

## VI. CLAIMS

What is claimed is:

1. A method of measuring the likelihood of recovery of an immune cell after a cell membrane  
5 damaging event, comprising assaying the level of an ADAM-17-cleaved surface receptor  
expressed on the immune cells, wherein an increase in the level of the surface receptor directly  
correlates with the likelihood of immune cell recovery.
2. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of claim 1, wherein the membrane damaging event comprises a freeze thaw  
10 cycle, gene editing, electroporation, magnetofection, detergent permeabilization, strotolysin O  
(SLO) exposure, physical morphology change (cell squeezing), and/or ethanol exposure.
3. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of claim 2, wherein the membrane damaging event comprises a freeze thaw  
cycle.
- 15 4. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of claim 3, wherein immune cells were thawed using a water bath.
5. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of any of claims 1-4, wherein the level of ADAM-17-cleaved surface receptor  
expression is assayed within 0 to 24 hours after the immune cell membrane damaging event.
- 20 6. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of any of claims 1-5, wherein the level of ADAM-17-cleaved surface receptor  
expression is assayed within 12 to 24 hours after the immune cell membrane damaging event.
7. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of any of claims 1-6, wherein the ADAM-17-cleaved surface receptor expressed  
25 on the immune cells comprises CD16, CD62L, or IL-15 receptor (IL-15R).
8. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of any of claims 1-7, wherein the immune cell comprises a T cell, Natural Killer  
(NK) cell, chimeric antigen receptor (CAR) T cell, CAR NK cell, macrophage, dendritic cell, natural  
killer T (NKT) cell, innate lymphoid cell (ILC), B cell,  $\gamma\delta$ T cell, or neutrophil.
- 30 9. The method of measuring the likelihood of recovery of an immune cell after a cell membrane  
damaging event of any of claims 1-8, wherein the immune cell is a Natural Killer (NK) cell or  
CAR NK cell, and the ADAM-17-cleaved surface receptor is CD16.

10. The method of measuring the likelihood of recovery of an immune cell after a cell membrane damaging event of claim 9, wherein the NK cell is an expanded NK cell.

11. The method of measuring the likelihood of recovery of an immune cell after a cell membrane damaging event of any of claims 1-8, wherein the immune cell is a T cell or CAR T cell and the ADAM-17-cleaved surface receptor comprises CD62L or IL-15R.

12. The method of measuring the likelihood of recovery of an immune cell after a cell membrane damaging event of any of claims 1-11, wherein the level of ADAM-17-cleaved surface receptor expression is assayed using flow cytometry.

13. The method of measuring the likelihood of recovery of an immune cell after a cell membrane damaging event of any of claims 1-12, wherein the level of ADAM-17-cleaved surface receptor expressed on the immune cells is expressed as a ratio of the level of surface receptor cleaved by ADAM17 expressed on the cell membrane damaged immune cell compared with the normal level of ADAM-17-cleaved surface receptor expressed on the immune cells.

14. ~~A method of administering an Immune cells for use in immunotherapy to~~ a subject in need thereof, comprising ~~wherein~~

a) ~~obtaining one or more~~ the immune cells are obtained after having previously subjected ~~following to~~ a cell membrane damaging event;

b) ~~assaying the immune cells are assayed~~ to determine an expression level of an ADAM-17-cleaved surface receptor; and

c) ~~administering to the subject a therapeutically effective amount of the~~ immune cells that for use in immunotherapy express an increased level of ADAM-17-cleaved surface receptor relative to a control immune cell.

15. ~~The method of administering an immunotherapy~~ immune cells for use of claim 14, further comprising ~~wherein manufacturing lots of immune cells for use in immunotherapy are screened or selected~~ using the assay results of expression level of an ADAM-17-cleaved surfaced receptor, ~~for screening or selecting manufacturing lots of an immune cell therapeutic; and~~

16. ~~The method of administering an immunotherapy~~ immune cells for use of claim 14 or 15, wherein the immunotherapy is anticancer treatment for a subject who has been diagnosed as having cancer.

17. ~~The method of administering an immunotherapy~~ immune cells for use of any of claims 14-16, wherein the cell membrane damaging event comprises a freeze thaw cycle, gene editing,

electroporation, magnetofection, detergent permeabilization, strotolysin O (SLO) exposure, physical morphology change (cell squeezing), and/or ethanol exposure.

18. The ~~method of administering an immunotherapy~~immune cells for use of claim 17, wherein the cell membrane damaging event comprises a freeze thaw cycle.

19. The ~~method of administering an immunotherapy~~immune cells for use of claim 18, wherein immune cells were thawed using a water bath.

20. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-19, wherein the level of ADAM-17-cleaved surface receptor expression is assayed within 0 to 24 hours after the cell membrane damaging event.

21. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-19, wherein the level of ADAM-17-cleaved surface receptor expression is assayed within 12 to 24 hours after the cell membrane damaging event.

22. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-21, wherein the ADAM-17-cleaved surface receptor expressed on the immune cells comprises CD16, CD62L, or IL-15 receptor (IL-15R).

23. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-22, wherein the immune cell comprises a T cell, Natural Killer (NK) cell, chimeric antigen receptor (CAR) T cell, CAR NK cell, macrophage, dendritic cell, natural killer T (NKT) cell, innate lymphoid cell (ILC), B cell,  $\gamma\delta$ T cell, or neutrophil.

24. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-23, wherein the immune cell is a Natural Killer (NK) cell or CAR NK cell, and the ADAM-17-cleaved surface receptor is CD16.

25. The ~~method of administering an immunotherapy~~immune cells for use of claim 24, wherein the NK cell is an expanded NK cell.

26. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-25, wherein the immune cell is a T cell or CAR T cell and the ADAM-17-cleaved surface receptor comprises CD62L or IL-15R.

27. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-26, wherein the level of ADAM-17-cleaved surface receptor expression is assayed using flow cytometry.

28. The ~~method of administering an immunotherapy~~immune cells for use of any of claims 14-27,

wherein the level of ADAM-17-cleaved surface receptor expressed on the immune cells is expressed as a ratio of the level of surface receptor cleaved by ADAM17 expressed on the cell membrane damaged immune cell compared with the normal level of ADAM-17-cleaved surface receptor expressed on the immune cells.

5     29. ~~Use in an immunotherapy of a~~ therapeutically effective amount of immune cells for use in an immunotherapy, wherein the immune cells were previously subjected following to a cell membrane damaging event, and wherein the cells express an increased level of ADAM-17-cleaved surface receptor relative to a control immune cell.

10     30. The immune cells for use of claim 29, wherein the immunotherapy is an anticancer treatment.

31. The immune cells for use of claim 29 or 30, wherein the cell membrane damaging event comprises a freeze thaw cycle, gene editing, electroporation, magnetofection, detergent permeabilization, strotolysin O (SLO) exposure, physical morphology change (cell squeezing), and/or ethanol exposure.

15     32. The immune cells for use of claim 29 or 30, wherein the cell membrane damaging event comprises a freeze thaw cycle.

33. The immune cells for use of claim 32, wherein the immune cells were thawed using a water bath.

20     34. The immune cells for use of any of claims 29-33, wherein the level of ADAM-17-cleaved surface receptor expression is determined within 0 to 24 hours after the cell membrane damaging event.

35. The immune cells for use of any of claims 29-33, wherein the level of ADAM-17-cleaved surface receptor expression is assayed within 12 to 24 hours after the cell membrane damaging event.

25     36. The immune cells for use of any of claims 29-35, wherein the ADAM-17-cleaved surface receptor expressed on the immune cells comprises CD16, CD62L, or IL-15 receptor (IL-15R).

37. The immune cells for use of any of claims 29-36, wherein the immune cells comprise T cell, Natural Killer (NK) cell, chimeric antigen receptor (CAR) T cell, CAR NK cell, macrophage,

dendritic cell, natural killer T (NKT) cell, innate lymphoid cell (ILC), B cell,  $\gamma\delta$ T cell, or neutrophil.

38. The immune cells for use of any of claims 29-37, wherein the immune cells comprise Natural Killer (NK) cells and/or CAR NK cells, and the ADAM-17-cleaved surface receptor is CD16.

5 39. The immune cells for use of claim 38 wherein the NK cells and/or CAR NK cells comprise expanded NK cells.

40. The immune cells for use of any of claims 29-36, wherein the immune cells comprise T cells and/or CAR T cells and the ADAM-17-cleaved surface receptor comprises CD62L or IL-15R.

10 41. The immune cells for use of any of claims 29-40, wherein the level of ADAM-17-cleaved surface receptor expression is assayed using flow cytometry.

42. The immune cells for use of any of claims 29-41, wherein the level of ADAM-17-cleaved surface receptor expressed on the immune cells is expressed as a ratio of the level of surface receptor cleaved by ADAM17 expressed on the cell membrane damaged immune cell compared with the normal level of ADAM-17-cleaved surface receptor expressed on the immune cells.

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43. An immunotherapeutic composition comprising a therapeutically effective amount of immune cells previously subjected ~~following to~~ a cell membrane damaging event, and expressing an increased level of ADAM-17-cleaved surface receptor relative to a control immune cell.

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44. The immunotherapeutic composition of claim 43, wherein the cell membrane damaging event comprises a freeze thaw cycle, gene editing, electroporation, magnetofection, detergent permeabilization, strotolysin O (SLO) exposure, physical morphology change (cell squeezing), and/or ethanol exposure.

25 45. The immunotherapeutic composition of claim 43 or 44, wherein the membrane damaging event comprises a freeze thaw cycle.

46. The immunotherapeutic composition of claim 45, wherein the immune cells were thawed using a water bath.

47. The immunotherapeutic composition of any of claims 43-46, wherein the level of ADAM-17-cleaved surface receptor expression is determined within 0 to 24 hours after the cell membrane damaging event.
- 5 48. The immunotherapeutic composition of any of claims 43-46, wherein the level of ADAM-17-cleaved surface receptor expression is assayed within 12 to 24 hours after the cell membrane damaging event.
49. The immunotherapeutic composition of any of claims 43-48, wherein the ADAM-17-cleaved surface receptor expressed on the immune cells comprises CD16, CD62L, or IL-15 receptor (IL-15R).
- 10 50. The immunotherapeutic composition of any of claims 43-49, wherein the immune cells comprise T cell, Natural Killer (NK) cell, chimeric antigen receptor (CAR) T cell, CAR NK cell, macrophage, dendritic cell, natural killer T (NKT) cell, innate lymphoid cell (ILC), B cell,  $\gamma\delta$ T cell, or neutrophil.
- 15 51. The immunotherapeutic composition of claim 50, wherein the immune cells comprise Natural Killer (NK) cells and/or CAR NK cells, and the ADAM-17-cleaved surface receptor is CD16.
52. The immunotherapeutic composition of claim 51, wherein the NK cells and/or CAR NK cells comprise expanded NK cells.
- 20 53. The immunotherapeutic composition of claim 50, wherein the immune cells comprise T cells and/or CAR T cells and the ADAM-17-cleaved surface receptor comprises CD62L or IL-15R.
54. The immunotherapeutic composition of any of claims 43-53, wherein the level of ADAM-17-cleaved surface receptor expression is assayed using flow cytometry.
- 25 55. The immunotherapeutic composition of any of claims 43-54, wherein the level of ADAM-17-cleaved surface receptor expressed on the immune cells is expressed as a ratio of the level of surface receptor cleaved by ADAM17 expressed on the cell membrane damaged immune cell compared with the normal level of ADAM-17-cleaved surface receptor expressed on the immune cells.
56. The immunotherapeutic composition of any of claims 43-55, further comprising a

pharmaceutically acceptable carrier.