

CLAIMS

1. A recombinant interleukin 12 subunit p40 (IL-12p40) variant comprising:
the amino acid sequence of a mature wild-type human IL-12p40 polypeptide as set forth in amino acid residues 23-328 of SEQ ID NO: 1, and further comprising one or more amino acid substitutions in the sequence of SEQ ID NO: 1,
wherein the recombinant IL-12p40 variant comprises amino acid residues 23-328 of an amino acid sequence selected from the group consisting of SEQ ID NOS: 3-8 and 13-16.
2. A recombinant IL-12p40 variant, wherein the recombinant IL-12p40 variant comprises the substitutions P39A/D40A/E81A/F82A as set forth in amino acid residues 23-328 of SEQ ID NO: 5.
3. The recombinant IL-12p40 variant of claim 1, wherein the recombinant IL-12p40 variant comprises the substitutions E81A/F82A as set forth in amino acid residues 23-328 of SEQ ID NO: 13.
4. The recombinant IL-12p40 variant of claim 1, wherein the recombinant IL-12p40 variant comprises the substitutions E81A/F82A/K106A as set forth in amino acid residues 23-328 of SEQ ID NO: 14.
5. The recombinant IL-12p40 variant of claim 4, wherein the recombinant IL-12p40 variant further comprises the K217A substitution.
6. The recombinant polypeptide of any one of claims 1-5, wherein:
(a) the recombinant polypeptide has an altered binding affinity for interleukin-12 receptor, subunit beta 1 (IL-12R β 1) compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or

- (b) the recombinant polypeptide has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or
- (c) the recombinant polypeptide has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).

7. The recombinant polypeptide of claim 6, wherein the recombinant polypeptide, when combined with an interleukin 12 subunit p35 (IL-12p35) polypeptide, has a reduced capability to stimulate STAT4 signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

8. The recombinant polypeptide of claim 6 or 7, wherein the recombinant polypeptide, when combined with an interleukin 23 subunit p19 (IL-23p19) polypeptide, has a reduced capability to stimulate STAT3 signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

9. The recombinant polypeptide of any one of claims 6-8, wherein the one or more amino acid substitution results in a cell-type biased signaling of the downstream signal transduction mediated through interleukin-12 (IL-12) and/or interleukin-23 (IL-23) compared to a reference polypeptide lacking the one or more amino acid substitution.

10. The recombinant polypeptide of claim 9, wherein:
- (a) the cell-type biased signaling comprises a reduced capability of the recombinant polypeptide to stimulate IL-12-mediated signaling in natural killer (NK) cells; and/or
 - (b) the cell-type biased signaling comprises a substantially unaltered capability of the recombinant polypeptide to stimulate IL-12 signaling in CD8⁺ T cells; and/or

- (c) the one or more amino acid substitution results in a reduced capability of the recombinant polypeptide to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8⁺ T cells.
11. The recombinant IL-12p40 variant of any one of claims 1-10, wherein the recombinant IL-12p40 variant further comprises a structural modification to increase half-life.
12. The recombinant IL-12p40 variant of claim 11, wherein the structural modification comprises one or more of the following: (a) fusion to a Fc antibody fragment, (b) fusion to albumin, and (c) PEGylation.
13. A recombinant nucleic acid molecule comprising a nucleic acid sequence encoding a recombinant IL-12p40 variant of any one of claims 1-10.
14. A recombinant cell comprising:
- a) a recombinant IL-12p40 variant according to any one of claims 1 to 10; and/or
 - b) a recombinant nucleic acid according to claim 13.
15. The recombinant cell of claim 14, wherein the recombinant cell further comprises (i) an IL-12p35 polypeptide and/or a second nucleic acid molecule encoding the IL-12p35 polypeptide.
16. The recombinant cell of claim 14, wherein the recombinant cell further comprises (i) an IL-23p19 polypeptide and/or (ii) a second nucleic acid molecule encoding the IL-23p19 polypeptide.
17. A cell culture comprising at least one recombinant cell of any one of claims 14 to 16 and a culture medium.

18. A method for producing a recombinant IL-12p40 variant, comprising:
- a) providing one or more recombinant cells of claim 14; 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.
19. The method of claim 18, further comprising structurally modifying the produced polypeptide to increase half-life.
20. The method of claim 19, wherein said modification comprises one or more alterations selected from the group consisting of (i) fusion to a human Fc antibody fragment, (ii) fusion to albumin, and (iii) PEGylation.
21. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and:
- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-12p35 polypeptide; and/or
 - b) a recombinant cell according to claim 15.
22. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and:
- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-23p19 polypeptide; and/or
 - b) a recombinant cell according to claim 16.
23. Use of a composition comprising:
- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-12p35 polypeptide;
 - b) a recombinant cell according to claim 15 and/or
 - c) a pharmaceutically composition according to claim 21,
- in the preparation of a medicament for modulating IL-12-mediated signal transduction in

a subject in need thereof, wherein the subject has or is suspected of having a health condition associated with IL-12 mediated signaling, and wherein the health condition is a cancer, an immune disease, or a chronic infection.

24. Use of a composition comprising:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-23p19 polypeptide ;
- b) a recombinant cell according to claim 16; and/or
- c) a pharmaceutically composition according to claim 22,

in the preparation of a medicament for modulating IL-23-mediated signal transduction in a subject in need thereof, wherein the subject has or is suspected of having a health condition associated with IL-23 mediated signaling, and wherein the health condition is a cancer, an immune disease, or a chronic infection.

25. Use of a composition comprising:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-12p35 polypeptide;
- b) a recombinant cell according to claim 15; and/or
- c) a pharmaceutically composition according to claim 21,

in the preparation of a medicament for the treatment of a health condition in a human subject in need thereof, wherein the subject has or is suspected of having a health condition associated with IL-12 mediated signaling, and wherein the health condition is a cancer, an immune disease, or a chronic infection.

26. Use of a composition comprising:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-23p19 polypeptide ;
- b) a recombinant cell according to claim 16; and/or
- c) a pharmaceutically composition according to claim 22,

in the preparation of a medicament for the treatment of a health condition in a human subject in need thereof, wherein the subject has or is suspected of having a health condition associated with IL-23 mediated signaling, and wherein the health condition is a cancer, an immune disease, or a chronic infection.

27. The use of any one of claims 23-26, wherein:

- (a) the recombinant IL-12p40 variant has an altered binding affinity for interleukin-12 receptor, beta 1 (IL-12R β 1) compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or
- (b) the recombinant IL-12p40 variant has a reduced binding affinity for IL-12R β 1 compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution; and/or
- (c) the recombinant IL-12p40 variant has binding affinity for IL-12R β 1 reduced by about 10% to about 100% compared to binding affinity of a reference polypeptide lacking the one or more amino acid substitution, as determined by surface plasmon resonance (SPR).

28. The use of claim 27, wherein the reduced binding affinity of the recombinant IL-12p40 variant to IL-12R β 1 receptor results in a reduction in STAT4-mediated signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

29. The use of claim 27 or 28, wherein the reduced binding affinity of the recombinant IL-12p40 variant to IL-12R β 1 receptor results in a reduction in STAT3-mediated signaling compared to a reference polypeptide lacking the one or more amino acid substitution.

30. The use of any one of claims 23-29, wherein the composition to be administered results in a cell-type biased signaling of the downstream signal transduction mediated by interleukin-12 (IL-

12) and/or by interleukin-23 (IL-23) compared to a reference polypeptide lacking the one or more amino acid substitution.

31. The use of claim 30, wherein:

- (a) the cell-type biased signaling comprises a reduced capability of the recombinant IL-12p40 variant to stimulate IL-12-mediated signaling in NK cells; and/or
- (b) the cell-type biased signaling comprises a substantially unaltered capability of the recombinant IL-12p40 variant to stimulate IL-12 signaling in CD8⁺ T cells; and/or
- (c) the composition to be administered results in a reduced capability of the recombinant IL-12p40 variant to stimulate IL-12 signaling in NK cells while substantially retains its capability to stimulate IL-12 signaling in CD8⁺ T cells; and/or
- (d) the composition to be administered substantially retains the recombinant IL-12p40 variant's capability to stimulate expression of interferon gamma (INF γ) in CD8⁺ T cells; and/or
- (e) the composition to be administered enhances antitumor immunity in a tumor microenvironment.

32. The use of any one of claims 23-31, wherein the composition is to be administered to the subject individually as a first therapy or in combination with a second therapy.

33. A kit for modulating IL-12p40 mediated signal transduction, modulating IL-12p40-mediated signal transduction, or treating a health condition in a subject in need thereof, the system comprising:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-12p35 polypeptide;
 - c) a recombinant cell according to claim 15; and/or
 - d) a pharmaceutical composition according to claim 21,
- wherein the health condition is a cancer, an immune disease, or a chronic infection.

34. A kit for modulating IL-12p40 mediated signal transduction, modulating IL-12p40-mediated signal transduction, or treating a health condition in a subject in need thereof, the system comprising:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-23p19 polypeptide;
 - b) a recombinant cell according to claim 16; and/or
 - c) a pharmaceutical composition according to claim 22,
- wherein the health condition is a cancer, an immune disease, or a chronic infection.

35. Use of the following for the manufacture of a medicament, for the treatment and/or prevention of a condition associated with a health condition associated with a perturbation in IL-12p40 mediated signal transduction:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12;
 - b) (i) an IL-12p35 polypeptide, and/or
(ii) an IL-23p19 polypeptide;
 - c) a recombinant cell according to claim 15 or 16; and/or
 - d) a pharmaceutical composition according to claim 21 or 22,
- wherein the health condition is a cancer, an immune disease, or a chronic infection.

36. Use of the following for the manufacture of a medicament, for the treatment and/or prevention of a condition associated with a health condition associated with a perturbation in IL-12p40 mediated signal transduction:

- a) a recombinant IL-12p40 variant according to any one of claims 1-12 and an IL-23p19 polypeptide;
 - b) a recombinant cell according to claim 16; and/or
 - c) a pharmaceutical composition according to claim 22,
- wherein the health condition is a cancer, an immune disease, or a chronic infection.