

CLAIMS:

1. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSC), wherein the population of MLPSCs are culture expanded from a cryopreserved intermediate MLPSC preparation and the culture expanded MLPSCs express a receptor which, upon activation via an inflammatory stimulus, phosphorylates NF- κ B, wherein the MLPSCs express a level of the receptor that is sufficient to increase phosphorylation of NF- κ B upon activation via the inflammatory stimulus at least 3.5 fold greater than unstimulated MLPSCs.
2. The composition of claim 1, wherein the MLPSCs express a level of the receptor that is sufficient to increase phosphorylation of NF- κ B upon activation via the inflammatory stimulus at least 4 fold greater than unstimulated MLPSCs.
3. The composition of claim 1 or claim 2, wherein the receptor is one or both of TNF-R1 or IL-1R.
4. The composition according to any one of claims 1 to 3, wherein activation of the MLPSCs via the inflammatory stimulus increases secretion of one or more of MCP-1, M-CSF and PGE2 under culture conditions.
5. The composition of claim 4, wherein activation of the MLPSCs via the inflammatory stimulus results in secretion of one or more of the following under culture conditions:
 - 18,000 pg/ml MCP-1;
 - 1,500 pg/ml M-CSF;
 - 40,000 pg/ml PGE2.
6. The composition according to any one of claims 1 to 5, wherein the receptor which, upon activation via an inflammatory stimulus, phosphorylates NF- κ B is TNF-R1 and the MLPSCs express at least about 200 pg/ml, at least about 225 pg/ml TNF-R1 under culture conditions.

7. The composition according to any one of claims 1 to 6, wherein the composition comprises at least 25×10^6 cells.
8. The composition according to any one of claims 1 to 7, wherein the phosphorylation of NF- κ B is determined after contacting the cells with TNF- α and/or IL-1 β .
9. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSC), wherein the population of MLPSCs are culture expanded from a cryopreserved intermediate MLPSC preparation and the culture expanded MLPSCs express at least about 200 pg/ml TNF-R1 under culture conditions.
10. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells, wherein the composition comprises at least 25×10^6 cells and, wherein the population of culture expanded cells has been selected for use in treatment of an inflammatory disease by determining expression of least about 200 pg/ml TNF-R1 under culture conditions.
11. The composition according to any one of claims 1 to 10, wherein the population of culture expanded cells express at least about 225 pg/ml TNF-R1, at least about 260 pg/ml TNF-R1, preferably at least about 270 pg/ml TNF-R1 under culture conditions.
12. The composition according to any one of claims 1 to 10, wherein the population of culture expanded cells express at least about 300 pg/ml TNF-R1, preferably at least about 350 pg/ml TNF-R1 under culture conditions.
13. The composition according to any one of claims 1 to 12, wherein the population of culture expanded cells are culture expanded in 3D culture.

14. The composition according to any one of claims 10 to 13, wherein the inflammatory disease is graft versus host disease (GvHD).
15. The composition according to any one of claims 1 to 14, further comprising a cryopreservative.
16. The composition according to any one of claims 1 to 15, wherein the MLPSCs inhibit IL-2R α by at least 55% under culture conditions.
17. A method for determining the therapeutic efficacy of mesenchymal lineage precursor or stem cells comprising:
- (i) obtaining a population comprising mesenchymal lineage precursor or stem cells;
 - (ii) culturing the cells in a culture medium; and
 - (iii) determining the amount of TNF-R1 expressed by the cells into the culture medium under culture conditions, wherein an amount of at least about 200 pg/ml TNF-R1 is indicative of therapeutic efficacy.
18. The method of claim 10, wherein at least about 225 pg/ml, at least about 260 pg/ml TNF-R1 is indicative of therapeutic efficacy.
19. The method of claim 11, wherein at least about 270 pg/ml TNF-R1 is indicative of therapeutic efficacy.
20. The method according to any one of claims 17 to 19, wherein the population of mesenchymal lineage precursor or stem cells is obtained from a 3D cell culture.
21. The method according to any one of claims 17 to 20, wherein the population of mesenchymal lineage precursor or stem cells are culture expanded in 3D cell culture from a cryopreserved intermediate MLPSC preparation.

22. A method of treating a subject with an inflammatory disease, the method comprising administering to a subject in need thereof a composition of mesenchymal lineage precursor or stem cells, wherein the cells express at least about 200 pg/ml TNF-R1 under culture conditions.
23. The method of claim 15, wherein the cells express at least about 225 pg/ml TNF-R1, at least about 260 pg/ml TNF-R1, preferably at least about 270 pg/ml TNF-R1, preferably at least about 300 pg/ml TNF-R1, preferably at least about 350 pg/ml TNF-R1 under culture conditions.
24. The method of claim 22 or 23, wherein the inflammatory disease is a T cell mediated inflammatory disease.
25. The method according to any one of claims 22 to 24, wherein the inflammatory disease is GvHD.
26. The method according to claim 25, wherein the GvHD is chronic GvHD.
27. The method according to any one of claims 22 to 26, wherein a treated subject has improved 100 day survival.
28. The method according to any one of claims 22 to 27, wherein the subject is refractory to steroid immunosuppressant and/or a biologic therapy.
29. The method according to any one of claims 22 to 28, wherein the subject receives at least two doses of the composition.
30. The method according to any one of claims 22 to 29, wherein the mesenchymal precursor lineage or stem cells are culture expanded from a cryopreserved intermediate MLPSC preparation.

31. The composition according to any one of 1 to 16 or the method of any one of claims 22 to 32, wherein the mesenchymal precursor lineage or stem cells are mesenchymal stem cells.

32. The composition according to any one of 1 to 16 or the method of any one of claims 22 to 31, wherein the composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO), human serum albumin (HSA).

33. The composition according to any one of 1 to 16 or the method of any one of claims 22 to 32, wherein the composition further comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

34. The composition according to any one of 1 to 16 or 31 to 33, or the method of any one of claims 15 to 26, wherein the composition comprises greater than 6.68×10^6 viable cells/mL.

35. The method of claim 29, wherein each dose comprises at least 2×10^6 cells/kg body weight of subject.

36. The method of claim 29 or 35, wherein the subject receives twice weekly dosing.

CLAIMS

1. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells (MLPSC), in which the population of MLPSCs are culture expanded from a cryopreserved intermediate MLPSC preparation and the culture expanded MLPSCs
5 express a receptor which, upon activation via an inflammatory stimulus, phosphorylates NF- κ B, in which the MLPSCs express a level of the receptor that is sufficient to increase phosphorylation of NF- κ B upon activation via the inflammatory stimulus at least 3.5 fold greater than unstimulated MLPSCs.
2. A composition according to Claim 1, in which the receptor is one or both of TNF-R1 or
10 IL-1R.
3. A composition according to Claim 1 or 2, in which activation of the MLPSCs via the inflammatory stimulus increases secretion of one or more of MCP-1, M-CSF and PGE2 under culture conditions.
4. A composition according to Claim 1, 2 or 3, in which the receptor which, upon
15 activation via an inflammatory stimulus, phosphorylates NF- κ B is TNF-R1 and the MLPSCs express at least 100 pg/ml to 225 pg/ml TNF-R1 under culture conditions.
5. A composition comprising a population of culture expanded mesenchymal lineage precursor or stem cells, in which the composition comprises at least 25×10^6 cells and, in which the population of culture expanded cells has been selected for use in treatment of an
20 inflammatory disease by determining expression of least about 200 pg/ml TNF-R1 under culture conditions.
6. A composition according to any preceding claim, in which the population of culture expanded cells express at least 100 pg/ml TNF-R1 to at least 270 pg/ml TNF-R1 under culture conditions.
- 25 7. A composition according to any preceding claim, in which the population of culture expanded cells are culture expanded in 3D culture.
8. A composition according Claim 5, 6 or 7, in which the inflammatory disease is graft versus host disease (GvHD).

9. A method for determining the therapeutic efficacy of mesenchymal lineage precursor or stem cells comprising:

- (i) obtaining a population comprising mesenchymal lineage precursor or stem cells;
- (ii) culturing the cells in a culture medium; and

5 (iii) determining the amount of TNF-R1 expressed by the cells into the culture medium under culture conditions, in which an amount of at least about 100 pg/ml TNF-R1 is indicative of therapeutic efficacy.

10. Use of a composition comprising mesenchymal lineage precursor or stem cells in the preparation of a medicament for the treatment of an inflammatory disease, in which the cells
10 express at least about 100 pg/ml TNF-R1 under culture conditions.

11. Use according to Claim 10, in which the inflammatory disease is a T cell mediated inflammatory disease.

12. Use according to Claim 10 or 11, in which the inflammatory disease is GvHD.

13. Use according to Claim 12, in which the GvHD is chronic GvHD.

15 14. Use according to any of Claims 10 to 13, in which a subject following the treatment has improved 100 day survival.

15. Use according to any of Claims 10 to 14, in which the treatment is for a subject that is refractory to steroid immunosuppressant and/or a biologic therapy.

20 16. Use according to any of Claims 10 to 15, in which the mesenchymal precursor lineage or stem cells are culture expanded from a cryopreserved intermediate MLPSC preparation.

17. A composition according to any of Claims 1 to 8, a method according to Claim 9 or use according to any of Claims 10 to 16, in which the mesenchymal precursor lineage or stem cells are mesenchymal stem cells.

25 18. A composition according to any of Claims 1 to 8 or use according to any of Claims 10 to 17, in which the composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO), human serum albumin (HSA).

19. A composition according to any of Claims 1 to 8 or use according to any of Claims 10 to 17, in which the composition comprises greater than 6.68×10^6 viable cells/mL.
20. A composition or use according to Claim 19, in which each dose comprises at least 2×10^6 cells/kg body weight of subject.