

tumors thus requires effective tumor infiltration and overcoming tumor-induced immunosuppression. In addition, solid tumors pose a challenge for selecting optimal immune targets – antigens whose targeting would enable tumor eradication by potent T cells, with minimal or tolerable toxicity to non-tumor tissues.

Accordingly, there are needs for novel therapeutic strategies to design CARs for treating cancers, particularly, solid tumors, which strategies capable of inducing potent tumor eradication with minimal toxicity and immunogenicity.

3. SUMMARY

In one aspect, the present disclosure refers to a polypeptide composition comprising:

i) a chimeric antigen receptor (CAR) comprising:

(a) an extracellular antigen-binding domain comprising: a heavy chain variable region that comprises a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; and a light chain variable region that comprises a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:79, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:80, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:81,

(b) an intracellular signaling domain comprising a modified CD3 ζ polypeptide comprising an ITAM2 variant and an ITAM3 variant, wherein each of the ITAM2 variant and the ITAM3 variant comprises two loss-of-function mutations; and

ii) a dominant negative form of programmed death 1 (PD-1 DN) comprising:

(a) at least a portion of an extracellular domain of programmed death 1 (PD-1) comprising a ligand binding region, and

(b) a first transmembrane domain.

In another aspect, the present disclosure refers to an immunoresponsive cell comprising a polypeptide composition as described herein.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of an immunoresponsive cell as described herein and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to a nucleic acid composition comprising a polynucleotide encoding the polypeptide composition as described herein.

In another aspect, the present disclosure refers to a vector comprising the nucleic acid composition as described herein.

5 In another aspect, the present disclosure refers to a method for producing an immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a polypeptide composition as described herein, a nucleic acid composition as described herein, or a vector as described herein.

10 In another aspect, the present disclosure refers to a kit comprising a polypeptide composition as described herein, a nucleic acid composition as described herein, a vector as described herein, an immunoresponsive cell as described herein, or a pharmaceutical composition as described herein.

15 In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for preventing and/or treating a neoplasm in a subject, wherein an effective amount of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject.

20 In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for reducing tumor burden in a subject, wherein an effective amount of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject.

25 In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for treating a subject having a relapse of a neoplasm, wherein an effective amount of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject

30 In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for increasing or lengthening survival of a subject having a neoplasm, wherein an effective amount of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject.

In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for increasing immune-activating cytokine production in response to a cancer cell or a pathogen in a subject, wherein an effective amount of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject

In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for preventing and/or treating an inflammatory disease in a subject, wherein the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject.

In yet another aspect, the present disclosure refers to a use of the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein in the manufacture of a medicament for preventing graft rejection in a subject who is a recipient of an organ transplant, wherein the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein is to be administered to the subject.

In yet another aspect, the present disclosure refers to the immunoresponsive cells as described herein, or a pharmaceutical composition as described herein for use in treating and/or preventing a neoplasm in a subject, treating a subject having a relapse of a neoplasm, reducing tumor burden in a subject, increasing or lengthening survival of a subject having a neoplasm, preventing and/or treating an inflammatory disease in a subject, and/or preventing graft rejection in a subject who is a recipient of an organ transplant.

The presently disclosed subject matter provides polypeptide compositions comprising (a) a chimeric antigen receptor (CAR) that specifically targets mesothelin (e.g., human mesothelin); and (b) a dominant negative form of programmed death 1 (PD-1 DN); immunoresponsive cells comprising such polypeptide compositions, and uses of these polypeptide compositions and immunoresponsive cells, e.g., for treating cancers.

The presently disclosed subject matter provides polypeptide compositions. In certain examples, the polypeptide composition comprises: i) a chimeric antigen receptor (CAR) and ii) a dominant negative form of programmed death 1 (PD-1 DN), wherein the CAR comprises (a) an extracellular antigen-binding domain and (b) an

intracellular signaling domain comprising a modified CD3 ζ polypeptide comprising an ITAM2 variant and an ITAM3 variant, wherein each of the ITAM2 variant and an ITAM3 variant comprises two loss-of-function mutations.

5 In certain examples, the extracellular antigen-binding domain comprises: a heavy chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:76, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:78; and a light chain variable region comprising a CDR1 comprising the amino acid sequence set forth in SEQ ID NO:79, a CDR2 comprising the amino acid sequence set forth in SEQ ID NO:80,
10 and a CDR3 comprising the amino acid sequence set forth in SEQ ID NO:81.

In certain examples, the PD-1 DN comprises: (a) at least a portion of an extracellular domain of programmed death 1 (PD-1) comprising a ligand binding region, and (b) a first transmembrane domain.

15 In certain examples, the first transmembrane domain of the PD-1 DN comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1

polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof. In certain examples, the first transmembrane domain of the PD-1 DN comprises a CD8 polypeptide. In certain examples, the CD8 polypeptide comprised in the first transmembrane domain of the PD-1 DN comprises amino acids 137 to 207 of SEQ ID NO: 86. In certain examples, the PD-1 DN lacks an intracellular domain. In certain examples, the PD-1 DN comprises amino acids 21 to 165 of SEQ ID NO: 48 and amino acids 137 to 207 of SEQ ID NO: 86.

In certain examples, the extracellular antigen-binding domain of the CAR specifically binds to human mesothelin with an EC50 value of from about 1 nM to about 25 nM. In certain examples, the extracellular antigen-binding domain of the CAR specifically binds to human mesothelin with an EC50 value of about 20 nM.

In certain examples, the extracellular antigen-binding domain of the CAR comprises a single-chain variable fragment (scFv), a Fab that is optionally crosslinked, or a F(ab)₂. In certain examples, the extracellular antigen-binding domain of the CAR comprises a human scFv. In certain examples, the extracellular antigen-binding domain of the CAR recognizes human mesothelin with a mesothelin expression level of about 1,000 or more mesothelin binding sites/cell.

In certain examples, the heavy chain variable region comprises an amino acid sequence that is at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% homologous to identical to the amino acid sequence set forth in SEQ ID NO:82. In certain examples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:82.

In certain examples, the light chain variable region comprises an amino acid sequence that is at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% homologous to identical to the amino acid sequence set forth in SEQ ID NO: 83. In

certain examples, the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 83.

In certain examples, the heavy chain variable region comprises an amino acid sequence that is at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% homologous to identical to the amino acid sequence set forth in SEQ ID NO:82, and the light chain variable region comprises an amino acid sequence that is at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% homologous to identical to the amino acid sequence set forth in SEQ ID NO: 83. In certain examples, the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:82, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 83.

In certain examples, the extracellular antigen-binding domain of the CAR comprises a linker between the heavy chain variable region and the light chain variable region.

In certain examples, a leader that is covalently joined to the N-terminus of the extracellular antigen-binding domain. In certain examples, the leader comprises a CD8 polypeptide. In certain examples, the CD8 polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 71. In certain examples, the at least a portion of the extracellular domain of PD-1 comprises amino acids 21 to 165 of SEQ ID NO: 48.

In certain examples, each of the loss-of-function mutations in the modified CD3 ζ polypeptide of the CAR is at a tyrosine amino acid residue. In certain examples, the ITAM2 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 29. In certain examples, the ITAM3 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33. In certain examples, the modified CD3 ζ polypeptide comprises a native ITAM1. In certain examples, the native ITAM1 comprises or consists of the amino acid sequence set forth in SEQ ID NO:

23. In certain examples, the modified CD3 ζ polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 35.

In certain examples, the CAR comprises or consists of the amino acid sequence set forth in SEQ ID NO: 56.

5 In certain examples, the CAR further comprises a second transmembrane domain. In certain examples, the second transmembrane domain of the CAR comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a
10 CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof. In certain examples, the second transmembrane domain of the CAR comprises a CD28 polypeptide

In certain examples, the intracellular signaling domain of the CAR further comprises a co-stimulatory signaling domain. In certain examples, the co-stimulatory
15 signaling region comprises a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide, a CD27 polypeptide, a CD40/My88 polypeptide, a NKGD2 polypeptide, or a combinations thereof. In certain examples, the co-stimulatory signaling region comprises a CD28 polypeptide.

The presently disclosed subject matter provides immunoresponsive cells
20 comprising a polypeptide composition disclosed herein. In certain examples, the PD-1 DN and/or the CAR is recombinantly expressed. In certain examples, the PD-1 DN and/or the CAR is expressed from a vector. In certain examples, the immunoresponsive cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a pluripotent stem cell from which lymphoid cells may be differentiated. In certain
25 examples, the pluripotent stem cell is an embryonic stem cell or an induced pluripotent stem cells. In certain examples, the immunoresponsive cell is a T cell. In certain examples, the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a regulatory T cell, and a Natural Killer T (NKT) cell. In certain examples, the immunoresponsive cell is autologous. In certain examples, the immunoresponsive cell is
30 allogenic.

The presently disclosed subject matter further provides compositions comprising an immunoresponsive cell disclosed herein. In certain examples, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain examples, the pharmaceutical composition comprises between

about 10^4 and 10^6 of the immunoresponsive cells. In certain examples, the pharmaceutical composition comprises at least about 10^5 of the immunoresponsive cells. In certain examples, the pharmaceutical composition comprises about 10^5 of the immunoresponsive cells. In certain examples, the pharmaceutical composition is for preventing and/or
5 treating a neoplasm in a subject, treating a subject having a relapse of a neoplasm, reducing tumor burden in a subject, increasing or lengthening survival of a subject having a neoplasm, preventing and/or treating an inflammatory disease in a subject, and/or preventing graft rejection in a subject who is a recipient of an organ transplant.

In addition, the presently disclosed subject matter provides nucleic acid
10 compositions comprising a polynucleotide encoding a polypeptide composition disclosed herein. In certain examples, the polynucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 123. In certain examples, the polynucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 124. The presently disclosed subject matter further provides vectors comprising the presently disclosed nucleic acid compositions. In
15 certain examples, the vector is a retroviral vector. In certain examples, the retroviral vector is a γ -retroviral vector or a lentiviral vector.

The presently disclosed subject matter provides methods for producing an immunoresponsive cell disclosed herein. In certain examples, the method comprises introducing into an immunoresponsive cell a presently disclosed polypeptide
20 composition, a presently disclosed nucleic acid composition, or a presently disclosed vector.

The presently disclosed subject matter provides kits comprising a presently disclosed polypeptide composition, a presently disclosed nucleic acid composition, a presently disclosed vector, an presently disclosed immunoresponsive cell, or a presently
25 disclosed pharmaceutical composition. In certain examples, the kit further comprises written instructions for treating and/or preventing a neoplasm.

Furthermore, the presently disclosed subject matter provides various methods of using the above-described immunoresponsive cell. For example, the presently disclosed subject matter provides methods of reducing tumor burden in a subject, wherein the
30 method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain examples, the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

The presently disclosed subject matter also provides methods of increasing or lengthening survival of a subject having a neoplasm, where the method comprises administering to the subject an effective amount of the presently disclosed immunoresponsive cell or a presently disclosed pharmaceutical composition.

5 In certain examples, the tumor or neoplasm is a solid tumor. In certain examples, the solid tumor is selected from the group consisting of mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, cholangiocarcinoma, cervical cancer, salivary
10 gland cancer, and a combination thereof.

The presently disclosed subject matter provides methods of treating a subject having a relapse of a neoplasm, the method comprising administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical composition disclosed herein. In certain examples, the subject received an immunotherapy prior to
15 said administration of the immunoresponsive cells or the composition.

Additionally, the presently disclosed subject matter provides methods of increasing immune-activating cytokine production in response to a cancer cell or a pathogen in a subject. In certain examples, the method comprises administering to the subject an effective amount of the immunoresponsive cells or the pharmaceutical
20 composition disclosed herein. In certain examples, the immune-activating cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor (GM-CSF), IFN- α , IFN- β , IFN- γ , TNF- α , IL-2, IL-3, IL-6, IL-11, IL-7, IL-12, IL-15, IL-21, interferon regulatory factor 7 (IRF7), and combinations thereof.

In accordance with the presently disclosed subject matter, the above-described
25 various methods can comprise administering at least one immunomodulatory agent. In certain examples, the at least one immunomodulatory agent is selected from the group consisting of immunostimulatory agents, checkpoint immune blockade agents, radiation therapy agents, chemotherapy agents, and combinations thereof. In some examples, the immunostimulatory agents are selected from the group consisting of IL-12, an agonist
30 costimulatory monoclonal antibody, and combinations thereof. In certain examples, the immunostimulatory agent is IL-12. In some examples, the agonist costimulatory monoclonal antibody is selected from the group consisting of an anti-4-1BB antibody, an anti-OX40 antibody, an anti-ICOS antibody, and combinations thereof. In certain examples, the agonist costimulatory monoclonal antibody is an anti-4-1BB antibody.

In certain examples, the checkpoint immune blockade agents are selected from the group consisting of anti-PD-L1 antibodies, anti-CTLA-4 antibodies, anti-PD-1 antibodies, anti-LAG3 antibodies, anti-B7-H3 antibodies, anti-TIM3 antibodies, and combinations thereof. In certain examples, the checkpoint immune blockade agent is an anti-PD-L1 antibody or an anti-PD-1 antibody. In certain examples, the subject is a human.

In certain examples, the immunoresponsive cell is pleurally or intrapleurally administered to the subject.

The presently disclosed subject matter further provides a method of preventing and/or treating an inflammatory disease in a subject. In certain examples, the method comprises administering the presently disclosed immunoresponsive cell or the pharmaceutical composition to the subject. In certain examples, the immunoresponsive cell is an immunoinhibitory cell. In certain examples, the immunoinhibitory cell is a regulatory T cell. In certain examples, the inflammatory disease is pancreatitis. In certain examples, the subject is a human. In certain examples, the subject is a recipient of an organ transplant. In certain examples, the subject is a recipient of a pancreas transplant.

The presently disclosed subject matter further provides a method of preventing graft rejection in a subject who is a recipient of an organ transplant. In certain examples, the method comprises administering the presently disclosed immunoresponsive cell or the pharmaceutical composition to the subject. In certain examples, the immunoresponsive cell is an immunoinhibitory cell. In certain examples, the immunoinhibitory cell is a regulatory T cell. In certain examples, the subject is a human. In certain examples, the subject is a recipient of an pancreas transplant.

4. BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific embodiments described, may be understood in conjunction with the accompanying drawings.

Figure 1 depicts a polypeptide composition in accordance with certain embodiments of the presently disclosed subject matter. The polypeptide composition comprises a CAR comprising an anti-mesothelin (MSLN) scFv, a CD28 transmembrane domain, a CD28 cytoplasmic signaling domain, a CD3zeta signaling domain (e.g., comprising an ITAM2 variant and an ITAM3 variant). The CAR is fused to the PD1DNR (and PD1 signaling domain) via a cleavable P2A peptide. SP: signaling peptide; scFv:

WHAT IS CLAIMED IS:

1. A polypeptide composition comprising:
 - i) a chimeric antigen receptor (CAR) comprising:
 - (a) an extracellular antigen-binding domain comprising: a heavy chain
5 variable region that comprises a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; and a light chain variable region that comprises a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:79, a CDR2 consisting of the amino acid sequence set
10 forth in SEQ ID NO:80, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:81,
 - (b) an intracellular signaling domain comprising a modified CD3 ζ polypeptide comprising an ITAM2 variant and an ITAM3 variant, wherein each of the ITAM2 variant and the ITAM3 variant comprises two loss-of-function mutations; and
15 ii) a dominant negative form of programmed death 1 (PD-1 DN) comprising:
 - (a) at least a portion of an extracellular domain of programmed death 1 (PD-1) comprising a ligand binding region, and
20 (b) a first transmembrane domain.
 2. The polypeptide composition of claim 1, wherein the extracellular antigen-binding
20 domain of the CAR specifically binds to human mesothelin with an EC50 value of from about 1 nM to about 25 nM.
 3. The polypeptide composition of claim 1 or 2, wherein the extracellular antigen-binding domain of the CAR comprises a single-chain variable fragment (scFv), a Fab that is optionally crosslinked, or a F(ab)₂.
 - 25 4. The polypeptide composition of claim 3, wherein the extracellular antigen-binding domain of the CAR comprises a human scFv.
 5. The polypeptide composition of any one of claims 1-4, wherein the extracellular antigen-binding domain of the CAR recognizes human mesothelin with a mesothelin expression level of about 1,000 or more mesothelin binding sites/cell.

6. The polypeptide composition of any one of claims 1-5, wherein the heavy chain variable region comprises an amino acid sequence that is at least about 80% homologous or identical to the amino acid sequence set forth in SEQ ID NO:82.
7. The polypeptide composition of any one of claims 1-6, wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:82.
8. The polypeptide composition of any one of claims 1-7, wherein the light chain variable region comprising an amino acid sequence that is at least about 80% homologous or identical to the amino acid sequence set forth in SEQ ID NO:83.
9. The polypeptide composition of any one of claims 1-8, wherein the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 83.
10. The polypeptide composition of any one of claims 1-9, wherein the heavy chain variable region comprises an amino acid sequence that is at least about 80% homologous or identical to the amino acid sequence set forth in SEQ ID NO:82, and the light chain variable region comprises an amino acid sequence that is at least about 80% homologous or identical to the amino acid sequence set forth in SEQ ID NO: 83.
11. The polypeptide composition of any one of claims 1-10, wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO:82, and the light chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 83.
12. The polypeptide composition of any one of claims 1-11, wherein the extracellular antigen-binding domain of the CAR comprises a linker between the heavy chain variable region and the light chain variable region.
13. The polypeptide composition of any one of claims 1-12, wherein a leader is covalently joined to a N-terminus of the extracellular antigen-binding domain.
14. The polypeptide composition of claim 13, wherein the leader comprises a CD8 polypeptide.
15. The polypeptide composition of claim 14, wherein the CD8 polypeptide consists of the amino acid sequence set forth in SEQ ID NO: 71.

16. The polypeptide composition of any one of claims 1-15, wherein the at least a portion of an extracellular domain of PD-1 comprises amino acids 21 to 165 of SEQ ID NO: 48.
17. The polypeptide composition of any one of claims 1-16, wherein the first
5 transmembrane domain of the PD-1 DN comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof.
- 10 18. The polypeptide composition of claim 17, wherein the first transmembrane domain of the PD-1 DN comprises a CD8 polypeptide.
19. The polypeptide composition of claim 18, wherein the CD8 polypeptide comprised in the first transmembrane domain of the PD-1 DN comprises amino acids 137 to 207 of SEQ ID NO: 86.
- 15 20. The polypeptide composition of any one of claims 1-28, wherein the PD-1 DN comprises amino acids 21 to 165 of SEQ ID NO: 48 and amino acids 137 to 207 of SEQ ID NO: 86.
21. The polypeptide composition of any one of claims 1-20, wherein each of the two loss-of-function mutations is at a tyrosine amino acid residue.
- 20 22. The polypeptide composition of any one of claims 1-21, wherein the ITAM2 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 29.
23. The polypeptide composition of any one of claims 1-22, wherein the ITAM3 variant comprises or consists of the amino acid sequence set forth in SEQ ID NO: 33.
24. The polypeptide composition of any one of claims 1-23, wherein the modified
25 CD3 ζ polypeptide comprises a native ITAM1.
25. The polypeptide composition of claim 24, wherein the native ITAM1 comprises or consists of the amino acid sequence set forth in SEQ ID NO: 23.

26. The polypeptide composition of any one of claims 1-25, wherein the modified CD3 ζ polypeptide comprises or consists of the amino acid sequence set forth in SEQ ID NO: 35.
27. The polypeptide composition of any one of claims 1-26, wherein the PD-1 DN
5 lacks an intracellular domain.
28. The polypeptide composition of any one of claims 1-27, wherein the CAR further comprise a second transmembrane domain.
29. The polypeptide composition of claim 28, wherein the second transmembrane domain of the CAR comprises a CD8 polypeptide, a CD28 polypeptide, a CD3 ζ
10 polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 polypeptide, a CD166 polypeptide, a CD8a polypeptide, a CD8b polypeptide, an ICOS polypeptide, an ICAM-1 polypeptide, a CTLA-4 polypeptide, a CD27 polypeptide, a CD40/My88 peptide, a NKGD2 peptide, or a combination thereof.
30. The polypeptide composition of claim 28 or 29, wherein the second
15 transmembrane domain of the CAR comprises a CD28 polypeptide
31. The polypeptide composition of any one of claims 1-30, wherein the intracellular signaling domain of the CAR further comprises a co-stimulatory signaling region.
32. The polypeptide composition of claim 31, wherein the co-stimulatory signaling region comprises a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an
20 ICOS polypeptide, a DAP-10 polypeptide, a CD27 polypeptide, a CD40/My88 polypeptide, a NKGD2 polypeptide, or a combinations thereof.
33. The polypeptide composition of claim 31 or 32, wherein the co-stimulatory signaling region comprises a CD28 polypeptide.
34. The polypeptide composition of any one of claims 1-33, wherein the CAR
25 comprises the amino acid sequence set forth in SEQ ID NO: 56.
35. An immunoresponsive cell comprising a polypeptide composition of any one of claims 1-34.

36. The immunoresponsive cell of claim 35, wherein the PD-1 DN and/or the CAR is recombinantly expressed.
37. The immunoresponsive cell of claim 35 or 36, wherein the PD-1 DN and/or the CAR is expressed from a vector.
- 5 38. The immunoresponsive cell of any one of claims 35-37, wherein the immunoresponsive cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.
39. The immunoresponsive cell of claim 38, wherein the immunoresponsive cell is a T cell.
- 10 40. The immunoresponsive cell of claim 39, wherein the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a regulatory T cell, and a Natural Killer T (NKT) cell.
41. The immunoresponsive cell of claim 38, wherein the pluripotent stem cell is an embryonic stem cell or an induced pluripotent stem cell.
- 15 42. The immunoresponsive cell of any one of claims 35-41, wherein the immunoresponsive cell is autologous.
43. The immunoresponsive cell of any one of claims 35-41, wherein the immunoresponsive cell is allogenic.
44. A pharmaceutical composition comprising an effective amount of an
20 immunoresponsive cell of any one of claims 35-43 and a pharmaceutically acceptable excipient.
45. The pharmaceutical composition of claim 44, comprising between about 10^4 and 10^6 of the immunoresponsive cells.
46. The pharmaceutical composition of claim 44 or 45, comprising at least about 10^5
25 of the immunoresponsive cells.
47. The pharmaceutical composition of any one of claims 44-46, comprising about 10^5 of the immunoresponsive cells.

48. The pharmaceutical composition of any one of claims 44-47, which is for preventing and/or treating a neoplasm in a subject, treating a subject having a relapse of a neoplasm, reducing tumor burden in a subject, increasing or lengthening survival of a subject having a neoplasm, preventing and/or treating an inflammatory disease in a subject, and/or preventing graft rejection in a subject who is a recipient of an organ transplant.
49. A nucleic acid composition comprising a polynucleotide encoding the polypeptide composition of any one of claims 1-34.
50. The nucleic acid composition of claim 49, wherein the polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 123.
51. The nucleic acid composition of claim 49, wherein the polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 124.
52. A vector comprising the nucleic acid composition of any one of claims 49-51.
53. The vector of claim 52, which is a retroviral vector.
54. The vector of claim 53, wherein the retroviral vector is a γ -retroviral vector or a lentiviral vector.
55. A method for producing an immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a polypeptide composition of any one of claims 1-34, a nucleic acid composition of any one of claims 49-51, or a vector of any one of claims 52-54.
56. A kit comprising a polypeptide composition of any one of claims 1-34, a nucleic acid composition of any one of claims 49-51, a vector of any one of claims 52-54, an immunoresponsive cell of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48.
57. The kit of claim 56, wherein the kit further comprises written instructions for treating and/or preventing a neoplasm in a subject, treating a subject having a relapse of a neoplasm, reducing tumor burden in a subject, increasing or lengthening survival of a subject having a neoplasm, preventing and/or treating an inflammatory disease in a

subject, and/or preventing graft rejection in a subject who is a recipient of an organ transplant.

58. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for preventing and/or treating a neoplasm in a subject, wherein an effective amount of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.

59. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for reducing tumor burden in a subject, wherein an effective amount of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.

60. The use of claim 59, wherein the medicament reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.

61. The use of any one of claims 58-60, wherein the neoplasm and/or the tumor is a solid tumor.

62. The use of claim 61, wherein the solid tumor is selected from the group consisting of mesothelioma, lung cancer, pancreatic cancer, ovarian cancer, breast cancer, colon cancer, pleural tumor, glioblastoma, esophageal cancer, gastric cancer, synovial sarcoma, thymic carcinoma, endometrial carcinoma, stomach cancer, cholangiocarcinoma, and a combination thereof.

63. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for treating a subject having a relapse of a neoplasm, wherein an effective amount of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.

64. The use of claim 63, wherein the subject received an immunotherapy prior to said administration of the immunoresponsive cells or the composition.

65. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for increasing or lengthening survival of a subject having a neoplasm, wherein an effective amount of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.
66. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for increasing immune-activating cytokine production in response to a cancer cell or a pathogen in a subject, wherein an effective amount of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.
67. The use of claim 66, wherein the immune-activating cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor (GM-CSF), IFN- α , IFN- β , IFN- γ , TNF- α , IL-2, IL-3, IL-6, IL-11, IL-7, IL-12, IL-15, IL-21, interferon regulatory factor 7 (IRF7), and combinations thereof.
68. The use of any one of claims 58-69, at least one immunomodulatory agent is to be further administered.
69. The use of claim 68, wherein the at least one immunomodulatory agent is selected from the group consisting of immunostimulatory agents, checkpoint immune blockade agents, radiation therapy agents, chemotherapy agents, and combinations thereof.
70. The use of claim 69, wherein the immunostimulatory agent is selected from the group consisting of IL-12, an agonist costimulatory monoclonal antibody, and combinations thereof.
71. The use of claim 70, wherein the immunostimulatory agent is IL-12.
72. The use of claim 69, wherein the agonist costimulatory monoclonal antibody is selected from the group consisting of an anti-4-1BB antibody, an anti-OX40 antibody, an anti-ICOS antibody, and combinations thereof.

73. The use of claim 72, wherein the agonist costimulatory monoclonal antibody is an anti-4-1BB antibody.
74. The use of claim 69, wherein the checkpoint immune blockade agents are selected from the group consisting of anti-PD-L1 antibodies, anti-CTLA-4 antibodies, anti-PD-1
5 antibodies, anti-LAG3 antibodies, anti-B7-H3 antibodies, anti-TIM3 antibodies, and combinations thereof.
75. The use of claim 74, wherein the checkpoint immune blockade agent is an anti-PD-L1 antibody or an anti-PD-1 antibody.
76. The use of any one of claims 58-75, wherein the subject is a human.
- 10 77. The use of any one of claims 58-75, wherein the immunoresponsive cell is to be pleurally or intrapleurally administered to the subject.
78. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a medicament for preventing and/or treating an inflammatory disease in a subject, wherein
15 the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.
79. The use of claim 78, wherein the immunoresponsive cell is an immunoinhibitory cell.
80. The use of claim 79, wherein the immunoinhibitory cell is a regulatory T cell.
- 20 81. The use of any one of claims 78-80, wherein inflammatory disease is pancreatitis.
82. The use of any one of claims 78-81, wherein the subject is a human.
83. The use of any one of claims 78-82, wherein the subject is a recipient of an organ transplant.
84. The use of any one of claims 78-83, wherein the subject is a recipient of a
25 pancreas transplant.
85. Use of the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 in the manufacture of a

medicament for preventing graft rejection in a subject who is a recipient of an organ transplant, wherein the immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 is to be administered to the subject.

5 86. The use of claim 85, wherein the immunoresponsive cell is an immunoinhibitory cell.

87. The use of claim 86, wherein the immunoinhibitory cell is a regulatory T cell.

88. The use of any one of claims 85-87, wherein the subject is a human.

89. The use of any one of claims 85-88, wherein the subject is a recipient of a
10 pancreas transplant.

90. The immunoresponsive cells of any one of claims 35-43, or a pharmaceutical composition of any one of claims 44-48 for use in treating and/or preventing a neoplasm in a subject, treating a subject having a relapse of a neoplasm, reducing tumor burden in a subject, increasing or lengthening survival of a subject having a neoplasm, preventing
15 and/or treating an inflammatory disease in a subject, and/or preventing graft rejection in a subject who is a recipient of an organ transplant.