effector T cell functions. However, lower affinity TCRs are dependent on CD8 co-receptors to trigger T cell activation.

Accordingly, a NeoTCR cell therapy that is engineered to have CD8 co-receptor expression could stabilize the TCR-pMHC interactions and increase the efficacy of NeoTCR cell therapies that comprise low affinity TCRs that are dependent on such CD8 co-receptors.

SUMMARY

In one aspect, the present disclosure refers to a composition comprising a peripheral T cell comprising:

a. an exogenous T cell receptor (TCR) recognizing a neoantigen; and

b. an exogenous CD8 comprising:

i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or

ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In another aspect, the present disclosure refers to a method of modifying a cell, the method comprising:

a. introducing into the cell a non-viral homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises:

i. first and second homology arms homologous to first and second target nucleic acid sequences;

ii. a TCR gene sequence positioned between the first and second homology arms;

iii. a CD8 gene sequence positioned between the first and second homology arms, wherein the CD8 gene sequence encodes:

1. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or

2. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain; and

b. recombining the HR template nucleic acid into an endogenous locus of the cell.

In another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for the treatment of cancer, wherein the composition comprises a therapeutically effective amount of a peripheral T cell comprising:

a. an exogenous T cell receptor (TCR) recognizing a neoantigen; and

b. an exogenous CD8 comprising:

i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or

ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In yet another aspect, the present disclosure refers to a composition comprising a peripheral T cell comprising:

a. an exogenous T cell receptor (TCR) recognizes a neoantigen; and

b. an exogenous CD8 comprising:

i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8B intracellular domain; or

ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain;

wherein the exogenous TCR is encoded by a polynucleotide integrated into a TRAC and/or TRBC locus of the cell.

In yet another aspect, the present disclosure refers to the composition as described herein for use in therapy.

In certain examples, the presently disclosed subject matter provides a cell, comprising an exogenous T cell receptor (TCR), and an exogenous CD8. In certain examples, the exogenous CD8 comprises at least one monomer. In certain examples, the at least one monomer of the exogenous CD8 comprises an extracellular domain, a transmembrane domain, an intracellular domain, fragments thereof, or combinations thereof. In certain examples, the extracellular domain comprises a CD8α extracellular domain or a CD8β extracellular domain. In certain examples, the transmembrane a CD8α transmembrane domain or a CD8β transmembrane domain. In certain examples, the intracellular domain comprises a CD8α intracellular domain or a CD8β intracellular domain. In certain examples, the intracellular domain comprises a CD4 intracellular domain.

In certain examples, the at least one monomer comprises a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain.

In certain examples, the at least one monomer comprises a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain. In certain examples, the at least one monomer comprises a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain. In certain examples, the at least one monomer comprises a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain. In certain examples, the at least one monomer comprises a signal peptide. In certain examples, the signal peptide is a CD8 signal peptide.

In certain examples, the extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 140, or SEQ ID NO: 145. In certain examples, the transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 141, or SEQ ID NO: 146. In certain examples, the intracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 142, SEQ ID NO: 147, or SEQ ID NO: 148.

In certain examples, the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 139, or SEQ ID NO: 144.

In certain examples, the exogenous CD8 comprises a 2A sequence. In certain examples, the exogenous CD8 comprises a linker. In certain examples, the linker comprises the amino acid sequence set forth in SEQ ID NO: 137. In certain examples, the exogenous CD8 comprises a protease cleavage site. In certain examples, the protease cleavage site is a Furin cleavage site.

In certain examples, the exogenous TCR is a patient derived TCR. In certain examples, the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and a TCR polypeptide sequence. In certain examples, the exogenous TCR recognizes a cancer antigen. In certain examples, the cancer antigen is a neoantigen. In certain examples, the cancer antigen is a patient specific antigen.

In certain examples, the cell is a primary cell. In certain examples, the cell is a patient-derived cell. In certain examples, the cell is a lymphocyte. In certain examples, the cell is a T cell. In certain embodiments, the cell if a young T cell. In certain examples, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain examples, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain examples, the cell is CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain examples, the cell further comprises a gene modification to enhance cell persistence and/or enhances memory cell differentiation. In certain examples, killing activity of the cell is increased between about 10% to about 500% as compared to killing activity of a cell that does not have the exogenous CD8. In certain examples, proliferation of the cell upon binding of the TCR to the antigen is increased between about 10% to about 500% as compared to proliferation of a cell that does not have the exogenous CD8. In certain examples, secretion of pro-inflammatory cytokine upon binding of the TCR to the antigen by the cell is increased between about 10% to about 500% as compared to secretion by a cell that does not have the exogenous CD8. In certain examples, LCK affinity of the cell is increased between about 10% to about 500% as compared to LCK affinity of a cell that does not have the exogenous CD8. In certain examples, persistence of the cell is increased between about 10% to about 500% as compared to persistence of a cell that does not have the exogenous CD8. In certain examples, tumor infiltration ability of the cell is increased between about 10% to about

500% as compared to tumor infiltration ability of a cell that does not have the exogenous CD8.

In certain examples, wherein the exogenous TCR is a CD8-dependent TCR. In certain examples, the exogenous TCR is a CD8-independent TCR. In certain examples, the exogenous CD8 is encoded by a CD8 Construct 1, a CD8 Construct 2, a CD8 Construct 3, or a CD8 Construct 4. In certain examples, the exogenous CD8 comprises: a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, and aCD8β intracellular domain; or a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain. In certain examples, the exogenous CD8 comprises: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO: 142); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), a CD8α intracellular domain (SEQ ID NO: 142), a CD8β signal peptide (SEQ ID NO:144), a CD8β extracellular domain (SEQ ID NO:145), a CD8β transmembrane domain (SEQ ID NO:146), and a CD8β intracellular domain (SEQ ID NO: 147); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8β intracellular domain (SEQ ID NO: 147); or a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD4 intracellular domain (SEQ ID NO: 148).

In certain examples, the presently disclosed subject matter provides a method of modifying a cell, the method comprising introducing into the cell a homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises first and second homology arms homologous to first and second target nucleic acid sequences, a TCR gene sequence positioned between the first and second homology arms, a CD8 gene sequence positioned between the first and the second homology arms, and recombining the HR template nucleic acid into an endogenous locus of the cell. In certain examples, the HR template comprises a first 2A-coding sequence positioned upstream of the CD8 gene sequence, a second 2A-coding sequence positioned downstream of the

CD8 gene sequence and upstream of the TCR gene sequence, and a third 2A-coding sequence positioned downstream of the TCR gene sequence; wherein the first, second, and third 2A-coding sequences code for the same amino acid sequence and are codon-diverged relative to each other. In certain examples, the HR template comprises a sequence coding for the amino acid sequence Gly Ser Gly positioned immediately upstream of the first, second, and/or third 2A-coding sequences. In certain examples, the HR template further comprises a sequence coding for a Furin cleavage site positioned upstream of the first, second, and/or third 2A-coding sequences. In certain examples, the HR template further comprises a sequence encoding a signal sequence positioned immediately upstream of the TCR gene sequence and/or the CD8 gene sequence.

In certain examples, the HR template comprises a second TCR sequence positioned between the third 2A-coding sequence and the second homology arm. In certain examples, the HR template comprises a sequence encoding a first signal sequence positioned immediately upstream the first TCR gene sequence, and a sequence encoding a second signal sequence positioned immediately upstream of the second TCR gene sequence. In certain examples, the HR template comprises a second CD8 gene sequence positioned between the first CD8 gene sequence and the second 2A-coding sequence. In certain examples, a 2A coding sequence is positioned between the first and second CD8 gene sequence. In certain examples, a sequence coding for the amino acid sequence Gly Ser Gly is positioned between the first and second CD8 gene sequences. In certain examples, a sequence coding for a Furin cleavage site is positioned between the first and second CD8 gene sequences.

In certain examples, the CD8 gene sequence comprises a sequence encoding an extracellular domain, a sequence encoding an intracellular domain, a sequence encoding an intracellular domain, fragments thereof, or combinations thereof. In certain examples, the sequence encoding an extracellular domain comprises a sequence encoding a CD8α extracellular domain or a CD8β extracellular domain. In certain examples, the sequence encoding a transmembrane domain comprises a sequence encoding a CD8α transmembrane domain or a CD8β transmembrane domain. In certain examples, the sequence encoding an intracellular domain comprises a sequence encoding a CD8α intracellular domain or a CD8β intracellular domain. In certain examples, the sequence encoding an intracellular domain comprises a sequence encoding a CD4 intracellular domain.

In certain examples, the CD8 gene sequence comprises a sequence encoding a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain. In certain examples, the CD8 gene sequence comprises a sequence encoding a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain. In certain examples, the CD8 gene sequence comprises a sequence encoding a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain. In certain examples, the CD8 gene sequence comprises a sequence encoding a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In certain examples, the HR template comprises a sequence encoding a first signal sequence positioned immediately upstream the first CD8 gene sequence, and a sequence encoding a second signal sequence positioned immediately upstream of the second CD8 gene sequence. In certain examples, the signal sequence is a CD8 signal sequence, a human growth hormone signal sequence, fragments thereof, or combinations thereof. In certain examples, the first and second homology arms of the HR template are each from about 300 bases to about 2,000 bases in length. In certain examples, the first and second homology arms of the HR template are each from about 600 bases to about 2,000 bases in length.

In certain examples, the exogenous TCR is a patient derived TCR. In certain examples, the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and a TCR polypeptide sequence. In certain examples, the exogenous TCR recognizes a cancer antigen. In certain examples, the cancer antigen is a neoantigen. In certain examples, the cancer antigen is a patient specific antigen. In certain examples, the HR template is non-viral. In certain examples, the HR template is a circular DNA. In certain examples, the HR template is a linear DNA. In certain examples, the introducing occurs via electroporation.

In certain examples, the recombining comprises cleavage of the endogenous locus by a nuclease, and recombination of the HR template nucleic acid sequence into the endogenous locus by homology directed repair. In certain examples, the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease, or derivative thereof. In certain examples, the nuclease further comprises a gRNA.

In certain examples, the method further comprises culturing the cell. In certain examples, the culturing is conducted in the presence of at least one cytokine. In certain examples, the culturing is conducted in the presence of IL2, IL7, IL15, or any

combination thereof. In certain examples, the culturing is conducted in the presence of IL7 and IL15. In certain examples, the method comprises a gene modification to enhance cell persistence and/or enhances memory cell differentiation.

In certain examples, the cell is a primary cell. In certain embodiments, the cell is a patient-derived cell. In certain examples, the cell is a lymphocyte. In certain examples, the cell is a T cell. In certain examples, the cell is a young T cell. In certain examples, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain examples, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain examples, the cell is CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain examples, killing activity of the cell is increased between about 10% to about 500% as compared to killing activity of a cell that does not have the CD8 gene sequence. In certain examples, proliferation of the cell upon binding of the TCR to the antigen is increased between about 10% to about 500% as compared to proliferation of a cell that does not have the CD8 gene sequence. In certain examples, secretion of pro-inflammatory cytokine upon binding of the TCR to the antigen by the cell is increased between about 10% to about 500% as compared to secretion by a cell that does not have the CD8 gene sequence. In certain examples, LCK affinity of the cell is increased between about 10% to about 500% as compared to LCK affinity of a cell that does not have the CD8 gene sequence. The method of any one of claims 43-95, wherein persistence of the cell is increased between about 10% to about 500% as compared to persistence of a cell that does not have the CD8 gene sequence. In certain examples, tumor infiltration ability of the cell is increased between about 10% to about 500% as compared to tumor infiltration ability of a cell that does not have the CD8 gene sequence.

In certain examples, the TCR gene encodes a CD8-dependent TCR. In certain examples, the TCR gene encodes a CD8-independent TCR. In certain examples, the CD8 gene sequence is encoded by a CD8 Construct 1, a CD8 Construct 2, a CD8 Construct 3, or a CD8 Construct 4. In certain examples, wherein the CD8 gene sequence comprises: a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or a CD8α extracellular domain, CD8α transmembrane domain, CD4 intracellular domain. In certain examples, the

CD8 gene sequence comprises: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO: 142); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), a CD8α intracellular domain (SEQ ID NO: 142), a CD8β signal peptide (SEQ ID NO:144), a CD8β extracellular domain (SEQ ID NO:145), a CD8β transmembrane domain (SEQ ID NO:146), and a CD8β intracellular domain (SEQ ID NO: 147); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8β intracellular domain (SEQ ID NO: 147); or a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD4 intracellular domain (SEQ ID NO: 148).

In certain examples, the presently disclosed subject matter provides a cell modified by any of the methods disclosed herein.

In certain examples, the presently disclosed subject matter provides a composition comprising an effective amount of a cell disclosed herein. In certain examples, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain examples, the composition is administered to a patient in need thereof for the treatment of cancer. In certain examples, the composition comprises a cryopreservation agent. In certain examples, the composition comprises serum albumin. In certain examples, the composition comprises Plasma-Lyte A, HSA, and CryoStor CS10.

In certain examples, the presently disclosed subject matter provides a method of treating cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of a cell or a composition disclosed herein.

In certain examples, prior to administering the therapeutically effective amount of cells disclosed herein, a non-myeloablative lymphodepletion regimen is administered to the subject. In certain examples, the cancer is a solid tumor. In certain examples, the cancer is a liquid tumor. In certain examples, the solid tumor is selected from the group consisting of melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+ cancer, esophageal cancer, thyroid cancer, gastric cancer, hepatocellular cancer,

cholangiocarcinomas, renal cell cancers, testicular cancer, sarcomas, and colorectal cancer. In certain examples, the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.

In certain examples, the presently disclosed subject matter provides a kit comprising a cell disclosed herein, reagents for performing a method disclosed herein, or a composition disclosed herein. In certain examples, the kit further comprises written instructions for treating a cancer.

In certain examples, the presently disclosed subject matter provides a cell, comprising: an exogenous T cell receptor (TCR); and an exogenous CD8, comprising: a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In certain examples, the presently disclosed subject matter provides a cell, comprising: an exogenous T cell receptor (TCR); and an exogenous CD8, comprising: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO: 142); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), a CD8α intracellular domain (SEQ ID NO: 142), a CD8β signal peptide (SEQ ID NO:144), a CD8β extracellular domain (SEQ ID NO:145), a CD8β transmembrane domain (SEQ ID NO:146), and a CD8β intracellular domain (SEQ ID NO: 147); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8β intracellular domain (SEQ ID NO: 147); or a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD4 intracellular domain (SEQ ID NO: 148).

In certain examples, the presently disclosed subject matter provides a method of modifying a cell, the method comprising: introducing into the cell a homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises: first and second homology arms homologous to first and second target nucleic acid sequences; a TCR gene sequence positioned between the first and second homology arms;

a CD8 gene sequence positioned between the first and the second homology arms; and recombining the HR template nucleic acid into an endogenous locus of the cell, wherein the CD8 gene sequence comprises: a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8β intracellular domain; or a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In certain examples, the presently disclosed subject matter provides a method of modifying a cell, the method comprising: introducing into the cell a homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises: first and second homology arms homologous to first and second target nucleic acid sequences; a TCR gene sequence positioned between the first and second homology arms; a CD8 gene sequence positioned between the first and the second homology arms; and recombining the HR template nucleic acid into an endogenous locus of the cell, wherein the CD8 gene sequence comprises: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO: 142); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), a CD8α intracellular domain (SEQ ID NO: 142), a CD8β signal peptide (SEQ ID NO:144), a CD8β extracellular domain (SEQ ID NO:145), a CD8β transmembrane domain (SEQ ID NO:146), and a CD8β intracellular domain (SEQ ID NO: 147); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8β intracellular domain (SEQ ID NO: 147); or a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD4 intracellular domain (SEQ ID NO: 148).

In certain examples, the presently disclosed subject matter provides a composition comprising a cell, wherein the cell comprises an exogenous T cell receptor (TCR) and an exogenous CD8, wherein the exogenous CD8 comprises: a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β

intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In certain examples, the presently disclosed subject matter provides a composition comprising a cell, wherein the cell comprises an exogenous T cell receptor (TCR) and an exogenous CD8, wherein the exogenous CD8 comprises: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO: 142); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), a CD8α intracellular domain (SEQ ID NO: 142), a CD8β signal peptide (SEQ ID NO:144), a CD8β extracellular domain (SEQ ID NO:145), a CD8β transmembrane domain (SEQ ID NO:146), and a CD8β intracellular domain (SEQ ID NO: 147); a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8β intracellular domain (SEQ ID NO: 147); or a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD4 intracellular domain (SEQ ID NO: 148).

In certain examples, the presently disclosed subject matter provides a method of treating cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of a cell, wherein the cell comprises an exogenous T cell receptor (TCR) and an exogenous CD8, wherein the exogenous CD8 comprises: a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8α intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, a CD8α intracellular domain, a CD8β extracellular domain, a CD8β transmembrane domain, and a CD8β intracellular domain; a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

In certain examples, the presently disclosed subject matter provides a method of treating cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of a cell, wherein the cell comprises an exogenous T cell receptor (TCR) and an exogenous CD8, wherein the exogenous CD8 comprises: a CD8α signal peptide (SEQ ID NO:139), a CD8α extracellular domain (SEQ ID NO:140), a CD8α transmembrane domain (SEQ ID NO:141), and a CD8α intracellular domain (SEQ ID NO:

WHAT IS CLAIMED IS:

1. A composition comprising a peripheral T cell comprising:
    a. an exogenous T cell receptor (TCR) recognizing a neoantigen; and
    b. an exogenous CD8 comprising:
        i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or
        ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

2. The composition of claim 1, wherein the exogenous CD8 comprises:
    a. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD8B intracellular domain set forth in SEQ ID NO: 147; or
    b. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD4 intracellular domain set forth in SEQ ID NO: 148.

3. The composition of claim 1, wherein the exogenous CD8 further comprises at least one of a 2A sequence, a linker, and a protease cleavage site.

4 The composition of claim 3, wherein the protease cleavage site is a Furin cleavage site.

5. The composition of claim 1, wherein the exogenous TCR is a patient derived TCR.

6. The composition of claim 1, wherein the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and a second TCR polypeptide sequence.

7. The composition of claim 1, wherein the cell is a patient-derived cell.

8. The composition of claim 1, wherein the peripheral T cell is:
    a. CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+;
    b. CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or

c. CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

9. The composition of claim 1, wherein the exogenous CD8 is encoded by a CD8 Construct 3 or a CD8 Construct 4.

10. The composition of claim 1, wherein the composition further comprises a pharmaceutically acceptable excipient.

11. The composition of claim 1, further comprising a cryopreservation agent, serum album, and a crystalloid solution.

12. A method of modifying a cell, the method comprising:

a. introducing into the cell a non-viral homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises:
    i. first and second homology arms homologous to first and second target nucleic acid sequences;
    ii. a TCR gene sequence positioned between the first and second homology arms;
    iii. a CD8 gene sequence positioned between the first and second homology arms, wherein the CD8 gene sequence encodes:
        1. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or
        2. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain; and
b. recombining the HR template nucleic acid into an endogenous locus of the cell.

13. The method of claim 12, wherein the CD8 gene sequence encodes:

a. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD8B intracellular domain set forth in SEQ ID NO: 147; or
b. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD4 intracellular domain set forth in SEQ ID NO: 148.

14. The method of claim 12, wherein the HR template comprises:

a. a first 2A-coding sequence positioned upstream of the CD8 gene sequence, a second 2A-coding sequence positioned downstream of the CD8 gene sequence and upstream of the TCR gene sequence, and a third 2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first, second and third 2A-coding sequences code for the same amino acid sequence and are codon-diverged relative to each other;
b. a sequence coding for the amino acid sequence Gly-Ser-Gly positioned immediately upstream of the first, second and/or third 2A-coding sequences; and
c. a sequence coding for a Furin cleavage site positioned upstream of the first, second and/or third 2A-coding sequences.

15. The method of claim 12, wherein the HR template comprises a second TCR gene sequence positioned between the third 2A-coding sequence and the second homology arm.

16. The method of claim 15, wherein the HR template comprises:
a. a sequence encoding a first signal sequence positioned immediately upstream of the first TCR gene sequence; and
b. a sequence encoding a second signal sequence positioned immediately upstream of the second TCR gene sequence.

17. The method of claim 12, wherein the first and second homology arms of the HR template are each from about 300 bases to about 2,000 bases in length.

18. The method of claim 12, wherein the exogenous TCR is a patient derived TCR.

19. The method of claim 12, wherein introducing into the cell comprises electroporation.

20. The method of claim 12, where the recombining comprises:
a. cleavage of the endogenous locus by a Cas9/gRNA ribonucleoprotein nuclease; and
b. recombination of the HR template nucleic acid sequence into the endogenous locus by homology directed repair.

21. The method of claim 12, further comprising culturing the cell in the presence of IL2, IL7, IL15, or a combination thereof.

22. The method of claim 12, wherein the CD8 gene sequence is encoded by a CD8 Construct 3 or a CD8 Construct 4.

23. Use of a composition in the manufacture of a medicament for the treatment of cancer, wherein the composition comprises a therapeutically effective amount of a peripheral T cell comprising:

a. an exogenous T cell receptor (TCR) recognizing a neoantigen; and
b. an exogenous CD8 comprising:
    i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8β intracellular domain; or
    ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain.

24. The use of claim 23, wherein the exogenous CD 8 comprises:

a. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD8B intracellular domain set forth in SEQ ID NO: 147; or
b. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD4 intracellular domain set forth in SEQ ID NO: 148.

25. The use of claim 23, wherein the composition is used after a non-myeloablative lymphodepletion regimen is to be administered to the subject.

26. The use of claim 23, wherein the cancer is selected from the group consisting of follicular lymphoma, leukemia, multiple myeloma, melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+ cancer, esophageal cancer, thyroid cancer, gastric cancer, hepatocellular cancer,

cholangiocarcinomas, renal cell cancers, testicular cancer, sarcomas, and colorectal cancer.

27. A composition comprising a peripheral T cell comprising:
    a. an exogenous T cell receptor (TCR) recognizes a neoantigen; and
    b. an exogenous CD8 comprising:
        i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8B intracellular domain; or
        ii. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD4 intracellular domain;

    wherein the exogenous TCR is encoded by a polynucleotide integrated into a TRAC and/or TRBC locus of the cell.

28. The composition of claim 27, wherein the exogenous CD8 comprises:
    a. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD8B intracellular domain set forth in SEQ ID NO: 147; or
    b. a CD8α signal peptide set forth in SEQ ID NO: 139, a CD8α extracellular domain set forth in SEQ ID NO: 140, a CD8α transmembrane domain set forth in SEQ ID NO: 141, and a CD4 intracellular domain set forth in SEQ ID NO: 148.

29. The composition of claim 27, wherein the exogenous CD8 is encoded by a polynucleotide integrated into the TRAC and/or TRBC locus of the cell.

30. The composition of claim 27, wherein the exogenous CD8 further comprises at least one of a 2A sequence, a linker, and a protease cleavage site.

31. The composition of claim 20, wherein the protease cleavage site is a Furin cleavage site.

32. The composition of claim 27, wherein the exogenous TCR is a patient derived TCR.

33. The composition of claim 27, wherein the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and a second TCR polypeptide sequence.

34. The composition of claim 27, wherein the cell is a patient-derived cell.

35. The composition of claim 27, wherein the peripheral T cell is:

a. CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+;

b. CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+; or

c. CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

36. The composition of claim 57, wherein the exogenous CD8 is encoded by a CD8 Construct 3 or a CD8 Construct 4.

37. The composition of claim 57, wherein the composition further comprises a pharmaceutically acceptable excipient.

38. The composition of claim 27, further comprising a cryopreservation agent, serum album, and a crystalloid solution.

39. The composition of claim 27, wherein the polynucleotide is non-virally integrated into the TRAC or TRBC locus.

40. The composition of claim 1 for use in therapy.