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

~~In one aspect, t~~The 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.

In one aspect, the disclosure provides a fusion protein comprising (i) a human IgG_1-subclass 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, wherein said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9 and the light chain variable region sequence of SEQ ID NO: 11, and wherein said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in the immunoglobulin heavy chains.

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

In another aspect, the disclosure provides a pharmaceutical composition comprising the fusion protein of the disclosure and a pharmaceutically acceptable carrier. In another aspect, the fusion protein or the pharmaceutical composition of the disclosure is also provided for use as a medicament. In another aspect, the fusion protein or the pharmaceutical composition of the disclosure is provided 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~~, in another aspect,~~ is the use of the fusion protein of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof. In another aspect, the disclosure provides the use of the fusion protein of the disclosure for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, wherein said disease is an autoimmune disease, transplant rejection or graft-versus-host disease. There is also provided 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 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 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 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. 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

Claims

1. A fusion protein comprising (i) ~~an~~ human IgG_1-subclass 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,

   wherein said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9 and the light chain variable region sequence of SEQ ID NO: 11, and

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

~~2. The fusion protein of claim 1, wherein said immunoglobulin molecule is an IgG-class immunoglobulin molecule, particularly an IgG_1-subclass immunoglobulin molecule.~~

~~3. The fusion protein of claim 1 or 2, wherein said immunoglobulin molecule is a human immunoglobulin molecule.~~

~~4. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule is not capable of specific binding to an antigen.~~

~~5. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises a heavy chain variable region sequence based on the human Vh3-23 germline sequence.~~

~~6. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the heavy chain variable region sequence of SEQ ID NO: 9.~~

~~7. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises a light chain variable region sequence based on the human Vk3-20 germline sequence.~~

~~8. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the light chain variable region sequence of SEQ ID NO: 11.~~

~~9~~2. The fusion protein of ~~any one of the preceding~~ claim~~s~~ 1, wherein said Fc receptor is an Fcγ receptor, particularly a human Fcγ receptor.

~~103~~.The fusion protein of ~~any one of the preceding~~ claims 1 or 2, wherein said Fc receptor is an activating Fc receptor.

~~114~~.The fusion protein of any one of the preceding claims, wherein said Fc receptor is selected from the group of FcγRIIIa (CD16a), FcγRI (CD64), FcγRIIa (CD32) and FcαRI (CD89).

~~12~~5. The fusion protein of any one of the preceding claims, wherein said Fc receptor is FcγRIIIa, particularly human FcγRIIIa.

~~13. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises an amino acid substitution at position 329 (EU numbering) of the immunoglobulin heavy chains.~~

~~14. The fusion protein of claim 13, wherein said amino acid substitution is P329G.~~

~~15. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises amino acid substitutions at positions 234 and 235 (EU numbering) of the immunoglobulin heavy chains.~~

~~16. The fusion protein of claim 15, wherein said amino acid substitutions are L234A and L235A (LALA).~~

~~17. The fusion protein of any one of the preceding claims, wherein said immunoglobulin molecule comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in the immunoglobulin heavy chains.~~

~~18~~6. The fusion protein of any one of the preceding claims, wherein said IL-2 molecules are wild-type IL-2 molecules.

~~19~~7. The fusion protein of any one of the preceding claims, wherein said IL-2 molecules are human IL-2 molecules.

~~20~~8. The fusion protein of any one of the preceding claims, wherein 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.

~~21~~9. The fusion protein of any one of the preceding claims, wherein 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.

~~22~~10. The fusion protein of any one of the preceding claims, wherein the fusion protein omprises the polypeptide sequences of SEQ ID NO: 17 and SEQ ID NO: 19.

~~23~~11. A polynucleotide encoding the fusion protein of any one of the preceding claims.

~~24~~12. A vector, particularly an expression vector, comprising the polynucleotide of claim ~~23~~11.

~~25~~13. A host cell comprising the polynucleotide of claim ~~23~~-11 or the vector of claim ~~24~~12.

~~26~~14. 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 claim ~~25~~-13 under conditions suitable for expression of the fusion protein, and (ii) recovering the fusion protein.

~~27~~15. 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~~14.

~~28~~16. A pharmaceutical composition comprising the fusion protein of any one of claims 1-~~22~~-10 or ~~27~~-15 and a pharmaceutically acceptable carrier.

~~29~~17. The fusion protein of any one of claims 1-~~22~~-10 or ~~27~~15, or the pharmaceutical composition of claim ~~28~~16, for use as a medicament.

~~30~~18. The fusion protein of any one of claims 1-~~22~~-10 or ~~27~~15, or the pharmaceutical composition of claim ~~28~~16, for use in the treatment or prophylaxis of an autoimmune disease.

~~31~~19. The fusion protein or the pharmaceutical composition of claim ~~30~~18, 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~~20. The fusion protein of any one of claims 1-~~22~~-10 or ~~27~~15, or the pharmaceutical composition of claim ~~28~~16, for use in the treatment or prophylaxis of transplant rejection or graft-versus-host disease.

~~33~~21. Use of the fusion protein of any one of claims 1-~~22~~-10 or ~~27~~-15 for the manufacture of a medicament for the treatment of a disease in an individual in need thereof, wherein said disease is an autoimmune disease, transplant rejection or graft-versus-host disease.

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

~~35~~22. The use of claim ~~34~~21, 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~~23. The use of ~~any one of~~ claims ~~33-36~~21 or 22, wherein said individual is a mammal, particularly a human.

~~38~~24. The fusion protein of any one of claims 1-~~22~~10 or ~~27~~15 for use in selective activation of regulatory T cells *in vitro* or *in vivo*.

~~39~~25. The fusion protein of claim ~~38~~24, wherein said activation comprises induction of proliferation of regulatory T cells and/or induction of IL-2 receptor signaling in regulatory T cells.

~~40~~26. The fusion protein of claim ~~38~~24 or ~~39~~25, 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.

~~41~~27. The fusion protein of claim ~~38~~24 or ~~39~~25, 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.