

What is claimed is:

1. An in vitro method of genetically modifying a primary NK cell comprising
 - a) providing a guide RNA specific for 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 complexed with the guide RNA that hybridizes to the target sequence within genomic DNA of the NK cell to obtain a genetically modified NK cell,
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.
- 10 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.
3. The method of claim 1, wherein the primary NK cell is expanded for 4 days in the presence of
15 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.
4. The method of claim 3, wherein the primary NK cell is expanded in the presence of irradiated feeder cells prior to electroporation, wherein the irradiated feeder cells comprise membrane bound
20 41BBL and membrane bound IL-21.
5. The method of claim 1, further comprising expanding the genetically modified NK cell with irradiated feeder cells following electroporation, wherein the irradiated feeder cells comprise membrane bound IL-21.
6. The method of claim 1, wherein the method further comprises forming the RNP complex by
25 diluting 36 μM of the class 2 CRISPR/Cas endonuclease into a solution of 200 μM crRNA and tracrRNA.
7. The method of claim 1, wherein the genetically modified NK cell comprises a knockout of the gene encoding the transforming growth factor- β receptor 2 (TGFB2) or hypoxanthine phosphoribosyltransferase 1 (HPRT1).
8. Use of an engineered NK cell modified by the method of claim 1 in the manufacture of a
30 medicament for the treatment of a 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.

5 9. The use of the engineered NK cell of claim 8, wherein the engineered NK cell is an autologous NK cell.

10. The use of the engineered NK cell of claim 8, wherein the engineered NK cell is from an allogeneic donor source.

11. The use of of the engineered NK cell claim 8, wherein the engineered NK cell is expanded with irradiated mbIL-21 expressing feeder cells prior to administration to the subject.

10 12. The use of the engineered NK cell of claim 8, wherein the RNP complex targets the TGFRB2 or HPRT1 gene.

13. The use of the engineered NK cell of claim 8, wherein the engineered NK cell has been modified to comprise a knockout of the TGFRB2 gene.