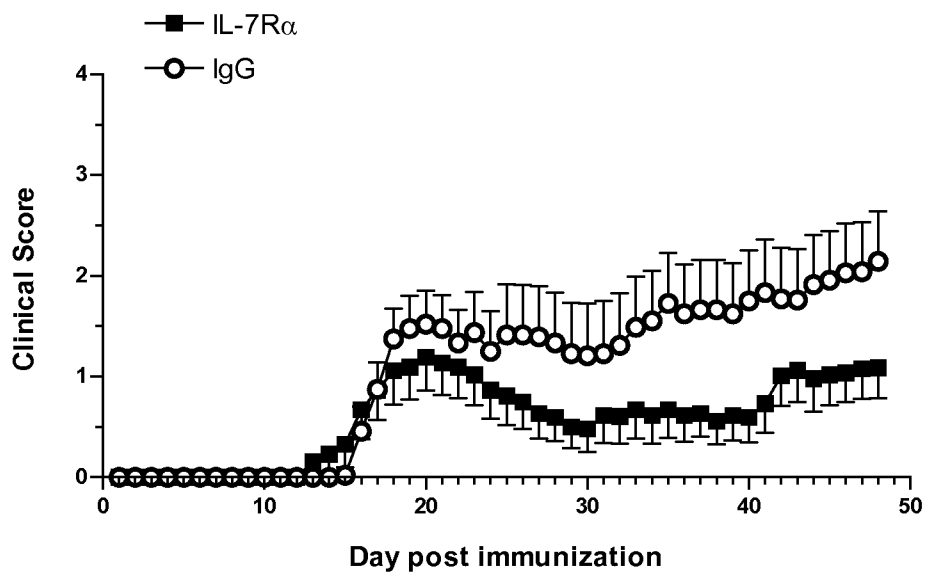


FIGURE 2B

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EAE Induction

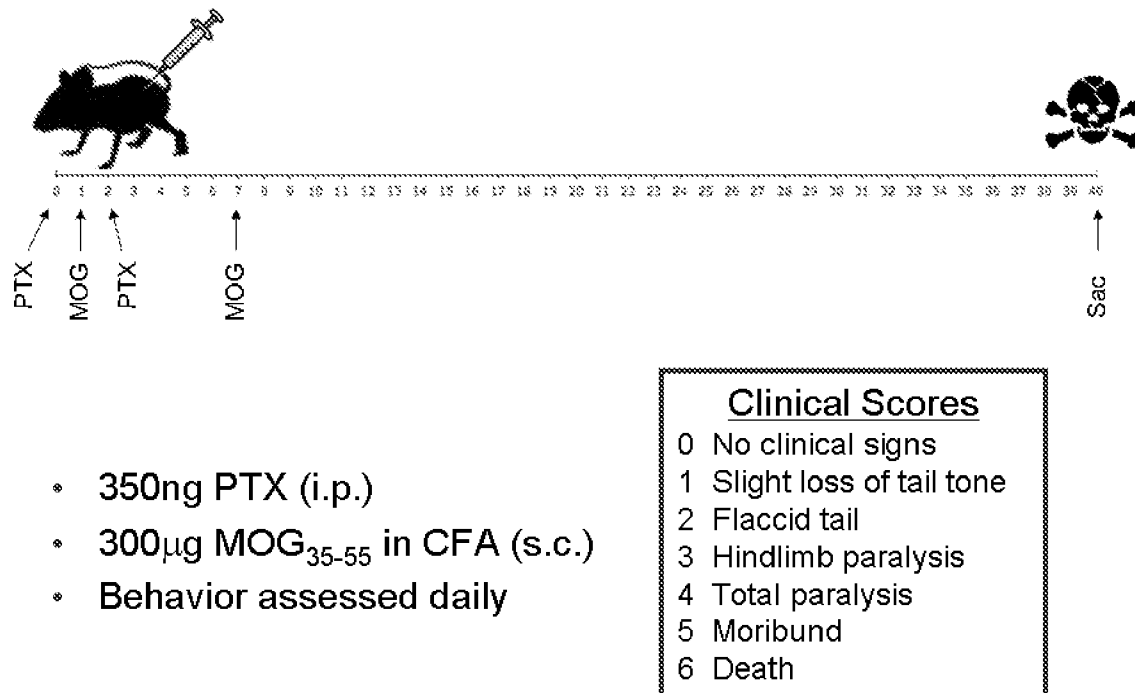


FIGURE 3

EAE in IL7R α Tg^{IL7R^{-/-}}

- IL7R α Tg^{IL7R^{-/-}} are less susceptible to EAE

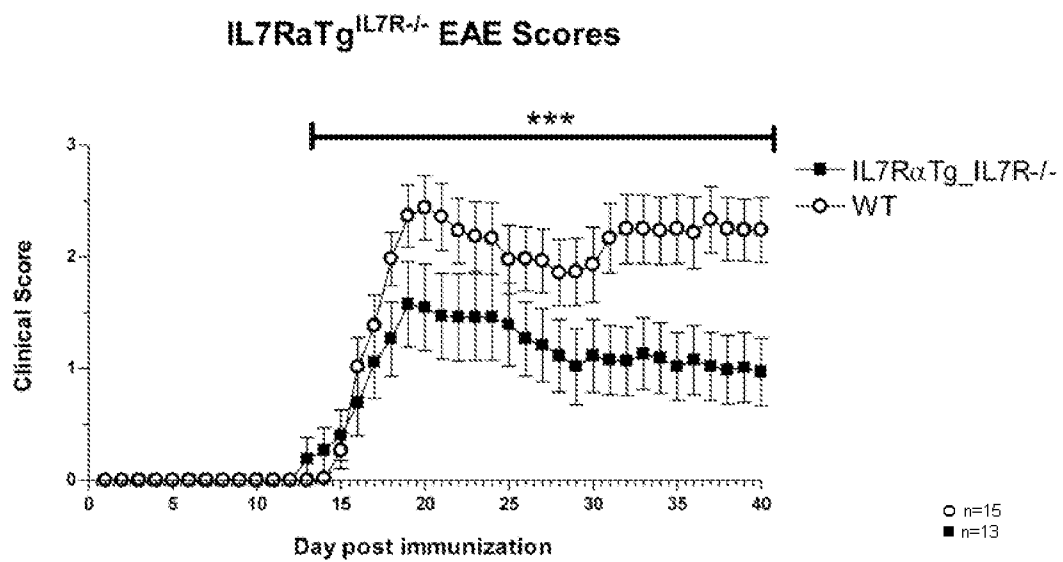


FIGURE 4

IL-7 Neutralization In Vitro

- IL7 and IL7R α neutralize T cell proliferation with equal capacities *in vitro*.

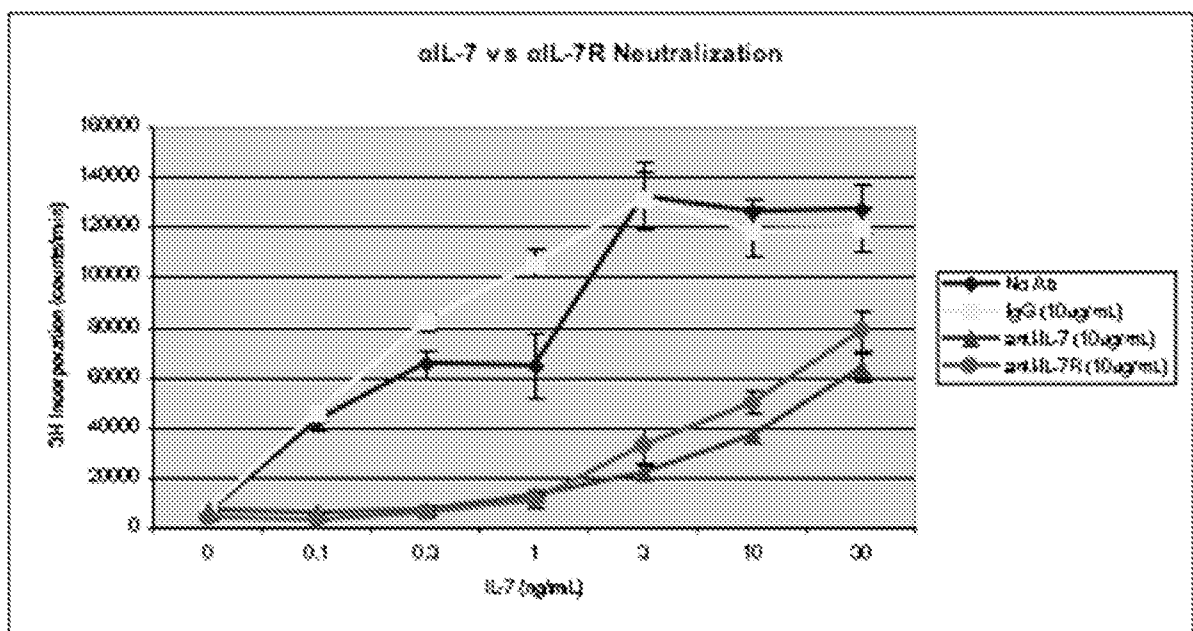


FIGURE 5

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Neutralizing Antibodies

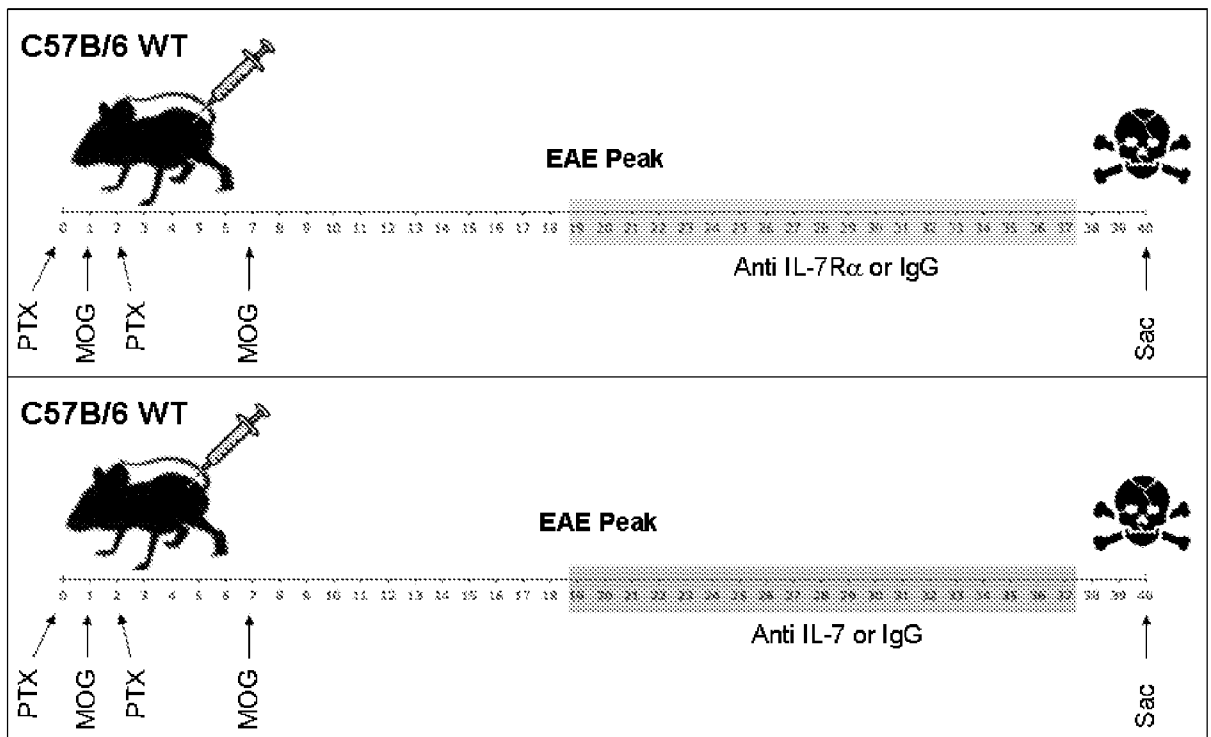
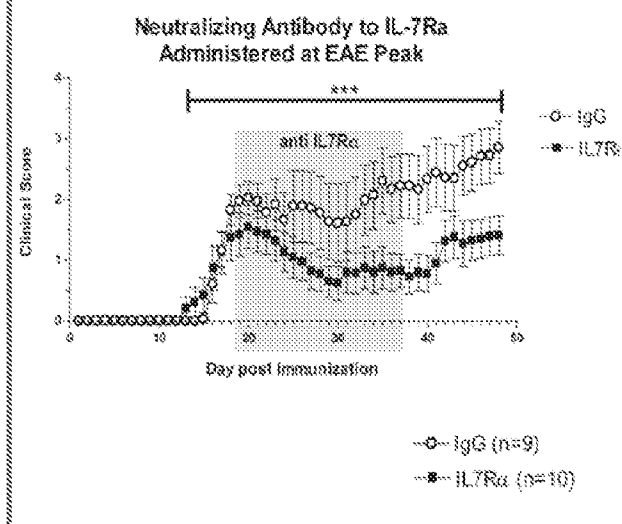


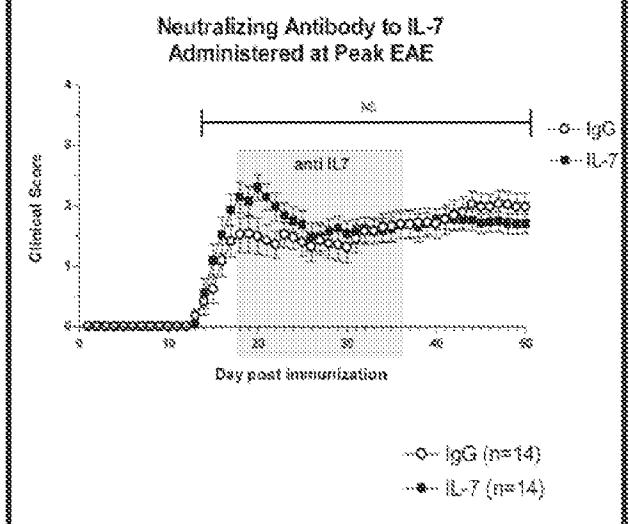
FIGURE 6

Ab Treatment at EAE Peak

- $IL7R\alpha$ neutralizing Ab leads to significant recovery, which holds even after cessation of treatment.



- $IL7$ neutralizing Ab does NOT improve recovery.



FIGURES 7A, 7B

Chimeric Mice

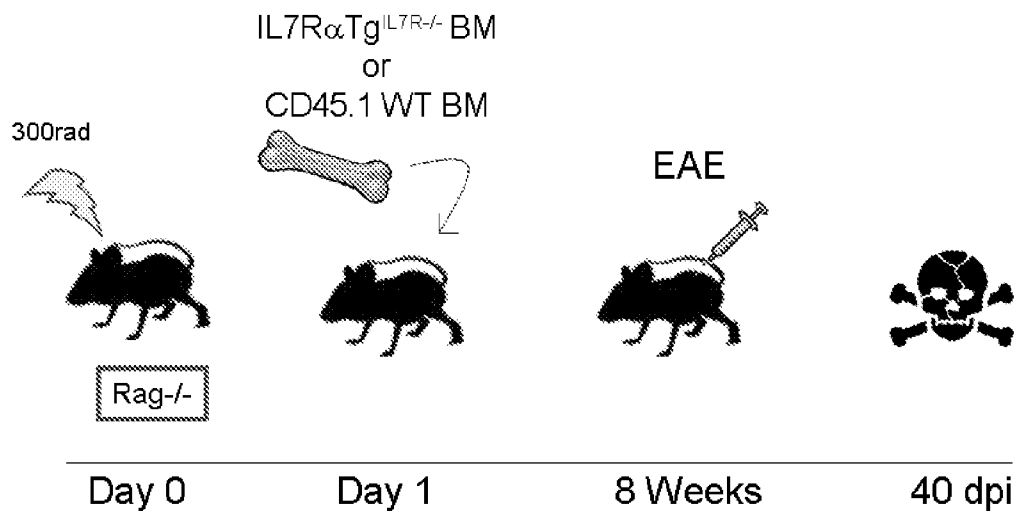


FIGURE 8

EAE in Chimeric Mice

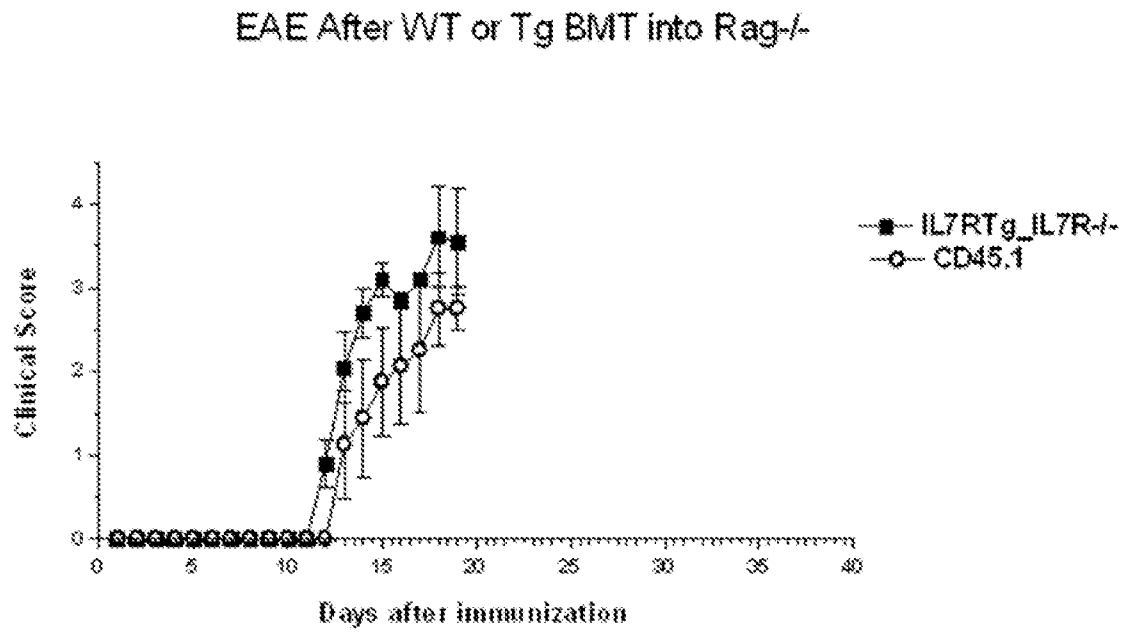


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/21799

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00, A61K 39/395, A61K 31/7088 (2010.01)

USPC - 514/2, 514/44R, 514/44A

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 -- 514/2, 514/44R, 514/44A, 514/\$

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 351, 6, 35, 65, 155; Google Scholar; USPTO Web Page; Search terms -- IL-7Ra, CD127, antibody, inhibitor, vivo/vitro, specific binding, IL-7 induced cell signaling, cancer, autoimmune disease, inhibition vs. control

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0131445 A1 (BLUESTONE et al.) 05 June 2008 (05.06.2008) para [0019], [0041], [0044], [0046], [0053], [0058], [0064], [0067], [0068], [0124], [0126], [0183]-[0185].	1-9
A	US 2006/0198822 A1 (BOOTH et al) 7 September 2006 (07.09.2006)	1-9
A	US 2004/0248157 A1 (AYALON-SOFFER et al.) 9 December 2004 (09.12.2004)	1-9

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

03 May 2010 (03.05.2010)

Date of mailing of the international search report

14 MAY 2010

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/21799

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.:
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:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-9, directed to a method of modulating IL-7 receptor or IL-7R.alpha. chain mediated signaling in vivo or in vitro comprising contacting IL-7R.alpha (CD127) or a molecule comprising IL-7R.alpha (CD127) with an agent which targets or binds to IL-7R.alpha. molecules and modulates IL-7R.alpha. mediated signaling.

Group II: claims 10-18, directed to a method for modulating a patient's immune system, comprising: administering to a patient and effective amount of an agent that modulates expression, function or activity of an IL-7R, or variant, isoform, mutant, allele or fragment thereof.

- Please see extra sheet for continuation -

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.:
1-9

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/21799

Continuation of Box III: Lack of Unity of Invention

Group III: claims 19-21, directed to a method of treating a patient suffering from an autoimmune disease or disorder, comprising administering a composition comprising a therapeutically effective amount of an agent that modulates IL-7R.alpha. mediated signaling.
Group IV: claims 22-25, directed to a pharmaceutical composition comprising an agent for modulating expression, function, signaling or activity of an IL-7 receptor, isoform, variant, mutant, allele or fragment thereof.

Group V: claims 26 and 27, directed to a fusion protein comprising at least one molecule which inhibits IL-7R.alpha. mediated signaling.
Group VI: claims 28 and 29, directed to a vector expressing a molecule which modulates IL-7R.alpha. mediated signaling, or a cell comprising said vector.

Group VII: claims 30-32, directed to a method of identifying candidate therapeutic agents, comprising: contacting an IL-7R.alpha. chain containing molecule or functional domain thereof, or a cell expressing said molecule with a candidate agent; measuring the signaling mediated by the IL-7R.alpha. chain as compared to a baseline control; and identifying a candidate therapeutic agent.

The inventions listed as Groups I - VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the Group I claims is a method of modulating IL-7 receptor or IL-7R.alpha. chain mediated signaling in vivo or in vitro comprising contacting IL-7R.alpha. (CD127) or a molecule comprising IL-7R.alpha. (CD127) with an agent which targets or binds to IL-7R.alpha. molecules and modulates IL-7R.alpha. mediated signaling. The special technical feature of the Group II claims is method for modulating a patient's immune system, comprising: administering to a patient and effective amount of an agent that modulates expression, function or activity of an IL-7R, or variant, isoform, mutant, allele or fragment thereof. The special technical feature of the Group III claims is a method of treating a patient suffering from an autoimmune disease or disorder, comprising administering a composition comprising a therapeutically effective amount of an agent that modulates IL-7R.alpha. mediated signaling. The special technical feature of the Group IV claims is a pharmaceutical composition comprising an agent for modulating expression, function, signaling or activity of an IL-7 receptor, isoform, variant, mutant, allele or fragment thereof. The special technical feature of the Group V claims is a fusion protein comprising at least one molecule which inhibits IL-7R.alpha. mediated signaling. The special technical feature of the Group VI claims is a vector expressing a molecule which modulates IL-7R.alpha. mediated signaling, or a cell comprising said vector. The special technical feature of the Group VII claims is a method of identifying candidate therapeutic agents, comprising: contacting an IL-7R.alpha. chain containing molecule or functional domain thereof, or a cell expressing said molecule with a candidate agent; measuring the signaling mediated by the IL-7R.alpha. chain as compared to a baseline control; and identifying a candidate therapeutic agent.

The only common technical element shared by the above groups is that they are related to modulation of IL-7R.alpha. (CD127). This common technical element does not represent an improvement over the prior art of US 2006/0198822 A1 to Booth et al. (see abstract, para [0006], [0036], [0037], [0049], [0060]). Therefore, the inventions of Groups I - VII lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.