

VI. CLAIMS

What is claimed is:

1. ~~A method of~~Use of an engineered NK cell in the manufacture of a medicament for
5 ~~treating a cancer, in a subject comprising administering to the subject an~~ wherein the engineered
NK cell that has been modified to comprise a knockout of the CD38 gene.
2. ~~The method of treating a cancer~~use of claim 1, wherein the cancer comprises multiple
myeloma, acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (T-ALL), or
Blastic plasmacytoid dendritic cell neoplasm (BPDCN).
- 10 3. ~~The method of treating a cancer~~use of claim 1 or 2, wherein the medicament further
~~comprises~~comprising administering to the subject an anti-cancer agent comprising a small
molecule, antibody, peptide, protein, or siRNA that targets CD38.
4. ~~The method of treating a cancer~~use of claim 3, wherein the anti-cancer agent comprises
Daratumumab, isatuximab, TAK-079, or MOR202.
- 15 5. ~~The method of treating a cancer~~use of claim 3 or 4, wherein the medicament further
~~comprises~~comprising administering to the subject an angiogenesis inhibitor and a steroid.
6. ~~The method of treating a cancer~~use of claim 5, ~~wherien~~ wherein the ~~angiogenesis~~
~~inhibitor~~angiogenesis inhibitor comprises Pomalidomide, Lenalidomide, or Apremilast.
7. ~~The method of treating a cancer~~use of claim 5, ~~wherien~~ wherein the steroid comprises a
20 glucocorticoid.
8. ~~The method of treating a cancer~~use of claim 7, ~~wherien~~ wherein the glucocorticoid
comprises dexamethasone, betamethasone, prednisolone, ~~methyln~~prednisolone~~method~~prednisolone,
triamcinolone, or fludrocortisone acetate.
9. ~~A method of adoptively transferring~~Use of an engineered NK cells ~~to a subject in need~~
25 ~~thereof~~in the manufacture of a medicament for treating a cancer, wherein the engineered NK cell
is obtained by a said method comprising
 - a) obtaining a target NK cell to be modified;
 - b) obtaining gRNA specific for the target DNA sequence;
 - c) introducing via electroporation into the target NK cell, a RNP complex comprising a
30 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 target
NK cell creating an engineered NK cell; ~~and~~

d) transferring the engineered NK cell into the subject.

~~10. The method of claim 9, wherein the subject has a cancer.~~

~~11.10. The method-use of claim 9, wherein the NK cell is a primary NK cell that has been modified ex vivo and after modification transferred to the subject.~~

5 ~~12.11. The method-use of claim 9, wherein the NK cell is an autologous NK cell.~~

~~13.12. The method-use of claim 9, wherein the NK cell is from an allogeneic donor source.~~

~~14.13. The method-use of claim 9, wherein the NK cell is expanded *ex vivo* with irradiated mbIL-21 expressing feeder cells prior to administration to the subject.~~

10 ~~15.14. The method-use of claim 9, wherein the medicament comprising the engineered NK cell is expanded in the subject following transfer of the NK cells to the subject via the prepared to be administered separately from administration of IL-21 or irradiated mbIL-21 expressing feeder cells.~~

~~16.15. The method-use of claim 9, wherein the RNP complex targets the CD38 gene.~~

15 ~~17.16. A genetically modified NK cell comprising a knockout of the gene encoding clustering of differentiation 38 (CD38) made by the a method of any of claims 9-16 comprising:~~

a) obtaining a target NK cell to be modified;

b) obtaining gRNA specific for the target DNA sequence; and

20 c) introducing via electroporation into the target NK cell, 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 target NK cell, thereby creating the genetically modified NK cell.

~~18.17. A method of treating a cancer in a subject comprising administering to the subject Use of the genetically modified NK cell of claim 17 16 in the manufacture of a medicament for treating cancer.~~

25 ~~19.18. The method-use of claim 18 17, further comprising administering to the subject wherein the medicament further comprises an anti-cancer agent comprising a small molecule, antibody, peptide, protein, or siRNA that targets CD38.~~

~~20.19. The method-use of treating a cancer of claim 19 18, wherein the anti-cancer agent comprises an anti-CD38 antibody.~~

~~21.20. The method of treating a cancer~~use of claim ~~20~~19, wherein the anti-CD38 antibody comprises Daratumumab, isatuximab, TAK-079, or MOR202.

~~22.21. The method of treating a cancer~~use of any of claims ~~18-21~~17-20, wherein the cancer comprises multiple myeloma, acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (T-ALL), or Blastic plasmacytoid dendritic cell neoplasm (BPDCN).

~~23.22. A method of reducing NK cell fratricide in a subject receiving anti-CD38 immunotherapy comprising administering to the subject~~Use the genetically modified NK cell of claim 1716 in the manufacture of a medicament for reducing NK cell fratricide in a subject receiving anti-CD38 immunotherapy.

