Claims

1. A method of preparing a transplantable NK cell fraction for transplantation into a subject in need thereof, the method comprising:

(a) *ex vivo* culturing a CD3-depleted NK cell fraction HLA-haploidentical or HLA-mismatched for said subject under conditions allowing for cell proliferation, wherein said conditions comprise providing nutrients, serum, IL-15 and 5 mM nicotinamide;

(b) supplementing said CD3- depleted NK cell fraction with fresh nutrients, serum, IL-15 and nicotinamide 8-10 days following step (b) to produce an expanded CD3- depleted NK cell fraction;

(c) harvesting said expanded CD3-depleted NK cell fraction 14-16 days following step (a), wherein said harvesting comprises harvesting a first portion of said expanded CD3-depleted NK cell fraction 14 days following step (a), and harvesting a second portion of said expanded CD3-depleted NK cell fraction 16 days following step (a), wherein said first portion comprises about 50% of said expanded CD3-depleted NK cell fraction and said second portion comprises the remainder of said expanded CD3-depleted NK cell fraction; and

(d) washing and concentrating said expanded CD3-depleted NK cell fraction of step (c),

(e) wherein said expanded CD3-depleted NK cells of step (d) are selected according to the following parameters:

(i) at least 70% CD56+/CD3- cells;

(ii) at least 70% viability;

(iii) fewer than $5.0X10^5$ CD3+ cells/Kg mass of patient, upon infusion;

(iv) no more than 5 EU endotoxin/Kg mass of patient, upon infusion; and

(v) no Gram-positive micro-organisms,

thereby producing a transplantable NK cell fraction for transplantation in said subject.

2. The method of claim 1, wherein said *ex-vivo* culturing is devoid of a feeder layer.

3. The method of claim 1, wherein said conditions for allowing for cell proliferation comprise providing 10% human serum.

4. The method of any one of claims 1-3, wherein said nicotinamide comprises 5.0 mM nicotinamide, said IL-15 comprises 20 ng/ml IL-15 and said nutrients comprise minimal essential culture medium.

5. The method of any one of claims 1-4, wherein said NK cell fraction is from an HLA-haploidentical or HLA-mismatched donor having at least:

(a) HLA matching at intermediate resolution DNA-based Class 1 typing of the A and B locus of at least 2/4 class 1 allele; and

(b) absence of (MFI $\leq$ 1000) recipient donor-specific anti-HLA antibodies, and

wherein said NK cells of step (a) comprise at least 40-90% CD56+/CD3- cells.

6. A transplantable NK cell fraction prepared according to any one of claims 1-5 comprising said first portion and said second portion of said expanded CD3 depleted NK cell fraction.

7. The method of claim 1, wherein selection parameter (i) of step (e) is at least 80% CD56+/CD3- cells.

8. The method of claim 1, wherein selection parameter (i) of step (e) is at least 85% CD56+/CD3- cells.

9. The method of claim 1, wherein selection parameter (i) of step (e) is at least 90% CD56+/CD3- cells.