

WHAT IS CLAIMED IS:

1. A population of nucleated cells comprising a plurality of NK cells, wherein the population comprises at least 1.0×10^6 nucleated cells, wherein at least about 70% of the cells in the population are viable and express an anti-Her2 chimeric antigen receptor (CAR), wherein:

- at least about 85% of cells in the population are CD56+;
- no more than about 0.5% of the cells in the population are CD3+;
- no more than about 10% of the cells in the population are CD19+;
- no more than about 10% of the cells in the population are CD14+;
- at least about 60% of the cells in the population are CD62L+;
- no more than about 20% of the cells in the population are LAG3+;

and wherein the CAR comprises an anti-Her2 scFv.

2. The population of nucleated cells of claim 1, wherein the CAR comprises at least one of:

- (i) a hinge domain selected from CD28 and CD8,
- (ii) a transmembrane domain selected from CD28, CD8, and NKG2D,
- (iii) a co-stimulatory domain selected from CD28, 4-1BB, 2B4, CD3zetaR, OX40, Lsk, ICOS, DAP10, and Fc fragment of IgE receptor Ig,
- (iv) an activation domain is selected from CD3 ζ , FcR- γ , and Fc-epsilon-R and
- (v) a signal peptide.

3. The population of nucleated cells of any one of claims 1-2, wherein the CAR is selected from SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, and SEQ ID NO: 38 and, optionally, wherein at least about 75% of the CD56+ cells comprise any one of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, or SEQ ID NO: 38.

4. A method of *ex vivo* producing natural killer (NK) cells of any one of claims 1-3, the method comprising:

- (a) expanding a population of NK cells by a method comprising:

- (i) culturing said population of NK cells under conditions allowing for cell proliferation, wherein said conditions comprise providing an effective amount of nutrients, serum, IL-15 and nicotinamide; and
 - (ii) supplementing said population of NK cells with an effective amount of fresh nutrients, serum, IL-15 and nicotinamide 5-10 days following step (i) to produce expanded NK cells;
- so as to obtain an *ex vivo* expanded population of NK cells, and
- (b) upregulating expression of a CAR capable of binding HER2 in said *ex vivo* expanded population of NK cells, thereby producing the NK cells expressing the CAR capable of binding the HER2.

5. The method of claim 4, wherein said population of NK cells is derived from cord blood, peripheral blood, bone marrow, CD34⁺ cells or iPSCs, optionally wherein said population of NK cells is deprived of CD3⁺ cells, and optionally wherein said population of NK cells comprises CD3⁻CD56⁺ cells.

6. The method of any one of claims 4 or 5, wherein said effective amount of said nicotinamide comprises an amount between 1.0 mM to 10 mM.

7. The method of any one of claims 4-6, wherein said expanding said population of NK cells is affected in the presence of feeder cells or a feeder layer.

8. The method of claim 7, wherein said feeder cells comprise irradiated cells.

9. The method of claim 7 or 8, wherein said feeder cells comprise T cells or PBMCs.

10. The method of claim 9, further comprising a CD3 agonist, and optionally, wherein said CD3 agonist is OKT3.

11. The method of any one of claims 4-10, wherein said expanding said population of NK cells is affected for 14-16 days.

12. The method of any one of claims 4-11, wherein said upregulating expression of said CAR or said tg-TCR is affected on day 12-14 from initiation of culture.
13. The method of any one of claims 4-12, wherein said upregulating expression of said CAR or said tg-TCR is affected with naked nucleic acid or with a vector.
14. The method of any one of claims 4-13, wherein said upregulating expression of said CAR or said tg-TCR is affected by mRNA electroporation and, optionally, wherein said CAR or said tg-TCR is transiently expressed.
15. An isolated population of NK cells of any one of claims 1-3 obtainable according to the method of any one of claims 4-14.
16. A pharmaceutical composition comprising the isolated population of NK cells of any one of claims 1-3 and a pharmaceutically active carrier.
17. A therapeutically effective amount of the isolated population of NK cells of claim 15 for use in the treatment of a disease associated with expression of HER2 in a subject in need thereof.
18. The isolated population of NK cells for use of claim 17, wherein the disease is a malignant disease.
19. The isolated population of NK cells for use of claim 18, wherein said malignant disease is a solid tumor or tumor metastasis.
20. The isolated population of NK cells for use of claim 19, wherein said malignant disease is selected from the group consisting of a breast cancer, a gastric cancer, a gastroesophageal cancer, an oesophageal cancer, an ovarian cancer, an endometrial cancer, a lung cancer, an urothelial cancer and a bladder cancer.
21. The isolated population of NK cells for use of claim 17-19, wherein the subject is a human subject.