TARGETED REPLACEMENT OF ENDOGENOUS T CELL RECEPTORS

PRIOR RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 62/578,153 filed on October 27, 2017, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] T cells are the most actively studied cell type in the growing field of adoptive cellular therapeutics. T cells interact specifically with the target of their T cell receptor (TCR), enabling highly specific responses with minimal side effects. These highly effective and specific responses can be engineered towards novel antigens and targets by inserting a new receptor with the desired specificity into a T cell. However, development of entirely new types of receptors is time consuming, expensive, and fails to take advantage of the fact that, through development of the endogenous T cell repertoire, the body naturally produces TCRs that bind almost any possible antigenic target. The ability to obtain human T cells and replace their endogenous TCR with a TCR having a desired antigen specificity could be transformative in the development and application of adoptive T cell therapies.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

[0003] The present disclosure is directed to compositions and methods for editing the genome of a human T cell. The inventors have discovered that a heterologous TCR can be inserted into a targeted region in the genome of a T cell, such that the heterologous TCR is under the control of an endogenous TCR promoter. The methods and compositions provided herein can be used to replace an endogenous TCR in a human T cell with a heterologous TCR having a desired antigen specificity. In some embodiments, the targeted region in the genome of a T cell is the native T cell receptor locus.

[0003a] In one aspect, the present disclosure is directed to a modified primary human T cell comprising: at least one nucleic acid sequence comprising at least one heterologous gene non-virally inserted into one or both of: an endogenous T cell receptor alpha subunit constant gene (TRAC); and an endogenous T cell receptor beta subunit constant gene (TRBC), wherein the at least one heterologous gene encodes at least one antigen-specific receptor that

specifically binds the target antigen expressed by a cancer, and wherein the at least one nucleic acid sequence is at least 500 bp in size.

[0003b] In another aspect, the present disclosure is directed to a population of cells comprising a plurality of the primary human T cell of the disclosure.

[0003c] In another aspect, the present disclosure is directed to the T cell of the disclosure or the population of cells of the disclosure for use in therapy.

[0003d] In another aspect, the present disclosure is directed to a use of the T cell of the disclosure or the population of cells of the disclosure in the manufacture of a medicament for treating a cancer that expresses the target antigen.

[0004] In some ~~embodiments~~examples, the present disclosure provides a method of editing the genome of a human T cell. In some ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a T cell receptor (TCR)-subunit constant gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a first heterologous TCR subunit chain, wherein the TCR

subunit chain comprises the variable region and the constant region of the TCR subunit; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a second heterologous TCR subunit chain; and (v) a portion of the N-terminus of the endogenous TCR subunit, wherein, if the endogenous TCR subunit is a TCR-alpha (TCR-α) subunit, the first heterologous TCR subunit chain is a heterologous TCR-beta (TCR-β) subunit chain and the second heterologous TCR subunit chain is a heterologous TCR-α subunit chain, and wherein if the endogenous TCR subunit is a TCR-β subunit, the first heterologous TCR subunit chain is a heterologous TCR-α subunit chain and the second heterologous TCR subunit chain is a heterologous TCR-β subunit chain. In some ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a TCR alpha subunit constant gene (TRAC) in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a heterologous TCR-β chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-α chain; and (v) a portion of the N-terminus of the endogenous TCR-α subunit.

[0005] In some ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a TCR-beta subunit constant gene (TRBC) in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a heterologous TCR-α chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-β chain; and (v) a portion of the N-terminus of the endogenous TCR-β subunit.

[0006] In some ~~embodiment~~examples, the nucleic acid is inserted by introducing a viral vector comprising the nucleic acid into the T cell. In some ~~embodiment~~examples, the nucleic is inserted by introducing a non-viral vector comprising the nucleic acid into the T cell. In some ~~embodiment~~examples, the nucleic acid is inserted into the T cell by introducing into the T cell, (a) a targeted nuclease that cleaves a target region in exon 1 of a TCR-α subunit constant gene (TRAC) to create an insertion site in the genome of the T cell; and (b) the nucleic acid sequence, wherein the nucleic acid sequence is incorporated into the insertion site by homology directed repair (HDR). In some ~~embodiment~~examples, the nucleic acid is inserted into the T cell by introducing into the T cell, (a) a targeted nuclease that cleaves a target region in exon 1 of a TCR-β subunit constant gene (TRBC) to create an insertion site in the genome of the T cell; and (b) the nucleic acid sequence, wherein the nucleic acid sequence is incorporated into the insertion site by homology directed repair (HDR). In some ~~embodiment~~examples, the 5' end and the 3' end of the nucleic acid comprise nucleotide sequences

that are homologous to genomic sequences flanking the target region. In some ~~embodiment~~examples, the 5' end and the 3' end of the nucleic acid comprise nucleotide sequences that are homologous to genomic sequences flanking the insertion site. In some ~~embodiment~~examples, the targeted nuclease introduces a double-stranded break at the insertion site. In some ~~embodiment~~examples, the nucleic acid sequence is introduced into the cell as a double-stranded or a single-stranded nucleic acid. In some ~~embodiment~~examples, the nucleic acid is introduced into the cell as a double-stranded or a single stranded DNA template. In some ~~embodiment~~examples, the nucleic acid sequence is introduced into the cell as a linear nucleic acid.

[0007] In some ~~embodiment~~examples, the first self-cleaving peptide and the second self-cleaving peptide are the same or different viral 2A peptides.

[0008] In some ~~embodiment~~examples, the targeted nuclease is selected from the group consisting of an RNA-guided nuclease domain, a transcription activator-like effector nuclease (TALEN), a zinc finger nuclease (ZFN) and a megaTAL. In some ~~embodiment~~examples, the RNA-guided nuclease is a Cpf1 nuclease or a Cas9 nuclease, and the method further comprises introducing into the cell a guide RNA that specifically hybridizes to the target region in exon 1 of the TRAC. In some ~~embodiment~~examples, the Cpf1 nuclease or the Cas9 nuclease, the guide RNA and the nucleic acid are introduced into the cell as a ribonucleoprotein complex (RNP)-DNA template complex, wherein the RNP-DNA template complex comprises: (i) the RNP, wherein the RNP comprises the Cpf1 nuclease or the Cas9 nuclease, and the guide RNA; and (ii) the DNA template.

[0009] In some ~~embodiment~~examples, the molar ratio of RNP to DNA template in the complex is from about 3:1 to about 100:1. In some ~~embodiment~~examples, the RNP-DNA template complex is formed by incubating the RNP with the DNA template for about ten to about thirty minutes, at a temperature of about 20° to 25° C. In some ~~embodiment~~examples, the RNP-DNA template complex and the cell are mixed prior to introducing the RNP-DNA template complex into the cell. In some ~~embodiment~~examples, the RNP-DNA template complex comprises at least two structurally different RNP complexes. In some ~~embodiment~~examples, the at least two structurally different RNP complexes contain structurally different guide RNAs. In some ~~embodiment~~examples, each of the structurally different RNP complexes comprises a Cas9 nickase, and wherein the structurally different guide RNAs hybridize to opposite strands of the target region.

[0010] In some ~~embodiment~~examples, the introducing comprises electroporation. In some ~~embodiment~~examples, the nucleic acid is introduced into a population of about 1×10^5 to about $2 \times$

10^6 T cells. In some examples, the targeted nuclease and the DNA template are introduced into a population of about 1×10^5 to about 2×10^6 T cells. In some ~~embodiment~~examples, at least two structurally different DNA templates are introduced into the cells. In some ~~embodiment~~examples, the at least two structurally-different DNA templates are non-viral templates. In some ~~embodiment~~examples, each of the at least two structurally different DNA templates encodes a unique combination of a variable region of a heterologous TCR-β chain of an antigen specific T cell receptor and a variable region of a heterologous TCR-α chain of an antigen specific T cell receptor. In some ~~embodiment~~examples, the T cell is a regulatory T cell, an effector T cell, or a naïve T cell. In some ~~embodiment~~examples, the T cell is an effector T cell, and wherein the effector T cell is a $CD8^+$ T cell. In some ~~embodiment~~examples, the T cell is an effector T cell, and wherein the effector T cell is a $CD4^+$ T cell. In some ~~embodiment~~examples, the effector T cell is a $CD4^+CD8^+$ T cell.

[0011] In some ~~embodiment~~examples, the method further comprises culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-α chain to form an antigen-specific T cell receptor. In some ~~embodiment~~examples, the method further comprises culturing the modified T cells under conditions effective for expanding the population of modified cells. In some ~~embodiment~~examples, the method further comprises purifying T cells that express the antigen-specific T cell receptor.

[0012] In some ~~embodiment~~examples, the present disclosure provides a method of editing the genome of a human T cell comprising: (a) inserting into a target region in exon 1 of a TCR alpha subunit constant gene (TRAC) in the human T cell a first nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a self-cleaving peptide sequence; (ii) a heterologous TCR-α chain of an antigen specific T cell receptor; and (iii) a portion of the N-terminus of exon 1 of the endogenous TCR alpha subunit; and (b) inserting into a target region in exon 1 of a TCR beta subunit constant gene (TRBC) in the human T cell a second nucleic acid sequence encoding, from the N-terminus to the C-terminus a second nucleic acid sequence encoding sequence encoding, from the N-terminus to the C-terminus,(i) a second self-cleaving peptide sequence; (ii) a heterologous TCR-β chain of an antigen specific T cell receptor; and (iii) a portion of the N-terminus of exon 1 of the endogenous TCR beta subunit.

[0013] In some ~~embodiment~~examples, the first and/or second nucleic acid sequence are inserted by introducing a viral vector comprising the first and/or second nucleic acid to the T cell. In

some ~~embodiment~~examples, the first and/or second nucleic acid sequence are inserted by introducing a non-viral vector comprising the first and/or second nucleic acid to the T cell. In some ~~embodiment~~examples, the first and second nucleic acids are inserted into the T cell by introducing (a) one or more targeted nucleases that create a first insertion site in exon 1 of a TRAC and a second insertion site in exon 1 of a TCR beta subunit constant gene (TRBC); and (b) the first nucleic acid sequence; and (c) the second nucleic acid sequence, wherein the first nucleic acid sequence is inserted into the first insertion site in exon 1 of the TRAC and the second nucleic acid sequence is inserted into the second insertion site in exon 1 of the TRBC by homology directed repair (HDR). In some ~~embodiment~~examples, the nucleic acid sequence is introduced into the cell as a double-stranded or a single-stranded DNA template. In some ~~embodiment~~examples, the nucleic acid sequence is introduced into the cell as a linear DNA template.

[0014] In some ~~embodiment~~examples, the 5' end and the 3' end of the first nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the target region in exon 1 of the TRAC gene. In some ~~embodiment~~examples, the 5' end and the 3' end of the first nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the first insertion site in exon 1 of the TRAC gene. In some ~~embodiment~~examples, the 5' end and the 3' end of the second nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the target region in exon 1 of the TRBC gene. In some ~~embodiment~~examples, the 5' end and the 3' end of the second nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the second insertion in exon 1 of the TRBC gene.

[0015] In some ~~embodiment~~examples, the one or more targeted nucleases introduce a double-stranded break at the first and second insertion sites. In some ~~embodiment~~examples, the first self-cleaving peptide and the second self-cleaving peptide are the same or different viral 2A peptides. In some ~~embodiment~~examples, the one or more targeted nucleases are selected from the group consisting of an RNA-guided nuclease domain, a transcription activator-like effector nuclease (TALEN), a zinc finger nuclease (ZFN) or a megaTAL.

[0016] In some ~~embodiment~~examples, the RNA-guided nuclease is a Cpf1 nuclease or a Cas9 nuclease and wherein the method further comprises introducing into the cell a first guide RNA that specifically hybridizes to a target region in exon 1 of the TRAC, and a second guide RNA that specifically hybridizes to a target region in exon 1 of the TRBC. In some ~~embodiment~~examples, the Cpf1 nuclease or the Cas9 nuclease, the first guide RNA and the first

nucleic acid are introduced into the cell as a ribonucleoprotein complex (RNP)-DNA template complex, wherein the RNP-DNA template complex comprises:(i) the RNP, wherein the RNP comprises the Cpf1 nuclease or the Cas9 nuclease, and the first guide RNA; and (ii) the first DNA template. In some ~~embodiment~~examples, the Cpf1 nuclease or the Cas9 nuclease, the second guide RNA, and the second nucleic acid are introduced into the cell as a RNP-DNA template complex, wherein the RNP-DNA template complex comprises: (i) the RNP, wherein the RNP comprises a Cpf1 nuclease or Cas9 nuclease, and the second guide RNA; and (ii) the second DNA template.

[0017] In some ~~embodiment~~examples, the molar ratio of RNP to DNA template in the complex is from about 3:1 to about 100:1. In some ~~embodiment~~examples, the RNP-DNA template complex is formed by incubating the RNP with the DNA template for about ten to about thirty minutes, at a temperature of about 20° to 25° C. In some ~~embodiment~~examples, the RNP-DNA template complex comprises at least two structurally different RNP complexes. In some ~~embodiment~~examples, each of the structurally different RNP complexes comprises a Cas9 nickase, and wherein the structurally different guide RNAs hybridize to opposite strands of the target region. In some ~~embodiment~~examples, the introducing comprises electroporation.

[0018] In some ~~embodiment~~examples, the first and second nucleic acids are introduced into about 1×10^5 to about 2×10^6 T cells. In some ~~embodiment~~examples, one or more targeted nucleases and the first and second nucleic acids are introduced into about 1×10^5 to about 2×10^6 T cells. In some ~~embodiment~~examples, at least two structurally different first DNA templates are introduced into the cells. In some ~~embodiment~~examples, the at least two structurally different first DNA templates comprise different variable regions of a TCR-α chain of an antigen specific T cell receptor. In some ~~embodiment~~examples, at least two structurally different second DNA templates are introduced into the cells. In some ~~embodiment~~examples, the at least two structurally different second DNA templates comprise different variable regions of a TCR-β chain of an antigen specific T cell receptor.

[0019] In some ~~embodiment~~examples, the T cell is a regulatory T cell, an effector T cell, or a naïve T cell. In some ~~embodiment~~examples, the T cell is an effector T cell, wherein the effector T cell is a $CD8^+$ T cell. In some ~~embodiment~~examples, the T cell is an effector T cell, wherein the effector T cell is a $CD4^+$ T cell. In some ~~embodiment~~examples, the effector T cell is a $CD4^+CD8^+$ T cell.

[0020] In some ~~embodiment~~examples, the method further comprises culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-

α chain to form an antigen-specific T cell receptor. In some ~~embodiment~~examples, the method further comprises culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-α chain to form an antigen-specific T cell receptor. In some ~~embodiment~~examples, the method further comprises culturing the modified T cells under conditions effective for expanding the population of modified cells. In some ~~embodiment~~examples, the method further comprises purifying T cells that express the antigen-specific T cell receptor.

[0021] In other ~~embodiment~~examples, the present disclosure provides a modified T cell comprising a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous T cell receptor (TCR)-β chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-α chain; and (v) a portion of the N-terminus of the endogenous TCR alpha subunit, wherein the nucleic acid sequence is integrated into exon 1 of the TRAC gene.

[0022] In some ~~embodiment~~examples, the present disclosure further provides a modified T cell comprising: a) a first nucleic acid sequence encoding, from N-terminus to C-terminus, (i) a first self-cleaving sequence, (ii) the variable region of a heterologous TCR-α chain, and (iii) a portion of the N-terminus of the endogenous TCR-α chain; and b) a second nucleic acid sequence encoding, from N-terminus to C-terminus, (i) a first self-cleaving sequence, (ii) the variable region of a heterologous TCR-β chain, and (iii) a portion of the N-terminus of the endogenous TCR-β chain, wherein the first nucleic acid sequence is integrated into exon 1 of the TRAC gene and the second nucleic sequence is integrated into exon 1 of the TRBC gene.

[0023] In some ~~embodiment~~examples, the present disclosure further provides a method of treating cancer in a human subject comprising: a) obtaining T cells from the subject; b) modifying the T cells to express a heterologous antigen-specific T cell receptor, wherein the T cell receptor recognizes a tumor-specific antigen in the subject; and c) administering the modified T cells to the subject.

[0024] Using the methods and compositions described herein for modifying T cells to express a heterologous TCR-α chain and a heterologous TCR-β chain, one can also edit a human gamma delta (γδ) T cell. For example, in some ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a T cell receptor gamma subunit constant (TRGC) gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous

TCR-β chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-α chain; and (v) a portion of the N-terminus of the endogenous TCR-α.

[0025] In other ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a T cell receptor gamma subunit constant (TRGC) gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous TCR-β chain; (iii) a second self-cleaving peptide sequence; (iv) a full length heterologous TCR-α chain; and (v) a stop codon, such that, upon insertion of the nucleic acid, the nucleic acids encoding the heterologous TCR-β and TCR-α sequences are under the control of the endogenous TCR-ɣ promoter.

[0026] In other ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a T cell receptor gamma subunit constant (TRGC) gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous TCR-δ chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-γ chain; and (v) a portion of the N-terminus of the endogenous TCR-γ subunit. Also provided is a method of editing the genome of a human T cell, comprising inserting into a target region in exon 1 of a TRAC gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous TCR-γ chain; (iii) a second self-cleaving peptide sequence; (iv) a variable region of a heterologous TCR-δ chain; and (v) a portion of the N-terminus of the endogenous TCR-α subunit. In other ~~embodiment~~examples, the method comprises inserting into a target region in exon 1 of a T cell receptor gamma subunit constant (TRAC) gene in the human T cell, a nucleic acid sequence encoding, from the N-terminus to the C-terminus, (i) a first self-cleaving peptide sequence; (ii) a variable region of a heterologous TCR-γ chain; (iii) a second self-cleaving peptide sequence; (iv) a full-length heterologous TCR-δ chain; and (v) a stop codon, such that upon insertion, of the nucleic acid, the heterologous TCR-γ and TCR-δ sequences are under the control of the endogenous TCR-α promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The present application includes the following figures. The figures are intended to illustrate certain embodiments and/or features of the compositions and methods, and to supplement any description(s) of the compositions and methods. The figures do not limit the

What is claimed is:

1. A modified primary human T cell comprising: at least one nucleic acid sequence comprising at least one heterologous gene non-virally inserted into one or both of:
an endogenous T cell receptor alpha subunit constant gene (TRAC); and
an endogenous T cell receptor beta subunit constant gene (TRBC),
wherein the at least one heterologous gene encodes at least one antigen-specific receptor that specifically binds the target antigen expressed by a cancer, and wherein the at least one nucleic acid sequence is at least 500 bp in size.

2. The T cell of claim 1, wherein the modified human T cell does not comprise a viral vector.

3. The T cell of claim 1 or 2, wherein the at least one nucleic acid sequence is at least 1.5 kb in size.

4. The T cell of any one of claims 1-3, wherein the at least one nucleic acid sequence is non-virally inserted into an exon of the T cell receptor alpha subunit constant gene (TRAC) or into an exon of the T cell receptor beta subunit constant gene (TRBC).

5. The T cell of any one of claims 1-4, wherein the at least one heterologous gene comprises at least one of: (a) a variable region of a heterologous T cell receptor alpha (TCR-α) chain and (b) a variable region of a heterologous T cell receptor beta (TCR-β) chain.

6. The T cell of any one of claims 1-5, wherein the at least one heterologous gene comprises at least one of:
(1) a) a variable region of a heterologous T cell receptor alpha (TCR-α) chain or b) a variable region and constant region of the heterologous TCR-α chain; and
(2) a) a variable region of a heterologous T cell receptor beta (TCR-β) chain or b) a variable region and constant region of the heterologous TCR-β chain.

7. The T cell of claim 6, wherein the at least one heterologous gene comprises each of:
(1) the a) variable region of the heterologous TCR-α chain or b) variable region and constant region of the heterologous TCR-α chain; and

(2) the a) variable region of the heterologous TCR-β chain or b) variable region and constant region of the heterologous TCR-β chain.

8. The T cell of claim 7, wherein one or more coding sequences for the heterologous TCR-α chain and the heterologous TCR-β chain are linked by a linker sequence or a multicistronic element.

9. The T cell of claim 8, wherein the linker sequence is a cleavable linker sequence that is cleaved to generate the heterologous TCR-α chain gene and the heterologous TCR-β chain gene.

10. The T cell of claim 8, wherein the heterologous gene encodes the TCR-α chain and the heterologous TCR-β chain and wherein the heterologous gene is inserted into TRAC.

11. The T cell of any one of claims 1-5, wherein the at least one heterologous gene is non-virally inserted into an endogenous TRAC, wherein the at least one heterologous gene comprises each of:
(1) a) a variable region of the heterologous TCR-α chain or b) a variable region and constant region of the heterologous TCR-α chain; and
(2) a) a variable region of the heterologous TCR-β chain or b) a variable region and constant region of the heterologous TCR-β chain,
wherein the heterologous TCR-α chain and the heterologous TCR-β chain are operably linked by a cleavable linker sequence that is cleaved to generate the heterologous TCR-α chain and the heterologous TCR-β chain, and wherein the heterologous TCR-α chain and the heterologous TCR-β form an antigen-specific T cell receptor (TCR) that recognizes the target antigen expressed by the cancer.

12. The T cell of any one of claims 1-11, wherein expression of the at least one heterologous gene is under the control of an endogenous promoter.

13. The T cell of any one of claims 1-12, wherein the modified human T cell is a CD8+ T cell or a CD4+ T cell.

14. The T cell of claim 13, wherein the CD8+ T cell or CD4+ T cell is an effector T cell or a naïve T cell.

15. The T cell of any one of claims 1-14, wherein the primary human T cell is obtained from a subject having cancer and modified to comprise the at least one nucleic acid sequence comprising at least one heterologous gene.

16. A population of cells comprising a plurality of the primary human T cell of any one of claims 1-15.

17. The T cell of any one of claims 1-15 or the population of cells of claim 16 for use in therapy.

18. Use of the T cell of any one of claims 1-15 or the population of cells of claim 16 in the manufacture of a medicament for treating a cancer that expresses the target antigen.

19. The use of claim 18, wherein the primary human T cell is obtained from a subject having the cancer and modified to comprise the at least one nucleic acid sequence comprising at least one heterologous gene, and the modified primary human T cell is to be administered to the subject.

20. The use of claim 18 or 19, wherein the modified human T cell is cultured under conditions that allow for expression of the at least one heterologous gene encoding the receptor that specifically binds the target antigen expressed by the cancer.

21. The use of any one of claims 18-20, wherein the modified human T cell is cultured under conditions effective for expanding the modified human T cell.

22. The use of any one of claims 18-21, wherein the modified human T cell that expresses the receptor is purified.

1. A method of editing the genome of a human T cell, the method comprising inserting into a target region in exon 1 of a T cell receptor (TCR) subunit constant gene in the human T cell,

a nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a first heterologous TCR subunit chain, wherein the TCR subunit chain comprises the variable region and the constant region of the TCR subunit;

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a second heterologous TCR subunit chain; and

(v) a portion of the N-terminus of the endogenous TCR subunit,

wherein, if the endogenous TCR subunit is a TCR alpha (TCR-α) subunit, the first heterologous TCR subunit chain is a heterologous TCR beta (TCR-β) subunit chain and the second heterologous TCR subunit chain is a heterologous TCR-α subunit chain, and wherein if the endogenous TCR subunit is a TCR-β subunit, the first heterologous TCR subunit chain is a heterologous TCR-α subunit chain and the second heterologous TCR subunit chain is a heterologous TCR-β subunit chain.

2. The method of claim 1, wherein a nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a heterologous TCR-β chain;

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a heterologous TCR-α chain; and

(v) a portion of the N-terminus of the endogenous TCR-α subunit, is inserted into exon 1 of a TCR alpha subunit constant gene (TRAC) in the human T cell.

3. The method of claim 1, wherein a nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a heterologous, TCR-α chain;

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a heterologous TCR-β chain; and

(v) a portion of the N-terminus of the endogenous TCR-β subunit, is inserted into exon 1 of a TCR-beta subunit constant gene (TRBC) in the human T cell.

4. The method of any one of claims 1-3, wherein the nucleic acid sequence is a double-stranded or single stranded nucleic acid sequence, and wherein the nucleic acid sequence is inserted by introducing the nucleic acid sequence into the T cell.

5. The method of any one of claims 1-4, wherein the nucleic acid is inserted by introducing a viral vector comprising the nucleic acid into the T cell.

6. The method of any one of claims 1, 2, 4 or 5, wherein the nucleic acid is inserted into the T cell by introducing into the T cell,

(a) a targeted nuclease that cleaves the target region in exon 1 of the TRAC gene to create an insertion site in the genome of the T cell; and

(b) the nucleic acid sequence, wherein the nucleic acid sequence is inserted into the insertion site by homology directed repair (HDR).

7. The method of any one of claims 1, 3, 4 or 5, wherein the nucleic acid is inserted into the T cell by introducing into the T cell,

(a) a targeted nuclease that cleaves the target region in exon 1 of the TRBC gene to create an insertion site in the genome of the T cell; and

(b) the nucleic acid sequence, wherein the nucleic acid sequence is inserted into the insertion site by homology directed repair (HDR).

8. The method of any one of claims 1-7, wherein the 5' end and the 3' end of the nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the target region.

9. The method of claim 8, wherein the 5' end and the 3' end of the nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the insertion site.

10. The method of any one of claims 6-9, wherein the targeted nuclease introduces a double-stranded break at the insertion site.

11. The method of any one of claims 1-10, wherein the nucleic acid sequence is a single-stranded DNA template.

12. The method of any one of claims 1-11, wherein the nucleic acid sequence is a linear DNA template.

13. The method of any one of claims 1-12, wherein the first self-cleaving peptide and the second self-cleaving peptide are the same or different viral 2A peptides.

14. The method of any one of claims 6-13, wherein the targeted nuclease is selected from the group consisting of an RNA-guided nuclease domain, a transcription activator-like effector nuclease (TALEN), a zinc finger nuclease (ZFN) and a megaTAL.

15. The method of claim 14, wherein the RNA-guided nuclease is a Cpf1 nuclease or a Cas9 nuclease and the method further comprises introducing into the cell a guide RNA that specifically hybridizes to the target region in exon 1 of the TRAC.

16. The method of claim 14, wherein the RNA-guided nuclease is a Cpf1 nuclease or a Cas9 nuclease and the method further comprises introducing into the cell a guide RNA that specifically hybridizes to the target region in exon 1 of the TRBC.

17. The method of claim 15 or 16, wherein the Cpf1 nuclease or the Cas9 nuclease, the guide RNA and the nucleic acid are introduced into the cell as a ribonucleoprotein complex (RNP)-DNA template complex, wherein the RNP-DNA template complex comprises:

(i) the RNP, wherein the RNP comprises the Cpf1 nuclease or the Cas9 nuclease and the guide RNA; and

(ii) the DNA template.

18. The method of claim 17, wherein the molar ratio of RNP to DNA template in the complex is from about 3:1 to about 100:1.

19. The method of claim 17 or 18, wherein the RNP-DNA template complex is formed by incubating the RNP with the DNA template for about ten to about thirty minutes, at a temperature of about 20° to 25° C.

20. The method of any one of claims 17-19, wherein the RNP-DNA template complex and the cell are mixed prior to introducing the RNP-DNA template complex into the cell.

21. The method of claim any one of claims 17-20, wherein the RNP-DNA template complex comprises at least two structurally different RNP complexes.

22. The method of claim 21, wherein the at least two structurally different RNP complexes contain structurally different guide RNAs.

23. The method of claim 22, wherein each of the structurally different RNP complexes comprises a Cas9 nickase, and wherein the structurally different guide RNAs hybridize to opposite strands of the target region.

24. The method of any one of claims 4-23, wherein the introducing comprises electroporation.

25. The method of any one of claims 4-24, wherein the nucleic acid or the RNP:DNA template complex is introduced into a population of about 1×10^5 to about 2×10^6 T cells.

26. The method of claim 25, wherein at least two structurally different DNA templates are introduced into the cells.

27. The method of claim 26, wherein each of the at least two structurally different DNA templates encodes a unique combination of a variable region of a heterologous TCR-β chain of an antigen specific T cell receptor and a variable region of a heterologous TCR-α chain of an antigen specific T cell receptor.

28. The method of any one of claims 1-27, wherein the T cell is a regulatory T cell, an effector T cell, or a naïve T cell.

29. The method of claim 28, wherein the T cell is an effector T cell, and wherein the effector T cell is a $CD8^+$ T cell or a CD4+ cell.

30. The method of claim 29, wherein the effector T cell is a $CD4^+CD8^+$ T cell.

31. The method of any one of claims 1-30, further comprising culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-α chain to form an antigen-specific T cell receptor.

32. The method of claim 31, further comprising culturing the modified T cells under conditions effective for expanding the population of modified cells.

33. The method of claim 31 or 32, further comprising purifying T cells that express the antigen-specific T cell receptor.

34. A modified T cell produced by any one of the methods of claims 1-33.

35. A modified T cell comprising a nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a first heterologousTCR subunit chain,

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a second, heterologous TCR subunit chain; and

(v) a portion of the N-terminus of the endogenous TCR subunit,

wherein, if the endogenous TCR subunit is a TCR-alpha (TCR-α) subunit, the first heterologous TCR subunit chain is a heterologous TCR-beta (TCR-β) subunit chain

and the second heterologous TCR subunit chain is a heterologous TCR-α subunit chain, and wherein if the endogenous TCR subunit is a TCR-β subunit, the first heterologous TCR subunit chain is a heterologous TCR-α subunit chain and the second heterologous TCR subunit chain is a heterologous TCR-β subunit chain.

36. The modified T cell of claim 35 wherein the nucleic acid sequence encodes, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a heterologous T cell receptor (TCR) β chain;

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a heterologous TCR-α chain; and

(v) a portion of the N-terminus of the endogenous TCR alpha subunit,

wherein the nucleic acid sequence is integrated into exon 1 of the TRAC gene.

37. The modified T cell of claim 35, wherein the nucleic acid sequence encodes, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a heterologous, T cell receptor (TCR) α chain;

(iii) a second self-cleaving peptide sequence;

(iv) a portion of the N-terminus of the endogenous TCR-β subunit,

wherein the nucleic acid sequence is integrated into exon 1 of the TRBC1 or TRBC2 gene.

38. A modified T cell comprising a nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a first self-cleaving peptide sequence;

(ii) a variable region of a heterologous T cell receptor (TCR) α chain;

(iii) a second self-cleaving peptide sequence;

(iv) a variable region of a heterologous TCR-β chain; and

(v) a portion of the N-terminus of the endogenous TCR beta subunit,

wherein the nucleic acid sequence is integrated into exon 1 of the TRBC gene.

39. A method of editing the genome of a T cell, the method comprising:

(a) inserting into a target region in exon 1 of a TCR alpha subunit constant gene (TRAC) in the human T cell a first nucleic acid sequence encoding, from the N-terminus to the C-terminus,

(i) a self-cleaving peptide sequence;

(ii) a heterologous TCR α chain of an antigen specific T cell receptor; and (iii) a portion of the N-terminus of exon 1 of the endogenous TCR alpha subunit; and

(b) inserting into a target region in exon 1 of a TCR beta subunit constant gene (TRBC) in the human T cell a nucleic acid sequence encoding, from the N-terminus to the C-terminus.

(i) a second self-cleaving peptide sequence;

(ii) a heterologous TCR β chain of an antigen specific T cell receptor; and (iii) a portion of the N-terminus of exon 1 of the endogenous TCR beta subunit.

40. The method of claim 39, wherein the first and/or second nucleic acid sequence is a double-stranded or single-stranded nucleic acid, and wherein the first and/or second nucleic acid is inserted by introducing the first and/or second nucleic acid into the T cell.

41. The method of claim 40, wherein the first and/or second nucleic acid sequence are inserted by introducing a viral vector comprising the first and/or second nucleic acid into the T cell.

42. The method of claim 40 or 41, wherein first and second nucleic acids are inserted into the T cell by introducing into the T cell,

(a) one or more targeted nucleases that that create a first insertion site in exon 1 of the TRAC gene and a second insertion site in exon 1 of the TRBC gene;

(b) the first nucleic acid sequence; and

(c) the second nucleic acid sequence.

43. The method of claim any one of claims 39-42, wherein the 5' and 3' ends of the first nucleic acid sequence comprise nucleotide sequences that are homologous to genomic sequences flanking the first target region in exon 1 of the TRAC.

44. The method of claim 43, wherein the 5' and 3' ends of the second DNA template comprise nucleotide sequences that are homologous to genomic sequences flanking the second target region in exon 1 of the TRBC.

45. The method of any one of claims 42-44, wherein the one or more targeted nucleases introduce a double-stranded break at the first and second insertion sites.

46. The method of any one of claims 39-45, wherein the first and/or second nucleic acid sequence is a single-stranded DNA template.

47. The method of any one of claims 39-46, wherein the first and/or second nucleic acid is a linear DNA template.

48. The method of any one of claims 39-47, wherein the first self-cleaving peptide and the second self-cleaving peptide are the same or different viral 2A peptides.

49. The method of any one of claims 42-48, wherein the one or more targeted nucleases are selected from the group consisting of an RNA-guided nuclease domain, a transcription activator-like effector nuclease (TALEN), a zinc finger nuclease (ZFN) or a megaTAL.

50. The method of claim 49, wherein the RNA-guided nuclease is a Cpf1 nuclease or a Cas9 nuclease, and wherein the method further comprises introducing into the cell a first guide RNA that specifically hybridizes to a target region in exon 1 of the TRAC, and a second guide RNA that specifically hybridizes to a target region in exon 1 of the TRBC.

51. The method of claim 50, wherein the Cpf1 nuclease or the Cas9 nuclease, the first guide RNA and the nucleic acid sequence are introduced into the cell as a ribonucleoprotein complex (RNP)-DNA template complex, wherein the RNP-DNA template complex comprises:

(i) the RNP, wherein the RNP comprises a Cas9 nuclease or a Cpf1 nuclease, and the first guide RNA; and

(ii) the first DNA template.

52. The method of claim 50, wherein the Cpf1 nuclease or the Cas9 nuclease, the second guide RNA and the second nucleic acid sequence are introduced into the cell as a RNP-DNA template complex, wherein the RNP-DNA template complex comprises:

(i) the RNP, wherein the RNP comprises the Cpf1 nuclease or the Cas9 nuclease and the second guide RNA; and

(ii) the second DNA template.

53. The method of claim 51 or 52, wherein the molar ratio of RNP to DNA template in the complex is from about 3:1 to about 100:1.

54. The method of any one of claims 51-53, wherein the RNP-DNA template complex is formed by incubating the RNP with the DNA template for about ten to about thirty minutes, at a temperature of about 20°C to about 25°C.

55. The method of any one of claims 51-54, wherein the RNP-DNA template complex comprises at least two structurally different RNP complexes.

56. The method of claim 55, wherein each of the structurally different RNP complexes comprises a Cas9 nickase, and wherein the structurally different guide RNAs hybridize to opposite strands of the target region.

57. The method of any one of claims 39-56, wherein the introducing comprises electroporation.

58. The method of any one of claims 39-57, wherein the nucleic acid sequence(s) or the RNP: DNA template complex(es) is introduced into about 1×10^5 to about 2×10^6 T cells.

59. The method of claim 58, wherein at least two structurally different first DNA templates are introduced into the cells.

60. The method of claim 59, wherein the at least two structurally different first DNA templates comprise different variable regions of a TCR-α chain of an antigen specific T cell receptor.

61. The method of claim 60, wherein at least two structurally different second DNA templates are introduced into the cells.

62. The method of claim 61, wherein the at least two structurally different second DNA templates comprise different variable regions of a TCR-β chain of an antigen specific T cell receptor.

63. The method of any one of claims 39-62, wherein the T cell is a regulatory T cell, an effector T cell, or a naïve T cell.

64. The method of claim 63, wherein the T cell is an effector T cell, and wherein the effector T cell is a $CD8^+$ T cell or a CD4+ cell.

65. The method of claim 64, wherein the effector T cell is a $CD4^+CD8^+$ T cell.

66. The method of any one of claims 39-65, further comprising culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-α chain to form an antigen-specific T cell receptor.

67. The method of claim 66, further comprising culturing the T cells under conditions that allow expression of the heterologous TCR-β chain and the heterologous TCR-α chain to form an antigen-specific T cell receptor.

68. The method of claim 67, further comprising culturing the modified T cells under conditions effective for expanding the population of modified cells.

69. The method of claim 67 or 68, further comprising purifying T cells that express the antigen-specific T cell receptor.

70. A modified T cell produced by any one of the methods of claims 39-69.

71. A modified T cell comprising:

a) a first nucleic acid sequence encoding, from N-terminus to C-terminus,

(i) a first self-cleaving sequence;

(ii) the variable region of a heterologous TCR-α chain; and

(iii) a portion of the N-terminus of the endogenous TCR-α chain; and

b) a second nucleic acid sequence encoding, from N-terminus to C-terminus,

(i) a first self-cleaving sequence;

(ii) the variable region of a heterologous TCR-β chain; and

(iii) a portion of the N-terminus of the endogenous TCR-β chain,

wherein the first nucleic acid sequence is integrated into exon 1 of the TRAC gene and the second nucleic sequence is integrated into exon 1 of the TRBC gene.

72. A method of treating cancer in a human subject comprising:

a) modifying the T cells of the subject using the method of any one of claims 1-33 or 39-69 to express an antigen-specific T cell receptor, wherein the T cell receptor recognizes a tumor-specific antigen in the subject.

73. The method of claim 72, wherein the T cells of the subject are modified *in vivo*.

74. The method of claim 72, wherein the method comprises

a) obtaining T cells from the subject;

b) modifying the T cells using the method of any one of claims 1-33 or 39-69 to express an antigen-specific T cell receptor, wherein the T cell receptor recognizes a tumor-specific antigen in the subject; and

c) administering the modified T cells to the subject.