

We claim:

1. A CAR-NK cell therapy product comprising NK cells genetically modified to express a CD19 CAR and an IL-15 formulated in a cryopreservation medium comprising PLASMA-LYTE A, trehalose, CS10 and human serum albumin (HSA).~~large-scale method of cryopreserving immune cells, the method comprising:~~

- ~~(a) providing a container comprising a sample comprising immune cells suspended in a cryopreservation medium, wherein the sample volume is at least 5 percent less than the full capacity volume of the container, and wherein the sample volume is at least 10 ml;~~
- ~~(b) cooling the container from a temperature above freezing temperature of the sample to a temperature of about or below -80°C in a multi-step process at a controlled rate to minimize latent heat of fusion;~~
- ~~(c) storing the cells in liquid nitrogen vapor phase, thereby cryopreserving the immune cells.~~

2. The cell therapy product method of claim 1, wherein the CD19 CAR comprises an anti-CD19 binding domain comprising a heavy chain variable region having at least 95% identity to SEQ ID NO: 2 and/or a light chain variable region having at least 95% identity to SEQ ID NO: 1.~~controlled rate to minimize latent heat of fusion comprises two or more steps of reducing the temperature at a rate between 0.75°C per minute to 30°C per minute, to a final temperature of -80°C or below.~~

3. The cell therapy product method of any one of the preceding claims 1-2, wherein the CD19 CAR comprises a heavy chain variable region having the sequence of SEQ ID NO: 2 and/or a light chain variable region having the sequence of SEQ ID NO: 1; and/or

wherein the transmembrane domain is selected from the group consisting of alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154; and/or

3. wherein the intracellular signaling domain is selected from the group consisting of FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3-zeta, CD3 epsilon, CD5, CD22, CD79a, CD79b and CD66d~~total time for achieving cryopreservation of the immune cells is less than 120 minutes, less than 110 minutes, less than 100 minutes, less than 90 minutes, less than 80 minutes, less than 70 minutes or less than 60 minutes.~~

4. The cell therapy product method of any one of the preceding claims, wherein the CD-19 CAR comprises a CD28 transmembrane domain having at least 95% identity to SEQ ID NO: 3, optionally

wherein the CD19 CAR comprises a CD28 transmembrane domain of SEQ ID NO: 3;
and/or

wherein the CD19 CAR comprises a CD3z signaling domain having at least 95% identity to SEQ ID NO: 4, optionally

4. wherein the CD19 CAR comprises a CD3z signaling domain of SEQ ID NO: 4~~immune cells are freshly isolated or at least once frozen and thawed.~~

5. ~~The method of claim 1, wherein the immune cells are naturally occurring or engineered natural killer (NK) cells, alpha beta T cells, gamma delta T cells, induced pluripotent stem cells (iPSCs), iPSC derived T or NK cells, regulatory T cells (Tregs), hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), dendritic cells, macrophages or B cells.~~

6. ~~The method of claim 1, wherein the immune cells are cord blood derived NK cells engineered with a chimeric antigen receptor (CAR).~~

7. ~~The method of claim 1, wherein the full capacity volume of container is about 50 ml.~~

8. ~~The method of claim 1, wherein the full capacity volume of container is about 50 ml and the sample volume is less than 40 ml.~~

9. — The method of claim 8, wherein the sample volume is about 10 ml, about 15 ml, about 20 ml, about 25 ml, about 30 ml, about 31 ml, about 32 ml, about 33 ml, about 34 ml, about 35 ml, about 36 ml, about 37 ml, about 38 ml, about 39 ml, about 40 ml, about 41 ml, about 42 ml, about 43 ml, about 44 ml or about 45ml.

5. The cell therapy product method of any one of the preceding claims 1-9, wherein the cell therapy product further comprises a suicide switch, wherein the suicide switch is iCaspase9; and/or

wherein the NK cells are derived from cord-blood, peripheral blood, T cells or iPS cells; and/or

wherein the cryopreservation medium comprises 25% v/v to 50% v/v PLASMA-LYTE A; and/or

wherein the trehalose is at a concentration of between about 10mM – 100 mM; and/or

wherein the HSA is at a concentration of between about 1.25% v/v to 25% v/v; and/or

10. wherein the cryopreservation medium comprises between about 40% v/v to 60% v/v CS10-container is a cryovial or a cryobag.

11. — The method of claim 10, wherein the container is a cryovial.

12. — The method of claim 11, wherein the cryovial has an interior dimension of between 10 mm and 18 mm.

13. — The method of claim 12, wherein the cryovial has an external dimension of about 13.5 mm.

14. — The method of claim 11, wherein the cryovial has a height of between about 40 mm and 50 mm.

15. — The method of claim 14, wherein the cryovial has a height of about 48.3 mm.

~~16. The cell therapy product method of any one of the preceding claims, 1-15 further comprising a cryoprotectant, wherein the cryoprotectant is DMSO wherein the container is resistant to DMSO.~~

~~17. A composition comprising the cell therapy product The method of any one of the preceding claims, wherein the immune cells are present at concentration of between about 6 and 120 million cells per mL.~~

~~18. —The method of any one of the preceding claims, wherein the cryopreservation medium comprises cryoprotectant, an albumin, a disaccharide and a non-pyrogenic and isotonic crystalloid solution~~

~~19. —The method of any one of claims 1-18, wherein the cryopreservation medium comprises human serum albumin (HSA), sodium chloride, sodium gluconate, sodium acetate trihydrate, potassium chloride, magnesium chloride, dimethyl sulfoxide (DMSO), and a trehalose~~

8. A cell therapy product comprising a CAR-NK cell population comprising NK cells genetically modified to express a CD-19 CAR and an IL-15 formulated in a cryopreservation medium comprising PLASMA-LYTE A, trehalose, CS10 and HSA, wherein the CD19 CAR comprises an anti-CD19 binding domain comprising a heavy chain variable region having at least 95% identity to SEQ ID NO: 2 and/or a light chain variable region having at least 95% identity to SEQ ID NO: 1, for use in treating cancer, such as a B-cell lymphoma.

920. A large-scale method of ~~thawing~~ cryopreserving immune cells, the method comprising:

- (a) providing a container comprising a sample comprising immune cells suspended in a cryopreservation medium, wherein the sample volume is at least 5 percent less than the

full capacity volume of the container, and wherein the sample volume is at least 10 ml; heating a water bath to a temperature ranging from 37°C and 70°C;

- (b) cooling the container from a temperature above freezing temperature of the sample to a temperature of about or below -80°C in a multi-step process at a controlled rate to minimize latent heat of fusion; transferring a container comprising cryopreserved immune cells according to claim 1 into the pre-heated water bath; and
- (c) storing the cells in liquid nitrogen vapor phase, thereby cryopreserving the sample; agitating the container at a speed of between about 100 and about 250 RPM for a suitable period of time, thereby to obtain thawed immune cells.

1024. The method of claim 920, wherein the cells are stored for 1 week, 2 weeks, 1 month, 6 months, 1 year, 2 years, 5 years, 10 years or more; and/or

wherein thawing the cryopreserved cells comprises: (a) heating a water bath to a temperature ranging from 37°C and 90°C; (b) transferring a container comprising cryopreserved cells to the pre-heated water bath; and (c) agitating the container at a speed of between about 100 and about 250 RPM for a suitable period of time, thereby thawing the cells; and/or

wherein the total viable cells post thawing is between about 200 million to about 800 million cells; suitable period of time is between 5-15 minutes.

1122. The method of claim 1024, wherein the method comprises changing temperature of a sample containing immune cells from a first temperature above the freezing temperature of the sample to a final temperature of less than or equal to -80°C, thereby cryopreserving the sample at the final temperature, the method comprising the steps of: suitable period of time is for about 10 minutes. (a) placing the sample at a first temperature above the freezing temperature of the sample;

(b) reducing the first temperature to a second temperature at a first controlled rate, where the second temperature is at least 2°C less than the first temperature;

(c) reducing the second temperature to a third temperature at a second controlled rate, where the third temperature is at least 40°C less than the second temperature;

(d) increasing the third temperature to a fourth temperature at a third controlled rate, where the fourth temperature is at least 20°C more than the third temperature;

(e) reducing the fourth temperature to a fifth temperature at a fourth controlled rate, where the fifth temperature is at least 10°C less than the fourth temperature; and

(f) reducing the fifth temperature to the final temperature at a fifth controlled rate, where the final temperature is less than or equal to -80°C.

1223. The method of claim 1120, wherein the ~~agitating occurs in an orbital shaker water bath~~ multi-step process comprises cooling the cells at the controlled rate of between 0.75°C per minute to 30°C per minute to a final temperature of -80°C or below, thereby cryopreserving the cells.

13. A cell therapy product comprising a CAR-NK cell population comprising NK cells genetically modified to express a B cell maturation antigen (BCMA) CAR and an IL-15 formulated in a cryopreservation medium comprising PLASMA-LYTE A, trehalose, CS10 and HSA.²⁴.~~—The method of claim 23, wherein agitating the cells in the orbital shaker water bath is at a speed of about 120–150 RPM.~~

14. The cell therapy product of any of claims 1-6 and 13, or the composition of claim 7, for use in treating cancer.²⁵.~~—The method of 23, wherein the orbital shaker water bath has a temperature of about 50°C, 55°C, 60°C, 65°C, 70°C, or 75°C.~~

15. A cryopreservation medium comprising a cryoprotectant, an albumin, a disaccharide and a non-pyrogenic and isotonic crystalloid solution; such as a medium comprising: PLASMA-LYTE A, human serum albumin (HSA), trehalose and a cryoprotectant.²⁶.~~The method of claim 23, wherein the orbital shaker water bath has a temperature of about 60°C.~~

~~27. —The method of claim 20, wherein the container has a full capacity volume of about 50 mL and a sample volume between about 8 mL and 45 mL.~~

~~28. —The method of claim 20, wherein the immune cells are naturally occurring or engineered natural killer (NK) cells, alpha beta T cells, gamma delta T cells, induced pluripotent stem cells (iPSC) derived NK or T cells, regulatory T cells (Tregs), macrophages, dendritic cells, hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), or B cells.~~

~~29. —The method of claim 20, wherein the cryopreserved immune cells are NK cells or T cells engineered to comprise a chimeric antigen receptor (CAR).~~

~~30. —The method of claim 20, wherein the thawed immune cells have a post thaw viability of 90%, 95%, 97% or more.~~

~~31. —The method of claim 20, wherein the thawed immune cells retain in vitro and/or in vivo function similar to that of freshly isolated immune cells.~~

~~32. —The method of claim 20, further comprising the step of administering the thawed immune cells to a subject in need thereof.~~

~~33. —A method of changing temperature of a sample containing immune cells from a first temperature above the freezing temperature of the sample to a final temperature of less than or equal to -80°C , thereby cryopreserving the sample at the final temperature, the method comprises the steps of:~~

- ~~(a) placing the sample at a first temperature above the freezing temperature of the sample;~~
- ~~(b) reducing the first temperature to a second temperature at a first controlled rate, where the second temperature is at least 2°C less than the first temperature;~~

- ~~(c) reducing the second temperature to a third temperature at a second controlled rate, where the third temperature is at least 40°C less than the second temperature;~~
- ~~(d) increasing the third temperature to a fourth temperature at a third controlled rate, where the fourth temperature is at least 20°C more than the third temperature;~~
- ~~(e) reducing the fourth temperature to a fifth temperature at a fourth controlled rate, where the fifth temperature is at least 10°C less than the fourth temperature; and~~
- ~~(f) reducing the fifth temperature to the final temperature at a fifth controlled rate, where the final temperature is less than or equal to -80°C.~~

34. ~~—The method of claim 33, wherein the first temperature is about 4°C to 1°C.~~

35. ~~—The method of claim 33, wherein the first controlled rate is between about 0.75°C and 1.25°C per minute.~~

36. ~~—The method of claim 33, wherein the second temperature is about -2°C.~~

37. ~~—The method of claim 33, wherein the second controlled rate is between about 20°C and 30°C per minute.~~

38. ~~—The method of claim 33, wherein the third temperature is about -60°C.~~

39. ~~—The method of claim 33, wherein the third controlled rate is between about 5°C and 15°C per minute.~~

40. ~~—The method of claim 33, wherein the fourth temperature is about -25°C.~~

41. ~~—The method of claim 33, wherein the fourth controlled rate is between 0.75°C and 1.25°C per minute.~~

42. ~~—The method of claim 33, wherein the fifth temperature is about -40°C.~~

43. — ~~The method of claim 33, wherein the fifth controlled rate is between 7°C and 15°C per minute.~~

44. — ~~The method of claim 33, wherein final temperature is less than or equal to -80°C.~~

45. — ~~A method comprising cryopreserving engineered immune cells suitable for cell therapy, the method comprising (1) providing a container comprising a sample comprising immune cells suspended in a cryopreservation medium, wherein sample volume is at least 5 percent less than the full capacity volume of the container, wherein the sample volume is at least 10 mL; and (2) stepwise freezing a population of engineered immune cells at a controlled rate to minimize impact of latent heat of fusion, where the stepwise freezing comprises cooling the cells at a rate of between 0.75°C per minute to 30°C minute to a final temp of -80 or below, thereby to cryopreserving the cells.~~