

CLAIMS

1. A method of measuring the likelihood of recovery of a Natural Killer cell after a freeze thaw cycle, the method comprising assaying the Natural Killer cell within 3 hours after thawing to
5 determine an expression level of CD16 expressed on the Natural Killer cell, wherein an increased level of CD16 expression compared to expression of CD16 on cell-surface-damaged negative control Natural Killer cell indicates low A disintegrin and metalloprotease domain 17 (ADAM-17) activity and positively correlates with the likelihood of Natural Killer cell recovery.
2. The method of claim 1, wherein the Natural Killer cell was thawed using a water bath.
- 10 3. The method of claim 1 or 2, wherein the Natural Killer (NK) cell comprises a chimeric antigen receptor (CAR) NK cell.
4. The method of any of claims 1-3, wherein the Natural Killer cell is an expanded Natural Killer cell.
5. The method of any of claims 1-4, wherein the level of CD16 expression is assayed using flow
15 cytometry.
6. Natural Killer cells for use in immunotherapy in a subject in need thereof, wherein
 - a) the Natural Killer cells are obtained after having previously subjected to a freeze thaw cycle;
 - b) the Natural Killer cells are assayed within 3 hours after thawing to determine an
20 expression level of CD16 on the Natural Killer cells; and
 - c) the Natural Killer cells for use in immunotherapy express an increased level of CD16 compared to a cell-surface-damaged negative control Natural Killer cell; and
 - d) the increased level of CD16 expression indicates low A disintegrin and metalloprotease domain 17 (ADAM-17) activity and positively correlates with the likelihood of
25 Natural Killer cell recovery.
7. The Natural Killer cells for use of claim 6, wherein the immunotherapy is anticancer treatment for a subject who has been diagnosed as having cancer.
8. The Natural Killer cells for use of claim 6 or 7, wherein Natural Killer cells were thawed using a water bath.
- 30 9. The Natural Killer cells for use of any of claims 6-8, wherein the Natural Killer cells comprise

chimeric antigen receptor (CAR) Natural Killer cells.

10. The Natural Killer cells for use of any of claims 6-9, wherein the Natural Killer cells are expanded Natural Killer cells.

5 11. The Natural Killer cells for use of any of claims 6-10, wherein the level of CD16 expression is assayed using flow cytometry.

12. An immunotherapeutic composition comprising a therapeutically effective amount of viable Natural Killer cells previously subjected to a freeze thaw cycle, and expressing an increased level of CD16 compared to a cell-surface-damaged negative control Natural Killer cell, wherein the Natural Killer cells are assayed within 3 hours after thawing to determine an expression level
10 of CD16 on the Natural Killer cells; and wherein the increased level of CD16 expression indicates low A disintegrin and metalloprotease domain 17 (ADAM-17) activity and positively correlates with the likelihood of Natural Killer cell recovery.

13. The immunotherapeutic composition of claim 12, wherein the Natural Killer cells were thawed using a water bath.

15 14. The immunotherapeutic composition of claim 12 or 13, wherein the Natural Killer (NK) cells comprise chimeric antigen receptor (CAR) Natural Killer cells.

15. The immunotherapeutic composition of any of claims 12-14, wherein the Natural Killer cells comprise expanded NK cells.

20 16. The immunotherapeutic composition of any of claims 12-15, wherein the level of CD16 expression is assayed using flow cytometry.

17. The immunotherapeutic composition of any of claims 12-16, further comprising a pharmaceutically acceptable carrier.

18. A method of selecting lots of a Natural Killer cell therapeutic suitable for use in anti-cancer immunotherapy comprising:

25 a) obtaining one or more Natural Killer (NK) cells previously subjected to a freeze thaw cycle;

b) assaying the Natural Killer cells within 3 hours after thawing to determine an expression level of CD16; and

c) selecting manufacturing lots of thawed Natural Killer cells from step b) that express CD16 at increased levels compared to cell-surface-damaged negative control Natural Killer cells for use as a Natural Killer cell therapeutic suitable for anti-cancer immunotherapy;

5 wherein the increased level of CD16 expression indicates low A disintegrin and metalloprotease domain 17 (ADAM-17) activity and positively correlates with the likelihood of Natural Killer cell recovery.

19. The method of claim 1, wherein the increased level of CD16 expression positively correlates with the likelihood of Natural Killer cell recovery within 24 hours after thawing.

10 20. The method of claim 1, wherein the likelihood of Natural Killer cell recovery is positively correlated with an expression level of CD16 on the Natural Killer cell within 3 hours after thawing that is 80% or more of an expression level of CD16 on a control Natural Killer cell that has not been frozen.