

CLAIMS

1. A method for preparing transduced lymphocytes, comprising
incubating a sample comprising lymphocytes, obtained from a donor subject, with a polynucleotide vector to transduce the lymphocytes to produce transduced lymphocytes; and
culturing the sample comprising the transduced lymphocytes for less than 72 hours before the lymphocytes are harvested to produce a harvested sample.
2. The method of claim 1, wherein the incubation is carried out in a closed system, and wherein the closed system has an inner surface area of at least 1500 cm².
3. The method of claim 1 or 2, wherein the closed system has an inner surface coated with a recombinant human fibronectin, wherein the coating is carried out with a solution that comprises about 1- 10 µg/ml of the recombinant human fibronectin.
4. The method of any one of claims 1 to 3, wherein the sample in the closed system comprises at least 1.5×10^8 lymphocytes.
5. The method of any one of claims 1 to 4, wherein the harvested sample comprises CD3+ cells, CD4+ T cells, and CD8+ T cells.
6. The method of claim 5, wherein at least 20% of the CD4+ T cells are naïve T cells, and no more than 12% of the CD4+ T cells are effector memory T cells.
7. The method of claim 5 or claim 6, wherein at least 10% of the CD8+ T cells are naïve T cells, and no more than 30% of the CD8+ T cells are effector memory T cells.
8. The method of any one of claims 5 to 7, wherein at least 80% of the CD4+ T cells are CCR7+ cells, and wherein at most 20% of the CD4+ T cells are a combination of effector memory T cells and effector T cells.
9. The method of any one of claims 5 to 7, wherein at least 60% of the CD8+ T cells are CCR7+ cells, and wherein at most 40% of the CD8+ T cells are a combination of effector memory T cells and effector T cells.
10. The method of any one of claims 1 to 9, further comprising contacting the sample with a lymphocyte stimulating agent to activate the lymphocytes.

11. The method of claim 10, wherein the activating the sample is prior to incubating the sample with the polynucleotide vector.
12. The method of claim 10, wherein the activating the sample is after the sample is incubated with the polynucleotide vector.
13. The method of any one of claims 1 to 12, wherein the harvested sample comprises a total of 10,000 to 1,000,000 harvested lymphocytes per kilogram.
14. The method of any one of claims 1 to 13, wherein the polynucleotide vector encodes a chimeric antigen receptor (CAR) or a T cell receptor (TCR).
15. The method of claim 14, wherein the CAR comprises an intracellular costimulatory domain, and wherein the intracellular costimulatory domain is a signaling region of CD28.
16. The method of claim 14 or claim 15, wherein the CAR recognizes a tumor antigen, and wherein the tumor antigen is CD19, CD20, and/or CLL-1.
17. A population of cells prepared from a blood sample of a donor subject, comprising CD4+ T cells and CD8+ T cells, wherein:
 - at least 20% of the CD4+ T cells are naïve T cells, and no more than 12% of the CD4+ T cells are effector memory T cells;
 - at least 10% of the CD8+ T cells are naïve T cells, and no more than 30% of the CD8+ T cells are effector memory T cells;
 - at least 50% of the cells are CD3+ T cells; and
 - at least 25% of all T cells are transduced with a polynucleotide vector encoding a CAR or TCR.
18. A population of cells prepared from a blood sample of a donor subject, comprising CD4+ T cells and CD8+ T cells, wherein:
 - at least 80% of the CD4+ T cells are CCR7+ cells;
 - at least 60% of the CD8+ T cells are CCR7+ cells;
 - at most 20% of the CD4+ T cells are a combination of effector memory T cells and effector T cells; and
 - at most 40% of the CD8+ T cells are a combination of effector memory T cells and effector T cells.

19. A pharmaceutical composition comprising the population of cells of claim 17 or claim 18.
20. Use of a harvested sample prepared by the method of any one of claims 1 to 16, a population of cells of claim 17 or claim 18, or the pharmaceutical composition of claim 19, in the manufacture of a medicament for administering T cells to a subject in need thereof.