activity of CAR transduced NK cells has been proven in the preclinical setting in different tumor models, and clinical trials of allogeneic CAR-transduced NK cells are currently underway (Mehta *et al.,* 2018). While immune checkpoint molecules, such as PD-1, are being targeted to enhance the function of CAR-T cells, no immune checkpoint molecule has been targeted in CAR-NK cells to date (Gay *et al.,* 2017).

[0006] Functional NK cells can be derived from several sources. Autologous NK cells can be reproducibly generated *in vitro*, but have limited activity against autologous tumors, which may not be overcome by CAR engineering. Cord blood (CB) is a readily available source of allogeneic NK cells with clear advantages. CB is available as an off-the-shelf frozen product, an advantage that has been bolstered by methods to generate large numbers of highly functional NK cells from frozen CB units *ex vivo*. The generation of CAR-transduced NK cells from frozen CB units stored in large global CB bank inventories holds promise for widespread scalability that cannot be replicated with individual adult donors who require screening and leukapheresis. However, a major disadvantage of NK cells is their lack of persistence after adoptive transfer in the absence of cytokine support. Therefore, engineering CAR-NK cells to express cytokines to enhance their persistence is important for their clinical activity. By doing so, CAR-NK cells can upregulate checkpoint molecules which could limit their functionality. Therefore, there is a need to delete checkpoint molecules in CAR engineered NK cells to enhance their potency and clinical activity.

SUMMARY

[0007] In ~~a first aspect~~one example, the present disclosure refers to 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 have essentially no expression of CISH. In one aspect, the present disclosure refers to 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), wherein the cell has disruption of expression of Cytokine Inducible SH2 Containing Protein (CISH). In some examples, the NK cell is engineered to express a CAR. In certain examples, the NK cell is engineered to express a TCR. In particular examples, the NK cell is engineered to express a CAR and TCR or 3 or 4 antigen receptors. In some examples, the NK cells are GMP-compliant. In certain examples, the NK cells are allogeneic or autologous.

[0008] In some examples, the NK cell is derived from cord blood, peripheral blood, bone marrow, $CD34^+$ cells, or iPSCs. In certain examples, the NK cell is derived from cord blood. In particular examples, the cord blood has previously been frozen.

[0009] In certain examples, 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, 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, ERBB2, WT-1, EGFRvIII, TRAIL/DR4, and/or VEGFR2.

[0010] In additional examples, the NK cell further expresses a second or third cytokine. In particular examples, the cytokine is IL-15, IL-21 or IL-12.

[0011] In another ~~aspect~~example, the present disclosure refers to a method for producing NK cells as disclosed herein comprising: (a) obtaining a starting population of NK cells; (b) culturing the starting population of NK cells in the presence of artificial presenting 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. In one aspect, the present disclosure refers to a method for producing NK cells as disclosed herein comprising: (a) obtaining a starting population of NK cells; (b) culturing the starting population of NK cells in the presence of antigen presenting 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.

[0012] In some examples, disrupting expression comprises using CRISPR-mediated gene silencing. In certain examples, CRISPR-mediated gene silencing comprises contacting the CAR NK cells with sgRNA and Cas9. In particular examples, the sgRNA targets exon 4 of CISH. In specific examples, the sgRNA comprises crRNA1 which has the sequence AGGCCACATAGTGCTGCACA (SEQ ID NO:1) and crRNA2 which has the sequence TGTACAGCAGTGGCTGGTGG (SEQ ID NO:2).

[0013] In some examples, the starting population of NK cells is obtained by isolating mononuclear cells using a ficoll-paque density gradient. In particular examples, the APCs are gamma-irradiated APCs. In some examples, the APCs are universal APCs (uAPCs). In specific examples, the APCs are engineered to express 41BB and IL-21. In some examples, the uAPCs are engineered to express (1) CD48 and/or CS1 (CD319), (2) membrane-bound interleukin-21 (mbIL-21), and (3) 41BB ligand (41BBL). In particular examples, the NK cells and APCs are present at a 1:1 to 1:10 ratio, such as a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10 ratio. In certain examples, the NK cells and APCs are present at a 1:2 ratio.

[0014] In certain examples, the NK cells in culture with APCs are expanded further in the presence of IL-2. In certain examples, the IL-2 is present at a concentration of 100-300 U/mL,

such as 100, 125, 150, 175, 200, 225, 250, 275, or 300 U/mL. In some examples, IL-2 is present at a concentration of 200 U/mL.

[0015] In some examples, introducing comprises transduction. In certain examples, the CAR and/or TCR expression construct is a lentiviral vector or retroviral vector.

[0016] In particular examples, 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, 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.

[0017] In some examples, the CAR and/or TCR expression construct further expresses a cytokine, such as 2 or 3 cytokines. In some examples, the cytokine is IL-15, IL-21, or IL-12.

[0018] In some examples, the NK cell has activated mammalian target of rapamycin (mTOR) signaling as compared to an NK cells with CISH expression. In certain examples, the NK cell has increased JAK/STAT signaling as compared to an NK cell with CISH expression.

[0019] In additional examples, the method further comprises cryopreserving the population of expanded NK cells.

[0020] In another ~~aspect~~example, the present disclosure refers to a pharmaceutical composition comprising a population of NK cells as disclosed herein or NK cells produced by the method as disclosed herein and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising a population of NK cells as disclosed herein and a pharmaceutically acceptable carrier. In another ~~aspect~~example, the present disclosure refers to a composition comprising an effective amount of NK cells as disclosed herein or NK cells produced by the method as disclosed herein ~~–~~for use in the treatment of a disease or disorder in a subject. In one aspect, the present disclosure refers to a composition comprising an effective amount of NK cells as disclosed herein for use in the treatment of a disease or disorder in a subject.

[0021] In another example, there is provided the use of a composition comprising an effective amount of NK cells of the examples or NK cells produced by the method of the examples for the treatment of an immune-related disorder in a subject.

[0021a] In another aspect, the present disclosure refers to use of a composition comprising an effective amount of NK cells as disclosed herein ~~or NK cells produced by the method as disclosed herein~~ in the manufacture of a medicament for the treatment of an immune-related disorder in a subject.

[0022] A further example provides a method of treating an immune-related disorder in a subject comprising administering an effective amount of NK cells of the examples or NK cells produced by the method of the examples to the subject.

[0022a] In another aspect, the present disclosure refers to use of NK cells as disclosed herein ~~or NK cells produced by the method as disclosed herein~~ in the manufacture of a medicament for treating an immune-related disorder in a subject.

[0023] In some examples of the above examples, the immune-related disorder is a cancer, autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition. In certain examples, the immune-related disorder is an inflammatory condition and the immune cells have essentially no expression of glucocorticoid receptor. In certain examples, the subject has been or is being administered a steroid therapy. In some examples, the NK cells are autologous or allogeneic. In certain examples, the immune-related disorder is a cancer. In some examples, the cancer is a solid cancer or a hematologic malignancy.

[0024] In additional examples of the above examples, the method further comprises administering at least a second therapeutic agent. In some examples, the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy. In some examples, 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.

[0025] In some examples, the NK cells with essentially no expression of CISH have enhanced function as compared to NK cells with expression of CISH. In certain examples, the enhanced function is measured by intracellular staining for IFN-γ and TNF-α, CD107a degranulation, and tumor killing by 51Cr release assay. In some examples, the enhanced function is measured by increased expression of granzyme-b, perforin, TRAIL, CD3z, Eomes, T-bet, DAP12, DNAM, CD25 and/or Ki67.

[0026] Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred examples of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

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), wherein the cell has disruption of ~~and to have essentially no~~ expression of Cytokine Inducible SH2 Containing Protein (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 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, 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, 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 NK cell of claim 1, wherein the NK cell further expresses a second cytokine.
11. The NK cell of claim 10, wherein the cytokine is IL-21 or IL-12.

12. The NK cell of any of claims 1-11, wherein the NK cell has activated mammalian target of rapamycin (mTOR) signaling.

13. The NK cell of claim 12, wherein the NK cell has increased JAK/STAT signaling.

14. A method for producing NK cells of any one of claims 1-13 comprising:

(a) obtaining a starting population of NK cells;

(b) culturing the starting population of NK cells in the presence of ~~artificial~~ antigen presenting 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.

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~~16, wherein the sgRNA targets exon 4 of CISH.

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~~19. The method of claim 14, wherein the APCs are gamma-irradiated APCs.

~~21~~20. The method of claim 14, wherein the APCs are universal APCs (uAPCs).

~~22~~21. The method of claim 14, wherein the APCs are engineered to express 41BB and IL-21.

~~23~~22. The method of claim ~~21~~20, 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~~23. The method of claim 14, wherein the NK cells and APCs are present at a 1:2 ratio.

~~25~~24. The method of claim 14, wherein the NK cells of step (b) and (d) are further expanded in the presence of IL-2.

~~26~~25. The method of claim ~~25~~24, wherein the IL-2 is present at a concentration of 100-300 U/mL.

~~27~~26. The method of claim ~~25~~24, wherein IL-2 is present at a concentration of 200 U/mL.

~~28~~27. The method of claim 14, wherein introducing comprises transduction.

~~29~~28. The method of claim 14, wherein the CAR and/or TCR expression ~~construct~~ vector is a lentiviral vector or retroviral vector.

~~30~~29. The method of claim 14, wherein the NK cells are GMP-compliant.

~~31~~30. The method of claim 14, wherein the NK cells are allogeneic.

~~32~~31. The method of claim 14, wherein the NK cells are autologous.

~~33~~32. 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, 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.

~~34~~33. The method of claim 14, wherein the CAR has antigenic specificity for CD19.

~~35~~34. The method of claim 14, wherein the CAR and/or TCR expression ~~construct~~ vector further expresses a cytokine.

~~36~~35. The method of claim ~~35~~34, wherein the cytokine is IL-15, IL-21, or IL-12.

~~37~~36. The method of claim 14, further comprising cryopreserving the population of expanded NK cells.

~~38~~37. 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~~38. 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 use in the treatment of a disease or disorder in a subject.

~~40~~39. 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~~ in the manufacture of a medicament for the treatment of an immune-related disorder in a subject.

~~41~~40. Use of NK cells of any one of claims 1-11 ~~or NK cells produced by the method of any one of claims 14-37~~ in the manufacture of a medicament for treating an immune-related disorder in a subject.

~~42~~41. The use of claim ~~41~~40, wherein the immune-related disorder is a cancer, autoimmune disorder, graft versus host disease, allograft rejection, or inflammatory condition.

~~43~~42. The use of claim ~~41~~40, wherein the immune-related disorder is an inflammatory condition and the ~~immune~~ NK cells have essentially no expression of glucocorticoid receptor.

~~44~~43. The use of claim ~~43~~42, wherein the subject is one that has been or is being administered a steroid therapy.

~~45~~44. The use of claim ~~41~~40, wherein the NK cells are autologous.

~~46~~45. The use of claim ~~41~~40, wherein the NK cells are allogeneic.

~~47~~46. The use of claim ~~41~~40, wherein the immune-related disorder is a cancer.

~~48~~47. The use of claim ~~47~~46, wherein the cancer is a solid cancer or a hematologic malignancy.

~~49~~48. The use of claim ~~41~~40, wherein at least a second therapeutic agent is to be further administered.

~~50~~49. The use of claim ~~49~~48, wherein the at least a second therapeutic agent comprises chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy.

~~51~~50. The use of claim ~~49~~48, wherein the NK cells and/or the at least a second therapeutic agent are to be administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

~~52~~51. The use of claim ~~49~~48, wherein the NK cells with essentially no expression of CISH have enhanced function as compared to NK cells with expression of CISH.

~~53~~52. The use of claim ~~52~~51, 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~~53. The use of claim ~~52~~51, wherein the enhanced function is measured by increased expression of granzyme-b, perforin, TRAIL, CD3z, Eomes, T-bet, DAP12, DNAM, CD25 and/or Ki67.