

What is claimed is:

1. An in vitro method of genetically modifying an a primary NK cell comprising
 - a) ~~obtaining~~ providing a guide RNA (gRNA)-specific for the a target DNA sequence; and
 - b) introducing via electroporation into a target NK cell, a ribonucleoprotein (RNP)

- 5 complex comprising a class 2 CRISPR/Cas endonuclease (Cas9)-complexed with a corresponding CRISPR/Cas the guide RNA that hybridizes to a the target sequence within the genomic DNA of the NK cell to obtain a genetically modified NK cell,

- 10 wherein the primary NK cell is incubated for 2, 3, or 4 days in the presence of IL-2 prior to electroporation; or wherein the primary NK cell is expanded for 4 days in the presence of irradiated feeder cells prior to electroporation.

2. The method of claim 1, wherein the genome of the genetically modified NK cell is modified by insertion or deletion of one or more base pairs, by insertion of a heterologous DNA fragment (e.g., the donor polynucleotide), by deletion of an endogenous DNA fragment, by inversion or translocation of an endogenous DNA fragment, or a combination thereof.

- 15 3. The method of claim 1, wherein the ~~NK cells are primary or expanded~~ NK cells is expanded for 4 days in the presence of irradiated feeder cells prior to electroporation and wherein the irradiated feeder cells comprise membrane bound IL-15, membrane bound 41BBL, or membrane bound IL-21 or any combination thereof.

- 20 ~~4. The method of claim 3, wherein the primary NK cells are incubated for 2, 3, or 4 days in the presence of IL-2 prior to electroporation.~~

54. The method of claim 3, wherein the primary NK cells ~~are~~ is expanded for 4 days in the presence of irradiated feeder cells prior to electroporation, wherein the irradiated feeder cells comprise membrane bound 41BBL and membrane bound IL-21.

- 25 65. The method of claim 1, further comprising expanding the genetically modified NK cells with irradiated ~~mbIL-21-expressing~~ feeder cells following electroporation, wherein the irradiated feeder cells comprise membrane bound IL-21.

76. The method of claim 1, wherein the method further comprises forming the RNP complex by diluting 36 μ M of the class 2 CRISPR/Cas endonuclease ~~cas9~~ into a solution of 200 μ M crRNA and ~~TracerRNA~~ tracrRNA.

~~8. An NK cell modified by the method of claim 1.~~

97. The method of claim 1, wherein the A genetically modified NK cell comprising comprises a knockout of the gene encoding the transforming growth factor- β receptor 2 (TGFB2) or hypoxanthine phosphoribosyltransferase 1 (HPRT1).

5 ~~108.~~ Use of an engineered NK cell modified by the method of claim 1 of claim 8 in the manufacture of a medicament for the treatment of a ~~disease~~ cancer in a subject in need thereof, wherein the engineered NK cell comprises a RNP complex comprising a class 2 CRISPR/Cas endonuclease (Cas9) complexed with a corresponding CRISPR/Cas guide RNA that hybridizes to a target sequence within the genomic DNA of the engineered NK cell.

10 ~~11. The use of claim 10, wherein the subject has a cancer.~~

~~12. The use of claim 10, wherein the NK cell is a primary NK cell that has been modified ex vivo and after modification is to be transferred to the subject.~~

~~139.~~ The use of the engineered NK cell of claim ~~108~~, wherein the engineered NK cell is an autologous NK cell.

15 ~~1410.~~ The use of the engineered NK cell of claim ~~108~~, wherein the engineered NK cell is from an allogeneic donor source.

~~1511.~~ The use of of the engineered NK cell claim ~~108~~, wherein the engineered NK cell is expanded with irradiated mbIL-21 expressing feeder cells prior to administration to the subject.

20 ~~1612.~~ The use of the engineered NK cell of claim ~~108~~, wherein the RNP complex targets the TGFB2 or HPRT1 gene.

~~1713.~~ The use of the engineered NK cell of claim ~~108~~, wherein the engineered NK cell has been modified to comprise a knockout of the TGFB2 gene.