CLAIMS

1. A population of human Treg cells, comprising at least about 1 x 10^8 human Treg cells that are:

(i) ≥ 60% $CD4^+CD25^+$; and

(ii) ≤ 10% $CD4^-CD8^+$;

wherein the human Treg cells are immunosuppressive.

2. A population of human Treg cells, comprising at least about 1 x 10^8 human Treg cells that are:

(i) ≥ 60% $CD4^+CD25^+$;

(ii) ≥ 60% $CD4^+CD25^+CXCR4^+$; and

(iii) ≤ 10% $CD4^-CD8^+$;

wherein the human Treg cells are immunosuppressive.

3. A population of human Treg cells, comprising at least about 1 x 10^8 human Treg cells that are:

(i) ≥ 60% $CD4^+CD25^+$;

(ii)) ≥ 60% $CD4^+CD25^+\alpha4\beta7^+$; and

(iii) ≤ 10% $CD4^-CD8^+$;

wherein the human Treg cells are immunosuppressive.

4. A population of human Treg cells, comprising at least about 1 x 10^8 human Treg cells that are:

(i) ≥ 60% $CD4^+CD25^+$;

(ii)) ≥ 60% $CD4^+CD25^+CD11a^+$; and

(iii) ≤ 10% $CD4^-CD8^+$;

wherein the human Treg cells are immunosuppressive.

5. The population of any one of claims 1-4, comprising at least about 1 x 10^9 human Treg cells.

6. The population of any one of claims 1-5, wherein the human Treg cells are determined to be immunosuppressive by an assay using carboxyfluorescein succinimidyl

ester intracellular staining dye or CellTrace™ Violet intracellular staining dye.

7. A method for producing an expanded population of activated human T regulatory (Treg) cells from at least one cryopreserved human umbilical cord blood unit, the method comprising:

a) thawing the cryopreserved human umbilical cord blood unit;

b) diluting and washing the thawed umbilical cord blood unit in a functionally closed system;

c) isolating naturally occurring Treg cells using a double selection method based on $CD25^+$ cell surface expression;

d) ex-vivo expanding the isolated $CD25^+$ Treg cells in a culture medium(s), in a gas permeable cultureware, in the presence of an effective amount of interleukin-2 (IL-2) and in the presence of a reagent that specifically binds to CD3 and CD28, for up to 10 days, up to 12 days or up to 14 days, wherein the culture medium is replaced about every 48 hours, to produce a population of activated $CD25^+$ Treg cells; and

e) harvesting the activated $CD25^+$ cells from the culture medium to produce an expanded population of activated human Treg cells.

8. The method of claim 7, wherein a single umbilical cord blood unit is used.

9. The method of claim 7, wherein between two and four pooled umbilical cord blood units are used.

10. The method of any one of claims 7-9, wherein the reagent that specifically binds to CD25 is an anti-CD25 antibody or an antigen-binding fragment thereof.

11. The method of any one of claims 7-10, wherein the reagent that specifically binds to CD25 is conjugated to a solid support.

12. The method of claim 11, wherein the solid support is a magnetic microbead.

13. The method of any one of claims 7-11, wherein the reagent that specifically binds to CD3 and CD28 comprises an anti-CD3 antibody or an antigen-binding fragment thereof and an anti-CD28 antibody or an antigen-binding fragment thereof.

14. The method of any one of claims 7-12, wherein the reagent that specifically binds to CD3 and CD28 comprises anti-CD3 coated beads and anti-CD28 coated beads.

15. The method of claim 14, wherein the anti-CD3 coated beads and the anti-CD28 coated beads are at a 1:1 ratio.

16. The method of claim 14 or 15, wherein the $CD25^+$ cells and the anti-CD3 and anti-CD28 coated beads are at a 1:1 ratio.

17. The method of any one of claims 7-16, wherein the effective amount of IL-2 is up to about 1000 IU/ml.

18. The method of any one of claims 7-17, wherein the effective amount of IL-2 is about 1000 IU/ml.

19. The method of any one of claims 7-18, wherein the $CD25^+$ Treg cells isolated in step c) are suspended in a culture medium comprising IL-2 at the immediate beginning of step d).

20. The method of any one of claims 7-19, wherein in step e), about 1 x 10^6 $CD25^+$ cells/ml are cultured.

21. The method of any one of claims 7-20, wherein in step e), the cells are initially cultured in gas-permeable cultureware that has a membrane surface area of 10 cm^2.

22. The method of claim 21, wherein the culture is subsequently transferred to gas-permeable cultureware that has a membrane surface area of 100 cm^2.

23. The method of any one of claims 7-22, wherein in step d), the culture is not mixed and resuspended.

24. The method of any one of claims 7-23, wherein from about 1 x 10^9 to about 2 x 10^9 activated $CD25^+$ cells are harvested following 14 days of culture.

25. The method of any one of claims 7-24, wherein in step a), the cryopreserved human umbilical cord blood unit is thawed in a single step in a water bath.

26. The method of any one of claims 7-25, wherein step b) does not comprise manual washing.

27. The method of any one of claims 7-26, wherein step b) takes place in a solution comprising PBS, EDTA, and about 0.5% human serum albumin.

28. The method of any one of claims 7-27, wherein a double ferromagnetic column method is used in step c) to isolate $CD25^+$ Treg cells.

29. A method for producing an expanded population of activated human T regulatory (Treg) cells from at least one cryopreserved human umbilical cord blood unit, the method comprising:

a) thawing the cryopreserved human umbilical cord blood unit in a single step in a water bath;

b) diluting and washing the thawed umbilical cord blood unit in a solution comprising PBS, EDTA, and 0.5% human serum albumin in a functionally closed system without manual washing;

c) isolating naturally occurring Treg cells using a double selection method based on $CD25^+$ cell surface expression using a double ferromagnetic column method;

d) ex-vivo expanding the isolated $CD25^+$ Treg cells in a culture medium(s), in a gas permeable cultureware, in the presence of about 1000 IU/ml of interleukin-2 (IL-2) and in the presence of anti-CD3 and anti-CD28 coated beads, for up to 10 days, up to 12 days or up to 14 days, wherein the culture medium is replaced about every 48 hours, to produce a population of activated $CD25^+$ Treg cells;

wherein the $CD25^+$ Treg cells and the anti-CD3 and anti-CD28 coated beads are at a 1:1 ratio;

wherein the culture is not mixed and resuspended; and

e) harvesting the activated $CD25^+$ cells from the culture medium to produce an expanded population of activated human Treg cells.

30. The method of any one of claims 7-29, the method further comprising cryopreserving

the expanded population of activated human Treg cells.

31. A population of activated human Treg cells produced by the method of any one of claims 7-30, wherein the Treg cells are at least 90% $CXCR4^+$.

32. The population of any one of claims 1-6, wherein the Treg cells are at least 90% $CXCR4^+$.

33. The population of any one of claims 1-6, 31 and 32, wherein the Treg cells are at least 95% $CXCR4^+$, at least 95% $CD45RA^+$ and at least 80% $CD45RO^+$.

34. The population of any one of claims 1-6 and 31-33, wherein the Treg cells are further at least 95% $CD95^+$, at least 95% $HLADR^+$, at least 95% $alpha4beta7^+$, at least 15% $CXCR3hi^+$, at least 95% $CCR6^+$, at least 95% $CD54^+$, at least 95% $CD11A^+$, at least 85% $CD45RARO^+$, at least 80% $CTLA4^+$, at least 80% $GPR83^+$ and at least 80% $CD62L^+$.

35. The population of any one of claims 1-6 and 31-34, wherein the Treg cells are at least 95% $CXCR4^+$, at least 95% $CD45RA^+$, at least 80% $CD45RO^+$, at least 95% $CD95^+$, at least 95% $HLADR^+$, at least 95% $alpha4beta7^+$, at least 15% $CXCR3hi^+$, at least 95% $CCR6^+$, at least 95% $CD54^+$, at least 95% $CD11A^+$, at least 85% $CD45RARO^+$, at least 80% $CTLA4^+$, at least 80% $GPR83^+$ and at least 80% $CD62L^+$.

36. The population of any one of claims 1-6 and 31-35, wherein the Treg cells exhibit high expression of FOXP3 and low expression of RORγt.

37. The population of any one of claims 1-6 and 31-36, wherein the Treg cells maintain their polyclonal T cell receptor Vβ (TCR Vβ) repertoire.

38. The population of any one of claims 1-6 and 31-37, wherein the Treg cells are cryopreserved prior to use.

39. A method for cryopreserving an expanded population of activated human T regulatory (Treg) cells produced from at least one cryopreserved human umbilical cord blood unit, the

method comprising:

a) thawing the cryopreserved human umbilical cord blood unit;

b) diluting and washing the thawed umbilical cord blood unit in a functionally closed system;

c) isolating naturally occurring Treg cells using a double selection method based on $CD25^+$ cell surface expression;

d) ex-vivo expanding the isolated $CD25^+$ Treg cells in a culture medium(s), in a gas permeable cultureware, in the presence of an effective amount of interleukin-2 (IL-2) and in the presence of a reagent that specifically binds to CD3 and CD28, for up to 10 days, up to 12 days or up to 14 days, wherein the culture medium is replaced about every 48 hours, to produce a population of activated $CD25^+$ Treg cells;

e) harvesting the activated $CD25^+$ cells from the culture medium to produce an expanded population of activated human Treg cells; and

f) cryopreserving the expanded population of activated human Treg cells.

40. ~~A method for treating or preventing graft versus host disease in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7~~5~~-28 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the treatment or prevention of graft versus host disease in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

41. ~~A method for treating or preventing graft versus host disease in a subject, the method comprising administering to the subject (i) an effective amount~~ Use of (i) the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 and (ii) ruxolitinib in the manufacture of a medicament for the treatment or prevention of graft versus host disease in a subject, wherein an effective amount of (i) and (ii) is to be administered to the subject.

42. ~~A method for treating or preventing a bone marrow failure syndrome in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the

treatment or prevention of bone marrow failure syndrome in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

43. The use~~method~~ of claim 42, wherein the bone marrow failure syndrome is aplastic anemia, primary myelofibrosis or myelodysplastic syndrome.

44. ~~A method for treating or preventing primary myelofibrosis in a subject, the method comprising administering to the subject (i) an effective amount~~ Use of (i) the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 and (ii) ruxolitinib in the manufacture of a medicament for the treatment or prevention of primary myelofibrosis in a subject, wherein an effective amount of (i) and (ii) is to be administered to the subject.

45. ~~A method for treating or preventing systemic lupus erythematosus (SLE) in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the treatment or prevention of systemic lupus erythematosus (SLE) in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

46. ~~A method for treating or preventing multiple myeloma in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the treatment or prevention of multiple myeloma in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

47. ~~A method for treating or preventing a neuro-inflammatory disorder in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the

treatment or prevention of neuro-inflammatory disorder in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

48. The use~~method~~ of claim 47, wherein the neuro-inflammatory disorder is Guillain-Barre Syndrome, amyotrophic lateral sclerosis, multiple sclerosis or demyelinating neuropathy.

49. ~~A method for treating or preventing a respiratory disease, disorder or condition associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the treatment or prevention of a respiratory disease, disorder or condition associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

50. The use~~method~~ of claim 49, wherein the respiratory disease, disorder or condition is COVID-19 (coronavirus disease) mediated acute respiratory distress syndrome (CoV-ARDS).

51. ~~A method for treating or preventing cytokine release syndrome (CRS) in a subject, the method comprising administering to the subject an effective amount~~ Use of the population of activated human Treg cells produced by the method of any one of claims 7-30 and 39 or the population of any one of claims 1-6 and 31-38 in the manufacture of a medicament for the treatment or prevention of cytokine release syndrome (CRS) in a subject, wherein an effective amount of the population of activated human Treg cells or the population is to be administered to the subject.

52. The use~~method~~ of claim 51, wherein the CRS is associated with chimeric antigen receptor T-cell therapy.

53. The use~~method~~ of any one of claims 40-52, wherein the effective amount of the

population of activated human Treg cells is to be administered intravenously to the subject.

54. The use~~method~~ of any one of claims 40-53, wherein the effective amount of the population of activated human Treg cells is between about $1x10^6$ and about $1x10^7$ Treg cells/kg of body weight of the subject.

55. The use~~method~~ of any one of claims 40-53, wherein the effective amount of the population of activated human Treg cells is between about $1x10^8$ Treg cells and about $3x10^8$ Treg cells.

56. The use~~method~~ of any one of claims 40-55, wherein multiple doses of an effective amount of the population of activated human Treg cells are to be administered to the subject.

57. The use~~method~~ of claim 56, wherein three doses or four doses are to be administered to the subject.

58. The use~~method~~ of claim 56 or 57, wherein the doses are to be administered to the subject about every 4-6 weeks.

59. The use~~method~~ of any one of claims 40-58, wherein, following the administration of the effective amount of the population of activated human Treg cells, circulating inflammatory cytokine levels in the subject are decreased compared to the circulating inflammatory cytokine levels in the subject prior to the administration.

60. The use~~method~~ of any one of claims 40-59, wherein, prior to treatment, serum biomarkers of the subject are examined in order to determine whether the subject will respond to the effective amount of the population of activated human Treg cells.

61. The use~~method~~ of any one of claims 40-60, wherein, following treatment, serum biomarkers of the subject are examined in order to determine a correlation with clinical response.

62. The use~~method~~ of claim 61, wherein the serum biomarkers are examined serially to examine whether subsequent retreatment with Treg cells is needed.

63. The use~~method~~ of any one of claims 40-62, wherein the population of activated human Treg cells is prepared from one or more umbilical cord blood units of a compatible blood type for the subject.

64. The use~~method~~ of any one of claims 40-63, wherein the population of activated human Treg cells is prepared from an umbilical cord blood unit that is at least a 3 out of 6 HLA (human leukocyte antigen) match for the subject.

65. The use~~method~~ of any one of claims 40-62, wherein the population of activated human Treg cells is prepared from an umbilical cord blood unit that is not an HLA match for the subject.

66. ~~Use of the~~The population of any one of claims 1-6 and 31-38 for use as~~in the preparation of~~ a medicament.