CLAIMS

1. An engineered cytokine-induced memory-like human NK cell or a population of said cells, wherein the engineered NK cell or population of said cells expresses a chimeric antigen receptor (CAR) polypeptide comprising an intracellular domain, a transmembrane domain and an extracellular binding domain, wherein the extracellular binding domain specifically binds to an antigen comprising an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein.

2. The engineered NK cell or population of cells of claim 1, wherein the extracellular binding domain does not bind to, or substantially does not bind to: (a) the MHC class I protein alone, and/or (b) a control peptide in complex with the MHC class I protein, optionally wherein the control peptide is an NY-ESO-1 epitope or influenza virus M1 epitope.

3. The engineered NK cell or population of cells of claim 1 or 2, wherein the NPM1c neoepitope comprises an amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, wherein X_1 is selected from A, V, L or I, wherein X_2 is selected from A, T, S, V, L, I, M or Q, wherein X_3 is selected from Q or N, wherein X_4 is selected from D or E, wherein X_5 is selected from L, I, V, M, A or F, wherein X_6 is selected from C, S, or A, wherein X_7 is selected from L, I, V, M, A, or F, wherein X_8 is selected from A, V, L or I, and wherein X_9 is selected from L, I, V, M or A.

~~4. The engineered NK cell or population of cells of claim 3, wherein X_1 is selected from A or V, wherein X_2 is selected from V, I, or L, wherein X_3 is selected from Q or N, wherein X_4 is selected from D or E, wherein X_5 is selected from L or I, wherein X_6 is selected from C or S, wherein X_7 is selected from V, L or I, wherein X_8 is selected from A or V, and wherein X_9 is selected from V, I, or L.~~

~~5. The engineered NK cell or population of cells of claim 4, wherein X_1 is A, wherein X_2 is selected from V, I, or L, wherein X_3 is Q, wherein X_4 is D, wherein X_5 is L, wherein X_6 is C, wherein X_7 is L, wherein X_8 is A, and wherein X_9 is selected from V, I, or L.~~

4.6. The engineered NK cell or population of cells of any one of claims 1-34, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: AIQDLCLAV (SEQ ID NO:1) or AIQDLCVAV (SEQ ID NO: 71).

57. The engineered NK cell or population of cells of claim 1 or 2, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: CLAVEEVSL (SEQ ID NO:72), VEEVSLRK (SEQ ID NO:73), AVEEVSLR (SEQ ID NO:74), AVEEVSLRK (SEQ ID NO:75), CLAVEEVSLRK (SEQ ID NO:76).

~~8. The engineered NK cell or population of cells of any one of claims 1-6, wherein the neoepitope comprises the amino acid sequence AIQDLCLAV (SEQ ID NO:1).~~

~~9. The engineered NK cell or population of cells of any one of claims 1-8, wherein the neoepitope is 7, 8, 9, 10, 11, or 12 amino acid residues in length.~~

~~10. The engineered NK cell or population of cells of any one of claims 1-9, wherein the MHC class I protein is an HLA-A*02 protein or is encoded by the HLA-A*02 allele group.~~

~~11. The engineered NK cell or population of cells of any one of claims 1-10, wherein the MHC class I protein is encoded by the HLA-A*02:01 allele.~~

612. The engineered NK cell or population of cells of any one of claims 1-511, wherein the extracellular domain comprises:

(i) a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2 and VH CDR3, said VH CDR1, VH CDR2 and VH CDR3 being the CDRs of a VH that has an amino acid sequence of SEQ ID NO: 5, and/or

(ii) a light chain variable region (VL) comprising VL complementarity determining region (CDR)1, VL CDR2 and VL CDR3, said VL CDR1, VL CDR2 and VL CDR3 being the CDRs of a VL that has an amino acid sequence of SEQ ID NO: 3.

~~13. The engineered NK cell or population of cells of any one of claims 1-12, wherein the extracellular domain comprises a VH comprising VH CDR1, VH CDR2 and VH CDR3, wherein the VH CDR1 has the amino acid sequence GFTFSSYA (SEQ ID NO: 9), the VH CDR2 has the amino acid sequence ISGSGGST (SEQ ID NO: 10), and the VH CDR3 has the amino acid sequence ARLGYPTTTLLPFDY (SEQ ID NO: 11).~~

~~14. The engineered NK cell or population of cells of claim 13, wherein the extracellular domain comprises a VL comprising VL CDR1, VL CDR2 and VL CDR3, wherein the VL CDR1 has the amino acid sequence QSISSY (SEQ ID NO: 6), the VL CD2 has the amino acid sequence AAS (SEQ ID NO: 7), and the VL CD3 has the amino acid sequence QQSYSTPLT (SEQ ID NO: 8).~~

~~15. The engineered NK cell or population of cells of any one of claims 1-14, wherein the extracellular domain comprises a VH and a VL, wherein the VH comprises an amino acid sequence which is at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 5, and/or wherein the VL comprises an amino acid sequence which is at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 3.~~

~~16. The engineered NK cell or population of cells of any one of claims 1-15, wherein the extracellular domain comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 5, and/or wherein the VL comprises the amino acid sequence of SEQ ID NO: 3.~~

~~17. The engineered NK cell or population of cells of any one of claims 1-16, wherein the extracellular domain comprises an scFv.~~

~~18. The engineered NK cell or population of cells of claim 17, wherein the scFv is a human scFv.~~

~~19. The engineered NK cell or population of cells of claims 17 or 18, wherein the scFv comprises a linker.~~

~~20. The engineered NK cell or population of cells of claim 19, wherein the linker is a peptide linker.~~

~~21. The engineered NK cell or population of cells of claim 20, wherein the peptide linker is a Gly-Ser linker.~~

7~~22~~. The engineered NK cell or population of cells of claim ~~21~~1 or 2, wherein the extracellular domain comprises a scFV, wherein the scFV comprises a Gly-Ser linker ~~is~~ selected from the group consisting of (Gly4Ser) (SEQ ID NO:58), (Gly4Ser)2 (SEQ ID NO:59), (Gly4Ser)3 (SEQ ID NO:60), and (Gly4Ser)4 (SEQ ID NO:61).

~~23. The engineered NK cell or population of cells of claim 21, wherein the Gly-Ser linker comprises the amino acid sequence SGSSGGSSSG (SEQ ID NO:4).~~

~~24. The engineered NK cell or population of cells of any one of claims 17-23, wherein the scFv has an amino acid sequence which is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 2; optionally wherein the scFv comprises: (a) a VH comprising VH CDR1, VH CDR2 and VH CDR3, wherein the VH CDR1 has the amino acid sequence GFTFSSYA (SEQ ID NO: 9), the VH CDR2 has the amino acid sequence ISGSGGST (SEQ ID NO: 10), and the VH CDR3 has the amino acid sequence ARLGYPTTTLLPFDY (SEQ ID NO: 11); and/or (b) a VL comprising VL CDR1, VL CDR2 and VL CDR3, wherein the VL CDR1 has the amino acid sequence QSISSY (SEQ ID NO: 6), the VL CD2 has the amino acid sequence AAS (SEQ ID NO: 7), and the VL CD3 has the amino acid sequence QQSYSTPLT (SEQ ID NO: 8).~~

~~25. The engineered NK cell or population of cells of any one of claims 17-24, wherein the scFv comprises the amino acid sequence of SEQ ID NO: 2.~~

8~~26~~. The engineered NK cell or population of cells, according to any one of claims 1-7~~25~~, wherein the antigen is on the surface of a cancer cell.

~~27. The engineered NK cell or population of cells of claim 26, wherein the cancer is Acute Myeloid Leukemia (AML).~~

~~28. The engineered NK cell or population of cells of any one of claims 1-27, wherein the extracellular domain binds to the antigen with an equilibrium dissociation constant (Kd) of 100 nM or less, 50 nM or less, 20 nM or less, 10 nM or less, from 0.5 nM to 100 nM, or from 1 nM to 15 nM.~~

9~~29~~. The engineered NK cell or population of cells of any one of claims 1-8~~28~~, wherein the transmembrane domain comprises the transmembrane domain of CD3-zeta, CD8, CD28, DAP12, 2B4, NKG2D, CD16, NKp44 or NKp46.

10~~30~~. The engineered NK cell or population of cells of any one of claims 1-9 ~~29~~, wherein the intracellular domain comprises one or more costimulatory domains of one or more costimulatory molecules selected from the group consisting of: CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, 2B4, DAP10, CD137 and DAP12.

~~31. The engineered NK cell or population of cells polypeptide of any one of the claims 1-30, wherein the intracellular domain comprises a CD3-zeta signaling domain and a 4-1BB costimulatory domain; wherein the transmembrane domain comprises a CD8 transmembrane domain, and wherein the CAR polypeptide further comprises a CD8 hinge region.~~

~~32. The engineered NK cell or population of cells of any one of the claims 1-31, wherein the intracellular domain comprises a CD3-zeta signaling domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a 4-1BB costimulatory domain comprising the amino acid sequence set forth in SEQ ID NO: 26; wherein the CAR polypeptide comprises a CD8 transmembrane domain and a CD8 hinge region, wherein the CD8 transmembrane domain and the CD8 hinge region comprise the amino acid sequence set forth in SEQ ID NO: 25; and wherein the extracellular binding domain comprises the antibody, or antigen binding fragment thereof, and a leading sequence comprising the amino acid sequence set forth in SEQ ID NO: 23.~~

~~33. The engineered NK cell or population of cells of any one of claims 1-32, wherein the extracellular binding domain is an scFv comprising the amino acid sequence set forth in SEQ ID NO: 24, or an amino acid sequence which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 24.~~

11~~34~~. The engineered NK cell or population of cells of any one of the claims 1-10~~33~~, wherein the intracellular domain further comprises a self-cleaving peptide sequence and a cytokine, wherein cleavage of the self-cleaving peptide releases the cytokine, ~~.~~

~~35. The engineered NK cell or population of cells of claim 34,~~ wherein the cytokine is IL-12, IL-7, IL-13, IL-15, TNF-α, IFN-γ, or CCL19.

~~36. The engineered NK cell or population of cells of anyone of claims 1-28, wherein the CAR polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 22, or an amino acid sequence which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO:22.~~

~~37. The engineered NK cell or population of cells of any one of claims 1-36, which is activated following exposure of a primary human NK cell or population of primary human NK cells to one or more of the following cytokines: IL-2, IL-12, IL-7, IL-15, IL-21, and IL-18, optionally wherein the cytokine is a recombinant human cytokine.~~

~~38. The engineered NK cell or population of cells of claim 37, which is activated following exposure to a combination of IL-12 and IL-15; IL-12 and IL-18; IL-15 and IL-18; or IL-12, IL-15 and IL-18.~~

~~39. The engineered NK cell or population of cells of claim 37 or 38, wherein the NK cell or population of said cells is exposed to: IL-12 in a concentration range from 1-20 ng/mL; IL-15 in a concentration range from 1-50 ng/mL and IL-18 in a concentration range from 10-100 ng/mL,~~

~~optionally wherein the NK cell or population of said cells is exposed to about 10 ng/mL IL-12, about 1 ng/mL IL-15, and about 50 ng/mL IL-18, for a period of about 3-48 hours, about 7-24 hours, about 16-20 hours, about 12-24 hours or about 14-16 hours, optionally for a period of about 16 hours, optionally wherein the NK cell or population of said cells is exposed to about 10 ng/mL IL-12, about 50 ng/mL IL-15, and about 50 ng/mL IL-18, for a period of about 3-48 hours, about 7-24 hours, about 16-20 hours, about 12-24 hours or about 14-16 hours, optionally for a period of about 16 hours.~~

~~40. The engineered NK cell or population of cells of any one of claims 37-39, wherein the CAR polypeptide is introduced following exposure of the primary human NK cell or population of said cells to the one or more cytokines.~~

12~~41~~. The engineered NK cell or population of cells of any one of claims 1-11~~40~~, which

(i) produces increased IFNγ in the presence of one or more cytokines and/or tumor targets relative to a control human NK cell or human NK cell line;

(ii) has enhanced antibody-dependent cellular cytotoxicity relative to a control human NK cell or human NK cell line; and/or

(iii) has enhanced anti-tumor efficacy relative to a control human NK cell or human NK cell line,

optionally wherein the control NK cell is a human NK cell activated in the presence of IL-15 alone, or a human NK cell line activated in the presence of IL-15 alone.

~~42. The engineered NK cell or population of cells of any one of claims 1-41, wherein expression of one or more of the following polypeptides is increased in the NK cell or population of said cells relative to a control human NK cell or human NK cell line: CD94/NKG2A, NKp30, NKp44, NKG2D, and CD25, optionally wherein the control human NK cell is a human NK cell activated in the presence of IL-15 alone, or the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.~~

~~43. The engineered NK cell or population of cells of any one of claims 1-42, wherein one or more of the following polypeptides is relatively unchanged in the NK cell or population of said~~

~~cells relative to a control human NK cell or human NK cell line: KIR, CD57, NKG2C, DNAM-1 and CD137, optionally wherein the control human NK cell is a human NK cell activated in the presence of IL-15 alone, or the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.~~

~~44. The engineered NK cell or population of cells of any one of claims 1-43, wherein expression of CD16 and/or CD11b is decreased in the NK cell or population of said cells relative to a control human NK cell or human NK cell line, optionally wherein the control human NK cell is a human NK cell activated in the presence of IL-15 alone, or the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.~~

~~45. The engineered NK cell or population of cells of any one of claims 1-44, wherein the NK cell or population of said cells is CD25+NKG2A+NKp30+NKp44+.~~

1346. The engineered NK cell or population of cells of any one of claims 1-1245, which expresses an IL-15 polypeptide, optionally a human IL-15 polypeptide, .

~~47. The engineered NK cell or population of cells of claim 46,~~ wherein the IL-15 polypeptide is a secreted IL-15 polypeptide or a membrane bound IL-15 polypeptide.

~~48. The engineered NK cell or population of cells of claim 47, wherein the membrane bound IL-15 polypeptide is a fusion of IL-15 to a heterologous transmembrane domain.~~

~~49. The engineered NK cell or population of cells of any one of claims 1-48, which expands 1.5-fold, 2-fold, 3-fold, or 4-fold following *in vivo* administration.~~

~~50. The engineered NK cell or population of cells of any one of claims 1-49, which exhibits an enhanced response to cytokine or activating receptor re-stimulation following *in vivo* administration.~~

~~51. The engineered NK cell or population of cells of claim 50, wherein the enhanced response is maintained for weeks to months, optionally for a period of 2 weeks to 3 months, a period of 3 weeks to 2 months, or about one month.~~

1452. The engineered NK cell or population of said cells of any one of claims~~1~~ 1-~~51~~13, wherein the engineered NK cell or population of said cells contacted with a target cell presenting the NPM1c neoepitope in complex with a MHC class I protein

(i) has increased expression of IFN gamma relative to a control population of NK cells;

(ii) has increased expression of granzyme B relative to a control population of NK cells;

(iii) has increased expression of one or more activation markers relative to a control population of NK cells, wherein the one or more activation markers are selected from: CD25, CD69, ICOS, CD226, CD107a, and CD62L;

(iv) has increased expression of one or more activating receptors relative to a control population of NK cells, wherein the one or more activating receptors are selected from: NKp30, NKG2D, NKp44;

(v) has increased expression of one or more maturation markers relative to a control population of NK cells, wherein the one or more maturation markers are selected from: CD56 and NKG2A;

(vi) has decreased expression of CD57 relative to a control population of NK cells;

(vii) has increased expression of TIGIT relative to a control population of NK cells; and/or

(viii) has decreased expression of TRAIL relative to a control population of NK cells;

optionally wherein the control population of NK cells is an untransduced population of cytokine-induced ML NK cells.

1553. A pharmaceutical composition, comprising the engineered cell or population of cells of any one of claims 1-1452, and a pharmaceutically acceptable carrier.

1654. A method for producing the engineered cell or population of cells of any one of claims 1-1552, wherein the method comprises:

(i) obtaining a primary human NK cell or population of said cells;

(ii) contacting the NK cell or population of said cells with an amount of IL-12, IL-15, IL-18, or any combination thereof, for a period of time sufficient to obtain a cytokine-induced memory-like NK cell or population of said cells;

(iii) contacting the NK cell or population of said cells of step (ii) with a lentiviral vector encoding the CAR polypeptide under conditions to transduce the NK cell or population of said cells;

(iv) isolating the NK cell expressing the CAR polypeptide or population of said cells; and

(v) optionally, expanding the isolated cell.

1755. The method of claim 1654, wherein the primary human NK cell or population of said cells is derived from an iPSC, cord blood, or PBMCs.

~~56. The method of claim 54 or 55, wherein the primary human NK cell or population of said cells is autologous or allogeneic.~~

~~57. The method of any one of claims 54-56, wherein the period of time in step (ii) is about 12-16 hours, optionally about 14-16 hours, optionally about 16 hours.~~

~~58. The method of any one of claims 54-57, wherein step (iii) further comprises resting the NK cell or population of said cells for a period of about 24-72 hours prior to (iv).~~

~~59. The method of any one of claims 54-58, wherein the lentiviral vector is a baboon envelope glycoprotein (BaEV-gp) pseudotyped lentivirus.~~

~~60. The method of any one of claims 54-58, wherein the cytokine-induced memory-like NK cell or population of said cells produces increased levels of IFNγ relative to a control human NK cell or NK cell line, optionally wherein the control human NK cell is a human NK cell activated in the presence of IL-15 alone, or the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.~~

1861. A method of producing a population of engineered cytokine-induced memory-like (ML) NK cell expressing a heterologous polypeptide, the method comprising:

contacting a population of cytokine-induced ML NK cells expressing ASCT-2 with a pseudotyped lentiviral vector encoding the heterologous polypeptide under conditions to transduce the population,

wherein the pseudotyped lentiviral vector comprises a glycoprotein that binds to ASCT-2,

thereby producing the population of engineered cytokine-induced ML NK cells expressing the heterologous polypeptide.

1962. The method of claim 1861, wherein the cytokine-induced ML NK cells are obtained from a population of primary human NK cells.

2063. The method of claim 1861 or 1962, wherein the population of primary human NK cells is derived from iPSCs, cord blood, or PBMCs.

2164. The method of any one of claims 18-2061-63, wherein the population of primary human NK cells is autologous or allogeneic.

2265. The method of any one of claims 18-2161-64, wherein expression of ASCT-2 is increased in the population of cytokine-induced ML NK cells relative to a control population of human NK cells by about 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, or 4-fold, optionally wherein the control population of human NK cells is activated in the presence of IL-15 alone.

2366. The method of any one of claims 18-2261-65, wherein the population of cytokine-induced ML NK cells is pre-activated by exposure to IL-12, IL-18, and IL-15.

~~67. The method of any one of claims 66, wherein the population of cytokine-induced ML NK cells is pre-activated for a period of time of about 8-24 hours, optionally 12-20 hours, optionally about 12-16 hours, optionally about 14-16 hours, optionally about 16 hours.~~

~~68. The method of any one of claims 61-65, wherein the population of cytokine-induced ML NK cells is pre-activated by exposure to about 10 ng/mL IL-12, about 50 ng/mL IL-15, and about 50 ng/mL IL-18 for a period of time of about 3-48 hours, about 7-24 hours, about 16-20 hours, about 12-24 hours, about 14-16 hours, or about 16 hours.~~

~~69. The method of any one of claims 61-68, wherein the population of cytokine-induced ML NK cells is rested for a period of time following the exposure to one or more cytokines and prior to the contacting, wherein the period of time is about 24-72 hours.~~

~~70. The method of any one of claims 61-69, wherein at least about 10% of the population of cytokine-induced ML NK cells is transduced.~~

~~71. The method of claim 70, wherein 10-90% of the population of cytokine-induced ML NK cells is transduced, optionally 35-80%, optionally about 40-60%, optionally about 60%.~~

2472. A method of producing a population of engineered cytokine-induced memory-like (ML) NK cell expressing a heterologous polypeptide, the method comprising:

(i) obtaining a population of primary human NK cells;

(ii) contacting the population of primary human NK cells with IL-12, IL-18, and IL-15 for a period of time sufficient to obtain a population of cytokine-induced ML NK cells expressing ASCT-2; and

(iii) contacting the population of (ii) with a pseudotyped lentiviral vector encoding the heterologous polypeptide under conditions to transduce the population of cytokine-induced ML NK cells;

wherein the pseudotyped lentiviral vector comprises a glycoprotein that binds to ASCT-2;

thereby producing a population of engineered cytokine-induced ML NK cells expressing the heterologous polypeptide.

2573. The method of claim 24~~any one of claims 61-72~~, wherein the glycoprotein is a retroviral glycoprotein.

2674. The method of claim 2573, wherein the retroviral glycoprotein is a baboon envelope (BaEV) glycoprotein.

2775. The method of any one of claims 24-2672-74, wherein the population of primary human NK cells is derived from iPSCs, cord blood, or PBMCs.

2876. The method of any one of claims 24-2772-75, wherein the population of primary human NK cells is autologous or allogeneic.

2977. The method of any one of claim 24-2872-76, wherein the period of time in step (ii) is about 12-16 hours, optionally about 14-16 hours, optionally about 16 hours.

3078. The method of any one of claims 18-2961-77, wherein the population of cytokine-induced ML NK cells

(i) produces increased IFNγ in the presence of one or more cytokines and/or tumor targets relative to a control human NK cell line;

(ii) has enhanced antibody-dependent cellular cytotoxicity relative to a control human NK cell line; and/or

(iii) has enhanced anti-tumor efficacy relative to a control human NK cell line,

optionally wherein the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.

~~79. The method of any one of claims 72-78, wherein the proportion of the population of cytokine-induced memory-like NK cells that is transduced as a result of (iii) is at least 10%.~~

~~80. The method of claim 79, wherein 10-90% of the population of cytokine-induced memory-like NK cells is transduced, optionally about 35-80%, optionally about 40-60%, optionally about 60%.~~

3181. The method of any one of claims 18-3061-80, wherein expression of one or more of the following polypeptides is increased in the population of cytokine-induced NK cells relative to a control human NK cell line: CD94/NKG2A, NKp30, NKp44, NKG2D, and CD25.

3282. The method of any one of claims 18-3161-81, wherein one or more of the following polypeptides is relatively unchanged in the population of cytokine-induced NK cells relative to a control human NK cell line: KIR, CD57, NKG2C, DNAM-1 and CD137.

3383. The method of any one of claims 18-3261-82, wherein expression of CD16 and/or CD11b is decreased in the population of cytokine-induced ML NK cells relative to a control human NK cell line.

3484. The method of any one of claims 18-3361-83, wherein a plurality of the population of cytokine-induced ML NK cells are CD25+NKG2A+NKp30+NKp44+.

~~85. The method of any one of claims 61-84, wherein the population of cytokine-induced ML NK cells expands 1.5-fold, 2-fold, 3-fold, or 4-fold following *in vivo* administration.~~

~~86. The method of any one of claims 61-85, wherein the population of cytokine-induced ML NK cells exhibits an enhanced response to cytokine or activating receptor re-stimulation following *in vivo* administration.~~

~~87. The method of any one of claims 61-86, wherein the enhanced response is maintained for weeks to months, optionally for a period of 2 weeks to 3 months, a period of 3 weeks to 2 months, or about one month.~~

3588. The method of any one of claims 18-3461-87, wherein the pseudotyped lentiviral vector further encodes an IL-15 polypeptide, optionally a human IL-15 polypeptide.

~~89. The method of claim 88, wherein the IL-15 polypeptide is a secreted IL-15 polypeptide or a membrane bound IL-15 polypeptide.~~

~~90. The method of claim 88 or 89, wherein the membrane bound IL-15 polypeptide is fused to a heterologous transmembrane domain, optionally a CD8 transmembrane domain.~~

~~91. The method of any one of claims 88-90, wherein expansion of the population of cytokine-induced ML NK cells is increased by about 1.5-fold, 2-fold, 3-fold, or 4-fold following the transducing relative to a control ML NK population transduced without the IL-15 polypeptide.~~

36~~92~~. The method of any one of claims 18-35~~61-91~~, wherein the method further comprises isolating the population of cytokine-induced ML NK cells; and optionally, expanding the population of cells.

~~93. The method of any one of claims 61-92, wherein the heterologous polypeptide is a CAR.~~

~~94. The method of any one of claims 93, wherein the CAR comprises an intracellular domain, a transmembrane domain and an extracellular binding domain, wherein the extracellular binding domain specifically binds to an antigen comprising an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein.~~

~~95. The method of claim 94, wherein the extracellular binding domain does not bind to, or substantially does not bind to: (a) the MHC class I protein alone, and/or (b) a control peptide in complex with the MHC class I protein, optionally wherein the control peptide is an NY-ESO-1 epitope or influenza virus M1 epitope.~~

~~96. The method of claims 94 or 95, wherein the NPM1c neoepitope comprises an amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, wherein X_1 is selected from A, V, L or I, wherein X_2 is selected from A, T, S, V, L, I, M or Q, wherein X_3 is selected from Q or N, wherein X_4 is selected from D or E, wherein X_5 is selected from L, I, V, M, A or F, wherein X_6 is selected from C, S, or A, wherein X_7 is selected from L, I, V, M, A, or F, wherein X_8 is selected from A, V, L or I, and wherein X_9 is selected from L, I, V, M or A.~~

97. The method of claim 96, wherein X_1 is selected from A or V, wherein X_2 is selected from V, I, or L, wherein X_3 is selected from Q or N, wherein X_4 is selected from D or E, wherein X_5 is selected from L or I, wherein X_6 is selected from C or S, wherein X_7 is selected from V, L or I, wherein X_8 is selected from A or V, and wherein X_9 is selected from V, I, or L.

98. The method of claim 97, wherein X_1 is A, wherein X_2 is selected from V, I, or L, wherein X_3 is Q, wherein X_4 is D, wherein X_5 is L, wherein X_6 is C, wherein X_7 is L, wherein X_8 is A, and wherein X_9 is selected from V, I, or L.

99. The method of any one of claims 94-98, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: AIQDLCLAV (SEQ ID NO:1) or AIQDLCVAV (SEQ ID NO: 71).

100. The method of claim 94 or 95, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: CLAVEEVSL (SEQ ID NO:72), VEEVSLRK (SEQ ID NO:73), AVEEVSLR (SEQ ID NO:74), AVEEVSLRK (SEQ ID NO:75), CLAVEEVSLRK (SEQ ID NO:76).

101. The method of any one of claims 94-99, wherein the neoepitope comprises the amino acid sequence AIQDLCLAV (SEQ ID NO:1).

102. The method of any one of claims 94-101, wherein the neoepitope is 7, 8, 9, 10, 11, or 12 amino acid residues in length.

103. The method of any one of claims 94-102, wherein the MHC class I protein is an HLA-A*02 protein or is encoded by the HLA-A*02 allele group.

104. The method of any one of claims 94-103, wherein the MHC class I protein is encoded by the HLA-A*02:01 allele.

105. The method of any one of claims 94-104, wherein the extracellular domain comprises:

(i) a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2 and VH CDR3, said VH CDR1, VH CDR2 and VH CDR3 being the CDRs of a VH that has an amino acid sequence of SEQ ID NO: 5, and/or

(ii) a light chain variable region (VL) comprising VL complementarity determining region (CDR)1, VL CDR2 and VL CDR3, said VL CDR1, VL CDR2 and VL CDR3 being the CDRs of a VL that has an amino acid sequence of SEQ ID NO: 3.

106. The method of any one of claims 94-105, wherein the extracellular domain comprises a VH comprising VH CDR1, VH CDR2 and VH CDR3, wherein the VH CDR1 has the amino acid sequence GFTFSSYA (SEQ ID NO: 9), the VH CDR2 has the amino acid sequence ISGSGGST (SEQ ID NO: 10), and the VH CDR3 has the amino acid sequence ARLGYPTTTLLPFDY (SEQ ID NO: 11).

107. The method of claim 106, wherein the extracellular domain comprises a VL comprising VL CDR1, VL CDR2 and VL CDR3, wherein the VL CDR1 has the amino acid sequence QSISSY (SEQ ID NO: 6), the VL CD2 has the amino acid sequence AAS (SEQ ID NO: 7), and the VL CD3 has the amino acid sequence QQSYSTPLT (SEQ ID NO: 8).

108. The method of any one of claims 94-107, wherein the extracellular domain comprises a VH and a VL, wherein the VH comprises an amino acid sequence which is at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 5, and/or wherein the VL comprises an amino acid sequence which is at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 3.

109. The method of any one of claims 94-108, wherein the extracellular domain comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 5, and/or wherein the VL comprises the amino acid sequence of SEQ ID NO: 3.

110. The method of any one of claims 94-109, wherein the extracellular domain comprises an scFv.

~~111. The method of claim 110, wherein the scFv is a human scFv.~~

~~112. The method of claims 110 or 112, wherein the scFv comprises a linker.~~

~~113. The method of claim 112, wherein the linker is a peptide linker.~~

~~114. The method of claim 113, wherein the peptide linker is a Gly-Ser linker.~~

~~115. The method of claim 114, wherein the Gly-Ser linker is selected from the group consisting of (Gly4Ser) (SEQ ID NO:58), (Gly4Ser)2 (SEQ ID NO:59), (Gly4Ser)3 (SEQ ID NO:60), and (Gly4Ser)4 (SEQ ID NO:61).~~

~~116. The method of claim 115, wherein the Gly-Ser linker comprises the amino acid sequence SGSSGGSSSG (SEQ ID NO:4).~~

~~117. The method of any one of claims 111-116, wherein the scFv has an amino acid sequence which is at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 2; optionally wherein the scFv comprises: (a) a VH comprising VH CDR1, VH CDR2 and VH CDR3, wherein the VH CDR1 has the amino acid sequence GFTFSSYA (SEQ ID NO: 9), the VH CDR2 has the amino acid sequence ISGSGGST (SEQ ID NO: 10), and the VH CDR3 has the amino acid sequence ARLGYPTTTLLPFDY (SEQ ID NO: 11); and/or (b) a VL comprising VL CDR1, VL CDR2 and VL CDR3, wherein the VL CDR1 has the amino acid sequence QSISSY (SEQ ID NO: 6), the VL CD2 has the amino acid sequence AAS (SEQ ID NO: 7), and the VL CD3 has the amino acid sequence QQSYSTPLT (SEQ ID NO: 8).~~

~~118. The method of any one of claims 111-117, wherein the scFv comprises the amino acid sequence of SEQ ID NO: 2.~~

~~119. The method of any one of claims 94-118, wherein the antigen is on the surface of a cancer cell.~~

~~120. The method of claim 119, wherein the cancer is Acute Myeloid Leukemia (AML).~~

~~121. The method of any one of claims 94-120, wherein the extracellular domain binds to the antigen with an equilibrium dissociation constant (Kd) of 100 nM or less, 50 nM or less, 20 nM or less, 10 nM or less, from 0.5 nM to 100 nM, or from 1 nM to 15 nM.~~

~~122. The method of any one of claims 94-121, wherein the transmembrane domain comprises the transmembrane domain of CD3-zeta, CD8, CD28, DAP12, 2B4, NKG2D, CD16, NKp44 or NKp46.~~

~~123. The method of any one of claims 94-122, wherein the intracellular domain comprises one or more costimulatory domains of one or more costimulatory molecules selected from the group consisting of: CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, 2B4, DAP10, CD137 and DAP12.~~

~~124. The method polypeptide of any one of the claims 94-123, wherein the intracellular domain comprises a CD3-zeta signaling domain and a 4-1BB costimulatory domain; wherein the transmembrane domain comprises a CD8 transmembrane domain, and wherein the CAR polypeptide further comprises a CD8 hinge region.~~

~~125. The method of any one of the claims 94-124, wherein the intracellular domain comprises a CD3-zeta signaling domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a 4-1BB costimulatory domain comprising the amino acid sequence set forth in SEQ ID NO: 26; wherein the CAR polypeptide comprises a CD8 transmembrane domain and a CD8 hinge region, wherein the CD8 transmembrane domain and the CD8 hinge region comprise the amino acid sequence set forth in SEQ ID NO: 25; and wherein the extracellular binding domain comprises the antibody, or antigen binding fragment thereof, and a leading sequence comprising the amino acid sequence set forth in SEQ ID NO: 23.~~

~~126. The method of any one of claims 94-125, wherein the extracellular binding domain is an scFv comprising the amino acid sequence set forth in SEQ ID NO: 24, or an amino acid sequence~~

~~which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 24.~~

~~127. The method of any one of claims 94-121, wherein the CAR polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 22, or an amino acid sequence which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO:22.~~

~~128. The method of any one of the claims 94-126, wherein the intracellular domain further comprises a self-cleaving peptide sequence and a cytokine, wherein cleavage of the self-cleaving peptide releases the cytokine.~~

~~129. The method of claim 128 wherein the cytokine is IL-12, IL-7, IL-13, IL-15, TNF-α, IFN-γ, or CCL19.~~

~~130. The method of claim 128 or 129, wherein the cytokine is an IL-15 polypeptide.~~

~~131. The method of claim 130, wherein the IL-15 polypeptide is secreted following expression.~~

~~132. The method of claim 130 or 131, wherein the CAR polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 102, or an amino acid sequence which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 102.~~

~~133. The method of claim 130, wherein the IL-15 polypeptide is fused to a heterologous transmembrane domain, optionally wherein the heterologous transmembrane domain is a CD8 transmembrane domain.~~

~~134. The method of claim 133, wherein the IL-15 polypeptide is expressed as a membrane-bound IL-15 polypeptide.~~

135. The method of claim 133 or 134, wherein the CAR polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 100, or an amino acid sequence which is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, or at least 95% identical, to the amino acid sequence of SEQ ID NO: 100.

136. A population of engineered cytokine-induced ML NK cells expressing a CAR comprising an extracellular binding domain that specifically binds to an antigen comprising an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein, the population prepared according to the method of any one of claims 94-135.

137. The population of engineered ML NK cells of claim 136, wherein in the presence of a target cell presenting the NPM1c neoepitope in complex with a MHC class I protein,

(i) has increased expression of IFNgamma;

(ii) has increased expression of granzyme B relative to a control population of NK cells;

(iii) has increased expression of one or more activation markers relative to a control population of NK cells, wherein the one or more activation markers are selected from: CD25, CD107a, CD69, ICOS, CD226, and CD62L;

(iv) has increased expression of one or more activating receptors relative to a control population of NK cells, wherein the one or more activating receptors are selected from: NKp30, NKG2D, NKp44;

(v) has increased expression of one or more maturation markers relative to a control population of NK cells, wherein the one or more maturation markers are selected from: CD56 and NKG2A;

(vi) has decreased expression of CD57 relative to a control population of NK cells;

(vii) has increased expression of TIGIT relative to a control population of NK cells; and/or

(viii) has decreased expression of TRAIL relative to a control population of NK cells;

optionally wherein the control population of NK cells is an untransduced population of cytokine-induced ML NK cells.

37138. A method of treating a cancer in a subject in need thereof, wherein the cell surface of cells comprising the cancer displays an NPM1c neoepitope in complex with a class I major

~~histocompatibility complex (MHC class I) protein, the method comprising administering to the subject~~ Use of the engineered cell or population of cells of any one of claims 1-~~14~~52, ~~the population of engineered ML NK cells of claims 136 or 137,~~ or the pharmaceutical composition of claim ~~15~~53, in the preparation of a medicament for treating~~an amount sufficient to treat the~~ cancer in a subject, wherein the cell surface of cells comprising the cancer displays an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein.

~~38~~139. The ~~method~~ use of claim ~~138~~ 37, wherein the cancer is AML.

~~140. The method of claim 138 or 139, wherein the method of treating cancer is a method of reducing cancer burden or a method of increasing survival in the subject.~~

~~141. A method of treating AML in a subject in need thereof, the method comprising administering to the subject the engineered cell or population of cells of any one of claims 1-52, the population of engineered ML NK cells of claim 136 or 137, or the pharmaceutical composition of claim 53, in an amount sufficient to treat AML.~~

~~142. The method of any one of claims 138-141, wherein the AML is a relapsed AML or a refractory AML.~~

~~143. A method of preventing relapse of AML in a subject in remission from AML, the method comprising administering to the engineered cell or population of cells of any one of claims 1-52, the population of engineered ML NK cells of claim 136 or 137, or the pharmaceutical composition of claim 53.~~

~~144. The method of any one of claims 138-143, comprising, before the administering step, detecting whether the subject expresses NPM1c or whether the subject has an *NPM1c* mutation in the *NPM1* gene, and if the subject expresses NPM1c or has an *NPM1c* mutation proceeding with the administering step.~~

39145. The ~~method~~ use of any one of claim~~s~~ 37 or 38 ~~138-144~~, wherein the engineered cell or population of cells exhibits enhanced expansion following *in vivo* administration.

~~146. The method of any one of claims 138-145, wherein the method further comprises administering one or more additional therapeutic agents or procedures.~~

~~147. Use of the engineered cell or population of cells of any one of claims 1-52, the population of engineered ML-NK cells of claim 136 or 137, or the pharmaceutical composition of claim 53, in the manufacture of a medicament for treating a cancer in a subject, wherein the cell surface of cells comprising the cancer displays an NPM1c neoepitope in complex with a MHC class I protein; optionally wherein the use is in combination with one or more additional therapeutic agents or procedures.~~

~~148. The method of any of claims 138-146 or the use of claim 147, wherein the subject is a human.~~

~~149. A kit comprising one or more containers comprising: (i) the engineered cell or population of cells of any one of claims 1-52, the population of engineered ML-NK cells of claim 136 or 137, or the pharmaceutical composition of claim 53; (ii) optionally, one or more additional therapeutic agents, and (iii) instructions for use in treating cancer in a subject.~~