

CLAIMS

1. A method of selecting universal donor NK cells, the method comprising: selecting candidate NK cells having (i) an HLA genotype carrying C1, C2, and Bw4 alleles and (ii) a KIR genotype or phenotype possessing inhibitory KIRs comprising 2DL1, 2DL2 or 2DL3, and 3DL1 as universal donor NK cells.
2. The method of claim 1, further comprising selecting candidate NK cells having a KIR genotype possessing at least 3 activating KIRs comprising 2DS1 and 3DS1 as universal donor NK cells.
3. The method of claim 1, further comprising:
 - a) evaluating expression of 2DL1, 2DL2 or 2DL3, and 3DL1 in the candidate donor NK cells;
 - b) obtaining an HLA genotype of the candidate NK cells; and/or
 - c) obtaining a KIR genotype of the candidate NK cells.
4. The method of claim 1, further comprising selecting a donor having a CMV seropositive profile as a source of the candidate NK cells.
5. The method of claim 4, wherein the universal donor NK cells selected are NKG2C+.
6. The method of any one of claims 1-5, wherein the universal donor NK cells selected are activated by incubating the universal donor NK cells in vitro in the presence of IL-21.
7. The method of claim 6, wherein the IL-21 comprises at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21), and IL-21 exosomes (EX21).
8. The method of claim 7, wherein the IL-21 present on FC21, PM21 and EX21 comprises a form of IL-21 selected from (a) an engineered membrane bound form for IL-21, (b) IL-21 chemically conjugated to the surface of FC21, PM21 or EX21, or (c) IL-21 in solution mixed to be in contact with the NK cells, and wherein any one of the FC21, PM21 or EX21 further comprises (a) one or more activating agents, stimulatory peptides, cytokines, and/or adhesion

molecules selected from 41BBL, IL-2, IL-12, IL-18, IL-15, IL-7, ULBP, MICA, LFA-1, 2B4, BCM/SLAMF2, CCR7, OX40L, NKG2D agonists, Delta-1, Notch ligands, NKp46 agonists, NKp44 agonists, NKp30 agonists, other NCR agonists, CD16 agonists; or (b) membrane bound TGF- β .

9. The method of claim 8, wherein any one of the FC21, PM21, and EX21 further comprises 41BBL.

10. A method for preparing a population of universal donor NK cells, the method comprising:

(a) selecting a NK cell donor having (i) an HLA genotype having C1, C2, and Bw4 alleles and (ii) a KIR genotype or phenotype possessing inhibiting KIRs comprising 2DL1, 2DL2 or 2DL3, and 3DL1; and

(b) exposing an initial population of NK cells from the NK cell donor to IL-21 in vitro for a time and under conditions sufficient to expand the initial population of NK cells to obtain a population of universal donor NK cells.

11. The method of claim 10, wherein the NK cell donor is further selected for having a KIR genotype possessing at least 3 activating KIRs comprising 2DS1 and 3DS1.

12. The method of claim 10, further comprising:

- i) evaluating expression of 2DL1, 2DL2 or 2DL3, and 3DL1 in the NK cell donor;
- ii) obtaining an HLA genotype of the NK cell donor; and/or
- iii) obtaining a KIR genotype of the NK cell donor.

13. The method of claim 10, wherein step (b) occurs for a time and under conditions to achieve at least one population doubling.

14. The method of claim 10, wherein the NK cell donor is further selected for having a CMV seropositive profile indicative of the presence of NKG2C⁺ NK cells.

15. The method of claim 10, wherein exposing the initial population of NK cells to IL-21 comprises contacting the initial population of NK cells in vitro with at least one of soluble IL-

21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21) and IL-21 exosomes (EX21).

16. The method of claim 15, wherein the IL-21 present on FC21, PM21 and EX21 comprises a form of IL-21 selected from (a) an engineered membrane bound form for IL-21, (b) IL-21 chemically conjugated to the surface of FC21, PM21 or EX21, or (c) IL-21 in solution mixed to be in co-contact with the NK cells, and wherein any one of the FC21, PM21 or EX21 further comprises (a) one or more activating agents, stimulatory peptides, cytokines, and/or adhesion molecules selected from 41BBL, IL-2, IL-12, IL-18, IL-15, IL-7, ULBP, MICA, LFA-1, 2B4, BCM/SLAMF2, CCR7, OX40L, NKG2D agonists, Delta-1, Notch ligands, NKp46 agonists, NKp44 agonists, NKp30 agonists, other NCR agonists, and CD16 agonists; or (b) membrane bound TGF- β .

17. The method of claim 16, wherein any one of the FC21, PM21, and EX21 further comprises 41BBL.

18. The method of any one of claims 10-17, wherein the population of universal donor NK cells has increased ability to produce and secrete anti-tumor cytokines of IFN γ or TNF α , increased expression of NKG2D, increased expression of CD16, increased expression of NKp46, and/or increased KIR expression compared to the initial population of NK cells.

19. An engineered NK cell or cell line, wherein the engineered NK cell or cell line has been transformed to express HLA C1, C2, and/or Bw4 alleles.

20. The engineered NK cell or cell line of claim 19, wherein the engineered NK cell or cell line has been transformed to express HLA C1, C2, and Bw4 alleles.

21. The engineered NK cell or cell line of claim 20, wherein the engineered NK cell or cell line has been further transformed to express one or more inhibitory KIRs comprising 2DL1, 2DL2, 2DL3, and/or 3DL1.

22. The engineered NK cell or cell line of claim 21, wherein the engineered NK cell or cell line has been further transformed to express inhibitory KIRs comprising 2DL1, 2DL2 or 2DL3, and 3DL1.

23. The engineered NK cell or cell line of claim 19, wherein the engineered NK cell or cell line has been further transformed to express one or more activating KIRs comprising 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4.
24. The engineered NK cell or cell line of claim 23, wherein the engineered NK cell or cell line has been further transformed to express three or more activating KIRs comprising 2DS1 and 3DS1.
25. The engineered NK cell or cell line of claim 19, wherein the engineered NK cell or cell line has been further engineered to activate NKG2C.
26. The engineered NK cell or cell line of any one of claims 19-25, wherein the engineered NK cell or cell line is activated by incubating the engineered NK cell or cell line in vitro in the presence of IL-21.
27. The engineered NK cell or cell line of claim 26, wherein the IL-21 comprises at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21), and IL-21 exosomes (EX21).
28. The engineered NK cell or cell line of claim 27, wherein the IL-21 present on FC21, PM21 and EX21 comprises a form of IL-21 selected from (a) an engineered membrane bound form for IL-21, (b) IL-21 chemically conjugated to the surface of FC21, PM21 or EX21, or (c) IL-21 in solution mixed to be in contact with the NK cells, and wherein any one of the FC21, PM21 or EX21 further comprise (a) one or more activating agents, stimulatory peptides, cytokines, and/or adhesion molecules selected from 41BBL, IL-2, IL-12, IL-18, IL-15, IL-7, ULBP, MICA, LFA-1, 2B4, BCM/SLAMF2, CCR7, OX40L, NKG2D agonists, Delta-1, Notch ligands, NKp46 agonists, NKp44 agonists, NKp30 agonists, other NCR agonists, CD16 agonists; or (b) membrane bound TGF- β .
29. The engineered NK cell or cell line of claim 28, wherein any one of the FC21, PM21, and EX21 further comprises 41BBL.
30. A population of NK cells for use in the treatment of a cancer or an infectious disease in a subject, wherein said population of NK cells comprises:

- (i) an HLA genotype carrying C1, C2 and Bw4 alleles; and
- (ii) a KIR genotype or phenotype possessing inhibitory KIRs comprising 2DL1, 2DL2 or 2DL3, and 3DL1.

31. The population of NK cells for use of claim 30, wherein the population of NK cells further comprises a KIR genotype possessing at least 3 activating KIRs comprising 2DS1 and 3DS1.

32. The population of NK cells for use of claim 30, wherein the population of NK cells is from a NK cell donor having a CMV seropositive profile.

33. The population of NK cells of claim 30, further comprising culturing the population of NK cells in vitro in the presence of IL-21 prior to said use.

34. The population of NK cells for use of claim 33, wherein the IL-21 comprises IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21), or IL-21 exosomes (EX21).

35. The population of NK cell for use of claim 34, wherein the IL-21 present on the FC21, PM21, and EX21 comprises a form of IL-21 selected from (a) an engineered membrane bound form for IL-21, (b) IL-21 chemically conjugated to the surface of the FC21, PM21 or EX21, or (c) IL-21 in solution mixed to be in co-contact with the NK cells, and wherein any one of the FC21, PM21 or EX21 further comprises (a) an NK stimulatory ligand one or more activating agents, stimulatory peptides, cytokines, and/or adhesion molecules selected from 41BBL, IL-2, IL-12, IL-18, IL-15, IL-7, ULBP, MICA, LFA-1, 2B4, BCM/SLAMF2, CCR7, OX40L, NKG2D agonists, Delta-1, Notch ligands, NKp46 agonists, NKp44 agonists, NKp30 agonists, other NCR agonists, and CD16 agonists; or (b) membrane bound TGF- β .

36. The population of NK cells for use in claim 35, wherein any one of the FC21, PM21, and EX21 further comprises 41BBL.

37. The population of NK cells for use of any one of claims 30-36, wherein the cancer is selected from a cancer of the blood, lung, esophagus, stomach, pancreas, liver, biliary tract, colon, rectum, breast, ovary, cervix uterus, endometrium, kidney, bladder, testes, prostate,

larynx, thyroid, brain or skin, and wherein the infectious disease is caused by a pathogen selected from a virus, bacterium or fungus.

38. A method of preparing a collection of NK cells from a donor, the method comprising
(i) selecting from one or more donors a universal donor having (i) an HLA genotype carrying C1, C2, and Bw4 alleles and (ii) a KIR genotype or phenotype possessing inhibitory KIRs comprising 2DL1, 2DL2 or 2DL3, and 3DL1; and

(ii) preparing a collection of NK cells from an ex vivo batch of NK-cells of said universal donor.

39. The method of claim 38, wherein the universal donor is further selected for having a KIR genotype possessing at least 3 activating KIRs comprising 2DS1 and 3DS1.

40. The method of claim 38, further comprising:

a) evaluating expression of 2DL1, 2DL2 or 2DL3, and 3DL1 in the one or more donors;

b) obtaining an HLA genotype of the one or more donors; and/or

c) obtaining a KIR genotype of the one or more donors.

41. The method of claim 38, wherein the universal donor is further selected for having a CMV seropositive profile indicative of presence of NKG2C⁺ NK cells.

42. The method of claim 38, further comprising activating the collection of NK cells by incubating the collection of NK cells in vitro in the presence of IL-21.

43. The method of claim 42, wherein the IL-21 comprises at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21), and IL-21 exosomes (EX21).

44. The method of claim 43, wherein the IL-21 present on the FC21, PM21, and EX21 comprises a form of IL-21 selected from (a) an engineered membrane bound form for IL-21, (b) IL-21 chemically conjugated to the surface of the FC21, PM21 or EX21, or (c) IL-21 in solution mixed to be in co-contact with the NK cells, and wherein any one of the FC21, PM21 or EX21 further comprises (a) an NK stimulatory ligand one or more activating agents,

stimulatory peptides, cytokines, and/or adhesion molecules selected from 41BBL, IL-2, IL-12, IL-18, IL-15, IL-7, ULBP, MICA, LFA-1, 2B4, BCM/SLAMF2, CCR7, OX40L, NKG2D agonists, Delta-1, Notch ligands, NKp46 agonists, NKp44 agonists, NKp30 agonists, other NCR agonists, and CD16 agonists; or (b) membrane bound TGF- β .

45. The method of claim 44, wherein any one of the FC21, PM21, and EX21 further comprises 41BBL.