

CLAIMS

~~What is claimed is:~~

~~1.~~ 1. A plasmid for use with clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) integration systems wherein the plasmid comprises in order a left homology arm, a polynucleotide sequence encoding a chimeric antigen receptor (CAR) polypeptide, and a right homology arm; wherein the left and right homology arms are each 30bp to 1000bp in length ~~or less.~~

~~2.~~ 2. The plasmid of claim ~~1,~~1, wherein the CAR polypeptide comprises a transmembrane domain, a co-stimulatory domain, a ~~CD3ζ~~CD3ζ signaling domain, and a single-chain variable fragment (scFV) that specifically binds to a receptor on a target cell~~.~~

~~1.~~ ~~The plasmid of claim 2, wherein the receptor comprises CD33.~~

~~2.~~ ~~The plasmid of claim 3, wherein scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof.~~

~~3.~~ ~~The plasmid of any one of claims 1-4,~~ wherein the transmembrane domain of the CAR polypeptide comprises a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a ~~CD3ζ~~CD3ζ transmembrane domain, or an NKG2D transmembrane domain~~.~~

~~4.~~ ~~The plasmid of any one of claims 1-5,~~ and wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof.

~~5.~~ ~~The plasmid of any one of claims 1-6, further comprising a polyadenylation signal between the transgene and the right homology arm.~~

~~6.~~ ~~The plasmid of any one of claims 1-7, wherein the left homology arm and right homology arm are the same length.~~

~~7.~~ 3. The plasmid of claim ~~8,~~2, wherein the ~~homology arms are each 30bp in length~~receptor

comprises CD33.

8. 4. The plasmid of claim 8,3, wherein ~~the homology arms are each 300bp in length~~scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof.

9. ~~The plasmid of claim 8, wherein the homology arms are each 600bp in length.~~

10. ~~The plasmid of claim 8, wherein the homology arms are each 1000bp in length.~~

11. ~~5.~~ The plasmid of any one of claims ~~1-7, wherein the left homology arm and right homology arm are different lengths.~~

12. ~~The plasmid of any one of claims 1-13,1-4,~~ wherein the homology arms specifically hybridize to the Adeno-Associated Virus Integration Site 1 (AAVS1) of chromosome 19 of humans.

13. ~~The plasmid of any one of claims 1-14, further comprising a murine leukemia virus-derived (MND) promoter.~~

14. 6. An Adeno-associated viral (AAV) vector comprising the plasmid of any one of claims ~~1-15,1-5.~~

15. 7. The AAV vector of claim ~~16,6,~~ wherein the serotype of the AAV comprises AAV6.

16. 8. The AAV vector of claim ~~16-or-17,7,~~ wherein the vector further comprises a plasmid encoding a crRNA, tracer RNA (trcrRNA), and a CAS endonuclease.

17. 9. The AAV vector of any of claims ~~16-18,6-8,~~ wherein the vector is a single stranded AAV (ssAAV).

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18. 10. The AAV vector of any of claims ~~16-18,6-8,~~ wherein the vector is a self-complimentary AAV (scAAV).

~~19.~~ 11. The AAV vector of any of claims ~~16-20,6-10~~, wherein the vector comprises a sequence at least 90% identical to SEQ ID NO: 22 or SEQ ID NO: 23 or a fragment thereof.-

~~20.~~ 12. A modified cell comprising the plasmid of any one of claims ~~1-15,1-5~~ or the AAV vector of any one of claims ~~16-21~~.

~~21.~~ ~~The modified cell of claim 22,6-11,~~ wherein the modified cell is a natural killer (NK) cell or NK T cell.

~~22.~~ 13. The modified cell of claim ~~23,12,~~ wherein the NK cell or NK T cell has been expanded in the presence of irradiated feeder cells, plasma membrane particles, or exosomes expressing membrane bound IL-21, membrane bound 4-1BBL, and/or membrane bound IL-15 or any combination thereof.-

~~A method~~

~~23.~~ 14. Use of a modified cell of claim 12 or 13 in the manufacture of a medicament for treating a cancer in a subject~~comprising administering to a subject with a cancer the modified cell of any one of claims 22-24.~~

~~24.~~ ~~The method of claim 25, wherein the cancer comprises leukemia.~~

~~25.~~ 15. A method of genetically modifying a Natural Killer (NK) cell ~~comprising~~
or a) NK T cell, comprising

a) obtaining a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease ~~(Cas9)~~ complexed with a corresponding CRISPR/Cas guide RNA and an AAV vector comprising a plasmid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR) polypeptide; wherein the polynucleotide sequence is flanked by homology arms; and wherein the homology arms are 800 bp in length or less; and-

b)-introducing the polynucleotide sequence encoding the CAR polypeptide and the RNP complex into the NK cell or NK T cell; wherein the polynucleotide sequence encoding the CAR polypeptide is introduced into the NK cell or NK T cell via infection with the AAV into the cell; wherein the RNP complex hybridizes to a target sequence

within the genomic DNA of the NK cell or NK T cells and the cell's DNA repair enzymes insert the polynucleotide sequence encoding the CAR polypeptide into the host genome at the target sequence within the genomic DNA of the NK cell or NK T cell thereby creating a modified NK cell or a modified NK T cell.

~~26.—16. The method of claim 27,15, wherein the NK cell or NK T cell is a primary cell or an expanded cell.~~

~~27.—The method of claim 28, wherein the primary cell is incubated for about 4 to 10 days in the presence of IL-2 prior to infection.~~

~~28.—The method of claim 28 or 29, or wherein the primary cell is expanded for about 4 to 10 days in the presence of irradiated feeder cells, plasma membrane particles, or exosomes prior to infection.~~

~~29.—The method of claim 30, wherein the irradiated feeder cells, plasma membrane particles, or exosomes express membrane bound 4-1BBL, membrane bound IL-21, or membrane bound IL-15, or any combination thereof.~~

~~30. The method of any one of claims 27-31, further comprising expanding the modified cell with irradiated feeder cells, plasma membrane particles, or exosomes following infection, wherein the irradiated feeder cells, plasma membrane particles, or exosomes express membrane bound 4-1BBL, membrane-bound IL-21, or membrane-bound IL-15, or any combination thereof.~~

~~31.—17. The method of any one of claims 27-32, further comprising expanding the modified cell with IL-2 following infection.~~

~~32.—The method of any one of claims 27-33, wherein the cell is infected with about 5 to 500,000 multiplicity of infection (MOI) of the AAV.~~

~~33.—The method of any one of claims 27-34, wherein the RNP complex is introduced into the cell via electroporation.~~

~~34. The method of any one of claims 27-35, wherein the RNP complex is introduced into the cell via transfection; and wherein the RNP complex is encoded on the same claim 15 or a different AAV.~~

~~35. The method of any one of claims 27-36, wherein the cell is a natural killer (NK) cell or NK T cell.~~

~~36. The method of any one of claims 27-37, 16, wherein the CAR polypeptide comprises a transmembrane domain, a co-stimulatory domain, a ~~CD3~~ζ~~CD3~~ζ signaling domain, and a single-chain variable fragment (scFV) that specifically binds to a receptor on a target cell.~~

~~37. The method of claim 38, wherein the receptor comprises CD33.~~

~~38. The method of 39, wherein scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof.~~

~~39. The method of any one of claims 27-40, 2, wherein the transmembrane domain of the CAR polypeptide comprises a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a ~~CD3~~ζ~~CD3~~ζ transmembrane domain, or an NKG2D transmembrane domain.~~

~~40. The method of any one of claims 27-41, and wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof.~~

18. The method of claim 17, wherein the receptor comprises CD33.

~~41. 19. The method of any one of claims 27-42, wherein the left homology arm and right homology arm are the same length.~~

~~42. The method of claim 43, wherein the homology arms are each 600bp in length.~~

~~43. The method of any one of claims 27-42, wherein the left homology arm and right homology arm are different lengths.~~

~~44. The method of any one of claims 27-45, 15-18, wherein the homology arms specifically~~

hybridize to the Adeno-Associated Virus Integration Site 1 (AAVS1) of chromosome 19 of humans.

~~45. The method of any one of claims 27-46, wherein the plasmid further comprises a murine leukemia virus derived (MND) promoter.~~

~~46. The method of any one of claims 27-47, wherein the serotype of the AAV comprises AAV6.~~

~~47. The method of any one of claims 27-48, wherein the vector is a single stranded AAV (ssAAV) or a self-complimentary AAV (scAAV).~~

~~48. The method of any of claims 27-49, wherein the vector comprises a sequence at least 90% identical to SEQ ID NO: 22 or SEQ ID NO: 23 or a fragment thereof.~~

49. 20. A method of creating a chimeric antigen receptor (CAR) natural killer (NK) cell or a CAR NK T cell comprising-

a) obtaining a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease ~~(Cas9)~~ complexed with a corresponding CRISPR/Cas guide RNA and an AAV vector comprising a plasmid comprising a polynucleotide sequence encoding a chimeric antigenreceptor (CAR) polypeptide; wherein the polynucleotide sequence is flanked by homology arms; and wherein the homology arms are 1000bp in length or less; and-

b) introducing the polynucleotide sequence encoding the CAR polypeptide and the RNP complex into an NK cell or an NK T cell; wherein the polynucleotide sequence encoding the CAR polypeptide is introduced into the NK cell or NK T cell via infection with the AAV into the NK cell or NK T cell; wherein the RNP complex hybridizes to a target sequence within the genomic DNA of the NK cell or NK T cell and the DNA repair enzymes of the NK cell or NK T cell insert the polynucleotide sequence encoding the CAR polypeptide into the host genome at the target sequence within the genomic DNA of the cell thereby creating a CAR NK cell or CAR NK T cell.

~~50.—21.~~ The method of claim ~~51,20,~~ wherein the NK cells or NK T cells are primary or expanded NK cells or NK T cells—

~~51.—The method of claim 52, and~~ wherein the primary NK cells or NK T cells are incubated for about 4 to 10 days in the presence of IL-2 prior to infection—

~~52. The method of claim 52 or 53, wherein the primary NK cells or NK T cells or~~ are expanded for about 4 to 10 days in the presence of irradiated feeder cells, plasma membrane particles, or exosomes prior to infection, wherein the irradiated feeder cells, plasma membrane particles, or exosomes express membrane bound 4-1BBL, membrane-bound IL-21, or membrane-bound IL-15 or any combination thereof.

~~53.—22.~~ The method of claim ~~54, wherein the irradiated feeder cells, plasma membrane particles,20 or exosomes express membrane bound 4-1BBL, membrane-bound IL-21, or membrane-bound IL-15 or any combination thereof.~~

~~54. The method of any one of claims 51-55, ,~~ further comprising expanding the CAR NK cell or CAR NK T cell with IL-2 or with irradiated feeder cells, plasma membrane particles, or exosomes following infection, wherein the irradiated feeder cells, plasma membrane particles, or exosomes express membrane bound 4-1BBL, membrane-bound IL-21, or membrane-bound IL-15 or any combination thereof.

~~55.—23.~~ The method of any one of claims ~~51-56, further comprising expanding the CAR-NK cell or CAR NK T cell with IL-2 following infection.~~

~~56. The method of any one of claims 51-57,20-22,~~ wherein the NK cell or NK T cell is infected with about 5 to 500K MOI of the AAV.

~~57.—24.~~ The method of any one of claims ~~51-58,20-23,~~ wherein the RNP complex is introduced into the NK cell or NK T cell via electroporation—

~~58. The method of any one of claims 51-59, wherein the RNP complex is introduced into the NK cell or NK T cell or~~ via transfection; and wherein the RNP complex is encoded on the same or a different

AAV from that which comprises the polynucleotide sequence.

~~59.—25.~~ The method of any one of claims ~~51-60,20-24~~, wherein the CAR polypeptide comprises a transmembrane domain, a co-stimulatory domain, a ~~CD3ζCD3ζ~~ signaling domain, and a single-chain variable fragment (scFV) that specifically binds to a receptor on a target cell.

~~60.—The method of claim 61, wherein the receptor comprises CD33.~~

~~61.—The method of claim 62, wherein scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof.~~

~~62.~~ The method of any one of claims ~~51-63,~~₂ wherein the transmembrane domain of the CAR polypeptide comprises a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a ~~CD3ζCD3ζ~~ transmembrane domain, or a NKG2D transmembrane domain and/or wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof.

26. The method of claim 25, wherein the receptor comprises CD33.

~~63.—27.~~ The method of any one of claims ~~51-64, wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof.~~

~~64.—The method of any one of claims 51-65, wherein the left homology arm and right homology arm are the same length.~~

~~65.—The method of claim 66, wherein the homology arms are each 600bp in length.~~

~~66.—The method of any one of claims 51-65, wherein the left homology arm and right homology arm are different lengths.~~

~~67.~~ The method of any one of claims ~~51-68,20-26~~, wherein the homology arms specifically hybridize to the Adeno-Associated Virus Integration Site 1 (AAVS1) of chromosome 19 of humans.

~~68.—28. The method of any one of claims 51-69, wherein the plasmid further comprises a murine leukemia virus derived (MND) promoter.~~

~~69. The method of any one of claims 51-70,20-27, wherein the serotype of the AAV comprises AAV6.-~~

~~70. 29. The method of any one of claims 51-71,20-28, wherein the vector is a single stranded AAV (ssAAV) or a self-complimentary AAV (scAAV).-~~

~~71. 30. The method of any one of claims 51-72,20-29, wherein the vector comprises a sequence at least 90% identical to SEQ ID NO: 22 or SEQ ID NO: 23 or a fragment thereof.~~

~~72.—A method of treating a cancer in a subject comprising administering to the subject a therapeutically effective amount of the CAR NK cell or the CAR NK T cell created by using the method of any one of claims 51-73.-~~

~~A method of treating a cancer in a subject comprising administering to the subject a therapeutically effective amount of a~~

~~73.—31. A natural killer (NK) cell or NK T cell for use in treating cancer, wherein the NK cell or NK T cell comprises a plasmid for use with clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) integration systems wherein the plasmid comprises in order a left homology arm, a polynucleotide sequence encoding a chimeric antigen receptor (CAR) polypeptide, and a right homology arm; wherein the left and right homology arms are each 30bp to 1000bp in length-~~or less.~~~~

~~74.—The method of claim 75, wherein the CAR polypeptide comprises a transmembrane domain, a co-stimulatory domain, a CD3ζCD3ζ signaling domain, and a single-chain variable fragment (scFV) that specifically binds to a receptor on a target cell.-~~

~~75.—The method of claim 76, wherein the receptor comprises CD33.—~~

~~76.—The method of claim 77, wherein scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof.-~~

~~77. The method of any one of claims 75-78,~~ wherein the transmembrane domain of the CAR polypeptide comprises a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a ~~CD3 ζ CD3 ζ~~ transmembrane domain, or an NKG2D transmembrane domain~~-, wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof, and wherein the receptor comprises CD33.~~

~~78. 32. The method of any one of claims 75-79, NK cell or NK T cell of claim 31, wherein the co-stimulatory domain of the CAR polypeptidescFV specifically binding to CD33 comprises a 2B4 domain, sequence at least 90% identical to SEQ ID NO: 29 or a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination~~fragment ~~thereof.~~

~~79. The method of any one of claims 75-80, further comprising a polyadenylation signal between the transgene and the right homology arm.~~

~~80. The method of any one of claims 75-81, wherein the left homology arm and right homology arm are the same length.~~

~~81. The method of claim 82, wherein the homology arms are each 30bp in length.~~

~~82. The method of claim 82, wherein the homology arms are each 300bp in length.~~

~~83. The method of claim 82, wherein the homology arms are each 600bp in length.~~

~~84. The method of claim 82, wherein the homology arms are each 1000bp in length.~~

~~85. The method of any one of claims 75-80, wherein the left homology arm and right homology arm are different lengths.~~

~~The method of any one of claims 75-87,~~

~~86. 33. The NK cell or NK T cell of claim 31 or 32,~~ wherein the homology arms specifically hybridize to the Adeno-Associated Virus Integration Site 1 (AAVS1) of chromosome 19 of humans.

~~87. The method of any one of claims 75-88, further comprising a murine leukemia virus-derived (MND) promoter.~~

~~88. The method of any one of claims 75-89, wherein the plasmid is transduced into the NK by an Adeno-associated viral (AAV) vector.~~

~~89. The method of claim 90, wherein the serotype of the AAV comprises AAV6.~~

~~90. The method of claim 90 or 91, wherein the vector further comprises a plasmid encoding a crRNA, tracer RNA (trcrRNA), and a CAS endonuclease.~~

~~91. The method of any one of claims 90-92, wherein the vector is a single stranded AAV (ssAAV).~~

~~92. The method of any one of claims 90-93, wherein the vector is a self-complementary AAV (scAAV).~~

~~93. The method of any one of claims 90-94, wherein the vector comprises a sequence at least 90% identical to SEQ ID NO: 22 or SEQ ID NO: 23 or a fragment thereof.~~

~~94. The method of any one of claims 75-95, wherein the cancer comprises acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), hairy cell leukemia (HCL), or myelodysplastic syndromes (MDS).~~

~~95.~~ 34. A plasmid for use with clustered regularly interspaced short palindromic repeat (CRISPR)/ CRISPR-associated 9 (Cas9) integration systems, wherein the plasmid comprises a polynucleotide sequence encoding a chimeric antigen receptor (CAR) polypeptide; wherein the polynucleotide sequence is adjacent to one protospacer adjacent motif (PAM) and one polynucleotide sequence encoding crRNA or flanked by two PAMs and two polynucleotide sequences that encode crRNAs.

~~96.~~ 35. The plasmid of claim ~~97,34, wherein~~ the plasmid comprises in order ~~onea first~~ PAM sequence and ~~onea first~~ polynucleotide sequence that encodes crRNA, the polynucleotide sequence encoding the CAR polypeptide, and ~~onea second~~ PAM sequence and ~~onea second~~ polynucleotide sequence that encodes crRNA.

~~97. The plasmid of claim 97 or 98,~~ wherein the CAR polypeptide comprises a transmembrane domain, a co-stimulatory domain, a ~~CD3 ζ CD3 ζ~~ signaling domain, and a single-chain variable fragment (scFV) that specifically binds to a receptor on a target cell.

~~98. The plasmid of claim 99, wherein the receptor comprises CD33.~~

~~99. The method of claim 100, wherein scFV specifically binding to CD33 comprises a sequence at least 90% identical to SEQ ID NO: 29 or a fragment thereof~~

~~100. The plasmid of any one of claims 97-101,~~ wherein the transmembrane domain of the CAR polypeptide comprises a CD4 transmembrane domain, a CD8 transmembrane domain, a CD28 transmembrane domain, a ~~CD3 ζ CD3 ζ~~ transmembrane domain, or an NKG2D transmembrane domain, and wherein the co-stimulatory domain of the CAR polypeptide comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-stimulatory domain, or any combination thereof.

~~101. 36. The plasmid of any one of claims 97-102, claim 35, wherein the co-stimulatory domain of the CAR polypeptide receptor comprises a 2B4 domain, a CD28 co-stimulatory domain, a 4-1 BB co-CD33.~~

~~102. The plasmid of any one of claims 97-103, further comprising a murine leukemia virus-derived (MND) promoter.~~

~~103. 37. An Adeno-associated viral (AAV) vector comprising the plasmid of any one of claims 97-104. 34-36.~~

~~104. The AAV vector of claim 105, wherein the serotype of the AAV comprises AAV6.~~

~~105. The AAV vector of claim 105 or 106, wherein the vector further comprises a plasmid encoding a crRNA, tracer RNA (trcrRNA), and a CAS endonuclease.~~

~~106. The AAV vector of any of claims 105-107, wherein the vector is a single stranded AAV (ssAAV) or a self-complimentary AAV (scAAV).~~

~~107. 38. A modified cell comprising the plasmid of any one of claims 97-104 34-36 or the AAV vector of any one of claims 105-108.~~

~~108. The modified cell of claim 109,37,~~ wherein the modified cell is a natural killer (NK) cell or NK T cell.

~~109. 39.~~ The modified cell of claim ~~110,38,~~ wherein the NK cell or NK T cell has been expanded in the presence of irradiated feeder cells, plasma membrane particles, or exosomes expressing membrane bound IL-21, membrane bound 4-1BBL, and/or membrane bound IL-15 or any combination thereof.

~~110. A method of treating a cancer in a subject comprising administering to a subject with a cancer the modified cell of any one of claims 109-111.~~

~~111. The method of claim 112, wherein the cancer comprises leukemia.~~

~~112. 40.~~ A method of creating a chimeric antigen receptor (CAR) natural killer (NK) cell or NK T cell comprising-

a)- obtaining a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease-~~(Cas9)~~ complexed with a corresponding CRISPR/Cas guide RNA and an AAV vector comprising a plasmid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR); wherein the polynucleotide sequence is adjacent to one protospacer adjacent motif (PAM) and one polynucleotide sequence encoding crispr RNA (crRNA) or flanked by two PAMs and two polynucleotide sequences that encode crRNAs; and-

b)- introducing the polynucleotide sequence encoding the CAR polypeptide and the RNP complex into the NK cell or NK T cell; wherein the plasmid is introduced into the cell via infection with the Adeno-associated virus (AAV) into a target cell; wherein in the ribonucleoprotein (RNP) complex hybridizes to a target sequence within the genomic DNA of the cell, and the cell's DNA repair enzymes insert the polynucleotide encoding the CAR into the host genome at the target sequence, thereby creating a CAR NK cell or CAR NK T cell.

~~113. The method of claim 114, wherein the plasmid comprises in order one PAM sequence and one polynucleotide sequence that encodes crRNA, the polynucleotide sequence encoding the~~

~~CAR polypeptide, and one PAM sequence and one polynucleotide sequence that encodes crRNA.~~

~~114.~~ 41. A method of genetically modifying a natural killer (NK) cell or NK T cell comprising

a) obtaining a ribonucleoprotein (RNP) complex comprising a class 2 CRISPR/Cas endonuclease ~~(Cas9)~~ complexed with a corresponding CRISPR/Cas guide RNA and an AAV vector comprising a plasmid comprising a polynucleotide sequence encoding a chimeric antigen receptor (CAR); wherein the polynucleotide sequence is adjacent to one PAM and one polynucleotide sequence encoding crRNA or flanked by two PAMs and two polynucleotide sequences encoding crRNAs; and

b) introducing the polynucleotide sequence encoding the CAR polypeptide ~~and the~~ RNP complex into the NK cell or NK T cell; wherein the polynucleotide sequence encoding the CAR polypeptide is introduced into the cell via infection with the Adeno-associated virus (AAV) into a target cell; wherein in the ribonucleoprotein (RNP) complex hybridizes to a target sequence within the genomic DNA of the cell, and the cell's DNA repair enzymes insert the polynucleotide sequence encoding the chimeric antigen receptor (CAR) into the host genome at the target sequence, thereby creating a modified cell.

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42. The method of claim ~~116,40 or 41~~, wherein the plasmid comprises in order ~~onea first~~ PAM sequence and ~~onea first~~ polynucleotide sequence encoding crRNAs, the polynucleotide sequence encoding the CAR polypeptide, and ~~onea second~~ PAM sequence and ~~onea second~~ polynucleotide sequence encoding crRNA.