

CLAIMS

1. A method of selecting universal donor NK cells for therapeutic administration to a subject in need thereof, the method comprising: determining a KIR phenotype of candidate NK cells from an NK cell donor, wherein the KIR phenotype is indicative of the presence of one or more variably inherited inhibitory KIRs 2DL1, 2DL2, 2DL3, and 3DL1 among the population of NK cells; and selecting the candidate NK cells as universal donor NK cells for therapeutic administration when the KIR phenotype indicates the presence of one or more variably inherited inhibitory KIRs 2DL1, 2DL2, 2DL3, and 3DL1.

2. A method of selecting universal donor NK cells for therapeutic administration to a subject in need thereof, the method comprising:

obtaining a HLA genotype of candidate NK cells from an NK cell donor, wherein the HLA genotype is indicative of the presence or absence of at least two HLA C1, C2, and Bw4 alleles, and thereby indicative of the presence of one or more variably inherited inhibitory KIRs 2DL1, 2DL2, 2DL3, and 3DL1 among the population of NK cells; and

selecting the candidate NK cells as universal donor NK cells for therapeutic administration when the HLA genotype of the candidate NK cells indicates the presence of at least two of the HLA C1, C2, and Bw4 alleles.

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

obtaining a KIR genotype of the candidate NK cells, wherein the KIR genotype is indicative of the presence or absence of at least three activating KIRs selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4, and wherein selecting the candidate NK cells as universal donor NK cells further comprises selecting the candidate NK cells comprising at least three of the activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4.

4. A method of selecting universal donor NK cells for therapeutic administration to a subject in need thereof, the method comprising:

obtaining a KIR genotype of candidate NK cells, wherein the KIR genotype is indicative of the presence or absence of at least three activating KIRs selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4; and selecting the candidate NK cells as universal donor NK cells for therapeutic administration when the KIR genotype indicates the presence of at least three of the activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4.

5. The method of claim 4, further comprising:

obtaining a HLA genotype of candidate NK cells, wherein the HLA genotype is indicative of the presence or absence of each of HLA C1, C2, and Bw4 alleles; and

selecting the candidate NK cells as universal donor NK cells for therapeutic administration when the HLA genotype of the candidate NK cells indicates the presence of at least two of the HLA alleles HLA C1, C2, and Bw4.

6. A method of screening a population of candidate NK cells from a donor to identify universal NK donor cells in the population for providing a source of NK cells for therapeutic administration to subjects in need thereof, the method comprising:

obtaining a HLA genotype of candidate NK cells from an NK cell donor, wherein the HLA genotype is indicative of the presence or absence of at least two of HLA C1, C2, and Bw4 alleles, wherein candidate NK cells comprising at least two HLA alleles of HLA C1, C2, and Bw4 are identified as universal donor NK cells.

7. The method of claim 6, further comprising obtaining a KIR genotype of the candidate NK cells, wherein the KIR genotype is indicative of the presence or absence of at least three activating KIRs selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4; wherein candidate NK cells comprising at least three activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4 are identified as universal NK cells.

8. The method of any one of claims 1-7, wherein the selected universal donor NK cells are histologically optimized for at least 50%-85% of recipient subjects.

9. The method of any one of claims 1-7, further comprising obtaining or having obtained the CMV seropositivity of the candidate NK cells, wherein selecting a candidate NK cell as a universal donor NK cell further comprises selecting a candidate NK cell seropositive for CMV or having high NKG2C expression compared to a reference level of NKG2C expression.

10. An isolated universal donor NK cell selected by the method of any one of claims 1-5, 8 or 9; or identified by screening by the method of claim 6 or 7.

11. The isolated universal donor NK cell of claim 10, wherein the isolated universal donor NK cell is NKG2C+.

12. The isolated universal donor NK cell of claim 10, wherein the isolated universal donor NK cell is activated by incubating the isolated universal donor NK cells in vitro in the presence of IL-21.

13. The isolated universal donor NK cell of claim 12, wherein the IL-21 used in the in vitro activation comprises at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21s), and IL-21 exosomes (EX21s).

~~14. A method of treating a cancer or an infectious disease in a subject comprising administering to the subject a donor NK cell selected by the method of any of claims 1-5, 8 or 9; or identified by screening by the method of any of claims 6 or 7; or administering the isolated universal NK cell of any of claims 10-13.~~

~~15. A method of treating a cancer or an infectious disease in a subject comprising (a) obtaining or having obtained a HLA genotype of candidate NK cells from an NK cell donor, wherein the HLA genotype is indicative of the presence or absence of each of HLA C1, C2, and Bw4 alleles and thereby indicative of the presence of one or more variably inherited inhibitory KIRs 2DL1, 2DL2, 2DL3, and 3DL1; (b) obtaining or having obtained a KIR~~

~~genotype of the candidate NK cells, wherein the KIR genotype is indicative of the presence or absence of each of activating KIRs selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4; and~~

~~(c) selecting a candidate NK cell as a universal donor NK cell for the therapeutic administration when (i) the HLA genotype indicates the presence of at least two of the HLA alleles HLA C1, C2, and Bw4; and (ii) the KIR genotype indicates the presence of at least three of the activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4.~~

~~16. — The method of claim 14 or 15, wherein the selected universal donor NK cells are histologically optimized for at least 50%–85% of recipient subjects.~~

~~17. — The method of any of any of claims 14–16, further comprising obtaining or having obtained the CMV seropositivity of the candidate NK cells; and wherein selecting an NK candidate cell further comprises selecting a candidate NK cell when the NK cell donor is seropositive for CMV or when the NK cells from the NK cell donor have high NKG2C expression compared to a reference level of NKG2C expression.~~

~~18. — The method of any of claims 14–16, further comprising obtaining or having universal donor NK cells in vitro in the presence of IL-21.~~

~~19. — The method of claim 18, wherein the IL-21 used in the in vitro culture comprises at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21s), and IL-21 exosomes (EX21s).~~

~~20. — The method of any of claims 14–19, 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.~~

~~21.~~ The method of any of claims ~~14-19~~, wherein the infectious disease is caused by a pathogen selected from a virus, bacterium or fungus.

~~22~~14. A method for preparing a population of universal donor NK cells for therapeutic administration to a subject in need thereof, the method comprising:

(a) obtaining an initial population of NK cells from a NK cell donor, wherein the NK cell donor has a genotype indicating the presence of (i) at least two of variably inherited activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4; and (ii) at least one of C1, C2, and Bw4 alleles; and

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

~~23~~15. The method of claim ~~22~~14, wherein the donor genotype indicates the presence of C1, C2, and Bw4 alleles.

~~24~~16. The method of claim ~~22~~14, wherein step (b) occurs for a time and under conditions to achieve at least one population doubling.

~~25~~17. The method of claim ~~22~~14, wherein the NK cell donor further has a CMV seropositive profile indicative of the presence of NKG2C+ NK cells.

~~26~~18. The method of claim any one of claims ~~22-25~~14-17, wherein exposing the initial population of NK cells to IL-21 comprises contacting the NK cells in vitro with at least one of soluble IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21s) and IL-21 exosomes (EX21s).

~~27~~19. The method of claim ~~26~~18, wherein the IL-21 present on feeder cells (FC21), IL-21 plasma membrane particles (PM21s) and IL-21 exosomes (EX21s) 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) or IL-21 in solution mixed to be in co-contact with the NK cells.

~~28~~20. The method of any one of claims ~~22-27~~ 14-19, wherein any one of the FC21, PM21 or EX21 further comprise (a) one or more activating agents, stimulatory peptides, cytokines, and/or adhesion molecules ~~an NK stimulatory ligand~~ 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~~21. A population of universal donor NK cells prepared by the method of any of claims ~~22-28~~ 14-20.

~~30~~22. A population of universal donor NK cells prepared by the method of any one of claims ~~22-29~~ 14-21, wherein the expanded population of NK cells is characterized by increased ability to produce and secrete anti-tumor cytokines of IFN γ or TNF α .

~~31~~23. A population of universal donor NK cells of claim ~~29-21~~ or ~~30~~ 22, wherein the expanded population of NK cells is characterized by increased expression of NKG2D, increased expression of CD16, increased expression of NKp46, increased KIR expression.

~~32~~24. An engineered NK cell or cell line, wherein the NK cells or cell line have been transformed to express one or more HLA alleles comprising C1, C2 or Bw4.

~~33~~25. The engineered NK cell or cell line of claim ~~32~~ 24, wherein the NK cells or cell line have been transformed to express C1, C2, and Bw4.

~~34~~26. The engineered NK cell or cell line of claims ~~32-24~~ or ~~33~~ 25, wherein the NK cells or cell line have been further transformed to express of one or more variably inherited activating

KIRs comprising 2DS1/2, 2DS3/5, 3DS1, or 2DS4.

~~35~~27. The engineered NK cell or cell line of any of claims ~~32-34~~ 24-26, wherein the NK cells or cell line have been further transformed to express of three or more variably inherited activating KIRs comprising 2DS1/2, 2DS3/5, 3DS1, or 2DS4.

~~36~~28. The method of ~~any one of claims 5, 11, or 34-35, 41 or claims 49-51~~ claim 13, wherein the IL-21 present on feeder cells (FC21), IL-21 plasma membrane particles (PM21s) and IL-21 exosomes (EX21s) 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.

29. The method of ~~any one of claims 5, 11, 34-36, 41 or claims 49-51~~ claim 28, wherein any one of the FC21, PM21 or EX21 further comprise (a) one or more activating agents, stimulatory peptides, cytokines, and/or adhesion molecules ~~an NK stimulatory ligand~~ 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 .

~~38~~30. A donor NK cell selected by the method of any one of claims 1-4, 7, or 8; or screened by the method of any one of claims 6-7; or the isolated universal NK cell of claims 10-13; or the population of universal donor NK cells of claims ~~29-31~~ 21-23; or the engineered NK cell or cell line of claims ~~32-35~~ 24-27 for use in the treatment of cancer or an infectious disease in a subject.

~~39~~31. A population of NK cells for use in the treatment of cancer or an infectious disease in a subject wherein said population of NK cells comprises:

(i) an HLA genotype comprising at least two HLA alleles selected from HLA C1, C2 and Bw4 indicative of the presence of one or more variably inherited inhibitory KIRs selected from 2DL1, 2DL2, 2DL3, and 3DL1; and

(ii) a KIR genotype comprising at least three activating KIRS selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4.

~~40~~32. An NK cell of claim ~~38~~30 or population of NK cells of claim ~~39~~31, wherein the NK cell or population of NK cells is histologically optimized for at least 50%-85% of the recipient subjects.

~~41~~33. An NK cell of claim ~~38~~30 or population of NK cells of claim ~~39~~31, wherein the donor of the NK cell or population of NK cells is seropositive for CMV, and/or the NK cell or population of NK cells have a high NKG2C expression compared to a reference level of NKG2C expression.

~~42~~34. The NK cell or population of NK cells of claims ~~38-41~~31-33, further comprising culturing the NK cell or the population of NK cells in vitro in the presence of IL-21 prior to said use in the treatment.

~~43~~35. The NK cell or population of NK cells of claim ~~42~~34, wherein the IL-21 in the in vitro culture comprises IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21s), or IL-21 exosomes.

~~44~~36. The NK cell or population of NK cells of claims ~~38-43~~30-35, 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.

~~45~~37. The NK cell or population of NK cells of claims ~~38-43~~30-35, wherein the infectious disease is caused by a pathogen selected from a virus, bacterium or fungus.

~~46~~38. The NK cell or population of NK cells of claims ~~38-43~~30-35, wherein the NK cell or

population of NK cells and/or the donor of said NK cell or population of NK cells is selected from a set comprising two or more cells, populations and/or donors of which said HLA genotype and said KIR genotype has been determined.

~~47~~39. A method of preparing a collection of NK cells from a donor comprising

(i) determining from one or more donors: (a) an HLA genotype indicative of the presence or absence of HLA C1, C2, and Bw4 alleles thereby indicative of the presence of one or more variably inherited inhibitory KIRs 2DL1, 2DL2, 2DL3, and 3DL1; and (b) a KIR genotype indicative of the presence or absence of activating KIRs selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4; and

(ii) selecting from said donors a universal donor NK for the therapeutic administration of NK cells when (i) the HLA genotype indicates the presence of at least two HLA alleles HLA C1, C2, and Bw4; and (ii) the KIR genotype indicates the presence of at least three activating KIRs 2DS1/2, 2DS3/5, 3DS1, and/or 2DS4; and

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

~~48~~40. A method according to claim ~~47~~39, wherein said selection as a universal donor NK cell for the therapeutic administration further comprises selecting a donor with a CMV-seropositive profile indicative of the presence of NKG2C+ NK cells.

~~49~~41. Use of a donor NK cell selected by the method of any one of claims 1-5, 8 or 9; or screened by the method of any one of claims 6-7; or the isolated universal NK cell of claims 10-13; or the population of universal donor NK cells of claims ~~29-31~~21-23; or the engineered NK cell or cell line of claims ~~32-35~~24-27 in the manufacture of a medicament for treating cancer or an infectious disease in a subject.

~~50~~42. Use of a population of NK cells in the manufacture of a medicament for treating cancer or an infectious disease in a subject wherein said population of NK cells comprises:

- (i) an HLA genotype comprising at least two HLA alleles selected from HLA C1, C2 and Bw4 indicative of the presence of one or more variably inherited inhibitory KIRs selected from 2DL1, 2DL2, 2DL3, and 3DL1; and
- (ii) a KIR genotype comprising at least three activating KIRS selected from the group consisting of 2DS1/2, 2DS3/5, 3DS1, and 2DS4.

~~51~~43. The use of claim ~~49~~ 41 or ~~50~~ 42, wherein the NK cell or population of NK cells is histologically optimized for at least 50%-85% of the recipient subjects.

~~52~~44. The use of any one of claims ~~49-51~~ 41-43, wherein the donor of the NK cell or population of NK cells is seropositive for CMV, or the NK cell or population of NK cells have a high NKG2C expression compared to a reference level of NKG2C expression.

~~53~~45. The use of any of claims ~~49-52~~ 41-44, further comprising culturing the NK cell or the population of NK cells in vitro in the presence of IL-21 prior to said use in the treatment.

~~54~~46. The use of claim ~~53~~ 45, wherein the IL-21 in the in vitro culture comprises IL-21, IL-21-expressing feeder cells (FC21), IL-21 plasma membrane particles (PM21s), or IL-21 exosomes.

~~55~~47. The use of any of claims ~~49-54~~ 41-46, 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.

~~56~~48. The use of any of claims ~~49-55~~ 41-47, wherein the infectious disease is caused by a pathogen selected from a virus, bacterium or fungus.

5749. The use of any of claims ~~49-56~~ 41-48, wherein the NK cell or population of NK cells and/or the donor of said NK cell or population of NK cells is selected from a set comprising two or more cells, populations and/or donors of which said HLA genotype and said KIR genotype has been determined.

50. The NK cell or population of NK cells of claim 46, wherein the IL-21 present on feeder cells (FC21), IL-21 plasma membrane particles (PM21s) and IL-21 exosomes (EX21s) 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) or IL-21 in solution mixed to be in co-contact with the NK cells.

51. The NK cell or population of NK cells of claim 50, 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 .