

WHAT IS CLAIMED IS:

1. A method for selecting a thawed umbilical cord blood unit for *ex-vivo* expansion and transplantation into a subject, the method comprising determining percent viability of the cells in said thawed umbilical cord blood unit and selecting units having about 40% to about 85% viability prior to separation of said cord blood unit into CD133+/CD34+ and CD133-/CD34- fractions, thereby selecting a thawed umbilical cord unit suitable for *ex-vivo* expansion and transplantation into said subject.

2. The method of claim 1, comprising selecting units having about 40% to about 70% viability.

3. The method of claims 1 or 2, further comprising:

(a) separating said selected thawed umbilical cord blood unit suitable for *ex-vivo* expansion transplantation into said subject into (i) a first, selected blood cell fraction comprising CD133+/CD34+ selected cells and (ii) a second, unselected blood cell fraction comprising CD133-/CD34- negative cells,

(b) determining in said first, selected CD133+/CD34+ fraction the following post-separation parameters:

- (i) about 20×10^5 to about 75×10^5 total cells;
- (ii) about 70% to about 85% viability;
- (iii) about 70% to about 85% CD133+ cells;
- (iv) about 70% to about 85% CD34+ cells;
- (v) about 0.02 to about 1% yield post CD133+/CD34+ selection,
- (vi) about 15 to about 75 CFU per 1000 cells, and
- (vii) less than 3 Eu/ml endotoxin, and

(c) selecting or excluding said first, selected CD133+/CD34+ fraction

according to said parameters,

thereby selecting a CD133+/CD34+ cord blood fraction suitable for *ex-vivo* expansion and transplantation into said subject.

4. The method of claim 3, wherein said separating of (a) comprises immunomagnetic bead selection for CD133+.

5. The method of claim 3, wherein the second, unselected blood cell fraction comprising CD133/CD34 negative cells comprises less than 0.5% CD133+/CD34+ cells.

6. The method of claim 3, wherein the second, unselected blood cell fraction comprising CD133/CD34 negative cells comprises less than 0.01% CD133+/CD34+ cells.

7. The method of claim 3, wherein the second, unselected blood cell fraction comprising CD133/CD34 negative cells comprises immune cells including T lymphocytes, B lymphocytes and Natural Killer (NK) cells.

8. A method for preparing an umbilical cord blood unit for transplantation into a subject, the method comprising:

(a) separating a single, thawed umbilical cord blood unit suitable for transplantation into said subject into (i) a first, selected blood cell fraction comprising CD133+/CD34+ selected cells and (ii) a second, unselected blood cell fraction comprising CD133/CD34 negative cells; and

(b) *ex vivo* culturing said first blood cell fraction comprising CD133+/CD34+ selected cells under conditions allowing for cell proliferation, said conditions comprising providing nutrients, serum and cytokines including each of stem cell factor, thrombopoietin, FLT3 ligand and IL-6 and nicotinamide in an amount between 1.0 mM to 10 mM.

9. The method of claim 8, wherein said first, selected CD133+/CD34+ blood cell fraction (a)(i) is a CD133+/CD34+ cord blood fraction selected suitable for *ex-vivo* expansion and transplantation into said subject according to the method of any one of claims 3 or 4.

10. The method of claim 8 or 9, further comprising excluding units having greater than 3.0 Eu/ml endotoxin, and/or bacterial, yeast or mold growth at day 7 or at day 14 of said *ex-vivo* expansion.

11. The method of any one of claims 8-10, wherein said single umbilical cord blood unit suitable for transplantation is characterized by the following pre-cryopreservation parameters:

(i) about 8×10^6 to about 15×10^6 total CD34+ cells;

- (ii) HLA-matched at at least 4 out of 6 HLA class I (HLA-A and HLA-B, low resolution) and HLA class II (HLA-DRB1, high resolution) loci with said subject;
- (iii) about 1.8×10^9 to about 3.0×10^9 pre-cryopreserved total nucleated cells; and
- (iv) about 1.5×10^7 to about 3.0×10^7 pre-cryopreserved total nucleated cells per kilogram subject weight.

12. The method of any one of claims 8-11, wherein said serum comprises 10% FBS.

13. The method of any one of claims 8-12, wherein said cytokines comprise 50 ng each of stem cell factor, thrombopoietin, FLt3 ligand and IL-6.

14. The method of any one of claims 8-13, wherein said nicotinamide is provided at 2.5 mM.

15. The method of any one of claims 8-14, wherein said ex-vivo culturing is affected for 18-25 days.

16. The method of any one of claims 8-14, further comprising harvesting said ex-vivo cultured first selected CD133+/CD34+ fraction after 19-23 days in culture.

17. The method of any one of claims 8-14, further comprising harvesting said ex-vivo cultured first selected CD133+/CD34+ fraction after 21 days in culture.

18. The method of any one of claims 8-17, further comprising separately cryopreserving said *ex-vivo* cultured first, selected blood cell fraction comprising CD133+/CD34+ selected cells and said second, unselected blood cell fraction comprising CD133/CD34 negative cells.

19. A method of selecting *ex-vivo* cultured umbilical cord blood cell fractions comprising CD133+/CD34+ selected cord blood cells for transplantation into a subject, comprising:

- (a) determining in said *ex-vivo* cultured umbilical cord blood fraction following *ex-vivo* expansion the following parameters:
 - (i) about 8×10^8 to about 15×10^8 total viable cells;

- (ii) about 70%-85% viability of the cells;
 - (iii) about 7-15% CD34+ cells;
 - (iv) about 5.6×10^7 to about 5×10^8 total CD34+ cells;
 - (v) about 2.4×10^7 to about 2×10^8 total CD133+ cells;
 - (vi) about 8×10^5 to about 25×10^5 CD133+/CD38- cells;
 - (vii) about 8×10^7 to about 15×10^8 total CD14+ cells,
 - (viii) about 2×10^8 to about 2×10^9 total CD15+ cells,
 - (ix) about 8×10^7 to about 8×10^9 total CD11b+ cells,
 - (x) about 3.2×10^7 to about 3×10^8 total CD1(a+c)+ cells,
 - (xi) No cultured mycoplasma or bacterial, yeast or mold growth, and
- (b) selecting or excluding said *ex-vivo* cultured umbilical cord blood fraction according to said parameters,

thereby selecting an *ex-vivo* cultured CD133+/CD34+ umbilical cord blood fraction suitable for transplantation into the subject.

20. The method of claim 19, wherein said *ex-vivo* cultured CD133+/CD34+ umbilical cord blood fraction is selected when (i) equals at least 8.0×10^8 viable cells.

21. The method of claim 19, wherein said *ex-vivo* cultured umbilical cord blood fraction was cultured from an umbilical cord blood unit selected for *ex-vivo* expansion according to the following pre-cryopreservation parameters:

- (i) about 8×10^6 to about 15×10^6 total CD34+ cells;
- (ii) HLA-matched at at least 4 out of 6 HLA class I (HLA-A and HLA-B, low resolution) and HLA class II (HLA-DRB1, high resolution) loci with said subject;
- (iii) about 1.8×10^9 to about 3.0×10^9 pre-cryopreserved total nucleated cells;
- (iv) about 1.5×10^7 to about 3.0×10^7 pre-cryopreserved total nucleated cells per kilogram subject weight.

22. The method of claim 20 or 21, further comprising selecting *ex-vivo* cultured umbilical cord blood fractions having a yellowish, cloudy to opaque color and free of white clumps and foreign particles.

23. A method of selecting unselected umbilical cord blood cell fractions comprising CD133/CD34 negative cells for transplantation into a subject, comprising:

(a) determining in said uncultured umbilical cord blood fraction following selection for CD133/CD34 negative cells the following parameters:

- (i) about 4×10^8 to about 15×10^8 total viable cells;
- (ii) about 70-85% viability of the cells;
- (iii) about 2.4×10^7 to about 8×10^7 CD3+ cells;
- (iv) no bacterial, yeast or mold growth, and

(b) selecting or excluding said unselected umbilical cord blood cell fraction according to said parameters,

thereby selecting an unselected umbilical cord blood cell fraction comprising CD133/CD34 negative cells suitable for transplantation into the subject.

24. The method of claims 22 or 23, further comprising selecting unselected umbilical cord blood cell fractions having a red, opaque color and free of clumps and foreign particles.