Tregs constitutively express the high affinity IL-2 receptor at higher levels than Teff, low doses of IL-2 preferentially support the maintenance of Tregs as compared to Teff cells.

With the preferential effect of IL-2 for activating Tregs *in vitro* and *in vivo*, the potential for low dose, long-lived IL-2 therapy would seem to have a high prospect for success in autoimmune diseases. A 200 patient, double blind, placebo controlled type 1 diabetes clinical trial with IL-2 (Proleukin®) is set to begin in late 2013. Recent clinical trials with daily low dose Proleukin® ameliorated some of the signs and symptoms of chronic graft-versus-host disease (GVHD) and hepatitis C virus-induced vasculitis (Koreth et al., New Engl J Med 365, 2055-2066 (2011), Saadoun et al., New Engl J Med 365, 2067-2077 (2011)). In both studies low dose Proleukin® induced Tregs and increased the Treg:Teff ratio. However, Proleukin®'s poor PK properties make it suboptimal for maintaining low, consistent levels of IL-2 in man. Other methods being tested in clinical trials are personalized expansion of Tregs *ex vivo* followed by reinfusion, but this approach is less than ideal and represents a challenging set of quality control issues.

Thus, a new therapeutic approach that re-establishes the natural regulatory T cell (Treg) mediated dominant immune tolerance would greatly enhance the ability to treat patients with autoimmune diseases such as type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, Crohn's disease as well as other autoimmune diseases and immune-based pro-inflammatory diseases such as chronic graft versus host disease, asthma, pulmonary fibrosis, chronic obstructive pulmonary disease, cardiovascular diseases such as atherosclerosis, and transplant rejection, both solid organ and bone marrow.

The IL-2 fusion proteins of the present invention preferentially activate Tregs, tipping the balance toward a higher Treg:Teff ratio and reduce the autoimmune response. They are long-lived, allowing convenient dosing schedules, and devoid of effector functions, reducing potential side effects and impairment of efficacy.

Summary ~~of the Invention~~

In one aspect, the ~~invention~~ disclosure provides a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules.

said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in the immunoglobulin heavy chains.

In one embodiment, said IL-2 molecules are wild-type IL-2 molecules. In one embodiment, said IL-2 molecules comprise an amino acid mutation that does not alter the binding affinity of said IL-2 molecules to an IL-2 receptor as compared to naturally occurring, native IL-2. In one embodiment, said IL-2 molecules comprise an amino acid mutation at a position corresponding to residue 125 of human IL-2. In a more specific embodiment, said amino acid mutation is the amino acid substitution C125A. In one embodiment, said IL-2 molecules are human IL-2 molecules. In a specific embodiment, said IL-2 molecules comprise the sequence of SEQ ID NO: 1 or SEQ ID NO: 3, particularly the sequence of SEQ ID NO: 3. In one embodiment, said IL-2 molecules have the sequence of SEQ ID NO: 1 or SEQ ID NO: 3, particularly the sequence of SEQ ID NO: 3. In one embodiment, said IL-2 molecules are each fused at their N-terminal amino acid to the C-terminal amino acid of one of the immunoglobulin heavy chains of said immunoglobulin molecule, optionally through a peptide linker.

In a specific embodiment, said fusion protein comprises the polypeptide sequences of SEQ ID NO: 17 and SEQ ID NO: 19. In a specific embodiment, said fusion protein comprises an immunoglobulin light chain of SEQ ID NO: 19 and an immunoglobulin heavy chain-IL-2 fusion polypeptide of SEQ ID NO: 17. In one embodiment, said fusion protein essentially consists of an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, two interleukin-2 (IL-2) molecules, and optionally one or more peptide linker. In one embodiment, said fusion protein essentially consists of two immunoglobulin light chains of SEQ ID NO: 19 and two immunoglobulin heavy chain-IL-2 fusion polypeptides of SEQ ID NO: 17.

The ~~invention~~ disclosure further provides, in another aspect, a polynucleotide encoding the fusion protein of the ~~invention~~disclosure. Further provided, in another aspect, is a vector, particularly an expression vector, comprising the polynucleotide of the ~~invention~~disclosure. In another aspect, the ~~invention~~ disclosure provides a host cell comprising the polynucleotide or the vector of the ~~invention~~disclosure. The ~~invention~~ disclosure also provides a method for producing a fusion protein of the ~~invention~~disclosure, comprising the steps of (i) culturing the host cell of the disclosure ~~invention~~ under conditions suitable for expression of the fusion

protein, and (i) recovering the fusion protein. Hence, in another aspect, there is provided a method for producing a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules, said method comprising the steps of (i) culturing the host cell of the disclosure under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein. Also provided, in another aspect, is a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as

compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules, produced by said method.

In ~~one~~ another aspect, the ~~invention~~ disclosure provides a pharmaceutical composition comprising the fusion protein of the disclosure ~~invention~~ and a pharmaceutically acceptable carrier. In another aspect, ~~T~~the fusion protein or the pharmaceutical composition of the disclosure ~~invention~~ is also provided for use as a medicament. In another aspect, the fusion protein or the pharmaceutical composition of the disclosure is provided ~~and~~ for use in the treatment or prophylaxis of an autoimmune disease. The autoimmune disease may, specifically be type 1 diabetes, multiple sclerosis (MS), systemic lupus erythematosus (SLE) or Crohn's disease, most specifically type 1 diabetes, or graft-versus-host disease or transplant rejection. In another aspect, there is provided the fusion protein or the pharmaceutical composition of the disclosure for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease. Further ~~provided~~ provide, in another aspect, is the use of the fusion protein of the ~~invention~~ disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. There is also provided, ~~and~~ a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the fusion protein of the ~~invention~~ disclosure in a pharmaceutically acceptable form. In one embodiment, said disease is an autoimmune disease. In a more specific embodiment, said autoimmune disease is type 1 diabetes, multiple sclerosis (MS), systemic lupus erythematosus (SLE) or Crohn's disease. In an even more specific embodiment, said autoimmune disease is type 1 diabetes. In another embodiment said disease is transplant rejection or graft-versus-host disease. In one embodiment, said individual is a mammal, particularly a human.

Further provided, in another aspect, is the fusion protein of the disclosure ~~invention~~ for use in selective activation of regulatory T cells *in vitro* or *in vivo*. In one embodiment, said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling, particularly phosphorylation of STAT5, in regulatory T cells. In one embodiment, said use is *in vitro* and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less. In another embodiment, said use is *in vivo* and said fusion protein is used at a dose of about 20 µg/kg body weight or less, particularly about 12 µg/kg body weight or less, more particularly about 6 µg/kg body weight or less.

The disclosure ~~invention~~ also provides a method for selective activation of regulatory T cells *in vitro* or *in vivo*, comprising contacting said regulatory T cells with the fusion protein of the disclosure~~invention~~. In one embodiment, said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling, particularly phosphorylation of STAT5, in regulatory T cells. In one embodiment, said method is *in vitro* and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less. In another

24. A vector, particularly an expression vector, comprising the polynucleotide of claim 23.

25. A host cell comprising the polynucleotide of claim 23 or the vector of claim 24.

26. A method for producing ~~the~~ a fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules~~of any one of claims 1-22~~, said method comprising the steps of (i) culturing the host cell of claim 25 under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein.

27. A fusion protein comprising (i) an immunoglobulin molecule comprising a modification reducing binding affinity of the immunoglobulin molecule to an Fc receptor as compared to a corresponding immunoglobulin molecule without said modification, and (ii) two interleukin-2 (IL-2) molecules, produced by the method of claim 38.

28. A pharmaceutical composition comprising the fusion protein of any one of claims 1-22 or 27 and a pharmaceutically acceptable carrier.

29. The fusion protein of any one of claims 1-22 or 27, or the pharmaceutical composition of claim 28, for use as a medicament.

30. The fusion protein of any one of claims 1-22 or 27, or the pharmaceutical composition of claim 28, for use in the treatment or prophylaxis of an autoimmune disease.

31. The fusion protein or the pharmaceutical composition of claim 30, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, eczema, dermatitis, asthma, and multiple sclerosis.

32. The fusion protein of any one of claims 1-22 or 27, or the pharmaceutical composition of claim 28, for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease.

33. Use of the fusion protein of any one of claims 1-22 or 27 for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

34. The use of claim 33, wherein said disease is an autoimmune disease.

35. The use of claim 34, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, eczema, dermatitis, asthma, and multiple sclerosis.

36. The use of claim 33, wherein said disease is transplant rejection or graft-versus-host disease.

37. The use of any one of claims 33-36, wherein said individual is a mammal, particularly a human.

~~38. A method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the fusion protein of any one of claims 1-22 or 27 in a pharmaceutically acceptable form.~~

~~39. The method of claim 38, wherein said disease is an autoimmune disease.~~

~~40. The method of claim 39, wherein said autoimmune disease is selected from the group of type 1 diabetes, systemic lupus erythematosus, Crohn's disease and multiple sclerosis.~~

~~41. The method of claim 38, wherein said disease is transplant rejection or graft-versus-host disease.~~

~~42. The method of any one of claims 38-41, wherein said individual is a mammal, particularly a human.~~

~~43~~38. The fusion protein of any one of claims 1-22 or 27 for use in selective activation of regulatory T cells *in vitro* or *in vivo*.

~~44~~39. The fusion protein of claim ~~43~~38, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.

~~45~~40. The fusion protein of claim ~~43~~ 38 or ~~44~~39, wherein said use is *in vitro* and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less.

~~46~~41. The fusion protein of claim ~~43~~ 38 or ~~44~~39, wherein said use is *in vivo* and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less, more particularly about 6 μg/kg body weight or less.

~~47. A method for selective activation of regulatory T cells *in vitro* or *in vivo*, comprising contacting said regulatory T cells with the fusion protein of any one of claims 1-22 or 27.~~

~~48. The method of claim 47, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.~~

~~49. The method of claim 47 or 48, wherein said method is *in vitro* and said fusion protein is used at a concentration of about 1 ng/mL or less, particularly about 0.1 ng/mL or less.~~

~~50. The method of claim 47 or 48, wherein said method is *in vivo* and said fusion protein is used at a dose of about 20 μg/kg body weight or less, particularly about 12 μg/kg body weight or less, more particularly about 6 μg/kg body weight or less.~~