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~~ a first exogenous T cell receptor (TCR) recognizing a neoantigen; and

b. an exogenous CD8 homodimer 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~~ a first exogenous T cell receptor (TCR) recognizing a neoantigen; and

b. an exogenous CD8 homodimer 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~~ a first exogenous T cell receptor (TCR) recognizes a neoantigen; and

b. an exogenous CD8 homodimer comprising:

i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8~~B~~β 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.

WHAT IS CLAIMED IS:

1. A composition comprising a peripheral T cell comprising:
    a. ~~an~~ a first exogenous T cell receptor (TCR) recognizing a neoantigen; and
    b. an exogenous CD8 homodimer 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 CD8~~B~~β 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 before the CD8α signal peptide, after the CD8β intracellular domain, or after the CD4 intracellular domain.

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 wherein the cell comprises 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 set forth in SEQ ID NO: 109 or a CD8 Construct 4 set forth in SEQ ID NO: 136.

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~~albumin, 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~~12. 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~~ a first exogenous T cell receptor (TCR) recognizing a neoantigen; and

b. an exogenous CD8 homodimer 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~~13. The use of claim ~~23~~12, 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 CD8~~B~~β 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~~14. The use of claim ~~23~~12, wherein the ~~composition~~ medicament is ~~used after~~ to be administered to a subject, wherein the subject has received or is to receive a non-myeloablative lymphodepletion regimen ~~is to be administered to the subject~~.

~~26~~15. The use of claim ~~23~~12, 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~~16. A composition comprising a peripheral T cell comprising:

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

b. an exogenous CD8 homodimer comprising:

i. a CD8α signal peptide, a CD8α extracellular domain, a CD8α transmembrane domain, and a CD8~~B~~β 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~~17. The composition of claim ~~27~~16, 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 CD8~~B~~β 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~~18. The composition of claim ~~27~~16, wherein the exogenous CD8 is encoded by a polynucleotide integrated into the TRAC and/or TRBC locus of the cell.

~~30~~19. The composition of claim ~~27~~16, wherein the exogenous CD8 further comprises at least one of a 2A sequence, a linker, and a protease cleavage site before the CD8α signal peptide, after the CD8β intracellular domain, or after the CD4 intracellular domain.

~~31~~20. The composition of claim ~~20~~19, wherein the protease cleavage site is a Furin cleavage site.

~~32~~21. The composition of claim ~~27~~16, wherein the exogenous TCR is a patient derived TCR.

~~33~~22. The composition of claim ~~27~~16, wherein the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and wherein the cell comprises a second TCR polypeptide sequence.

~~34~~23. The composition of claim ~~27~~16, wherein the cell is a patient-derived cell.

~~35~~24. The composition of claim ~~27~~16, 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~~25. The composition of claim ~~57~~16, wherein the exogenous CD8 is encoded by a CD8 Construct 3 set forth in SEQ ID NO: 109 or a CD8 Construct 4 set forth in SEQ ID NO: 136.

~~37~~26. The composition of claim ~~57~~16, wherein the composition further comprises a pharmaceutically acceptable excipient.

~~38~~27. The composition of claim ~~27~~16, further comprising a cryopreservation agent, serum ~~albuma~~albumin, and a crystalloid solution.

~~39~~28. The composition of claim ~~27~~16, wherein the polynucleotide is non-virally integrated into the TRAC or TRBC locus.

~~40~~29. The composition of claim 1 for use in therapy.