editing methods were identified in a patient with remission and accounted for nearly all edited cells two (2) months post profusion.

Cell therapies designed to express a NeoTCR with a concurrent knockout or knockdown of the TET2 gene have not been previously pursued. Furthermore, there is an unmet need to develop cell therapies that have long persistence times and/or ideally full engraftment as memory stem cells in order to allow for cell therapies to be single or infrequently dosed therapies.

However, given the endogenous suppression of TET2 and its inherent anti-tumor activity, therapies that modulate the expression of TET2 on T cells need to be engineered precisely in order to prevent oncogenic phenotypes of such therapies.

Accordingly, there is an unmet need of a cell therapy that can specifically target tumors through NeoTCR engagement and that persist for extended periods of time within the patient post infusion. Such therapies are that include a knockout or knockdown TET2 that is specific to T cells are described herein.

SUMMARY

The present disclosures described herein provide for cells that were engineered to modulate the endogenous expression of the TET2 gene.

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

a) introducing into a cell a homologous recombination (HR) template nucleic acid sequence, optionally wherein the introducing occurs via electroporation;

b) recombining the HR template nucleic acid into an endogenous locus of the cell, optionally wherein the endogenous locus is within an endogenous TCR gene; and

c) disrupting a TET2 locus of the cell;

wherein the HR template comprises a TCR gene sequence, a first 2A-coding sequence positioned upstream of the TCR gene sequence, and a second 2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first 2A-coding sequence and second 2A-coding sequence encode for the same amino acid sequence and are codon-diverged relative to each other.

In another aspect, the present disclosure refers to a cell produced by the method as described herein, wherein the cell comprises an exogenous TCR and a gene disruption of a Tet2 locus.

In another aspect, the present disclosure refers to a composition comprising a cell as described herein, optionally wherein the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure refers to a kit comprising the cell as described herein~~, reagents for performing the method as described herein,~~ or a composition as described herein, optionally wherein the kit further comprises written instructions for treating a cancer.

In yet another aspect, the present disclosure refers to a use of a cell as described herein, ~~reagents for performing the method as described herein,~~ a composition as described herein, or the kit as described herein for the manufacture of a medicament for treating a cancer.

In yet another aspect, the present disclosure refers to the cell as described herein, ~~reagents for performing the method as described herein,~~ a composition as described herein~~,~~, or the kit as described herein for use in therapy

In certain aspects, the presently disclosed subject matter provides a cell, comprising an exogenous T cell receptor (TCR), and a gene disruption of a TET2 locus. In certain examples, the gene disruption comprises a substitution, a deletion, an insertion, or any combination thereof. In certain examples, the gene disruption comprises a missense mutation, a nonsense mutation, a non-frameshift deletion, a frameshift deletion, a non-frameshift insertion, a frameshift insertion, or any combination thereof. In certain examples, the gene disruption of the TET2 locus results in a non-functional TET2 protein. In certain examples, the gene disruption of the TET2 locus results in knockout of the TET2 gene expression.

In certain examples, the cell comprises a gRNA and a Cas9 nuclease. In certain examples, the gRNA comprises a nucleotide sequence set forth in SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, or SEQ ID NO.5.

In certain examples, the gene disruption of the TET2 locus enhances cell persistence. In certain examples, the gene disruption of the TET2 locus enhances memory cell differentiation.

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

WHAT IS CLAIMED IS:

~~1. A cell, comprising:~~

~~a) an exogenous T cell receptor (TCR); and~~

~~b) a gene disruption of a TET2 locus.~~

~~2. The cell of claim 1, wherein:~~

~~a) the gene disruption comprises a substitution, a deletion, an insertion, or any combination thereof;~~

~~b) the gene disruption comprises a missense mutation, a nonsense mutation, a non-frameshift deletion, a frameshift deletion, a non-frameshift insertion, a frameshift insertion, or any combination thereof;~~

~~c) the gene disruption of the TET2 locus results in a non-functional TET2 protein;~~

~~d) the gene disruption of the TET2 locus results in knockout of the TET2 gene expression;~~

~~e) the gene disruption of the TET2 locus enhances cell persistence; and/or~~

~~f) the gene disruption of the TET2 locus enhances memory cell differentiation.~~

~~3. The cell of claim 1 or 2, comprising a gRNA and a Cas9 nuclease, optionally wherein the gRNA comprises a nucleotide sequence set forth in SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.3, SEQ ID NO.4, or SEQ ID NO.5.~~

~~4. The cell of any one of claims 1-3, wherein the cell is a primary cell, a patient-derived cell, a lymphocyte, or a T cell, optionally wherein the 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+.~~

~~5. The cell of any one of claims 1-5, wherein the exogenous TCR comprises a signal sequence, a first and second 2A sequence, and a TCR polypeptide sequence, optionally wherein the exogenous TCR is a patient-derived TCR, optionally wherein the exogenous TCR recognizes a cancer antigen, optionally wherein the cancer antigen is a neoantigen or a patient specific antigen.~~

~~6~~1. A method of modifying a cell, the method comprising:

a) introducing into a cell a homologous recombination (HR) template nucleic acid sequence, optionally wherein the introducing occurs via electroporation;

b) recombining the HR template nucleic acid into an endogenous locus of the cell, optionally wherein the endogenous locus is within an endogenous TCR gene; and

c) disrupting a TET2 locus of the cell;

wherein the HR template comprises a TCR gene sequence, a first 2A-coding sequence positioned upstream of the TCR gene sequence, and a second 2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first 2A-coding sequence and second 2A-coding sequence encode for the same amino acid sequence and are codon-diverged relative to each other.

~~7~~2. The method of claim ~~6~~1, wherein the HR template comprises:

a) first and second homology arms homologous to first and second target nucleic acid sequences, optionally wherein the first and second homology arms are each from ~~about~~ 300 bases to ~~about~~ 2,000 bases in length, optionally wherein the first and second homology arms are each from ~~about~~ 600 bases to ~~about~~ 2,000 bases in length; and

b) ~~a~~ the TCR gene sequence is positioned between the first and second homology arms.

~~8~~3. The method of claim ~~6 or 7~~2, wherein the HR template comprises

~~a) a first 2A-coding sequence positioned upstream of the TCR gene sequence and a second 2A-coding sequence positioned downstream of the TCR gene sequence, wherein the first and second 2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;~~

~~b~~a) a sequence coding for the amino acid sequence Gly Ser Gly is positioned immediately upstream of the 2A-coding sequences;

~~c~~b) a sequence coding for a Furin cleavage site positioned upstream of the second 2A-coding sequence;

~~d~~c) a signal sequence positioned between the first 2A-coding sequence and the TCR gene sequence, optionally wherein the signal sequence is a human growth hormone signal sequence; and/or

~~e~~d) a second TCR sequence positioned between the second 2A-coding sequence and the second homology arm.

~~9~~4. The method of claim ~~8~~3, wherein the HR template comprises:

a) a first signal sequence positioned between the first 2A-coding sequence and the first TCR gene sequence; and

b) a second signal sequence positioned between the second 2A-coding sequence and the second TCR gene sequence;

wherein the first and the second signal sequences encode for the same amino acid sequence and are codon diverged relative to each other, optionally wherein the signal sequence is a human growth hormone signal sequence.

~~10~~5. The method of any one of claims ~~6-9~~1-4, wherein the HR template is a circular DNA or a linear DNA.

~~11~~6. The method of any one of claims ~~6-10~~1-5, wherein the recombining comprises:

a) cleavage of the endogenous locus by a nuclease, optionally wherein the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease, or derivative thereof; and

b) recombination of the HR template nucleic acid sequence into the endogenous locus by homology directed repair.

~~12~~7. The method of any one of claims ~~6-11~~1-6, wherein the disrupting comprises

a) introducing a substitution, a deletion, an insertion, or any combination thereof;

b) introducing a missense mutation, a nonsense mutation, a non-frameshift deletion, a frameshift deletion, a non-frameshift insertion, a frameshift insertion, or any combination thereof;

c) generating a non-functional TET2 protein;

d) knockout of the TET2 gene expression;

e) cleavage of the TET2 locus by a nuclease, optionally wherein the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease, or derivative thereof, optionally wherein the nuclease is expressed by a vector, optionally wherein the vector is a viral vector or a non-viral vector;

f) enhancing cell persistence; and/or

g) enhancing memory cell differentiation.

~~13~~8. The method of any one of claims ~~6-12~~1-7, wherein the cell is a primary cell, a patient-derived cell, a lymphocyte, or a T cell.

~~14~~9. The method of claim ~~13~~8, wherein the T cell is a patient-derived cell, optionally wherein the T cell is a young T cell, optionally wherein the 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+.

~~15~~10. The method of any one of claims ~~6-14~~1-9, wherein the TCR gene sequence encodes for a TCR that recognizes a tumor antigen, a neoantigen, and/or a patient specific neoantigen, optionally wherein the TCR gene sequence is a patient specific TCR gene sequence.

~~16~~11. The method of any one of claims ~~6-15~~1-10, wherein the method further comprises culturing the cell optionally wherein the culturing is conducted in the presence of at least one cytokine, optionally wherein the culturing is conducted in the presence of IL2, IL7, IL15, or any combination thereof, optionally wherein the culturing is conducted in the presence of IL7 and IL15.

~~17~~12. A cell produced by the method of any one of claims ~~6-16~~1-11, wherein the cell comprises an exogenous TCR and a gene disruption of a Tet2 locus.

~~18~~13. A composition comprising a cell of ~~any one of claims 1-5 or~~ claim ~~17~~12, optionally wherein the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.

~~19~~14. The composition of claim ~~18~~13, wherein the composition comprises Plasma-Lyte A, human serum albumin (HSA), and CryoStor CS10.

~~20~~15. A kit comprising the cell of ~~any one of claims 1-5 or 17~~claim 12~~, reagents for performing the method of any one of claims 6-16,~~ or a composition of claim ~~18 or 19~~13 or 14, optionally wherein the kit further comprises written instructions for treating a cancer.

~~21~~16. Use of a cell of ~~any one of claims 1-5 or 17~~claim 12~~, reagents for performing the method of any one of claims 6-16,~~ a composition of claim ~~18 or 19~~13 or 14, or the kit of claim ~~20~~ 15 for the manufacture of a medicament for treating a cancer.

~~22~~17. The use of claim ~~21~~16, wherein the cancer is

a) a solid tumor, optionally wherein 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; or

b) a liquid tumor, optionally wherein the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.

~~23~~18. The cell of ~~any one of claims 1-5 or 17~~claim 12~~, reagents for performing the method of any one of claims 6-16,~~ a composition of claim ~~18 or 19~~13 or 14, or the kit of claim ~~20~~ 15 for use in therapy.