

What Is Claimed Is:

1. A method for improving a clinical outcome in a subject receiving a chimeric antigen receptor (CAR) T cell therapy, the method comprising: administering to the subject an effective amount of a population of engineered human T cells expressing a CAR (CAR T cells), wherein the engineered human CAR T cells comprise disrupted MHC class I, and wherein the subject has received or is receiving an effective amount of an NK cell inhibitor, thereby improving a clinical outcome in the subject.

2. A method for improving a clinical outcome in a subject receiving a chimeric antigen receptor (CAR) T cell therapy, the method comprising: administering to the subject an effective amount of an NK cell inhibitor, wherein the subject has received or is receiving an effective amount of a population of engineered human T cells expressing a CAR (CAR T cells), and wherein the engineered human CAR T cells comprise disrupted MHC class I, thereby reducing NK cell activity in the subject, thereby improving a clinical outcome in the subject.

3. A method for improving a clinical outcome in a subject receiving a chimeric antigen receptor (CAR) T cell therapy, the method comprising administering to the subject: an effective amount of:

- (a) an effective amount of an NK cell inhibitor; and
- (b) an effective amount of a population of engineered human T cells expressing a CAR (CAR T cells), wherein the engineered human CAR T cells comprise disrupted MHC class I, thereby improving a clinical outcome in the subject.

4. The method of any one of claims 1-3, wherein the improved clinical outcome comprises one or more of the following:

- (i) reducing natural killer (NK) cell activity in the subject;
- (ii) increasing a clinical response to the CAR-T therapy in the subject, which optionally is increased relative to the CAR-T therapy alone or relative to the therapy comprising the NK cell inhibitor alone;
- (iii) increasing persistence of the engineered human CAR-T cells in the subject; and

(iv) decreasing cell lysis of the engineered human CAR-T cells in the subject, optionally wherein cell lysis of the engineered human CAR-T cells is reduced by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in the subject relative to a subject receiving the engineered human CAR T cells without the NK cell inhibitor.

5. The method of claim 4, wherein in (ii), the clinical response increase is additive or synergistic.

6. The method of any one of claims 1-5, wherein the subject is a cancer patient, and wherein the improved clinical outcome comprises one or more of the following:

- (i) reducing tumor size or tumor cell numbers in the subject; and
- (ii) increasing an anti-tumor response in the subject.

7. The method of any one of claims 1-6, wherein expression of the MHC class I by the engineered human CAR-T cells is inhibited.

8. The method of any one of claims 1-7, wherein the engineered human CAR T cells comprise a disrupted beta-2-microglobulin (B2M) gene.

9. The method of any one of claims 1-8, wherein the engineered human CAR T cells comprise a disrupted HLA-A, HLA-B or HLA-C gene.

10. The method of any one of claims 1-6, wherein the engineered human CAR T cells comprise:

- (i) a disrupted T cell receptor alpha chain constant region (TRAC) gene;
- (ii) a disrupted B2M gene; and
- (iii) a nucleic acid encoding the CAR.

11. The method of any one of claims 1-10, wherein the CAR comprises an ectodomain that comprises an antigen-binding fragment, which binds a tumor antigen.

12. The method of claim 11, wherein the tumor antigen is CD19, CD33, CD70 or BCMA.
13. The method of any one of claims 8-12, wherein at least 50% of the engineered human CAR T cells do not express a detectable level of B2M surface protein.
14. The method of any one of claims 1-13, wherein the NK cell inhibitor reduces the number of NK cells, inhibits an activity of the NK cells, or both.
15. The method of claim 14, wherein the NK cells inhibitor reduces the number of NK cells by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.
16. The method of any one of claims 1-15, wherein the NK cell inhibitor reduces the number of NK cells by antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), apoptosis, or any combinations thereof.
17. The method of any one of claims 1-16, wherein the NK cell inhibitor is a small molecule, a monoclonal antibody or an antigen-binding fragment thereof, a polypeptide, a polynucleotide, or combinations thereof.
18. The method of any one of claim 1-16, wherein the NK cell inhibitor is an antibody that specifically binds CD38.
19. The method of claim 18, wherein the antibody is daratumumab, SAR650984, or MOR202, or an antigen-binding fragment thereof.
20. The method of claim 19, wherein the antibody is an antibody that binds to the same epitope as daratumumab and/or competes with daratumumab for binding to CD38.

21. The method of claim 20, wherein the antibody comprises the same heavy chain and light chain complementary determining regions as daratumumab.

22. The method of claim 21, wherein the antibody comprises the same heavy chain variable region and the same light chain variable region as daratumumab.

23. The method of any one of claims 1-22, wherein the NK cell inhibitor does not significantly reduce endogenous T cell numbers.

24. The method of any one of claims 1-23, wherein the NK inhibitor does not activate the engineered human CAR T cells.

25. The method of any one of claims 1-24, wherein the NK cell inhibitor is administered concurrently with administration of the population of engineered human CAR T cells.

26. The method of any one of claims 1-24, wherein the population of engineered human CAR T cells is administered prior to administration of the NK cell inhibitor.

27. The method of any one of claims 1-24, wherein the NK cell inhibitor is administered prior to administration of the population of engineered human CAR T cells.

28. The method of any one of claims 1-27, wherein the method further comprises a pre-conditioning regimen prior to administration of the population of engineered human CAR T cells.

29. The method of claim 28, wherein the pre-conditioning regimen comprises a lymphodepletion regimen.

30. The method of claim 29, wherein the population of engineered human CAR T cells is administered at least 48 hours after the lymphodepletion regimen.

31. The method of claim 30, wherein the population of engineered human CAR T cells is administered at least two days, at least three days, at least four days, at least five days, at least six days, or at least seven days after the lymphodepletion regimen.

32. The method of claim 30 or claim 31, wherein the population of engineered human CAR T cells is administered no more than seven days after the lymphodepletion regimen.

33. The method of any one of claims 29-32, wherein the lymphodepletion regimen is administered for at least one day, at least two days, at least three days, or at least four days.

34. The method of any one of claims 29-33, wherein the population of engineered human CAR T cells is administered between 48 hours and seven days after the lymphodepletion regimen, and wherein the lymphodepletion regimen is administered for two to three days.

35. The method of any one of claims 1-34, further comprising administering to the subject a subsequent dose of the NK cell inhibitor.

36. The method of claim 35, wherein the subsequent dose of the NK cell inhibitor is administered to the subject when NK cell numbers in the subject recover to about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of NK cell numbers prior to administration of the NK cell inhibitor.

37. The method of any one of claims 1-36, wherein the population of engineered human CAR T cells is administered by one or more intravenous infusions.

38. The method of any one of claims 1-36, wherein the population of engineered human CAR T cells is administered by a single intravenous infusion.

39. The method of any one of claims 1-36, wherein the population of engineered human CAR T cells is administered by more than one intravenous infusion.

40. The method of any one of claims 1-39, wherein the NK cell inhibitor is administered by one or more intravenous infusions.
41. The method of claim 40, wherein the NK cell inhibitor is daratumumab, which is administered at a dose of 1 to 24 mg/kg.
42. The method of claim 40 or claim 41, wherein the NK cell inhibitor is daratumumab, which is administered as a single dose infusion at 16 mg/kg.
43. The method of claim 42, wherein the NK cell inhibitor is daratumumab, which is administered as a split dose infusion at 8 mg/kg.
44. The method of claim 43, wherein the split dose is administered on consecutive days.
45. The method of any one of claims 29-44, wherein the lymphodepletion regimen comprises administering at least one chemotherapeutic agent.
46. The method of claim 45, wherein the at least one chemotherapeutic agent is cyclophosphamide, fludarabine, or a combination thereof.
47. A method for improving a clinical outcome in a subject, the method comprising: administering to the subject a chimeric antigen receptor (CAR) T cell therapy comprising a population of engineered human T cells expressing a CAR (CAR T cells), wherein the engineered human CAR T cells comprise (i) a disrupted B2M gene; (ii) a disrupted TRAC gene; and (iii) a nucleic acid encoding a CAR, and wherein the subject has received or is receiving an effective amount of an anti-CD38 antibody, thereby improving a clinical outcome in the subject.
48. A method for improving a clinical outcome in a subject, the method comprising: administering to the subject an effective amount of an anti-CD38 antibody, wherein the subject has received or is receiving a chimeric antigen receptor (CAR) T cell therapy comprising a

population of engineered human T cells expressing a CAR (CAR T cells), wherein the engineered human CAR T cells comprise (i) a disrupted B2M gene; (ii) a disrupted TRAC gene; and (iii) a nucleic acid encoding a CAR, thereby improving a clinical outcome in the subject.

49. A method for improving a clinical outcome in a subject, the method comprising administering to the subject:

(a) an effective amount of a population of engineered human CAR T cells, wherein the engineered human CAR T cells comprise (i) a disrupted B2M gene; (ii) a disrupted TRAC gene; and (iii) a nucleic acid encoding a CAR; and

(b) an effective amount of an anti-CD38 monoclonal antibody, thereby improving a clinical outcome in the subject.

50. The method of any one of claims 47-49, wherein the improved clinical outcome comprises one or more of the following:

(i) reducing natural killer (NK) cell activity in the subject;

(ii) increasing a clinical response to the CAR-T therapy in the subject, which optionally is increased relative to the CAR-T therapy alone or relative to the therapy comprising the NK cell inhibitor alone;

(iii) increasing persistence of the engineered human CAR-T cells in the subject; and

(iv) decreasing cell lysis of the engineered human CAR-T cells in the subject, optionally wherein cell lysis of the engineered human CAR-T cells is reduced by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% in the subject relative to a subject receiving the engineered human CAR T cells without the NK cell inhibitor.

51. The method of claim 50, wherein in (ii), the clinical response increase is additive or synergistic.

52. The method of any one of claims 47-51, wherein the subject is a cancer patient, and wherein the improved clinical outcome comprises one or more of the following:

(i) reducing tumor size or tumor cell numbers in the subject; and

(ii) increasing an anti-tumor response in the subject.

53. The method of any one of claims 47-52, wherein the CAR comprises an ectodomain, which comprises an antigen-binding fragment binding to CD19, CD33, BCMA or CD70.

54. The method of claim 53, wherein the antigen-binding fragment binds BCMA.

55. The method of claim 54, wherein the anti-BCMA antigen-binding fragment is an anti-BCMA scFv.

56. The method of claim 54 or claim 55, wherein the anti-BCMA antigen-binding fragment is a humanized anti-BCMA antigen-binding fragment.

57. The method of any one of claims 47-56, wherein the anti-CD38 monoclonal antibody is daratumumab or an antigen-binding fragment thereof.

58. The method of any one of claims 47-57, further comprising an administration of a lymphodepletion regimen comprising a combination of fludarabine and cyclophosphamide administered via intravenous infusion.

59. The method of claim 58, wherein the subject is administered a dose of about  $1 \times 10^7$  -  $3 \times 10^8$  engineered human CAR T cells expressing a detectable level of the CAR at least 48 hours but no more than seven days after the lymphodepletion therapy.

60. The method of any one of claims 47-59, wherein the disrupted B2M gene comprises an insertion, deletion and/or substitution of at least one nucleotide base pair.

61. The method of claim 60, wherein the disrupted B2M gene of the engineered human CAR-T cells comprises at least one nucleotide sequence selected from the group consisting of: SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 13; and SEQ ID NO: 14.

62. The method of any one of claims 1-61, wherein the engineered human CAR-T cells comprise a deletion of the nucleotide sequence of SEQ ID NO: 26 in the TRAC gene relative to unmodified T cells.

63. The method of any one of claims 1-62, wherein at least 50% of the engineered human CAR T cells express a detectable level of the CAR, and wherein less than 0.5% of the population of cells express a detectable level of TCR.

64. The method of any one of claims 1-63, wherein the nucleic acid encoding the CAR is located inside the disrupted TRAC gene.

65. A composition comprising a population of engineered human chimeric antigen receptor (CAR) T cells for use in a CAR T cell therapy in combination with an effective amount of an NK cell inhibitor, for the treatment of a cancer, optionally wherein the engineered human CAR-T cells are set forth in any one of claims 1-3, 7-13, 47-49, 53-56, and 60-64, and/or optionally wherein the NK cell inhibitor is set forth in any one of claims 14-24 and 57.

66. Use of the composition according to claim 65 in a CAR T cell therapy for treating a cancer in a subject in need thereof, wherein a first medicament comprises the population of cells comprising engineered human CAR T cells, and wherein the first medicament is administered in combination with a second medicament comprising an NK cell inhibitor and optionally a pharmaceutically acceptable carrier.

67. A kit comprising a first medicament comprising the composition of claim 65, and a package insert comprising instructions for administration of the composition in combination with a second medicament comprising a composition that comprises an NK cell inhibitor as set forth in claim 65, and an optional pharmaceutically acceptable carrier, to a subject in need thereof.

70. A kit comprising a first composition comprising the population of engineered human CAR T cells set forth in claim 65, a second composition comprising the NK cell inhibitor

set forth in claim 65, and a package insert comprising instructions for administration of the first composition in combination with the second composition to a subject in need thereof.

71. A kit comprising a first composition comprising a population of engineered human CAR T cells as set forth in claim 65, for use in a CAR T cell therapy, a second composition comprising an NK cell inhibitor as set forth in claim 65, and a package insert comprising instructions for administration of the first composition in combination with the second composition, to a subject for the treatment of cancer.