

USE OF CHIMERIC ANTIGEN RECEPTOR T CELLS AND NK CELL INHIBITORS FOR TREATING CANCER

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Application No. 62/867,764, filed June 27, 2019, and U.S. Provisional Application No. 62/951,732, filed December 20, 2019. The entire contents of each of the prior applications are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Chimeric antigen receptor (CAR) T cell therapies are adoptive T cell therapeutics used to treat human malignancies. Although CAR T cell therapy has led to tremendous clinical success, including durable remission in relapsed/refractory non-Hodgkin lymphoma (NHL) and pediatric acute lymphoblastic leukemia (ALL), the approved products are autologous and require patient-specific cell collection and manufacturing. Because of this, some patients have experienced disease progression or death while awaiting treatment. Allogeneic CAR T cell therapy, comprising disrupted MHC-1 complexes, presents an attractive off-the-shelf option to autologous CAR T cell therapy. The disrupted MHC class I in the allogeneic T cells, however, renders the CAR T cells susceptible to elimination by the host immune system, for example, by natural killer (NK) cell-mediated immune response. Accordingly, there remains a need for improved CAR T cell therapy

SUMMARY ~~OF THE INVENTION~~

The present disclosure is based, at least in part, on the unexpected discoveries that an anti-CD38 antibody (daratumumab), which is an exemplary NK cell inhibitor, successfully depleted NK cells both *in vitro* and *in vivo* but did not affect T cell numbers, including numbers of genetically engineered T cells expressing a chimeric antigen receptor (CAR), and did not activate CAR T cells. Further, it was found that, unexpectedly, daratumumab pre-treatment significantly reduced NK cell-mediated CAR T cell lysis (*e.g.*, by approximately 50%) and preserves the viability and number of allogeneic CAR T cells. Moreover, combined therapy of daratumumab and CAR-T cells exhibited synergistic effect in reducing tumor burden and extending survival rates in a xenograft mouse model, even in the presence of NK cells.

In one aspect, the present disclosure provides a use of a population of engineered human T cells expressing a CAR (CAR T cells) in the manufacture of a medicament for improving a clinical outcome in a subject, 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.

In another aspect, the present disclosure provides a use of an NK cell inhibitor in the manufacture of a medicament for improving a clinical outcome in a subject, 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.

In another aspect, the present disclosure provides a use of a population of engineered human T cells expressing a CAR (CAR T cells) in the manufacture of a first medicament and use of an NK cell inhibitor in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to improve a clinical outcome in a subject, wherein the engineered human CAR T cells comprise disrupted MHC class I.

In yet another aspect, the present disclosure provides a use of a population of engineered human T cells expressing a CAR (CAR T cells) in the manufacture of a medicament for use in combinatorial therapy for improving a clinical outcome in a subject, wherein said medicament is to be administered in combination with an NK cell inhibitor, wherein the engineered human CAR T cells comprise disrupted MHC class I.

In a further aspect, the present disclosure provides a kit comprising a first medicament comprising the population of genetically engineered human T cells as described herein and a second medicament comprising an NK cell inhibitor as described herein and an optional pharmaceutically acceptable carrier, as a combined pharmaceutical formulation for use in treating cancer.

Accordingly, one ~~aspect~~example of the present disclosure features a combined therapy to improve a clinical outcome, wherein the combined therapy involves genetically engineered T cells expressing a chimeric antigen receptor (“CAR” and “CAR T cells”) and an NK cell inhibitor (e.g., an anti-CD38 antibody such as daratumumab or a functional variant thereof).

In some ~~embodiment~~examples, provided herein is 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 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.

In some examples, a method for improving a clinical outcome in a subject, comprises: administering to the subject a chimeric antigen receptor (CAR) T cell therapy comprising 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 wherein the subject has received or is receiving an effective amount of an anti-CD38 antibody, thereby improving a clinical outcome in the subject.

In some embodiment examples, provided herein is 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 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.

In some examples, a method for improving a clinical outcome in a subject comprises: 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 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.

In some embodiment examples, provided herein is 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 CAR T cells, wherein the engineered human CAR T cells comprise disrupted MHC class I, thereby improving a clinical outcome in the subject.

In some examples, 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.

In any of the methods disclosed herein, the improved clinical outcome may comprise 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; (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. In some embodiment examples, the subject is a cancer patient, and 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.

In some examples, a clinical response to the CAR-T therapy (*e.g.*, anti-tumor response) in the subject may be increased relative to the CAR-T therapy alone. Alternatively, the clinical response to the CAR-T therapy in the subject may be increased relative to the therapy comprising the NK cell inhibitor alone. In yet other examples, the clinical response increase may be additive. Alternatively, the clinical response increase may be synergistic.

In some examples, cell lysis of the engineered human CAR-T cells can be 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.

In any of the methods disclosed herein, expression of the MHC class I in the engineered human CAR-T cells is inhibited. In some instances, the engineered human CAR T cells may comprise a disrupted beta-2-microglobulin (B2M) gene. Alternatively or in addition, the engineered human CAR T cells may comprise a disrupted HLA-A, HLA-B or HLA-C gene.

In some embodiment examples, the engineered human CAR T cells comprise: (i) a disrupted T cell receptor alpha chain constant region (TRAC) gene; (ii) a disrupted B2M gene (*e.g.*, as those described herein); and (iii) a nucleic acid encoding a CAR. For example, the disrupted B2M gene may comprise an insertion, deletion and/or substitution of at least one nucleotide base pair. In specific examples, the disrupted B2M gene of the engineered human CAR-T cells may comprise at least one nucleotide sequence 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. Alternatively or in

addition, the engineered human T cells may comprise a deletion of the nucleotide sequence of SEQ ID NO: 29 in the TRAC gene relative to unmodified T cells. In some instances, the nucleic acid encoding the CAR may be located inside the disrupted TRAC gene.

In some [embodiment examples](#), at least 50% of the engineered human T cells may express a detectable level of the CAR. Alternatively or in addition, less than 0.5% of the population of cells expresses a detectable level of TCR. Further, at least 50% of the engineered human CAR T cells may not express a detectable level of B2M surface protein.

In some examples, the engineered human CAR T cells express a CAR comprising an ectodomain that comprises an antigen-binding fragment, which binds a tumor antigen. Exemplary tumor antigens include, but are not limited to, CD19, CD33, CD70, and BCMA. In some examples, the tumor antigen is CD19 and the anti-CD19 antigen-binding fragment in a CAR is an anti-CD19 scFv. In some instances, the anti-CD19 antigen-binding fragment is a human or humanized anti-CD19 antigen-binding fragment. In some examples, the tumor antigen is BCMA and the anti-BCMA antigen-binding fragment in a CAR is an anti-BCMA scFv. In some instances, the anti-CD19 antigen-binding fragment is a human or humanized anti-BCMA antigen-binding fragment. In some examples, the tumor antigen is CD70 and the anti-CD70 antigen-binding fragment in a CAR is an anti-CD70 scFv. In some instances, the anti-CD70 antigen-binding fragment is a human or humanized anti-CD70 antigen-binding fragment. In some examples, the tumor antigen is CD33 and the anti-CD33 antigen-binding fragment in a CAR is an anti-CD33 scFv. In some instances, the anti-CD33 antigen-binding fragment is a human or humanized anti-CD33 antigen-binding fragment.

In any of the methods disclosed herein, the NK cell inhibitor may reduce the number of NK cells, inhibits an activity of the NK cells, or both. In some examples, the NK cells inhibitor may reduce the number of NK cells by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. Without being bound by theory, 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.

In some [embodiment examples](#), the NK cell inhibitor does not significantly reduce endogenous T cell numbers. Alternatively or in addition, the NK inhibitor does not significantly activate the engineered human CAR T cells.

In some embodiment examples, the NK cell inhibitor may be a small molecule, a monoclonal antibody or an antigen-binding fragment thereof, a polypeptide, a polynucleotide, or combinations thereof. In some examples, the NK cell inhibitor can be an antibody that specifically binds CD38. For example, the NK cell inhibitor can be daratumumab, SAR650984, or MOR202, or an antigen-binding fragment thereof. In some instances, the NK cell inhibitor is an antibody that binds to the same epitope as daratumumab and/or competes with daratumumab for binding to CD38. In specific examples, the antibody may comprise the same heavy chain and light chain complementary determining regions as daratumumab. For example, the antibody may comprise the same heavy chain variable region and the same light chain variable region as daratumumab.

In any of the methods disclosed herein, the NK cell inhibitor can be administered concurrently with administration of the population of engineered human CAR T cells. Alternatively, the population of engineered human CAR T cells is administered prior to administration of the NK cell inhibitor. In other examples, the NK cell inhibitor is administered prior to administration of the population of engineered human CAR T cells.

Any of the methods disclosed herein may further comprise a pre-conditioning regimen prior to administration of the population of engineered human CAR T cells. Such a pre-conditioning regimen comprises a lymphodepletion regimen. In some examples, a lymphodepletion regimen may comprise administering at least one chemotherapeutic agent. Examples include cyclophosphamide, fludarabine, or a combination thereof. In specific examples, a lymphodepletion regimen may comprise a combination of fludarabine and cyclophosphamide administered via intravenous infusion.

In some instances, the population of engineered human CAR T cells can be administered at least 48 hours after the lymphodepletion regimen. In some examples, the population of engineered human CAR T cells can be administered at least two days, at least three days (*e.g.*, at least four days, at least five days, at least six days, or at least seven days) after the lymphodepletion regimen. In some examples, the population of engineered human CAR T cells can be administered no more than seven days after the lymphodepletion regimen.

In some instances, the lymphodepletion regimen can be administered for at least one day (*e.g.*, at least two days, at least three days, or at least four days). In specific examples, the population of engineered human CAR T cells can be administered between 48 hours and seven

days after the lymphodepletion regimen, and the lymphodepletion regimen can be administered for two to three days.

In some embodiment examples, the population of engineered human CAR T cells can be administered by one or more intravenous infusions. In some examples, the population of engineered human CAR T cells is administered by a single intravenous infusion. Alternatively, the population of engineered human CAR T cells is administered by more than one intravenous infusion. In some examples, a single container comprises a dose of the population of engineered human CAR T cells. In other examples, more than one container comprises the dose of the population of engineered human CAR T cells.

In some embodiment examples, the NK cell inhibitor can be administered by one or more intravenous infusions. In some examples, the NK cell inhibitor is daratumumab, which can be administered at a dose of 1 to 24 mg/kg. In specific examples, the NK cell inhibitor is daratumumab, which is administered as a single dose infusion at 16 mg/kg. In further examples, the NK cell inhibitor is daratumumab, which is administered as a split dose infusion at 8 mg/kg. The split dose is administered on consecutive days.

In some instances, any of the method disclosed herein may comprise administering to the subject a subsequent dose of the NK cell inhibitor. In some examples, 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.

In one example, the subject is administered a dose of about 1×10^7 - 3×10^8 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.

Further, the present disclosure provides a composition comprising a population of engineered human chimeric antigen receptor (CAR) T cells as those disclosed herein for use in a CAR T cell therapy in combination with an effective amount of an NK cell inhibitor as also disclosed herein, for the treatment of a cancer. The present disclosure also provides uses of the composition comprising a population of engineered human chimeric antigen receptor (CAR) T cells as those disclosed herein for manufacturing a first medicament for use in a CAR T cell therapy for treating a cancer in a subject in need thereof. The first medicament comprises the population of cells comprising engineered human CAR T cells and the first medicament is

administered in combination with a second medicament comprising an NK cell inhibitor and optionally a pharmaceutically acceptable carrier.

Also within the scope of the present disclosure are:

(a) a kit comprising a first medicament comprising the composition comprising the population of CAR-T cells as disclosed herein, 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 also disclosed herein, and an optional pharmaceutically acceptable carrier, to a subject in need thereof;

(b) a kit comprising a first composition comprising the population of engineered human CAR T cells as disclosed herein, a second composition comprising the NK cell inhibitor as also disclosed herein, and a package insert comprising instructions for administration of the first composition in combination with the second composition to a subject in need thereof; and

(c) a kit comprising a first composition comprising a population of engineered human CAR T cells as disclosed herein, for use in a CAR T cell therapy, a second composition comprising an NK cell inhibitor as also disclosed herein, 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.

The details of one or more embodiment examples of the invention disclosure are set forth in the description below. Other features or advantages of the present invention disclosure will be apparent from the following drawings and detailed description of several embodiment examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein.

What Is Claimed Is:

1. ~~Use of 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) in the manufacture of a medicament for improving a clinical outcome in a subject, 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. ~~Use of 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 in the manufacture of a medicament for improving a clinical outcome in a subject, 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. ~~Use of 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) in the manufacture of a first medicament and use of an NK cell inhibitor in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to improve a clinical outcome in a subject, wherein the engineered human CAR T cells comprise disrupted MHC class I, ~~thereby improving a clinical outcome in the subject.~~

4. Use of a population of engineered human T cells expressing a CAR (CAR T cells) in the manufacture of a medicament for use in combinatorial therapy for improving a clinical

outcome in a subject, wherein said medicament is to be administered in combination with an NK cell inhibitor, wherein the engineered human CAR T cells comprise disrupted MHC class I.

~~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.~~

65. The ~~method-use~~ of any one of claims 1-~~54~~, 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.~~

86. The ~~method-use~~ of any one of claims 1-~~75~~, wherein the engineered human CAR T cells comprise a disrupted beta-2-microglobulin (B2M) gene, and/or a disrupted HLA-A, HLA-B or HLA-C 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.~~

~~107.~~ The ~~method-use~~ of any one of claims 1-~~65~~, 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, wherein the CAR comprises an ectodomain that comprises an antigen-binding fragment, which binds a tumor antigen.

~~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.~~

~~812.~~ The ~~method-use~~ of claim ~~511~~, wherein the tumor antigen is CD19, CD33, CD70 or BCMA.

~~913.~~ The ~~method-use~~ of any one of claims ~~6-88-12~~, wherein at least 50% of the engineered human CAR T cells in the population 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.~~

~~1710.~~ The ~~method-use~~ of any one of claims 1-~~169~~, 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.

~~1811.~~ The ~~method-of-any-one-of-claim-1-16~~use of any one of claims 1-9, wherein the NK cell inhibitor is an antibody that specifically binds CD38.

~~1249.~~ The ~~method-use~~ of claim ~~1811~~, 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.

~~1321.~~ The ~~method-use~~ of claim ~~1220~~, wherein the antibody comprises the same heavy chain and light chain complementary determining regions as daratumumab.

~~1422.~~ The ~~method-use~~ of claim ~~1321~~, 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.~~

1528. The ~~method-use~~ of any one of claims 1-~~1427~~, wherein the subject is to be treated ~~method further comprises~~ a pre-conditioning regimen prior to administration of the population of engineered human CAR T cells, and wherein the pre-conditioning regimen comprises a lymphodepletion regimen.

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

1630. The ~~method-use~~ of claim 1529, wherein the population of engineered human CAR T cells is to be 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.~~

1732. The ~~method-use~~ of claim ~~30 or claim 31~~16, wherein the population of engineered human CAR T cells is to be 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.~~

1837. The ~~method-use~~ of any one of claims 1-~~17~~36, wherein the population of engineered human CAR T cells is to be 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.~~

1940. The ~~method-use~~ of any one of claims 1-~~18~~39, wherein the NK cell inhibitor is to be 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.~~

2042. The ~~method of claim 40 or claim 41~~use of claim 19, wherein the NK cell inhibitor is daratumumab, ~~which is and wherein daratumumab is to be~~ administered as a single dose infusion at 16 mg/kg or as a split dose infusion at 8 mg/kg on two consecutive days.

~~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.~~

2145. The ~~usemethod~~ of any one of claims ~~15-2029-44~~, wherein the lymphodepletion regimen comprises ~~administering~~ at least one chemotherapeutic agent.

2246. The ~~usemethod~~ of claim ~~4521~~, 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.~~

5423. The ~~method-use~~ of claim ~~538~~, 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.~~

5924. The ~~method-use~~ of any one of claims 581-23, wherein the engineered human CAR T cells are to be administered to the subject ~~is administered at~~ a dose of about 1×10^7 - 3×10^8 ~~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.~~

2561. The ~~method-of-claim-60~~ use of any one of claims 6-24, 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.

6226. The ~~method-use~~ of any one of claims ~~1-61~~ 7-25, 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.

6327. The ~~method-use~~ of ~~any one of claims 1-~~ 6226, wherein at least 50% of the engineered human CAR T cells in the population express a detectable level of the CAR, and wherein less than 0.5% of the engineered human CAR T cells in the population express a detectable level of TCR.

~~6428.~~ The ~~method-use~~ of any one of claims ~~1-637-27~~, 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.~~

~~2966. The use Use of the composition according to claim 65 of any one of claims 1-28 in a CAR T cell therapy for treating a cancer in thea 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.~~

~~3067. A kit comprising a first medicament comprising the population of genetically engineered human T cells set forth in any one of claims 1-4, 6-9, 23, and 25-28 and a second medicament comprising 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 any one of claims 6510-14, and an optional pharmaceutically acceptable carrier, as a combined pharmaceutical formulation for use in treating cancer 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.