[6] However, to date all of these approaches have seen limited success. Therefore, there remains a need for the development of better therapeutic approaches for immunotherapy.

Summary

[6.1] In one aspect, the present disclosure refers to a modified natural killer (NK) cell, wherein the modified NK cell: (a) does not express endogenous CD3, CD4, and/or CD8; and (b) expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof; wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and (c) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

[6.2] In another aspect, the present disclosure refers to modified natural killer (NK) cell, wherein the modified cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and further comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16)

[6.3] In another aspect, the present disclosure refers to a population of cells comprising the modified NK cell as disclosed herein, or the modified cell disclosed herein.

[6.4] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the population of NK cells as disclosed herein.

[6.5] In another aspect, the present disclosure refers to an isolated *in vitro* population of natural killer (NK) cells, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or 100% of the NK cells in the population: (a) comprise a rearranged T-cell receptor (TCR) locus; (b) do not express endogenous CD3; (c) express endogenous CD56 (NCAM), CD49, and/or CD45; (d) comprise a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH); (e) comprise at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16); and (f) expresses at least one endogenous gene encoding: (i) FcγRIII (CD16); (ii) natural killer group-2 member D (NKG2D); (iii) CD69; (iv) a natural cytotoxicity receptor; or any combination of two or more thereof.

[6.6] In another aspect, the present disclosure refers to the use of the NK cell as disclosed herein, the modified NK cell as disclosed herein, the population of NK cells as disclosed herein, the pharmaceutical composition as disclosed herein, or the isolated *in vitro* population of NK cells as disclosed herein, in the manufacture of a medicament for treating a proliferative disease.

[6.7] In another aspect, the present disclosure refers to a method of generating the NK cell as disclosed herein, the modified NK cell as disclosed herein, the population of NK cells as disclosed herein, or the isolated *in vitro* population of NK cells as disclosed herein, the method comprising: (a) obtaining an induced pluripotent stem cell (iPSC); (b) modifying the iPSC, or an undifferentiated or differentiated daughter cell thereof, to comprise express at least one exogenous nucleic acid expression construct of (1) and/or to comprise a loss of function in at least one gene of (2); (c) directing differentiation of the iPSC to hematopoietic lineage cells, wherein the hematopoietic lineage cells retain the edited genetic loci comprised in the iPSCs.

[6.8] In another aspect, the present disclosure refers to a method of generating a modified NK cell, the method comprising: differentiating a genetically modified pluripotent stem cell into a lymphocyte, wherein the genetically modified pluripotent stem cell comprises: a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

[6.9] In another aspect, the present disclosure refers to a method of generating the modified NK cell as disclosed herein, the method comprising: reprogramming a donor cell to a pluripotent state; editing a target locus in the donor cell genome; and differentiating the reprogrammed donor cell into the modified NK cell.

[6.10] In another aspect, the present disclosure refers to a modified NK cell as disclosed herein, the modified NK cell as disclosed herein, or the population of NK cells as disclosed herein for use in therapy.

[6.11] In yet another aspect, the present disclosure refers to the use of the modified NK cell as disclosed herein, the modified NK cell as disclosed herein, or the population of NK cells as disclosed herein in the manufacture of a medicament for treating a proliferative disease.

[6.12] In another aspect, the present disclosure refers to a modified NK cell, wherein the modified NK cell: (a) does not express endogenous CD3, CD4, and/or CD8; and (b) expresses at least one endogenous gene encoding: (i) CD56 (NCAM), CD49, and/or CD45; (ii) NK cell receptor (cluster of differentiation 16 (CD16)); (iii) natural killer group-2 member D (NKG2D); (iv) CD69; (v) a natural cytotoxicity receptor; or any combination of two or more thereof; wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

[6.13] In another aspect, the present disclosure refers to a modified NK cell, wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

[6.14] In another aspect, the present disclosure refers to an isolated population of NK cells, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the NK cells in the population: (a) comprise a rearranged T-cell receptor (TCR) locus; (b) do not express endogenous CD3; (c) express endogenous CD56 (NCAM), CD49, and/or CD45; and (d) expresses at least endogenous gene encoding: (i) NK cell receptor (cluster of differentiation 16 (CD16)); (ii) natural killer group-2 member D (NKG2D); (iii) CD69; (iv) a natural cytotoxicity receptor; or any combination of two or more thereof; and wherein the modified lymphocyte comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

[6.15] In another aspect, the present disclosure refers to a method, the method comprising: differentiating a genetically modified pluripotent stem cell into an NK cell, wherein the genetically modified pluripotent stem cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

CLAIMS

1. A modified natural killer (NK) cell, wherein the modified NK cell:

(a) does not express endogenous CD3, CD4, and/or CD8; and

(b) expresses at least one endogenous gene encoding:

(i) CD56 (NCAM), CD49, and/or CD45;

(ii) NK cell receptor immunoglobulin gamma Fc region receptor III (FcγRIII, cluster of differentiation 16 (CD16));

(iii) natural killer group-2 member D (NKG2D);

(iv) CD69;

(v) a natural cytotoxicity receptor;

or any combination of two or more thereof;

wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and

(c) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

2. The modified NK cell of claim 1, wherein the modified NK cell further:

(1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) a chimeric antigen receptor (CAR);

(ii) interleukin 15 (IL-15);

(iii) IL-15 receptor (IL-15R), or a variant thereof;

(iv) interleukin 12 (IL-12);

(v) IL-12 receptor (IL-12R), or a variant thereof;

(vi) human leukocyte antigen G (HLA-G);

(vii) human leukocyte antigen E (HLA-E);

(viii) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);
(iii) β-2 microgobulin (B2M);
(iv) programmed cell death protein 1 (PD-1);
(v) class II, major histocompatibility complex, transactivator (CIITA);
(vi) natural killer cell receptor NKG2A (natural killer group 2A);
(vii) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;
(viii) cluster of differentiation 32B (CD32B, FCGR2B);
(ix) T cell receptor alpha constant (TRAC);
or any combination of two or more thereof.

3. The modified NK cell of claim 1, wherein the NK cell further exhibits a loss of function of :
(i) TIGIT, ADORA2A, or NKG2A;
(ii) TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or
(iii) TIGIT, ADORA2A and NKG2A.

4. The modified NK cell of any one of claims 1 to 3, wherein the NK cell comprises a rearranged endogenous T-cell receptor (TCR) locus.

5. The modified NK cell of claim 4, wherein the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

6. The modified NK cell of any one of claims 1 to 5 wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

7. The modified NK cell of any of claims 2-6, wherein the IL-15R variant is a constitutively active IL-15R variant and/or wherein the IL12-R variant is a constitutively active IL12-R variant.

8. The modified NK cell of claim 7, wherein the constitutively active IL-15R variant is a fusion between IL-15R and an IL-15R agonist (IL-15RA), and/or wherein the constitutively active IL-12R variant is a fusion between IL-12R and an IL-12R agonist (IL-12RA).

9. The modified NK cell of claim 8, wherein the IL-15R agonist is IL-15, or an IL-15R-binding variant thereof; and/or wherein the IL-12R agonist is IL-12, or an IL-12R-binding variant thereof.

10. The modified NK cell of any one of claims 1-9, wherein the loss of TGFβR2 is associated with exogenous expression of a dominant-negative variant of TGFβ receptor II (DN-TGFβR2).

11. The modified NK cell of claim 1, wherein the CAR is capable of binding mesothelin, EGFR, HER2, MICA/B, BCMA, CD19, CD22, CD20, CD33, CD123, androgen receptor, PSMA, PSCA, Muc1, HPV viral peptides (ie. E7), EBV viral peptides, CD70, WT1, CEA, EGFRvIII, IL13Rα2, GD2, CA125, CD7, EpCAM, Muc16, or CD30,

12. The modified NK cell of any one of claims 1-11, wherein the NK cell is derived from a pluripotent or multipotent stem cell.

13. The modified NK cell of claim 12, wherein the multipotent stem cell is a hematopoietic stem cell (HSC).

14. The modified NK cell of claim 12, wherein the pluripotent stem cell is an induced pluripotent stem cell (iPSC).

15. The modified NK cell of claim 12, wherein the pluripotent stem cell is an embryonic stem cell (ESC).

16. The modified NK cell of any one of claims 2 or 4 to 12, wherein the NK cell is derived from a pluripotent or multipotent stem cell that comprises at least one or more exogenous nucleic acid constructs encoding any of (1)(i)-(1)(viii), or any combination thereof; and/or at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(ix), or any combination thereof, in the lymphocyte.

17. The modified NK cell of claim 16, wherein the NK cell is derived from a pluripotent or multipotent stem cell that comprises at least one genomic alteration that effects the loss-of-function of any of (2)(i)-(2)(ix), or any combination thereof, in the NK cell.

18. The modified NK cell of claim 17, wherein the at least one genomic alteration that effects the loss-of-function of any of one or more (2)(i)-(2)(ix) in the NK cell comprises an insertion of an exogenous nucleic acid construct.

19. The modified NK cell of claim 18, wherein the exogenous nucleic acid construct encodes any of (1)(i)-(1)(viii), or any combination thereof.

20. The modified NK cell of any one of claims 2-19, wherein the NK cell exhibits a loss-of-function in two or more of the genes/proteins listed under (2).

21. The modified NK cell of any one of claims 2-20, wherein the NK cell comprises an indel or an insertion of an exogenous nucleotide construct into a genomic locus harboring a gene or encoding a protein under (2).

22. The modified NK cell of any one of claims 2-21, wherein the NK cell comprises an indel or an insertion of an exogenous nucleotide construct into two or more genomic loci harboring a gene or encoding a protein under (2).

23. The modified NK cell of any one of claims 1-22, wherein the NK cell is obtained by editing a genomic locus with an RNA-guided nuclease.

24. The modified NK cell of claim 23, wherein the RNA-guided nuclease is a CRISPR/Cas nuclease.

25. The modified NK cell of claim 23, wherein the RNA-guided nuclease is selected from the group consisting of SpCas9, SaCas9, (KKH) SaCas9, AsCpf1 (AsCas12a), LbCpf1, (LbCas12a), CasX, CasY, Cas12h1, Cas12i1, Cas12c1, Cas12c2, eSpCas9, Cas9-HF1, HypaCas9, dCas9-Fokl, Sniper-Cas9, xCas9, AaCas12b, evoCas9, SpCas9-NG, VRQR, VRER, NmeCas9, CjCas9, BhCas12b, and BhCas12b V4.

26. The modified NK cell of any one of claims 2-25, wherein the NK cell is obtained by editing two or more genomic loci harboring genes encoding any of the proteins under (2).

27. The modified NK cell of claim 26, wherein at least two of the two or more genomic loci harboring genes encoding any of the proteins under (2) have been edited by a different RNA-guided nuclease.

28. The modified NK cell of claim 26, wherein at least one of the two or more genomic loci harboring genes encoding any of the proteins under (2) has been edited by Cas9, and wherein at least one of the loci has been edited by Cpf1.

29. The modified NK cell of any one of claims 1-28, wherein the modified NK cell expresses endogenous CD56, CD49, and CD45.

30. A modified natural killer (NK) cell, wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and further comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

31. The modified NK cell of claim 30, wherein the modified NK cell further

(1) comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) a chimeric antigen receptor (CAR);

(ii) interleukin 15 (IL-15);

(iii) IL-15 receptor (IL-15R), or a variant thereof;

(iv) interleukin 12 (IL-12);

(v) IL-12 receptor (IL-12R), or a variant thereof;

(vi) human leukocyte antigen G (HLA-G);

(vii) human leukocyte antigen E (HLA-E);

(viii) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof~~;~~

and/or

(2) exhibits a loss of function of at least one of:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iii) β-2 microgobulin (B2M);

(iv) programmed cell death protein 1 (PD-1);

(v) class II, major histocompatibility complex, transactivator (CIITA);

(vi) natural killer cell receptor NKG2A (natural killer group 2A);

(vii) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;

(viii) cluster of differentiation 32B (CD32B, FCGR2B);

(ix) T cell receptor alpha constant (TRAC)

or any combination of two or more thereof.

32. The modified NK cell of claim 31, wherein the modified NK cell exhibits a loss of function of:

(i) TIGIT, ADORA2A, or NKG2A;

(ii) TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or

(iii) TIGIT, ADORA2A and NKG2A.

33. The modified NK cell of claim 31, wherein the cell is a differentiated daughter cell derived from a pluripotent stem cell.

34. The modified NK cell of claim 33, wherein the modified cell does not express an endogenous T-cell co-receptor.

35. The modified NK cell of claim 30, wherein the NK cell comprises a rearranged endogenous TCR locus, wherein the rearranged TCR comprises TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

36. The modified NK cell of any one of claims 30-32, 33 or 35, wherein the modified NK cell expresses at least one endogenous gene encoding:

(i) CD56 (NCAM), CD49, and/or CD45;

(ii) FcγRIII (CD16);

(iii) natural killer group-2 member D (NKG2D);

(iv) CD69;

(v) a natural cytotoxicity receptor;

or any combination of two or more thereof.

37. The modified NK cell of claim 36, wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

38. A population of natural killer (NK) cells comprising the modified NK cell of any one of claims 1-29, or the modified NK cell of any one of claims 30-37.

39. A pharmaceutical composition comprising the population of NK cells of claim 38.

40. An isolated *in vitro* population of nautral killer (NK) cells, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or 100% of the NK cells in the population:

(a) comprise a rearranged T-cell receptor (TCR) locus;

(b) do not express endogenous CD3;

(c) express endogenous CD56 (NCAM), CD49, and/or CD45;

(d) comprise a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH);

(e) comprise at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16); and

(f) expresses at least one endogenous gene encoding:

(i) FcγRIII (CD16);

(ii) natural killer group-2 member D (NKG2D);

(iii) CD69;

(iv) a natural cytotoxicity receptor;

or any combination of two or more thereof.

41. The isolated *in vitro* population of natural killer (NK) cells of claim 40, wherein at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or 100% of the NK cells in the population further

(1) comprise at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding:

(i) chimeric antigen receptor (CAR);

(ii) interleukin 15 (IL-15);

(iii) IL-15 receptor (IL-15R), or a variant thereof;

(iv) interleukin 12 (IL-12);

(v) IL-12 receptor (IL-12R), or a variant thereof;

(vi) human leukocyte antigen G (HLA-G);

(vii) human leukocyte antigen E (HLA-E);

(viii) leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof~~;~~

and/or

(2) exhibit a loss of function of at least one of:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iii) β-2 microgobulin (B2M);

(iv) programmed cell death protein 1 (PD-1);

(v) class II, major histocompatibility complex, transactivator (CIITA);
(vi) natural killer cell receptor NKG2A (natural killer group 2A);
(vii) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;
(viii) cluster of differentiation 32B (CD32B, FCGR2B);
(ix) T cell receptor alpha constant (TRAC);
or any combination of two or more thereof

42. The isolated *in vitro* population of NK cells of claim 41, wherein the modified NK cell exhibits a loss of function of :
(i) TIGIT, ADORA2A, or NKG2A;
(ii) TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or
(iii) TIGIT, ADORA2A and NKG2A.

43. The isolated *in vitro* population of NK cells of any one of claims 40 to 42, wherein the rearranged TCR locus comprises of TCRα VJ and/or TCRβ V(D)J section rearrangements and complete V-domain exons.

44. The isolated *in vitro* population of NK cells of claim 43, wherein the rearranged endogenous TCR locus consists of no more than two rearranged alleles.

45. The isolated *in vitro* population of NK cells of claim 40 or 41, wherein the natural cytotoxicity receptor is NKp30, NKp44, NKp46, and/or CD158b.

46. The isolated *in vitro* population of NK cells of any one of claims 40-45, wherein the population does not comprise more than 1%, more than 0.1%, more than 0.001%, more than 0.0001%, more than 0.00001%, more than 0.000001%, more than 0.0000001%, more than 0.00000001%, more than 0.000000001%, more than 0.0000000001%, or more than more than 0.00000000001% of cells expressing a reprogramming factor from an exogenous nucleic acid construct.

47. The isolated *in vitro* population of NK cells of claim 46, wherein the population does not comprise a cell expressing a reprogramming factor from an exogenous nucleic acid construct.

48. The isolated *in vitro* population of NK cells of claim 46 or claim 47, wherein the reprogramming factor is Oct-4 and/or Sox-2.

49. The isolated *in vitro* population of NK cells of claim 40 or 41, wherein the population does not comprise cells harboring episomal expression constructs encoding a reprogramming factor.

50. The isolated *in vitro* population of NK cells of any one of claims 41-49, wherein each cell in the population of cells comprises the same combination of (1) and (2).

51. The isolated *in vitro* population of NK cells of any one of claims 40-50, wherein the cell population comprises less than 0.001%, less than 0.002%, less than 0.003%, less than 0.004%, less than 0.005%, less than 0.006%, less than 0.007%, less than 0.008%, less than 0.009%, less than 0.01%, less than 0.02%, less than 0.03%, less than 0.04%, less than 0.05%, less than 0.06%, less than 0.07%, less than 0.08%, less than 0.09%, less than 0.1%, less than 0.2%, less than 0.3%, less than 0.4%, less than 0.5%, less than 0.6%, less than 0.7%, less than 0.8%, less than 0.9%, less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% cell that harbor a chromosomal translocation.

52. The NK cell of any one of claims 1-29, the modified NK cell of any one of claims 30-37, the population of NK cells of claim 38, the pharmaceutical composition of claim 39, or the isolated *in vitro* population of NK cells of any one of claims 40-51, for use in therapy.

53. Use of the NK cell of any one of claims 1-29, the modified NK cell of any one of claims 30-37, the population of NK cells of claim 38, the pharmaceutical composition of claim 39, or the isolated *in vitro* population of NK cells of any one of claims 40-51, in the manufacture of a medicament for treating a proliferative disease.

54. The use of claim 53, wherein the proliferative disease is cancer.

55. The use of claim 54, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, gastric cancer, renal cell carcinoma (RCC), or non-small cell lung cancer (NSCLC), solid tumors, bladder cancer, hepatocellular carcinoma, prostate cancer, ovarian/uterine cancer, pancreatic cancer, mesothelioma, melanoma, glioblastoma, HPV-associated and/or HPV-positive cancers such as cervical and HPV+ head and neck cancer, oral cavity cancer, cancer of the pharynx, thyroid cancer, gallbladder cancer, soft tissue sarcomas, and hematological cancers like ALL, CLL, NHL, DLBCL, AML, CML, multiple myeloma (MM).

56. A method of generating the NK cell of any one of claims 2-29, the modified NK cell of any one of claims 31-37, the population of NK cells of claim 38, or the isolated *in vitro* population of NK cells of any one of claims 41-51, the method comprising:

(a) obtaining an induced pluripotent stem cell (iPSC);

(b) modifying the iPSC, or an undifferentiated or differentiated daughter cell thereof, to comprise the at least one exogenous nucleic acid expression construct and/or to comprise the loss of function in at least one gene of of TGFβR2 and CISH;

(c) directing differentiation of the iPSC to hematopoietic lineage cells,

wherein the hematopoietic lineage cells retain the edited genetic loci comprised in the iPSCs.

57. The method of claim 56, wherein directing differentiation comprises:

(i) contacting iPSCs with a composition comprising a BMP pathway activator, and optionally bFGF, to obtain mesodermal cells; and

(ii) contacting the mesodermal cells with a composition comprising a BMP pathway activator, bFGF, and a WNT pathway activator, to obtain mesodermal cells having definitive hemogenic endothelium (HE) potential, wherein the mesodermal cells having definitive hemogenic endothelium (HE) potential are capable of providing hematopoietic lineage cells;

wherein mesodermal cells and mesodermal cells having definitive HE potential are obtained in steps (i) and (ii) without the step of forming embryoid bodies;

wherein the hematopoietic lineage cells comprise definitive hemogenic endothelium cells, hematopoietic stem and progenitor cells (HSC), hematopoietic multipotent progenitor cell (MPP), pre-T cell progenitor cells, pre-NK cell progenitor cells, T cell progenitor cells, NK cell progenitor cells, T cells, NK cells, NKT cells, or B cells.

58. The method of claim 57, wherein directing differentiation of iPSCs to hematopoietic lineage cells further comprises: contacting the mesodermal cells having definitive HE potential with a composition comprising bFGF and a ROCK inhibitor to obtain definitive HE cells.

59. The method of claim 56, claim 57, or claim 58, further comprising: contacting the definitive HE cells with a composition comprising a BMP activator, and optionally a ROCK inhibitor, and one or more growth factors and cytokines selected from the group consisting of TPO, IL3, GMCSF, EPO, bFGF, VEGF, SCF, IL6, Flt3L and IL11 to obtain hematopoietic multipotent progenitor cells (MPP).

60. The method of any one of claims 56-59, further comprising: contacting the definitive HE cells with a composition comprising one or more growth factors and cytokines selected from the group consisting of SCF, Flt3L, TPO, IL7 and IL15, and optionally one or more of a BMP activator, a ROCK inhibitor, VEGF and bFGF to obtain pre-NK cell progenitors, NK cell progenitors, and/or NK cells.

61. The method of any one of claims 56-60, further comprising: prior to step c), contacting the pluripotent stem cells with a composition comprising a MEK inhibitor, a GSK3 inhibitor, and a ROCK inhibitor, to seed and expand the cells.

62. The method of any one of claims 56-60, further comprising detecting a rearranged T-cell receptor (TCR) locus in the hematopoietic lineage cells.

63. The method of claim 62, further comprising selecting the hematopoietic lineage cells comprising the rearranged TCR locus based on the TCR encoded by the rearranged TCR locus binding an antigen of interest.

64. The method of claim 63, wherein the antigen of interest is a tumor antigen.

65. A method of generating the modified NK cell of any one of claims 1-29, the method comprising:

reprogramming a donor cell to a pluripotent state;

editing a target locus in the donor cell genome; and

differentiating the reprogrammed donor cell into the modified NK cell.

66. The method of claim 65, wherein the editing is performed before or during the step of reprogramming of the donor cell to a pluripotent state.

67. The method of claim 65 or 66, wherein the donor cell is a fibroblast, a peripheral blood cell, a lymphocyte, or a T cell.

68. A method of generating a modified NK cell, the method comprising:

differentiating a genetically modified pluripotent stem cell into an NK cell,

wherein the genetically modified pluripotent stem cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

69. The method of claim 68, wherein the modified NK cell further comprises:

(1) an exogenous nucleic acid expression construct comprising:

(i) a nucleic acid sequence encoding a chimeric antigen receptor (CAR);

(ii) a nucleic acid sequence encoding interleukin 15 (IL-15);

(iii) a nucleic acid sequence encoding interleukin 15 receptor (IL-15R) or a variant thereof;

(iv) a nucleic acid sequence encoding interleukin 12 (IL12);

(v) a nucleic acid sequence encoding interleukin-12 receptor (IL-12R) or a variant thereof;

(vi) a nucleic acid sequence encoding human leukocyte antigen G (HLA-G);

(vii) a nucleic acid sequence encoding human leukocyte antigen E (HLA-E);

(viii) a nucleic acid sequence encoding leukocyte surface antigen cluster of differentiation CD47 (CD47);

or any combination of two or more thereof; and

(2) an indel, or an insertion of an exogenous nucleic acid in one or more of the following genetic loci:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);
(iii) β-2 microgobulin (B2M);
(iv) programmed cell death protein 1 (PD-1, CD279);
(v) class II, major histocompatibility complex, transactivator (CIITA);
(vi) natural killer cell receptor NKG2A (natural killer group 2A);
(vii) two or more HLA class II histocompatibility antigen alpha chain genes, and/or two or more HLA class II histocompatibility antigen beta chain genes;
(viii) cluster of differentiation 32B (CD32B, FCGR2B);
(ix) T cell receptor alpha constant (TRAC);
or any combination of two or more thereof,
wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.

70. The method of claim 69, wherein the indel, or the insertion of an exogenous nucleic acid is in the following genetic loci :
(i) TIGIT, ADORA2A, or NKG2A;
(ii) TIGIT and ADORA2A, TIGIT and NKG2A, or ADORA2A and NKG2A; or
(iii) TIGIT, ADORA2A and NKG2A,
wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.

71. The method of claim 69, wherein the exogenous nucleic acid of (2) is the exogenous nucleic acid of (1).

72. The method of claim 68 or claim 71, wherein the pluripotent stem cell is an iPS cell.

73. The method of claim 68 or claim 71, wherein the differentiating comprises contacting the pluripotent stem cell with a differentiation medium or a sequence of differentiation media.

74. The modified NK cell of any one of claims 1-29, wherein the two or more HLA class II histocompatibility antigen alpha chain genes are selected from HLA-DQA1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA2, and HLA-DOA.

75. The modified NK cell of any one of claims 2-29, wherein the two or more HLA class II histocompatibility antigen beta chain genes are selected from HLA-DMB, HLA-DOB, HLA-DPB1, HLA-DQB1, HLA-DQB3, HLA-DQB2, HLA-DRB1, HLA-DRB3, HLA-DRB4, and HLA-DRB5.

76. The modified NK cell of any one of claims 2-29 and 74-75, the modified NK cell of any one of claims 31-37, the population of NK cells of claim 38, the pharmaceutical composition of claim 39, or the isolated *in vitro* population of NK cells of any one of claims 41-51, wherein the exogenous nucleic acid expression construct comprises the nucleic acid sequence encoding the non-naturally occurring variant of FcγRIII (CD16) under the control of a heterologous promoter.

77. The modified NK cell, modified NK cell, population of NK cells, pharmaceutical composition, or isolated *in vitro* population of NK cells of claim 76, wherein the heterologous promoter is an NK-cell specific promoter.

78. The modified NK cell, modified NK cell, population of NK cells, pharmaceutical composition, or isolated *in vitro* population of NK cells of claim 76, wherein the NK-cell specific promoter is a promoter of a gene known to be expressed specifically in NK cells, or a variant thereof.

79. The modified NK cell, modified NK cell, population of NK cells, pharmaceutical composition, or isolated *in vitro* population of NK cells, of claim 76, wherein the NK-cell specific promoter is a CD56 (NCAM), CD49, or CD45 promoter, or a variant thereof.

80. The modified NK cell, modified NK cell, population of NK cells, pharmaceutical composition, or isolated in vitro population of NK cell, of claim 76, wherein the NK-cell specific promoter is an FcγRIII promoter, an NKG2D promoter, a CD69 promoter, or a natural cytotoxicity receptor promoter, or a variant thereof.

81. The modified NK cell of any one of the claims 74-76, the modified NK cell of any one of the claims 76-80, or the population of NK cells of any one of the claims 76-80 for use in therapy.

82. Use of the modified NK cell of any one of the claims 74-76, the modified NK cell of any one of the claims 76-80, or the population of NK cells of any one of the claims 76-80 in the manufacture of a medicament for treating a proliferative disease.

83. The use of claim 82, wherein the proliferative disease is cancer.

84. The use of claim 83, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, gastric cancer, renal cell carcinoma (RCC), or non-small cell lung cancer (NSCLC), solid tumors, bladder cancer, hepatocellular carcinoma, prostate cancer, ovarian/uterine cancer, pancreatic cancer, mesothelioma, melanoma, glioblastoma, HPV-associated and/or HPV-positive cancers such as cervical and HPV+ head and neck cancer, oral cavity cancer, cancer of the pharynx, thyroid cancer, gallbladder cancer, soft tissue sarcomas, and hematological cancers like ALL, CLL, NHL, DLBCL, AML, CML, multiple myeloma (MM).

85. A modified NK cell, wherein the modified NK cell:

(a) does not express endogenous CD3, CD4, and/or CD8; and

(b) expresses at least one endogenous gene encoding:

(i) CD56 (NCAM), CD49, and/or CD45;

(ii) NK cell receptor (cluster of differentiation 16 (CD16));

(iii) natural killer group-2 member D (NKG2D);

(iv) CD69;

(v) a natural cytotoxicity receptor;

or any combination of two or more thereof;

wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

86. The modified NK cell of claim 85, wherein the modified NK cell further

(1) comprises at least one exogenous nucleic acid construct encoding:

(i) a chimeric antigen receptor (CAR);

(ii) interleukin 15 (IL-15);

(iii) IL-15 receptor (IL-15R), or a variant thereof;
(iv) interleukin 12 (IL-12);
(v) IL-12 receptor (IL-12R), or a variant thereof;
(vi) human leukocyte antigen G (HLA-G);
(vii) human leukocyte antigen E (HLA-E);
or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:
(i) adenosine A2a receptor (ADORA2A);
(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);
(iii) β-2 microgobulin (B2M);
(iv) programmed cell death protein 1 (PD-1);
(v) class II, major histocompatibility complex, transactivator (CIITA);
or any combination of two or more thereof.

87. A modified NK cell, wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

88. The modified NK cell of claim 90, wherein the modified NK cell further

(1) comprises at least one exogenous nucleic acid construct encoding:
(i) a chimeric antigen receptor (CAR);
(ii) interleukin 15 (IL-15);
(iii) IL-15 receptor (IL-15R), or a variant thereof;
(iv) interleukin 12 (IL-12);
(v) IL-12 receptor (IL-12R), or a variant thereof;
(vi) human leukocyte antigen G (HLA-G);
(vii) human leukocyte antigen E (HLA-E);
or any combination of two or more thereof;

and/or

(2) exhibits a loss of function of at least one of:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iii) β-2 microgobulin (B2M);

(iv) programmed cell death protein 1 (PD-1);

(v) class II, major histocompatibility complex, transactivator (CIITA);

or any combination of two or more thereof.

89. An isolated population of NK cells, wherein the population comprises at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the NK cells in the population:

(a) comprise a rearranged T-cell receptor (TCR) locus;

(b) do not express endogenous CD3;

(c) express endogenous CD56 (NCAM), CD49, and/or CD45; and

(d) expresses at least endogenous gene encoding:

(i) NK cell receptor (cluster of differentiation 16 (CD16));

(ii) natural killer group-2 member D (NKG2D);

(iii) CD69;

(iv) a natural cytotoxicity receptor;

or any combination of two or more thereof; and

wherein the modified NK cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

90. The isolated population of NK cells of claim 89, wherein at least $1x10^3$, at least $1x10^4$, at least $1x10^5$, at least $2x10^5$, at least $3x10^5$, at least $4x10^5$, at least $5x10^5$,at least $1x10^6$, at least $2x10^6$, at least $3x10^6$, at least $4x10^6$, at least $5x10^6$,at least $1x10^7$, at least $1x10^7$, at least $2x10^7$, at least $3x10^7$, at least $4x10^7$, at least $5x10^7$,at least $1x10^8$, at least $2x10^8$, at least $3x10^8$, at least $4x10^8$, at least $5x10^8$,at least $1x10^9$, at least $1x10^9$, at least $2x10^9$, at least $3x10^9$, at least $4x10^9$, at least $5x10^9$,at least $1x10^{10}$, at least $2x10^{10}$, at least $3x10^{10}$, at least $4x10^{10}$, at least $5x10^{10}$, at least $1x10^{11}$, or at least $1x10^{12}$ cells, and wherein at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.9%, at least 99.99%, at least 99.999%, or virtually 100% of the NK cells in the population further

(1) comprise at least one exogenous nucleic acid construct encoding:

(i) chimeric antigen receptor (CAR);

(ii) interleukin 15 (IL-15);

(iii) IL-15 receptor (IL-15R), or a variant thereof;

(iv) interleukin 12 (IL-12);

(v) IL-12 receptor (IL-12R), or a variant thereof;

(vi) human leukocyte antigen G (HLA-G);

(vii) human leukocyte antigen E (HLA-E);

or any combination of two or more thereof;

and/or

(2) exhibit a loss of function of at least one of:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iii) β-2 microgobulin (B2M);

(iv) programmed cell death protein 1 (PD-1);

(v) class II, major histocompatibility complex, transactivator (CIITA);

or any combination of two or more thereof.

91. A method, the method comprising:

differentiating a genetically modified pluripotent stem cell into an NK cell,

wherein the genetically modified pluripotent stem cell comprises a loss of function of transforming growth factor beta receptor 2 (TGFβR2) and cytokine inducible SH2 containing protein (CISH), and comprises at least one exogenous nucleic acid expression construct

comprising a nucleic acid sequence encoding a non-naturally occurring variant of FcγRIII (CD16).

92. The method of claim 91, wherein the genetically modified pluripotent stem cell further comprises

(1) an exogenous nucleic acid comprising:

(i) a nucleic acid encoding a chimeric antigen receptor (CAR);

(ii) a nucleic acid encoding interleukin 15 (IL-15) and/or interleukin 12 (IL12);

(iii) a nucleic acid encoding human leukocyte antigen G (HLA-G);

(iv) a nucleic acid encoding human leukocyte antigen E (HLA-E);

or any combination of two or more thereof; and

(2) an indel, or an insertion of an exogenous nucleic acid in one or more of the following genetic loci:

(i) adenosine A2a receptor (ADORA2A);

(ii) T cell immunoreceptor with Ig and ITIM domains (TIGIT);

(iii) β-2 microgobulin (B2M);

(iv) programmed cell death protein 1 (PD-1, CD279);

(v) class II, major histocompatibility complex, transactivator (CIITA);

or any combination of two or more thereof,

wherein the indel or insertion results in a loss-of-function of a gene product encoded by the respective genetic locus or loci.