

CLAIMS

What is Claimed is:

1. An isolated natural killer (NK) cell engineered to express (1) a chimeric antigen receptor (CAR) and/or a T cell receptor (TCR) and (2) human IL-15 (hIL-15) and to
5 have essentially no expression of CISH.
2. The NK cell of claim 1, wherein the NK cell is engineered to express a CAR.
3. The NK cell of claim 1, wherein the NK cell is engineered to express a TCR.
4. The NK cell of claim 1, wherein the NK cell is engineered to express a CAR and TCR.
5. The NK cell of claim 1, wherein the NK cell is derived from cord blood, peripheral
10 blood, bone marrow, CD34⁺ cells, or iPSCs.
6. The NK cell of claim 1, wherein the NK cell is derived from cord blood.
7. The NK cell of claim 6, wherein the cord blood has previously been frozen.
8. The NK cell of claim 1, wherein the CAR and/or TCR has antigenic specificity for
CD19, CD319/CS1, ROR1, CD20, CD5, CD7, CD22, CD70, CD30, BCMA, CD25,
15 NKG2D ligands, MICA/MICB, carcinoembryonic antigen, alphafetoprotein, CA-125,
MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated
ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120,
HIV-1 envelope glycoprotein gp41, GD2, CD123, CD33, CD30, CD56, c-Met,
mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4,
20 ERBB2, WT-1, EGFRvIII, TRAIL/DR4, and/or VEGFR2.
9. The NK cell of claim 1, wherein the CAR has antigenic specificity for CD19.
10. The method of claim 1, wherein the NK cell further expresses a second cytokine.
11. The method of claim 10, wherein the cytokine is IL-21 or IL-12.
12. The method of any of claims 1-11, wherein the NK cell has activated mammalian target
25 of rapamycin (mTOR) signaling.

13. The method of claim 12, wherein the NK cell has increased JAK/STAT signaling.
14. A method for producing NK cells of any one of claims 1-14 comprising:
 - (a) obtaining a starting population of NK cells;
 - (b) culturing the starting population of NK cells in the presence of artificial presenting
5 cells (APCs);
 - (c) introducing a CAR and/or TCR expression vector into the NK cells;
 - (d) expanding the NK cells in the presence of APCs, thereby obtaining expanded NK cells; and
 - (e) disrupting the expression of CISH in the expanded NK cells.
- 10 15. The method of claim 14, wherein disrupting expression comprises using CRISPR-mediated gene silencing.
16. The method of claim 15, wherein CRISPR-mediated gene silencing comprises contacting the CAR NK cells with sgRNA and Cas9.
17. The method of claim 11, wherein the sgRNA targets exon 4 of CISH.
- 15 18. The method of claim 17, wherein the sgRNA comprises SEQ ID NOs:1-2.
19. The method of claim 14, wherein the starting population of NK cells is obtained by isolating mononuclear cells using a ficoll-paque density gradient.
20. The method of claim 14, wherein the APCs are gamma-irradiated APCs.
21. The method of claim 14, wherein the APCs are universal APCs (uAPCs).
- 20 22. The method of claim 14, wherein the APCs are engineered to express 41BB and IL-21.
23. The method of claim 21, wherein the uAPCs are engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL).
24. The method of claim 14, wherein the NK cells and APCs are present at a 1:2 ratio.

25. The method of claim 14, wherein the NK cells of step (b) and (d) are further expanded in the presence of IL-2.
26. The method of claim 25, wherein the IL-2 is present at a concentration of 100-300 U/mL.
- 5 27. The method of claim 25, wherein IL-2 is present at a concentration of 200 U/mL.
28. The method of claim 14, wherein introducing comprises transduction.
29. The method of claim 14, wherein the CAR and/or TCR expression construct is a lentiviral vector or retroviral vector.
30. The method of claim 14, wherein the NK cells are GMP-compliant.
- 10 31. The method of claim 14, wherein the NK cells are allogeneic.
32. The method of claim 14, wherein the NK cells are autologous.
33. The method of claim 14, wherein the CAR and/or TCR has antigenic specificity for CD19, CD319/CS1, ROR1, CD20, CD5, CD7, CD22, CD70, CD30, BCMA, CD25, NKG2D ligands, MICA/MICB, carcinoembryonic antigen, alphafetoprotein, CA-125, 15 MUC-1, epithelial tumor antigen, melanoma-associated antigen, mutated p53, mutated ras, HER2/Neu, ERBB2, folate binding protein, HIV-1 envelope glycoprotein gp120, HIV-1 envelope glycoprotein gp41, GD2, CD123, CD23, CD30, CD56, c-Met, mesothelin, GD3, HERV-K, IL-11Ralpha, kappa chain, lambda chain, CSPG4, ERBB2, WT-1, EGFRvIII, TRAIL/DR4, and/or VEGFR2.
- 20 34. The method of claim 14, wherein the CAR has antigenic specificity for CD19.
35. The method of claim 14, wherein the CAR and/or TCR expression construct further expresses a cytokine.
36. The method of claim 35, wherein the cytokine is IL-15, IL-21, or IL-12.
37. The method of claim 14, further comprising cryopreserving the population of expanded 25 NK cells.

38. A pharmaceutical composition comprising a population of NK cells of any one of claims 1-11 or NK cells produced by the method of any one of claims 14-37 and a pharmaceutically acceptable carrier.
39. A composition comprising an effective amount of NK cells of any one of claims 1-11
5 or NK cells produced by the method of any one of claims 14-37 for use in the treatment of a disease or disorder in a subject.
40. The use of a composition comprising an effective amount of NK cells of any one of claims 1-11 or NK cells produced by the method of any one of claims 14-37 for the treatment of an immune-related disorder in a subject.
- 10 41. A method of treating an immune-related disorder in a subject comprising administering an effective amount of NK cells of any one of claims 1-11 or NK cells produced by the method of any one of claims 14-37 to the subject.
42. The method of claim 41, wherein the immune-related disorder is a cancer, autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition.
- 15 43. The method of claim 41, wherein the immune-related disorder is an inflammatory condition and the immune cells have essentially no expression of glucocorticoid receptor.
44. The method of claim 43, wherein the subject has been or is being administered a steroid therapy.
- 20 45. The method of claim 41, wherein the NK cells are autologous.
46. The method of claim 41, wherein the NK cells are allogeneic.
47. The method of claim 41, wherein the immune-related disorder is a cancer.
48. The method of claim 47, wherein the cancer is a solid cancer or a hematologic malignancy.

49. The method of claim 41, further comprising administering at least a second therapeutic agent.
50. The method of claim 49, wherein the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy.
- 5 51. The method of claim 49, wherein the NK cells and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.
52. The method of claim 49, wherein the NK cells with essentially no expression of CISH
10 have enhanced function as compared to NK cells with expression of CISH.
53. The method of claim 52, wherein the enhanced function is measured by intracellular staining for IFN- γ and TNF- α , CD107a degranulation, and tumor killing by ^{51}Cr release assay.
54. The method of claim 52, wherein the enhanced function is measured by increased
15 expression of granzyme-b, perforin, TRAIL, CD3z, Eomes, T-bet, DAP12, DNAM, CD25 and/or Ki67.

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