

# GENETICALLY ENGINEERED T CELLS HAVING IMPROVED PERSISTENCE IN CULTURE

## CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/897,016, filed September 06, 2019, U.S. Provisional Patent Application No. 62/927,764, filed October 30, 2019, and U.S. Provisional Patent Application No. 63/034,646, filed June 04, 2020. Each of the prior applications is hereby incorporated by reference in its entirety.

## BACKGROUND OF THE INVENTION

10 Chimeric antigen receptor (CAR) T-cell therapy uses genetically-modified T cells to more specifically and efficiently target and kill cancer cells. After T cells have been collected from the blood, the cells are engineered to include CARs on their surface. The CARs may be introduced into the T cells using CRISPR/Cas9 gene editing technology.

15 When these allogeneic CAR T cells are injected into a patient, the receptors enable the T cells to kill cancer cells.

T cells having improved persistence in culture are desired in CAR-T therapy. Such T cells live longer in both *in vitro* and *in vivo*, thereby conferring benefits in CAR-T cell manufacturing and clinical applications. However, it remains challenging to improve

20 persistence of T cells in culture.

## SUMMARY ~~OF THE INVENTION~~

25 The present disclosure is based, at least in part, on the development of a T cell bank comprising T cells with genetic editing of certain genes (*e.g.*, genes involved in cell self-renewal such as *TET2*, apoptosis such as *FAS*, T cell exhaustion or replicative senescence such as *CD70*, or a combination thereof) to improve T cell persistence in cell culture. The genetically edited T cells disclosed herein showed enhanced cell expansion and proliferation capacity in culture, reduced apoptosis (*e.g.*, induced by *FAS* ligand), enhanced frequency of activation (*e.g.*, enhanced cytotoxicity), and enhanced CAR-T efficacy in animal models (via,

30 *e.g.*, longer persistence). For example, CAR-T cells having a disrupted *TET2* gene showed both growth advantage and CAR-T enrichment effects *in vitro* and allows CAR-T cells to persist longer in animal models having both liquid and solid tumors. Further, the genetically edited T cells having a disrupted *TET2* gene showed cytokine-dependent growth, indicating safety. Such a T cell bank (*e.g.*, having a disrupted *TET2* gene) can be used for making

35 therapeutic T cells, for example, CAR-T cells.

In one aspect, the present disclosure provides a population of genetically engineered T cells, comprising: (i) a disrupted *TET2* gene; (ii) a disrupted T cell receptor alpha chain constant region (*TRAC*) gene; and (iii) a disrupted beta-2-microglobulin ( $\beta 2M$ ) gene.

Accordingly, one aspect example of the present disclosure provides a population of genetically engineered T cells (a T cell bank), comprising: (i) a mutated gene involved in cell self-renewal; (ii) a disrupted gene involved in apoptosis; (iii) a disrupted gene involved in regulation of T cell exhaustion or replicative senescence; or (iv) a combination of any one of (i)-(iii). The population of genetically engineered T cells, as compared to the non-engineered T counterparts, has one or more of the following superior features: (a) enhanced expansion capacity in culture, (b) enhanced proliferation capacity, (c) a reduced apoptosis level, and (d) an enhanced frequency of activation. The T cell bank as disclosed herein may be derived from primary T cells of one or more donors, for example, one or more human donors.

In some embodiment examples, genes involved in cell self-renewal may comprise the *TET2* gene. A mutated *TET2* gene may be a disrupted *TET2* gene, which does not express a functional TET2 protein. Alternatively, a mutated *TET2* gene may be a modulated *TET2* gene that expresses a truncated version of *TET2* (e.g., a truncated version having a molecular weight of around 170 kDa). Any of the mutated *TET2* genes may be genetically edited by CRISPR/Cas mediated gene editing, for example, using a guide RNA (gRNA) targeting a desired site in the *TET2* gene (either coding region or non-coding region). In some examples, the mutated *TET2* gene may have one or more genetic editing events in one or more of exon 1, exon 3, exon 4, exon 5 and exon 6. In particular examples, the mutated *TET2* gene is genetically edited using a gRNA comprising SEQ ID NO: 14, 18, 22, 26, 112, 116, or 120.

In some embodiment examples, genes involved in apoptosis may comprise *FAS*, and/or genes involved in T cell exhaustion may comprise *CD70*. Such genes may be disrupted, for example, via CRISPR/Cas-mediated gene editing. Exemplary gRNAs targeting *FAS* may comprise SEQ ID NO: 69, 73, 77, 81, or 85. Exemplary gRNAs targeting *CD70* may comprise SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.

In some embodiment examples, the population of genetically engineered T cells described herein may comprise a combination at least one mutated gene involved in cell self-renewal (e.g., *TET2*), at least one gene involved in apoptosis (e.g., *FAS*), and/or at least one gene involved in T cell exhaustion (e.g., *CD70*).

Any of the population of genetically engineered T cells as disclosed herein may further comprise a disrupted beta-2-microglobulin ( $\beta 2M$ ) gene, a disrupted T cell receptor alpha chain constant region (*TRAC*) gene, or a combination thereof. In some

~~embodiment~~examples, the T cells may be further engineered to express a chimeric antigen receptor (CAR), for example, comprising a nucleic acid encoding the CAR. In some examples, the nucleic acid is inserted in the genome of the T cells. In specific examples, the genetically engineered T cells

may have a disrupted *TRAC* gene, in which a nucleotide acid encoding a chimeric antigen receptor may be inserted. In some embodimentexamples, the CAR may target a tumor antigen. Examples include CD19, B cell maturation antigen (BCMA), or CD70.

In another aspect, the present disclosure provides a method for preparing the population of genetically engineered T cells as described herein, the method comprising: (a) providing a plurality of cells, which are T cells or precursor cells thereof; (b) genetically editing the *TET2* gene in the plurality of cells; and (c) producing the population of genetically engineered T cells; wherein the plurality of cells in step (a) comprises a disrupted *TRAC* gene and a disrupted *b2M* gene; or wherein step (b) further comprises genetically editing the *TRAC* gene and the *b2M* gene in the plurality of cells.

In another aspectexample, the present disclosure provides a method for preparing T cell bank as disclosed herein. Such a method may comprise (a) providing a plurality of cells, which are T cells or precursor cells thereof; (b) genetically editing at least one gene involved in cell self-renewal (*e.g.*, *TET2*), at least one gene involved in apoptosis (*e.g.*, *FAS*), and/or at least one gene involved in regulation of T cell exhaustion (*e.g.*, *CD70*); and (c) producing the population of genetically engineered T cells.

In some embodimentexamples, the T cells of step (a) may be derived from one or more suitable donors, for example, one or more human donors. In some embodimentexamples, the T cells show cytokine-dependent growth.

In some embodimentexamples, step (b) can be performed by delivering to the cells of (a) one or more RNA-guided nucleases, and one or more gRNAs specific to the one or more target genes disclosed herein. In some examples, the RNA-guided nuclease can be a Cas9 nuclease, for example, a *S. pyogenes* Cas9 nuclease. In some examples, the RNA-guided nuclease and the one or more gRNAs can be complexed in a ribonucleoprotein particle (RNP). Step (b) may be performed by a single electroporation event. Alternatively, step (b) may be performed by two sequential electroporation events.

In some embodimentexamples, the target gene comprises *TET2*. Exemplary gRNA targeting *TET2* may be specific to exon 1, exon 3, exon 4, exon 5, and/or exon 6 of the *TET2* gene. Such gRNAs may comprise the nucleotide sequence of SEQ ID NO: 14, 18, 22, or 26. Alternatively or in addition, the target genes may comprise *FAS* and/or *CD70*. Exemplary gRNAs targeting *FAS* may comprise the nucleotide sequence of SEQ ID NO: 69, 73, 77, 81, or 85. Exemplary gRNAs targeting *CD70* may comprise the nucleotide sequence of SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.

In yet another ~~aspect~~example, the present disclosure provides a method for preparing genetically engineered T cells expressing a chimeric antigen receptor (CAR) using genetically engineered T cells from any of the T cell banks disclosed herein. Such a method may comprise: (a) providing a plurality of T cells from a T cell bank, which may comprise  
5 genetically engineered T cells having at least one gene involved in cell self-renewal (*e.g.*, *TET2*), at least one gene involved in apoptosis (*e.g.*, *FAS*), and/or at least one gene involved in regulation of T cell exhaustion (*e.g.*, *CD70*); (b) delivering to the plurality of the T cells a

nucleic acid encoding a CAR; and (c) producing genetically engineered T cells expressing the CAR.

In some embodimentexamples, the plurality of T cells from the T cell bank further comprises a disrupted  $\beta 2M$  gene. In other embodimentexamples, the method may further  
5 comprise genetically editing a  $\beta 2M$  gene, for example, delivering to the plurality of the T cells a gRNA targeting a  $\beta 2M$  gene.

In some embodimentexamples, the plurality of T cells from the T cell bank further comprises a disrupted *TRAC* gene. In other embodimentexamples, the method may further  
10 comprise genetically editing a *TRAC* gene, for example, delivering to the plurality of the T cells a gRNA targeting a *TRAC* gene.

In any of the methods disclosed herein, a RNA-guided nuclease may be delivered to the plurality of the T cells from the T cell bank. In some examples, the RNA-guided nuclease can be a Cas9 nuclease, for example, a *S. pyogenes* Cas9 nuclease. In some examples, the RNA-guided nuclease and the gRNA(s) can be complexed in a ribonucleoprotein particle  
15 (RNP).

The genetically edited T cells prepared by any of the preparation methods disclosed herein are also within the scope of the present disclosure.

Any of the CAR-T cells disclosed herein may be used for therapeutic purposes (*e.g.*, eliminating disease cells targeted by the CAR polypeptide on the CAR-T cells) at a dose  
20 lower than a standard dose of CAR-T therapy, which refers to the dose of CAR-T cells expressing the same CAR polypeptide and lacking the generic edits for enhancing T cell persistence as disclosed herein (*e.g.*, with no genetic edits to *TET2*, *FAS*, and/or *CD70*).

In yet another aspect, the present disclosure provides a genetically engineered T cell population, which is prepared by a method as described herein.

In a further aspect, the present disclosure provides a use of a population of genetically engineered T cells in the manufacture of a medicament for eliminating undesired cells in a subject, wherein the genetically engineered T cells are set forth as described herein.

In a further aspect, the present disclosure provides a guide RNA (gRNA) targeting a *TET2* gene, comprising: a nucleotide spacer sequence specific to exon 3, exon 4, exon 5, or  
30 exon 6 of the *TET2* gene; and a scaffold sequence. Also within the scope of the present disclosures is a guide RNA (gRNA) targeting a *TET2* gene. In some embodimentexamples, the gRNA comprises a nucleotide sequence specific to exon 5 of the *TET2* gene. In some examples, a *TET2* gene edited by the gRNA may express a truncated version of TET2. Such a gRNA may target the site of GGGATGTCCTATTGCTAAGT (SEQ ID NO: 125) in

exon 5 of the *TET2* gene. In one example, the gRNA may comprise the nucleotide sequence of SEQ ID NO: 18. Alternatively, the gRNA may target the site of AGGGATGTCCTATTGCTAAG (SEQ ID NO: 126) in exon 5 of the *TET2* gene. In one example, the gRNA may comprise the nucleotide sequence of SEQ ID NO: 22.

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In other embodiment examples, the gRNA may comprise a nucleotide sequence targeting exon 3 of the *TET2* gene. For example, the gRNA may comprise a nucleotide sequence targeting GATTCCGCTTGGTGAAAACG (SEQ ID NO: 129) in exon 3 of the *TET2* gene.

Alternatively, the gRNA may comprise a nucleotide sequence targeting CAGGACTCACACGACTATTC (SEQ ID NO: 131) in exon 3 of the *TET2* gene. In another example, the gRNA may comprise a nucleotide sequence targeting TTCCGCTTGGTGAAAACGAG (SEQ ID NO: 133) in exon 3 of the *TET2* gene. Exemplary gRNAs may comprise the nucleotide sequence of SEQ ID NO: 112, 116, or 120.

In some embodiment examples, the gRNA may comprise a nucleotide sequence targeting exon 4 of the *TET2* gene. For example, the gRNA may comprise a nucleotide sequence targeting CATTAGGACCTGCTCCTAGA (SEQ ID NO: 124) in exon 4 of the *TET2* gene. In one example, the gRNA may comprise the nucleotide sequence of SEQ ID NO: 14.

In some embodiment examples, the gRNA may comprise a nucleotide sequence targeting exon 6 of the *TET2* gene. For example, the gRNA may comprise a nucleotide sequence targeting ACGGCACGCTCACCAATCGC (SEQ ID NO: 127) in exon 6 of the *TET2* gene. In one example, the gRNA may comprise the nucleotide sequence of SEQ ID NO: 26.

Any of the gRNAs disclosed herein may further comprise a scaffold sequence. In some instances, the gRNA may comprise one or more modified nucleotides, for example, one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA. Exemplary gRNAs targeting *TET2* are provided in **Table 3** (including unmodified sequences and modified sequences), all of which are within the scope of the present disclosure and can be used in any of the methods disclosed herein for genetic editing of the *TET2* genes in a host cell such as a T cell.

The details of one or more embodiment examples of the invention disclosure are set forth in the description below. Other features or advantages of the present invention disclosure will be apparent from the following drawings and detailed description of several embodiment examples, and also from the appended claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

**FIGs. 1A-1D** include diagrams showing that mutations leading to either *TET2* gene knockout (KO) or protein truncation increased proliferation and expansion, in primary human T cells. **FIG. 1A:** a picture showing presence of TET2 proteins in primary human T cells edited by gRNAs target exon 5 of the *TET2* gene as determined by a Western Blot assay. No protein was detected in cells transfected with one of the gRNAs including TET2 exon 5\_T1, and TET2 exon 5\_T2. TET2 exon 5\_T1 gRNA produced a truncated form of the TET2 protein. **FIG. 1B:** a picture showing presence of TET2 proteins in primary human T cells edited by gRNAs target exon 4 or exon 6 of the *TET2* gene as determined by a Western Blot

## What Is Claimed Is:

1. A population of genetically engineered T cells, comprising:
  - (i) a disrupted TET2 gene; ~~involved in cell self-renewal;~~
  - ~~(ii) a disrupted T cell receptor alpha chain constant region (TRAC) gene; and~~
  - ~~(iii) a disrupted beta-2-microglobulin ( $\beta 2M$ ) gene.~~

~~(ii) a disrupted gene involved in apoptosis;~~

~~(iii) a disrupted gene involved in regulation of T cell exhaustion; or~~

~~(iv) a combination of any one of (i) (iii);~~

~~wherein the population of genetically engineered T cells, as compared to the non-engineered T counterparts, have one or more of the following features: (a) enhanced expansion capacity in culture, (b) enhanced proliferation capacity, (c) a reduced apoptosis level, and (d) an enhanced frequency of activation.~~
- ~~2. The population of genetically engineered T cells of claim 1, wherein the T cells comprise a combination of (i) (iii).~~
- ~~3. The population of genetically engineered T cells of claim 1 or claim 2, wherein (i) comprises TET2, and optionally wherein the genetically engineered T cells do not comprise (ii), (iii) or both.~~
42. The population of genetically engineered T cells of ~~any one of~~ claims 1-3, wherein the disrupted TET2 gene is genetically edited in an exon selected from the group consisting of ~~exon 1, exon 3, exon 4, exon 5, and exon 6, or a combination thereof, optionally wherein the mutated TET2 is genetically edited in~~ exon 3, exon 4, exon 5, ~~and/or~~ exon 6.
53. The population of genetically engineered T cells of ~~any one of~~ claims 1-4 or claim 2, wherein the disrupted TET2 gene is genetically edited by CRISPR/Cas-mediated gene editing.

**64.** The population of genetically engineered T cells of claim **53**, wherein the disrupted *TET2* gene is genetically edited by CRISPR/Cas-mediated gene editing with a guide RNA (gRNA) comprising a nucleotide sequence of SEQ ID NO: 14, 18, 22, 26, 112, 116, or 120.

~~7. — The population of genetically engineered T cells of any one of claims 1-6; wherein (ii) comprises FAS.~~

~~8. — The population of genetically engineered T cells of any one of claims 1-7; wherein (iii) comprises CD70.~~

~~9. — The population of genetically engineered T cells of claim 7 or claim 8, wherein the disrupted FAS and/or CD70 gene is genetically edited by CRISPR/Cas-mediated gene editing.~~

~~10. — The population of genetically engineered T cells of claim 9, wherein the disrupted FAS gene is genetically edited by CRISPR/Cas-mediated gene editing with a guide RNA (gRNA) comprising the nucleotide sequence of SEQ ID NO: 69, 73, 77, 81, or 85, and/or wherein the disrupted CD70 gene is genetically edited by CRISPR/Cas-mediated gene editing with a gRNA comprising the nucleotide sequence of SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.~~

~~11. — The population of genetically engineered T cells of any one of claims 1-10; wherein the T cells further comprise a disrupted beta-2-microglobulin ( $\beta$ 2M) gene.~~

~~12. — The population of genetically engineered T cells of any one of claims 1-11; wherein the T cells further comprise a disrupted T cell receptor alpha chain constant region (TRAC) gene.~~

**135.** The population of genetically engineered T cells of any one of claims 1-~~124~~, wherein the T cells are further engineered to express a chimeric antigen receptor (CAR).

146. The population of genetically engineered T cells of claim 135, wherein the CAR targets a tumor antigen.

157. The population of genetically engineered T cells of claim 146, wherein the tumor antigen is CD19, B cell maturation antigen (BCMA), or CD70.

168. The population of genetically engineered T cells of any one of claims 13-155-7, wherein the T cells comprise a nucleic acid encoding the CAR, and wherein the nucleic acid is inserted in the genome of the T cells.

179. The population of genetically engineered T cells of claim 168, wherein the disrupted TRAC gene has an insertion of the nucleotide acid encoding the chimeric antigen receptor.

1810. The population of genetically engineered T cells of any one of claims 1-179, wherein the T cells are derived from primary T cells of one or more human donors.

~~19. The population of genetically engineered T cells of any one of claims 1-18, wherein the T cells show cytokine-dependent growth.~~

2011. A method for preparing the population of genetically engineered T cells of any one of claims 1-104, the method comprising:

(a) providing a plurality of cells, which are T cells or precursor cells thereof;

(b) genetically editing the *TET2* gene in the plurality of cells;

~~(b) genetically editing one or more of the (i) (iii) genes; and~~

(c) producing the population of genetically engineered T cells;

wherein the plurality of cells in step (a) comprises a disrupted *TRAC* gene and a disrupted *β2M* gene; or

wherein step (b) further comprises genetically editing the *TRAC* gene and the *β2M* gene in the plurality of cells.

2412. The method of claim 2011, wherein step (b) is performed by delivering to the plurality of cells a RNA-guided nuclease, and one or more gRNAs targeting the *TET2* gene, and optionally the *TRAC* gene and the *β2M* gene. ~~one or more of the (i)-(iii) genes.~~

2213. The method of claim 2412, wherein ~~(i) comprises TET2 and optionally wherein~~ the gRNA targeting TET2 is specific to an exon of the *TET2* gene selected from the group consisting of ~~exon 1~~, exon 3, exon 4, exon 5, and exon 6.

2314. The method of claim 2213, wherein the guide RNA (gRNA) targeting TET2 comprises a nucleotide sequence of SEQ ID NO: 14, 18, 22, 26, 112, 116, or 120.

~~24. The method of any one of claims 20-23, wherein (ii) comprises FAS.~~

~~25. The method of any one of claims 20-24, wherein (iii) comprises CD70.~~

~~26. The method of claim 24 or claim 25, wherein the gRNA targeting FAS comprises the nucleotide sequence of SEQ ID NO: 69, 73, 77, 81, or 85, and/or wherein the gRNA targeting CD70 comprises the nucleotide sequence of SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.~~

2715. The method of any one of claims 20-2611-14, wherein the cells of step (a) are primary T cells of one or more human donors.

~~28 The method of any one of claims 20-27, wherein in step (b), gRNAs targeting a combination of (i)-(iii) are delivered to the cells.~~

2916. The method of any one of claims 20-2812-15, wherein in step (b), the RNA-guided nuclease is a Cas9 nuclease.

3017. The method of claim 2916, wherein the Cas9 nuclease is a *S. pyogenes* Cas9 nuclease.

~~3418.~~ The method of any one of claims ~~20-30~~12-17, wherein the RNA-guided nuclease and the one or more gRNAs are complexed in a ribonucleoprotein particle (RNP).

~~3219.~~ The method of any one of claims ~~20-34~~12-18, wherein step (b) is performed by a single electroporation event or by two sequential electroporation events.

~~33.—The method of any one of claims 20-32, wherein step (b) is performed by two sequential electroporation events.~~

~~3420.~~ The method of any one of claims 11-19, further comprising A method for preparing genetically engineered T cells expressing a chimeric antigen receptor (CAR), the method comprising:

~~(a)—providing a plurality of T cells from a cell bank comprising genetically engineered T cells set forth in any one of claims 1-10;~~

~~(b)~~ delivering to the plurality of the T cells a nucleic acid encoding a CAR to produce; and

~~(c)—producing the genetically engineered T cells expressing the CAR.~~

~~35.—The method of claim 34, wherein the genetically engineered T cells in the cell bank further comprises a disrupted  $\beta 2M$  gene.~~

~~36.—The method of claim 34, wherein the method further comprises genetically editing a  $\beta 2M$  gene.~~

~~37.—The method of claim 36, wherein the genetically editing step comprises delivering to the plurality of the T cells a gRNA targeting a  $\beta 2M$  gene.~~

~~38.—The method of any one of claims 34-37, wherein the genetically engineered T cells in the cell bank further comprises a disrupted TRAC gene.~~

~~39. The method of any one of claims 34-38, wherein the method further comprises genetically editing a TRAC gene.~~

~~40. The method of any one of claims 34-39, wherein the genetically engineered T cells in the cell bank are derived from primary T cells of one or more human donor.~~

~~41. The method of any one of claims 34-40, wherein a RNA-guided nuclease is delivered to the T cells.~~

~~42. The method of claim 41, wherein the RNA-guided nuclease is a Cas9 nuclease.~~

~~43. The method of claim 42, wherein the Cas9 nuclease is a *S. pyogenes* Cas9 nuclease.~~

~~44. The method of any one of claims 41-43, wherein the RNA-guided nuclease and the gRNA(s) are complexed in a ribonucleoprotein particle (RNP).~~

4521. A genetically engineered T cell population, which is prepared by a method of any one of claims ~~2011-2044~~.

4622. Use of aA population of genetically engineered T cells in the manufacture of a medicament ~~method~~ for eliminating undesired cells in a subject, ~~the method comprising administering to a subject in need thereof T cells expressing a chimeric antigen receptor targeting to the undesired cells,~~ wherein the genetically engineered T cells are set forth in any one of claims ~~1-19 and 455-10 and 21~~.

4723. The ~~usemethod~~ of claim 4622, wherein the undesired cells are cancer cells.

4824. A guide RNA (gRNA) targeting a *TET2* gene, comprising:  
a nucleotide spacer sequence specific to exon 3, exon 4, ~~or~~ exon 5, or exon 6 of the *TET2* gene; and

a scaffold sequence.

~~49. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence specific to exon 5.~~

~~5025. The gRNA of claim 4924, wherein the nucleotide spacer sequence targets:~~  
~~(i) the site of GGGATGTCCTATTGCTAAGT (SEQ ID NO: 125) or AGGGATGTCCTATTGCTAAG (SEQ ID NO: 126) in exon 5 of the *TET2* gene;~~  
~~(ii) the site of GATTCCGCTTGGTGAAAACG (SEQ ID NO: 129), CAGGACTCACACGACTATTC (SEQ ID NO: 131), or TTCCGCTTGGTGAAAACGAG (SEQ ID NO: 133) in exon 3 of the *TET2* gene;~~  
~~(iii) the site of CATTAGGACCTGCTCCTAGA (SEQ ID NO: 124) in exon 4 of the *TET2* gene; or~~  
~~(iv) the site of ACGGCACGCTCACCAATCGC (SEQ ID NO: 127) in exon 6 of the *TET2* gene.~~

~~2654. The gRNA of claim 2550, wherein the nucleotide spacer sequence comprises any one of SEQ ID NOs: 14, 18, or 22, 26, 112, 116, and 120.~~

~~52. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence targeting exon 3 of the *TET2* gene.~~

~~53. The gRNA of claim 52, wherein the nucleotide sequence target GATTCCGCTTGGTGAAAACG (SEQ ID NO: 129), CAGGACTCACACGACTATTC (SEQ ID NO: 131), or TTCCGCTTGGTGAAAACGAG (SEQ ID NO: 133) in exon 3 of the *TET2* gene.~~

~~54. The gRNA of claim 53, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 112, 116, or 120.~~

~~55. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence targeting exon 4 of the *TET2* gene.~~

~~56. The gRNA of claim 55, wherein the nucleotide sequence targets CATTAGGACCTGCTCCTAGA (SEQ ID NO: 124) in exon 4 of the TET2 gene.~~

~~57. The gRNA of claim 56, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 14.~~

~~58. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence targeting exon 6 of the TET2 gene.~~

~~59. The gRNA of claim 58, wherein the nucleotide sequence targets ACGGCACGCTCACCAATCGC (SEQ ID NO: 127) in exon 6 of the TET2 gene.~~

~~60. The gRNA of claim 59, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 26.~~

~~61. The gRNA of any one of claims 48-60, wherein the gRNA further comprises a scaffold sequence.~~

6227. The gRNA of any one of claims ~~24-26~~48-61, wherein the gRNA comprises one or more modified nucleotides.

6328. The gRNA of claim 6227, wherein the gRNA comprises one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA.

6429. The gRNA of ~~any one of claims 61-63~~claim 24, which comprises the nucleotide sequence of SEQ ID NO: 12, 13, 16, 17, 20, 21, 24, 25, 114, 115, 118, 119, 122, or 123.