

CLAIMS

1. A nucleic acid molecule comprising a nucleotide sequence encoding a membrane-bound homodimeric IL-10 (mem-IL10) comprising a homodimeric IL-10 linked to a transmembrane-intracellular region.
2. The nucleic acid molecule of claim 1, wherein the homodimeric IL-10 comprises a first and a second IL-10 monomer connected in a single-chain configuration such that the C-terminus of the first IL-10 monomer is linked to the N-terminus of the second IL-10 monomer via a first flexible linker.
3. The nucleic acid molecule of claim 2, wherein the first flexible linker has the amino acid sequence GSTSGSGKPGSGEGSTKG (SEQ ID NO: 1).
4. The nucleic acid molecule of any one of claims 1–3, wherein the homodimeric IL-10 is linked to the transmembrane-intracellular region through a hinge.
5. The nucleic acid molecule of claim 4, wherein the hinge comprises: (i) a polypeptide selected from a hinge region of CD8 α , a hinge region of a heavy chain of IgG, a hinge region of a heavy chain of IgD; (ii) an extracellular region of an IL-10R β chain; and (iii) a second flexible linker comprising an amino acid sequence of up to 28 amino acids and comprising the amino acid sequence of Gly₄Ser(Gly₃Ser)₂ (SEQ ID NO: 13).
6. The nucleic acid molecule of claim 5, wherein the second flexible linker comprises the amino acid sequence Gly₄Ser(Gly₃Ser)₂ (SEQ ID NO: 13).
7. The nucleic acid molecule of claim 5, wherein the second flexible linker comprises the amino acid sequence Gly₄Ser(Gly₃Ser)₂Ser₂(Gly₃Ser)₃ (SEQ ID NO: 15).
8. The nucleic acid molecule of any one of claims 5 to 7, wherein the polypeptide further comprises an amino acid bridge of the sequence SSQPTIPI (SEQ ID NO: 17).

9. The nucleic acid molecule of any one of claims 1 to 8, wherein the transmembrane-intracellular region is derived from a heavy chain of a human MHC class I molecule selected from an HLA-A, HLA-B or HLA-C molecule; human CD28; or human IL-10R β chain.
10. The nucleic acid of claim 9, wherein the transmembrane-intracellular region is derived from a heavy chain of an HLA-A2.
11. The nucleic acid molecule of any one of claims 1 to 10, wherein the mem-IL10 comprises the amino acid sequence of SEQ ID NO: 25.
12. The nucleic acid molecule of any one of claims 1 to 10, wherein the mem-IL10 comprises the amino acid sequence of SEQ ID NO: 27
13. The nucleic acid molecule of any one of claims 1 to 10, wherein the mem-IL10 comprises the amino acid sequence of SEQ ID NO: 29.
14. A membrane-bound homodimeric IL-10 (mem-IL10) comprising a homodimeric IL-10 linked to a transmembrane-intracellular region.
15. A mem-IL10 encoded by the nucleic acid of any one of claims 1 to 13.
16. A composition comprising the nucleic acid molecule of any one of claims 1 to 13.
17. A viral vector comprising the nucleic acid molecule of any one of claims 1 to 13.
18. The viral vector of claim 17, which is a modified virus derived from a virus selected from a retrovirus, lentivirus, gammavirus, adenovirus, adeno-associated virus, poxvirus, alphavirus, and herpesvirus.
19. A composition comprising the viral vector of claim 17 or 18.
20. A mammalian regulatory T cell (Treg) comprising the nucleic acid molecule of any one of claims 1 to 13 or the viral vector of claim 17 or 18.
21. The mammalian Treg of claim 20, expressing on its surface a homodimeric IL-10 that is linked to a transmembrane-intracellular region.

22. The mammalian Treg of claim 20 or 21, which is a human Treg.
23. The mammalian Treg of any one of claims 20 to 22 having a stable Tr1 phenotype exhibiting the cell-surface markers CD49b and LAG-3.
24. The mammalian Treg of claim 23, further exhibiting PD-1, 4-1BB, CD25 and IL-10R α .
25. The mammalian Treg of any one of claims 20-24, wherein the Treg is manipulated to express a tissue-targeting protein, a T cell receptor, or a chimeric antigen receptor.
26. A method of preparing allogeneic or autologous Tregs with a stable Tr1 phenotype, the method comprising contacting CD4⁺ T cells with (i) the nucleic acid molecule of any one of claims 1 to 13; (ii) the viral vector of claim 17 or 18; or (iii) the homodimeric IL-10 of claim 14 or 15, thereby endowing the CD4⁺ T cells with a stable Tr1 phenotype, and thus preparing Tregs with a stable Tr1 phenotype.
27. Use of the mammalian Treg of any one of claims 20 to 25, in the manufacture of a medicament for increasing immune suppression in a subject in need thereof.
28. Use of the mammalian Treg of any one of claims 20 to 25, in the manufacture of a medicament for treating or preventing a disease, disorder or condition, manifested in excessive activity of the immune system.
29. Use of the mammalian Treg of any one of claims 20–25, in the manufacture of a medicament for treating or preventing a disease, disorder or condition selected from an autoimmune disease, allergy, asthma, and organ and bone marrow transplantation.
30. The use of claim 29, wherein the autoimmune disease is selected from type 1 diabetes; rheumatoid arthritis; psoriasis; psoriatic arthritis; multiple sclerosis; systemic lupus erythematosus; inflammatory bowel disease; Addison's disease; Graves' disease; Sjögren's syndrome; Hashimoto's thyroiditis; myasthenia gravis; vasculitis; pernicious anemia; celiac disease; and atherosclerosis.
31. The use of claim 30, wherein the autoimmune disease is type 1 diabetes.

32. The use of claim 30, wherein the autoimmune disease is inflammatory bowel disease.
33. The use of claim 30, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.
34. The use of claim 30, wherein the autoimmune disease is atherosclerosis.
35. The use of any one of claims 29 to 34, wherein the mammalian Treg is a human Treg and the medicament is for treating or preventing the disease, disorder, or condition in a human subject.
36. The use of claim 35, wherein the human Treg is an allogeneic Treg.