

What is claimed is:

1. An NK cell fraction selected comprising a population of nucleated cells,
wherein the population comprises at least 1.0×10^6 nucleated cells,
wherein at least about 70% of the cells in the population are viable,
wherein:
 - at least about ~~8570~~85% of the cells in the population are CD56+;
 - no more than about 0.5% of the cells in the population are CD3+;
 - no more than about 10% of the cells in the population are CD19+;
 - no more than about 10% of the cells in the population are CD14+;
 - ~~at least about 44% of the cells in the population are CD49a+;~~
 - ~~no more than about 27% of the cells in the population are LAG3+;~~
 - ~~no more than about 32% of the cells in the~~
population are CD200R+;
 - ~~no more than about 25% of the cells in the~~
population are CD57+;
 - at least about 10% of the cells in the population are CD16+; ~~and~~
 - at least about 10% of the cells in the population are CD62L;
 - said population is sterile and devoid of mycoplasma, and
 - said population has no more than 0.5 EU/ml endotoxin.
2. The NK cell fraction of claim 1, wherein:
 - the target cell death in a K562 cell potency assay is at least about 10%, and
 - the target cell death in Antibody-Dependent Cellular Cytotoxicity (ADCC) Raji
cell potency assay is greater than the value for Raji cells without the antibody.
3. The NK fraction of claim 1, wherein the percentage of viable cells stained with an
anti-CD107a antibody in a degranulation assay is at least 10%.
2. ~~The NK cell fraction of claim 1, wherein at least about 90% of the cells in the~~
~~population are CD56+.~~

3. ~~— The NK cell fraction of any one of the preceding claims, wherein about 90 to about 95% of the cells in the population are CD56+.~~

4. ~~— The NK cell fraction of any one of the preceding claims, wherein about 0.2 to about 0.3% of the cells in the population are CD3+.~~

45. The NK cell fraction of claim 1 ~~any one of the preceding claims~~, wherein
at least about 90-99~~70~~% of the cells in the population are CD56+/CD3-;
and
no more than about 0.5% of the cells in the population are CD56-/CD3+.

6. ~~— The NK cell fraction of any one of the preceding claims, wherein at least about 99% of the cells in the population are CD56+/CD3-.~~

57. The NK cell fraction of one of the preceding claims, wherein at least about 15% of the cells in the population are CD62L+.

8. ~~— The NK cell fraction of any one of the preceding claims, wherein about 18% to about 70% of the cells in the population are CD62L+.~~

69. The NK cell fraction of any one of the preceding claims, wherein at least about 20% of the cells in the population are CD16~~+~~++.

10. ~~— The NK cell fraction of any one of the preceding claims, wherein about 20% to about 60% of the cells in the population are CD16+.~~

11. The NK cell fraction of any one of the preceding claims, wherein
no more than about 1% of the cells in the population are LAG3+;
~~— no more than about 1.5% of the cells in the population are CD200R+;~~
~~— no more than about 2.5% of the cells in the population are CD57+~~

12. — ~~The NK cell fraction of any one of the preceding claims, wherein:~~
 ~~no more than about 3% of the cells in the population are CD56+/LAG3+;~~
 ~~no more than about 11% of the cells in the population are~~
 ~~CD56+/CD200R+;~~
 ~~no more than about 4% of the cells in the population are CD56+/CD57+.~~
13. — ~~The NK cell fraction of any one of the preceding claims, wherein at least about~~
~~43% of the cells in the population are CD56+/CD16+.~~
14. — ~~The NK cell fraction of any one of the preceding claims, wherein at least about~~
~~57% of the cells in the population are CD56+/CD16+.~~
15. — ~~The NK cell fraction of any one of the preceding claims, wherein at least about~~
~~78% of the cells in the population are CD56+/CD62L+.~~
16. — ~~The NK cell fraction of any one of the preceding claims, wherein no more than~~
~~about 77% of the cells in the population are NKp80+, preferably wherein no more than~~
~~about 15.77% of cells in the population are NKp80+.~~
17. — ~~The NK cell fraction of any one of the preceding claims, wherein the NK cell~~
~~fraction comprises:~~
 ~~i) at least about 17.5×10^8 nucleated cells;~~
 ~~ii) at least about 35×10^8 nucleated cells;~~
 ~~iii) at least about 2.5×10^9 nucleated cells;~~
 ~~iv) at least about 5×10^9 nucleated cells;~~
 ~~v) at least about 1.25×10^7 nucleated cells;~~
 ~~vi) at least about 2.5×10^7 nucleated cells;~~
 ~~vii) at least about 5×10^7 nucleated cells; or~~
 ~~viii) at least about 1×10^8 nucleated cells.~~

~~18. The NK cell fraction of any one of the preceding claims, wherein the NK cell fraction had been previously frozen and subsequently thawed.~~

~~19. A cryopreserved NK cell fraction, comprising:~~

- ~~——— i) the NK cell fraction of any one of claims 1-17; and~~
- ~~——— ii) DMSO, wherein the concentration of DMSO is about 10% v/v.~~

~~20. The cryopreserved NK cell fraction of claim 18, wherein the cryopreserved NK cell fraction is stable:~~

- ~~i) at about -80°C for at least about six weeks; and/or~~
- ~~ii) at about -150°C for at least about 12 months.~~

~~721. The~~A method of preparing the NK cell fraction of any one of claims 1-67,
wherein the NK cells are prepared by the method comprising:

- (a) obtaining a human~~n~~ apheresis product comprising NK cells and CD3+ cells allogeneic to subject;
- (b) separating the apheresis product into a CD3-depleted cell fraction and a CD3+ cell fraction;
- (c) inactivating cells of said CD3+ cell fraction by irradiation;
- (d) *ex vivo* culturing said CD3-depleted cell fraction with the inactivated, irradiated CD3+ cell fraction at a ratio of 1:1 under conditions allowing for cell proliferation, wherein said conditions comprise providing nutrients, serum, IL-15, OKT-3~~a~~-CD3 ~~agonist~~ and nicotinamide in an amount between 1.0 mM to 10 mM;
- (e) supplementing said combined CD3- depleted and CD3+ cell fractions with fresh nutrients, serum, IL-15 and nicotinamide 6-10 days following step (d) to produce an expanded CD3- depleted cell fraction;
- (f) harvesting said combined CD3-depleted and CD3+ cell fractions 14-16 days following step (d); and
- (g) washing and concentrating said combined CD3-depleted and CD3+ cell fraction of step (f),
thereby producing the NK cell fraction.

~~22. The method of claim 21, wherein said apheresis product is a human apheresis product comprising human NK and CD3+ cell fractions.~~

~~23. The method of claim 21 or claim 22, wherein said CD3 agonist is OKT3.~~

~~24. The method of any one of claims 21-23, wherein said conditions for allowing for cell proliferation comprise:~~

- ~~i) human serum at a concentration of about 10% (v/v)~~
- ~~ii) IL-15 at a concentration of about 20 ng/ml;~~
- ~~iii) OKT3 at a concentration of about 1 µg/ml;~~
- ~~iv) nicotinamide at a concentration of about 7.0 mM;~~
- ~~v) nutrients comprising minimal essential cell culture medium.~~

~~25. The method of any one of claims 21-24, wherein said CD3-depleted and said irradiated CD3+ cells are seeded at a 1:1 ratio in step (d).~~

826. The method of ~~any one of claims 721-25~~, wherein said culturing of step (d) is affected in flasks at

- i) $0.30\text{-}0.40 \times 10^6$ CD3-depleted and $0.30\text{-}0.40 \times 10^6$ irradiated CD3+ cells/ml;
- ii) 0.35×10^6 CD3-depleted and 0.35×10^6 irradiated CD3+ cells/ml;
- iii) $400\text{-}900 \times 10^6$ CD3-depleted and $400\text{-}900 \times 10^6$ irradiated CD3+ cells per flask; or
- iv) 700×10^6 CD3-depleted and 700×10^6 irradiated CD3+ cells per flask.

927. A method for cryopreservation of a NK cell fraction comprising:

- (a) suspending the NK cell fraction of any one of claims 1-~~617~~ or the NK cell fraction produced by the methods of any one of claims 21-26 in a DMSO-free cryopreservation buffer;
- (b) adding DMSO to 10% v/v;
- (c) reducing temperature of the cells to -120°C ; and

(d) storing the cryopreserved NK cell at a temperature of less than or equal to -120°C .

10. A cryopreserved NK cell fraction, comprising:

i) the NK cell fraction of any one of claims 1-6; and

ii) DMSO, wherein the concentration of DMSO is about 10% v/v, and

wherein the cryopreserved NK cell fraction is stable:

i) at about -80°C for up to six weeks; and/or

ii) at about -150°C for up to about 12 months.

28. ~~A method for preparing a thawed NK cell fraction comprising:~~

~~(a) thawing the cryopreserved NK cell fraction of any one of claims 19-20 or the cryopreserved NK cell fraction produced by the method of claim 27 in a 37°C water bath;~~

~~(b) diluting said cryopreserved NK cell fraction thawed in step (a) with infusion solution~~

~~thereby producing a thawed, cryopreserved NK cell fraction.~~

1129. A cryopreserved NK fraction for use in method of treating a disease selected from the group consisting of a hematological disease, a solid tumor and an autoimmune disease in a human subject in need thereof, wherein said treating comprises the method comprising:

(a) administering at least one anti-cancer monoclonal antibody is to be administered to said subject;

(b) administering at least one immunosuppressive agent is to be administered to said subject;

(c) ~~thawing and administering~~ the cryopreserved NK cell fraction of any one of claims 10 is to be thawed and administered 18, the NK cell fraction produced by the method of any one of claims 21-26, or the thawed NK cell fraction produced by the method of claim 28; and

(d) administering IL-2 is to be administered to said subject.

1230. The cryopreserved NK fraction for use method of claim 1129, wherein step (c) comprises ~~administering~~ a first dose of said NK cell fraction or thawed NK cell fraction to be administered, and two days later ~~administering~~ a second dose of said NK cell fraction or thawed NK cell fraction to be administered.

1331. The cryopreserved NK fraction for use method of claim 1230, wherein:

(i) said first dose and said second dose each comprise at least about 1.25×10^7 cells/kg, for a total dose of 2.5×10^7 cells/kg;

(ii) said first dose and said second dose each comprise at least about 2.5×10^7 cells/kg, for a total dose of at least about 5×10^7 cells/kg;

(iii) said first dose and said second dose of said NK cell fraction each comprise 5×10^7 cells/kg, for a total dose of 1×10^8 cells/kg; or

(iv) said first dose and said second dose of said NK cell fraction each comprise 1×10^8 cells/kg, for a total dose of 2×10^8 cells/kg.

~~32. The method of any one of claims 29-31, wherein said immunosuppressive agent is a chemotherapeutic immunosuppressive agent, irradiation or any combination thereof.~~

1433. The cryopreserved NK cell fraction for use method of any one of claims 11-1329-32, wherein said hematological disease is a hematologic malignancy.

1534. The cryopreserved NK cell fraction for use method of any one of claim 11-14s 29-33, wherein the hematological disease is multiple myeloma.

1635. The cryopreserved NK cell fraction for use method of any one of claims 11-1429-34, wherein said hematological disease is non-Hodgkins lymphoma (NHL), optionallypreferably wherein the NHL is:

- i) CD20 positive B cell NHL;
- ii) follicular lymphoma (FL);
- iii) high grade B-cell lymphoma (HGBCL);

- iv) HGBCL, not otherwise specified (HGBCL, NOS);
- v) primary mediastinal large B-cell lymphoma (PMBCL); or
- vi) diffuse large B cell lymphoma (DLBCL) .

1736. The cryopreserved NK cell fraction for use ~~method of claim 1635~~, wherein said NHL is characterized by at least one of:

- a) relapsed/refractory disease that has failed conventional therapy;
- b) patient having received at least two prior lines of therapy, preferably wherein at least one of the at least two prior lines of therapy comprised the administration of chemotherapy and at least one of the at least two prior lines of therapy comprise the administration of an anti-CD20 monoclonal antibody;
- c) measurable disease as defined by the Lugano response criteria;
- d) wherein said NLH is FL transformed to HGBCL, the subject has previously received at least one line of therapy after transformation to HGBCL.

1837. The cryopreserved NK cell fraction for use of ~~method of any one of any one of~~ claims 11-1429-36, wherein said hematologic disease is NHL and said anticancer monoclonal antibody is rituximab, preferably wherein the rituximab is to be administered at least once at a dose of about 375mg/m², preferably wherein rituximab is to be administered at least three times:

~~38. The method of any one of claims 29-37, wherein the at least one anti-cancer monoclonal antibody is administered to the subject at least three times, preferably wherein the at least one anti-cancer monoclonal antibody is administered:~~

- ~~at about 10 days before the first administration of the NK cell fraction or thawed NK cell fraction~~ is to be administered;
- ~~at about 3 days before the first administration of the NK cell fraction or thawed NK cell fraction~~ is to be administered;

and at 12-16 days ~~following the first administration of~~ after the NK cell fraction or thawed NK cell fraction is to be administered.

~~1939.~~ The cryopreserved NK cell fraction for use of ~~method of~~ any one of claims ~~11-1829-38~~, wherein said at least one immunosuppressive agent comprises cyclophosphamide and/or fludarabine, optionally:-

40. — ~~The method of claim 39~~, wherein:

said at least one immunosuppressive agent comprises both cyclophosphamide (400 mg/m²) and fludarabine (30 mg/m²),

wherein the cyclophosphamide is to be administered at a dose of 400 mg/m² and the fludarabine is to be administered at a dose of 30 mg/m², and

wherein said cyclophosphamide and said fludarabine are to be administered:

at about 5 days before ~~the first administration of~~ the NK cell fraction or thawed NK cell fraction is to be administered;

at about 4 days before ~~the first administration of~~ the NK cell fraction or thawed NK cell fraction is to be administered; and

at about 4 days before ~~the first administration of~~ the NK cell fraction or thawed NK cell fraction is to be administered.

41. — ~~The method of any one of claims 29-40~~, wherein ~~step (d) comprises administering 6 x 10⁶ units IL-2 one each one of following transfusion of said allogeneic thawed cryopreserved expanded NK cells~~:

(i) ~~on the day of the first administration of the NK cell fraction or thawed NK cell fraction~~;

(ii) ~~two days following the first administration of the NK cell fraction or thawed NK cell fraction~~; and

(iii) ~~four days following the first administration of the NK cell fraction or thawed NK cell fraction~~.

20. The cryopreserved NK cell fraction for use of claim 11, wherein said disease is the autoimmune disease systemic lupus erythematosus (SLE or lupus).