

CLAIMS

~~WHAT IS CLAIMED IS:~~

1. A recombinant polypeptide comprising:
an amino acid sequence having at least 70% sequence identity to an interleukin-10 (IL-10) polypeptide having the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 16;
and further comprising one or more amino acid substitution at a position corresponding to an amino acid residue selected from the group consisting of X25, X14, X18, X24, X28, X74, X90, X92, X96, X100 and X104 of SEQ ID NO: 1 or SEQ ID NO: 16.
2. The recombinant polypeptide of claim 1, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of X25, and X96 of SEQ ID NO: 1 or SEQ ID NO: 16.
3. The recombinant polypeptide of any one of claims 1 to 2, wherein the amino acid sequence further comprising at least one additional amino acid substitution at a position corresponding to an amino acid residue selected from the group consisting of X21, X22, X32, and X93 of SEQ ID NO: 1 or SEQ ID NO: 16.
4. The recombinant polypeptide of any one of claims 1 to 3, wherein the recombinant polypeptide has an altered binding affinity for IL-10 receptor beta (IL-10R β) compared to binding affinity of a reference IL-10 polypeptide lacking the one or more amino acid substitution.
5. The recombinant polypeptide of claim 4, wherein the recombinant polypeptide has a reduced binding affinity for IL-10R β compared to binding affinity of the reference IL-10 polypeptide.
6. The recombinant polypeptide of any one of claims 4 to 5, wherein the recombinant polypeptide has a reduced capability to stimulate STAT3 signaling compared to the reference IL-10 polypeptide.
7. The recombinant polypeptide of claim 4, wherein the recombinant polypeptide has an increased binding affinity for IL-10R β compared to binding affinity of the reference IL-10 polypeptide.

8. The recombinant polypeptide of any one of claims 4 to 7, wherein the recombinant polypeptide confers a cell-type biased signaling of the downstream signal transduction mediated through IL-10 compared to the reference IL-10 polypeptide.
9. The recombinant polypeptide of any one of claims 1 to 8, wherein the one or more amino acid substitution is independently selected from the group consisting of an alanine substitution, an aspartic acid substitution, a histidine substitution, a glutamic acid substitution, a lysine substitution, a serine substitution, a tryptophan substitution, a tyrosine substitution, a valine substitution, and combinations of any thereof.
10. The recombinant polypeptide of any one of claims 1 to 9, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of D25, H14, N18, R24, D28, E74, H90, N92, E96, T100 and R104 of SEQ ID NO: 1 or SEQ ID NO: 16.
11. The recombinant polypeptide of claim 10, wherein the one or more amino acid substitution is at a position corresponding to an amino acid residue selected from the group consisting of D25 and E96 of SEQ ID NO: 1 or SEQ ID NO: 16.
12. The recombinant polypeptide of any one of claims 10 to 11, wherein the amino acid sequence further comprising at least one additional amino acid substitution at a position corresponding to an amino acid residue selected from the group consisting of N21, M22, R32, and S93 of SEQ ID NO: 1 or SEQ ID NO: 16.
13. The recombinant polypeptide of any one of claims 1 to 12, comprising an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 16, and further comprising the amino acid substitutions corresponding to the following amino acid substitutions:
- a) N18Y/N92Q/T100D/R104W;
 - b) N18Y/N21H/N92Q/E96D/T100V/R104W;
 - c) N18Y/N21H/E96H/T100V/R104W;
 - d) N18Y/D25A/N92Q/T100D/R104W;
 - e) N18Y/D25K/N92Q/T100D/R104W; and
 - f) N18Y/D25A/N92Q/E96A/T100D/R104W.

14. The recombinant polypeptide of any one of claims 1 to 12, comprising an amino acid sequence having at least 70% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 16, and further comprising the amino acid substitutions corresponding to the following amino acid substitutions:

- a) a) D25A;
- b) D25K;
- c) E96A;
- d) E96K;
- e) D25A/E96A;
- f) N21A/R104A;
- g) N21A/D25A;
- h) N21A/D25A/E96A; and
- i) N21A/M22A/D25A.

15. A recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide that comprises an amino acid sequence having at least 70% sequence identity to the amino acid sequence of the polypeptide of any one of 1-14 and 23.

16. The nucleic acid molecule of claim 15, wherein the nucleic acid sequence is operably linked to a heterologous nucleic acid sequence.

17. The nucleic acid molecule of any one of claims 15-16, wherein the nucleic acid molecule is incorporated into an expression cassette or an expression vector.

18. A recombinant cell comprising:

- a) a recombinant polypeptide according to any one of claims 1-14 and 23; and/or
- b) a recombinant nucleic acid of any one of claims 15-17.

19. The recombinant cell of claim 18, wherein the recombinant cell is a eukaryotic cell.

20. The recombinant cell of claim 19, wherein the eukaryotic cell is a mammalian cell.

21. A cell culture comprising at least one recombinant cell of any one of claims 18, and a culture medium.

22. A method for producing a polypeptide comprising:

- a) providing one or more recombinant cells of any one of claim 18; and

- b) culturing the one or more recombinant cells in a culture medium such that the cells produce the polypeptide encoded by the recombinant nucleic acid molecule.
23. The method of claim 22, further comprising isolating and/or purifying the produced polypeptide.
24. The method of any one of claims 22-23, further comprising structurally modifying the produced polypeptide to increase half-life.
25. The method of claim 24, wherein said modification comprises one or more alterations selected from the group consisting of fusion to a human Fc antibody fragment, fusion to albumin, and PEGylation.
26. A recombinant polypeptide produced by the method of any one of claims 22.
27. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and:
a) a recombinant polypeptide according to any one of claims 1-14 and 23;
b) a recombinant nucleic acid of any one of claims 15-17; and/or
c) a recombinant cell of any one of claims 18.
28. The pharmaceutical composition of claim 27, wherein the composition comprises a recombinant polypeptide according to any one of claims 1-14 and 23, and a pharmaceutically acceptable carrier.
29. The pharmaceutical composition of claim 27, wherein the composition comprises a recombinant nucleic acid according to any one of claims 15-17, and a pharmaceutically acceptable carrier.
30. The pharmaceutical composition of claim 27, wherein the composition comprises a recombinant cell according to any one of claims 16-20, and a pharmaceutically acceptable carrier.
31. ~~Use of~~A method for modulating IL-10-mediated signaling in a subject, the method comprising administering to the subject a composition comprising:
a) a recombinant polypeptide according to any one of claims 1-14 and 23;
b) a recombinant nucleic acid of any one of claims 15-17;
c) a recombinant cell of any one of claims 18-20; and/or

- d) a pharmaceutical composition claim 27-30,
for the preparation of a medicament for modulating IL-10-mediated signaling in a subject.
32. ~~Use of~~A method for the treatment of a health condition in a subject in need thereof, the method comprising administering to the subject a composition comprising:
- a) a recombinant polypeptide according any one of claims 1-14 and 23;
 - b) a recombinant nucleic acid of any one of claims 15-17;
 - c) a recombinant cell of any one of claims 18-20; and/or
 - d) a pharmaceutical composition of any one of claims 27-30,
for the preparation of a medicament for the treatment of a health condition in a subject.
33. The ~~method-use~~ of any one of claims 31 to 32, wherein the medicament to be administered ~~composition~~ confers a cell-type biased signaling of the downstream signal transduction mediated through IL-10 compared to a reference IL-10 polypeptide lacking the one or more amino acid substitution.
34. The ~~method-use~~ of claim 33, wherein the cell-type biased IL-10 signaling comprises a reduction of STAT1- or STAT3-mediated pro-inflammatory function in B cells, T cells, and NK cells while substantially retaining its STAT3-mediated anti-inflammatory function in monocytes and macrophages.
35. The ~~method-use~~ of claim 34, wherein the STAT3-mediated signaling is determined by an assay selected from the group consisting of by a gene expression assay, a phospho-flow signaling assay, and an enzyme-linked immunosorbent assay (ELISA).
36. The ~~method-use~~ of any one of claims 34-35, wherein the STAT3-mediated pro-inflammatory function is selected from the group consisting of cytokine production, chemokine production, immune cell proliferation, and immune cell recruitment.
37. The ~~method-use~~ of any one of claims 34-36, wherein the STAT3-mediated pro-inflammatory function is reduced from about 20% to about 100%.
38. The ~~method-use~~ of any one of claims 32-37, wherein the medicament to be administered ~~composition~~ results in a reduced capacity to induce expression of a pro-inflammatory gene

selected from IFN- γ , granzyme B, granzyme A, perforin, TNF- α , GM-CSF, and MIP1 α in the subject.

39. The ~~methods-use~~ use of claim 32, wherein the medicament to be administered composition stimulates expression of interferon gamma (INF γ) in CD8+ T cells.

40. The ~~method-use~~ use of claim 39, wherein the health condition is a cancer.

41. A kit for modulating IL-10-mediated signaling in a subject, or treating a health condition in a subject in need thereof, the system comprising:

- a) a recombinant polypeptide according to any one of claims 1-14 and 23;
- b) a recombinant nucleic acid of any one of claims 15-17;
- c) a recombinant cell of any one of claims 18-20; and/or
- d) a pharmaceutical composition of any one of claims 27-30.