

the function of a gene. Early technologies developed to insert a transgene into a living cell were often limited by the random nature of the insertion location of the new sequence into the genome. Random insertions into the genome may result in disruption of normal regulation of neighboring genes leading to severe unintended effects. Furthermore, random integration technologies offer little reproducibility, as there is no guarantee that the sequence would be inserted at the same place in two different cells. Common genome engineering strategies, such as ZFNs, TALENs, HEs, and MegaTALs, allow a specific area of the DNA to be modified, thereby increasing precision of the correction or insertion compared to earlier technologies. These platforms offer a greater degree of reproducibility, but limitations remain.

Despite efforts from researchers and medical professionals worldwide to address genetic disorders, and despite the promise of previous genome engineering approaches, there remains a long-felt need to develop safe and effective universal donor cells in support of cell therapy treatments involving regenerative medicine and/or immuno-oncology related indications.

SUMMARY

In one aspect, the present disclosure refers to a population of cells comprising engineered human primary T cells, wherein the engineered human primary T cells comprise:

- (a) a disrupted T cell receptor alpha chain constant region (TRAC) gene comprising a deletion of the nucleotide sequence of SEQ ID NO: 76; (b) a nucleic acid comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (i) an ectodomain that comprises an anti-CD19 single-chain variable fragment (scFv), which comprises a variable heavy chain comprising the amino acid sequence of SEQ ID NO: 1595, and a variable light chain comprising the amino acid sequence of SEQ ID NO: 1596, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 co-stimulatory domain and a CD3z co-stimulatory domain, and wherein the nucleic acid is inserted in the disrupted TRAC gene; and (c) a disrupted beta-2-microglobulin (B2M) gene.

In another aspect, the present disclosure refers to a composition comprising the population as described herein, and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a population of cells comprising engineered human primary T cells, wherein the engineered human primary T cells comprise: (a) a disrupted TRAC gene comprising a deletion of the nucleotide sequence of SEQ ID NO: 76; (b) a

nucleic acid comprising a nucleotide sequence encoding a CAR, wherein the CAR comprises: (i) an ectodomain that comprises an anti-CD19 scFv, which comprises the amino acid sequence of SEQ ID NO: 1334, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 co-stimulatory domain and a CD3z co-stimulatory domain, and wherein the nucleic acid is inserted in the disrupted TRAC gene; and (c) a disrupted B2M gene. In a further aspect, the present disclosure refers to a composition comprising the population as described herein, and a pharmaceutically acceptable carrier.

Provided herein, in some examples, are cells, methods, and compositions (*e.g.*, nucleic acids, vectors, pharmaceutical compositions) used for the treatment of certain malignancies. The gene editing technology of the present disclosure, in some examples, is used to engineer immune cell therapies targeting tumor cells that express the CD19, CD70, or BCMA antigens. Surprisingly, the immune cell therapies engineered according to the methods of the present disclosure are capable of reducing tumor volume *in vivo*, in some examples, by at least 80%, relative to untreated controls. Data from animal models, as provided herein, demonstrates that the engineered immune cell therapies, in some examples, eliminate the presence of detectable tumor cells just 30 days following *in vivo* administration, and the effect in these animal models, following a single dose of the cell therapy, persists for at least 66 days. Further, in some examples, the engineered immune cell therapies of the present disclosure are capable of increasing the survival rate of subject by at least 50% relative to untreated controls.

Further still, these cells are engineered to block both host-versus-graft disease and graft-versus-host disease, which renders them suitable for use as allogeneic cell transplantation therapeutics.

Moreover, genetic constructs and methods provided herein may be used, in some examples, to engineer immune cell populations with gene modification efficiencies high enough that the cell populations do not require purification or enrichment prior to administration *in vivo*. For example, at least 80% of the immune cells of an exemplary engineered cell population of the present disclosure lack surface expression of both the T cell receptor alpha constant gene and the $\beta 2$ microglobulin gene, and at least 50% of the immune cells also express the particular chimeric antigen receptor of interest (*e.g.*, targeting CD19, CD70, or BCMA).

Thus, provided herein, in some examples, are populations of cells comprising engineered T cells that comprise a T cell receptor alpha chain constant region (TRAC) gene

disrupted by insertion of a nucleic acid encoding a chimeric antigen receptor (CAR) comprising (i) an ectodomain that comprises an anti-CD19 antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, and a disrupted beta-2-microglobulin (B2M) gene, wherein at least 70% of the engineered T cells do not express a detectable level of TCR surface protein and do not express a detectable level of B2M surface protein, and/or wherein at least 50% of the engineered T cells express a detectable level of the CAR.

Other examples provide populations of cells comprising engineered T cells that comprise a TRAC gene disrupted by insertion of a nucleic acid encoding a CAR comprising (i) an ectodomain that comprises an anti-CD70 antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, and a disrupted B2M gene, wherein at least 70% of the engineered T cells do not express a detectable level of TCR surface protein and do not express a detectable level of B2M surface protein, and/or wherein at least 50% of the engineered T cells express a detectable level of the CAR.

Yet other examples provide populations of cells comprising engineered T cells that comprise a TRAC gene disrupted by insertion of a nucleic acid encoding a CAR comprising (i) an ectodomain that comprises an anti-BCMA antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, and a disrupted B2M gene, wherein at least 70% of the engineered T cells do not express a detectable level of TCR surface protein and do not express a detectable level of B2M surface protein, and/or wherein at least 50% of the engineered T cells express a detectable level of the CAR.

Some examples of the present disclosure provide methods for producing an engineered T cell suitable for allogenic transplantation, the method comprising (a) delivering to a composition comprising a T cell a RNA-guided nuclease, a gRNA targeting a TRAC gene, a gRNA targeting a B2M gene, and a vector comprising a donor template that comprises a nucleic acid encoding a CAR, wherein the CAR comprises (i) an ectodomain that comprises an anti-CD19 antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, wherein the nucleic acid encoding the CAR is flanked by left and

right homology arms to the TRAC gene locus and (b) producing an engineered T cell suitable for allogeneic transplantation.

Other examples of the present disclosure provide methods for producing an engineered T cell suitable for allogeneic transplantation, the method comprising (a) delivering to a composition comprising a T cell a RNA-guided nuclease, a gRNA targeting a TRAC gene, a gRNA targeting a B2M gene, and a vector comprising a donor template that comprises a nucleic acid encoding a CAR, wherein the CAR comprises (i) an ectodomain that comprises an anti-CD70 antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the TRAC gene locus and (b) producing an engineered T cell suitable for allogeneic transplantation.

Yet other examples of the present disclosure provide methods for producing an engineered T cell suitable for allogeneic transplantation, the method comprising (a) delivering to a composition comprising a T cell a RNA-guided nuclease, a gRNA targeting a TRAC gene, a gRNA targeting a B2M gene, and a vector comprising a donor template that comprises a nucleic acid encoding a CAR, wherein the CAR comprises (i) an ectodomain that comprises an anti-BCMA antibody fragment, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 or 41BB co-stimulatory domain and optionally a CD3z co-stimulatory domain, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the TRAC gene locus and (b) producing an engineered T cell suitable for allogeneic transplantation.

In some examples, the engineered T cells are unpurified and/or unenriched. In some examples, the population of cells is unpurified and/or unenriched.

In some examples, the anti-CD19 antibody fragment is an anti-CD19 scFv antibody fragment. In some examples, the anti-CD70 antibody fragment is an anti-CD70 scFv antibody fragment. In some examples, the anti-BCMA antibody fragment is an anti-BCMA scFv antibody fragment.

In some examples, the antibody fragment (*e.g.*, scFv fragment) is humanized. In some examples, the humanized anti-CD19 antibody fragment is encoded by the nucleotide sequence of SEQ ID NO: 1333 and/or wherein the humanized anti-CD19 antibody fragment comprises the amino acid sequence of SEQ ID NO: 1334. In some examples, the humanized anti-CD19 antibody fragment comprises a heavy chain that comprises the amino acid

sequence of SEQ ID NO: 1595. In some examples, the humanized anti-CD19 antibody fragment comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 1596. In some examples, the humanized anti-CD70 antibody fragment is encoded by the nucleotide sequence of SEQ ID NO: 1475 or 1476 and/or wherein the humanized anti-CD70 antibody fragment comprises the amino acid sequence of SEQ ID NO: 1499 or 1500. In some examples, the humanized anti-CD70 antibody fragment comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 1592. In some examples, the humanized anti-CD70 antibody fragment comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 1593. In some examples, the humanized anti-BCMA antibody fragment is encoded by the nucleotide sequence of SEQ ID NO: 1479 or 1485 the humanized anti-BCMA antibody fragment comprises the amino acid sequence of SEQ ID NO: 1503 or 1509. In some examples, the humanized anti-BCMA antibody fragment comprises a heavy chain that comprises the amino acid sequence of SEQ ID NO: 1589 or 1524. In some examples, the humanized anti-BCMA antibody fragment comprises a light chain that comprises the amino acid sequence of SEQ ID NO: 1590 or 1526.

In some examples, the ectodomain of the CAR further comprises a signal peptide, optionally a CD8 signal peptide. In some examples, the CAR further comprises a hinge domain, optionally a CD8 hinge domain, located between the anti-CD19 antibody fragment and the CD8 transmembrane domain. In some examples, the CAR comprises the following structural arrangement from N-terminus to C-terminus: the ectodomain that comprises an anti-CD19 antibody fragment, a CD8 hinge domain, the CD8 transmembrane domain, and the endodomain that comprises a CD28 or 41BB co-stimulatory domain and a CD3z co-stimulatory domain.

In some examples, the CAR (anti-CD19 CAR) is encoded by the nucleotide sequence of SEQ ID NO: 1316 and/or wherein the CAR comprises the amino acid sequence of SEQ ID NO: 1338. In some examples, the CAR (anti-CD70 CAR) is encoded by the nucleotide sequence of SEQ ID NO: 1423, 1424, or 1275, and/or wherein the CAR comprises the amino acid sequence of SEQ ID NO: 1449, 1450, or 1276. In some examples, the CAR (anti-BCMA CAR) is encoded by the nucleotide sequence of SEQ ID NO: 1427, 1428, 1434, or 1435, and/or wherein the CAR comprises the amino acid sequence of SEQ ID NO: 1453, 1454, 1460, or 1461.

In some examples, at least 70% (*e.g.*, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%) of the engineered T cells do not express a detectable level of TCR and/or B2M surface protein.

5 In some examples, at least 50% (*e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%) of the engineered T cells express a detectable level of the CAR.

In some examples, at least 50% (*e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, or at least 80%) of the engineered T cells express a detectable level of the CAR and do not express a detectable level of TCR surface protein or B2M surface protein (*e.g.*, detectable by flow cytometry).

10 In some examples, co-culture of the engineered T cell with CD19+ B cells results in lysis of at least 50% (*e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%) of the CD19+ B cells. In some examples, co-culture of the engineered T cell with CD70+ B cells results in lysis of at least 50% (*e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%) of the CD70+ B cells. In some examples, co-culture of the
15 engineered T cell with BCMA+ B cells results in lysis of at least 50% (*e.g.*, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%) of the BCMA+ B cells.

In some examples, the engineered T cells produce interferon gamma in the presence of CD19+ cells. In some examples, the engineered T cells produce interferon gamma in the presence of CD70+ cells. In some examples, the engineered T cells produce interferon
20 gamma in the presence of BCMA+ cells.

In some examples, the engineered T cells do not proliferate in the absence of cytokine stimulation, growth factor stimulation, or antigen stimulation.

In some examples, the population of cells further comprises a disrupted programmed cell death protein 1 (PD1) gene. In some examples, at least 70% (*e.g.*, at least 75%, at least
25 80%, at least 85%, or at least 90%) of the engineered T cells do not express a detectable level of PD1 surface protein.

In some examples, the population of cells further comprises a disrupted cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) gene. In some examples, at least 70% (*e.g.*, at least 75%, at least 80%, at least 85%, or at least 90%) of the engineered T cells do not
30 express a detectable level of CTLA-4 surface protein.

In some examples, the population of cells further comprises a gRNA targeting the TRAC gene, a gRNA targeting the B2M gene, and Cas9 protein (*e.g.*, a *S. pyogenes* Cas9 protein).

In some examples, the gRNA targeting the TRAC gene comprises the nucleotide sequence of any one of SEQ ID NOs: 83-158. In some examples, the gRNA targeting the TRAC gene targets the nucleotide sequence of any one of SEQ ID NOs: 7-82. In some examples, the gRNA targeting the B2M gene comprises the nucleotide sequence of any one of SEQ ID NOs: 458-506. In some examples, the gRNA targeting the B2M gene targets the nucleotide sequence of any one of SEQ ID NOs: 409-457. In some examples, the gRNA targeting the TRAC gene comprises the nucleotide sequence of SEQ ID NO: 152. In some examples, the gRNA targeting the TRAC gene targets the nucleotide sequence of SEQ ID NO: 76. In some examples, the gRNA targeting the B2M gene comprises the nucleotide sequence of SEQ ID NO: 466. In some examples, the gRNA targeting the B2M gene targets the nucleotide sequence of SEQ ID NO: 417.

In some examples, the population of cells further comprises a gRNA targeting the PD1 gene. In some examples, the gRNA targeting the PD1 gene comprises the nucleotide sequence of any one of SEQ ID NOs: 1083-1274 and/or targets the nucleotide sequence of any one of SEQ ID NOs: 891-1082. In some examples, the gRNA targeting the PD1 gene comprises the nucleotide sequence of SEQ ID NOs: 1086. In some examples, the gRNA targeting the PD1 gene targets the nucleotide sequence of SEQ ID NO: 894.

In some examples, the population of cells further comprises a gRNA targeting the CTLA-4 gene. In some examples, the gRNA targeting the CTLA-4 gene comprises the nucleotide sequence of any one of SEQ ID NOs: 1289-1298. In some examples, the gRNA targeting the CTLA-4 gene targets the nucleotide sequence of any one of SEQ ID NOs: 1278-1287. In some examples, the gRNA targeting the CTLA-4 gene comprises the nucleotide sequence of SEQ ID NO: 1292. In some examples, the gRNA targeting the CTLA-4 gene targets the nucleotide sequence of SEQ ID NO: 1281.

In some examples, engineered T cells of the population of cells comprise a deletion of the nucleotide sequence of SEQ ID NO: 76, relative to unmodified T cells.

In some examples, the disrupted B2M gene comprises an insertion of at least one nucleotide base pair and/or a deletion of at least one nucleotide base pair.

In some examples, a disrupted B2M gene of the engineered T cells comprises at least one nucleotide sequence selected from the group consisting of: SEQ ID NO: 1560; SEQ ID NO: 1561; SEQ ID NO: 1562; SEQ ID NO: 1563; SEQ ID NO: 1564; and SEQ ID NO: 1565.

In some examples, at least 16% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1560; at least 6% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1561; at least 4% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1562; at least 2% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1563; at least 2% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1564; and at least 2% of the cells comprise a B2M gene edited to comprise the nucleotide of SEQ ID NO: 1565.

In some examples, the vector is an adeno-associated viral (AAV) vector. In some examples, the AAV vector is an AAV serotype 6 (AAV6) vector. In some examples, the AAV vector comprise the nucleotide sequence of any one of SEQ ID NOs: 1354-1357. In some examples, the AAV vector comprise the nucleotide sequence of SEQ ID NO: 1354. In some examples, the AAV vector comprise the nucleotide sequence of any one of SEQ ID NOs: 1358-1360. In some examples, the AAV vector comprise the nucleotide sequence of SEQ ID NO: 1360. In some examples, the AAV vector comprise the nucleotide sequence of any one of SEQ ID NOs: 1365, 1366, 1372, or 1373. In some examples, the AAV vector comprise the nucleotide sequence of SEQ ID NOs: 1366 or 1373.

In some examples, the donor template comprises the nucleotide sequence of any one of claims 1390-1393. In some examples, the donor template comprises the nucleotide sequence of SEQ ID NO: 1390. In some examples, the donor template comprises the nucleotide sequence of any one of SEQ ID NOs: 1394-1396. In some examples, the donor template comprises the nucleotide sequence of SEQ ID NO: 1396. In some examples, the donor template comprises the nucleotide sequence of any one of SEQ ID NOs: 1401, 1402, 1408, or 1409. In some examples, the donor template comprises the nucleotide sequence of SEQ ID NO: 1402 or 1409. It is understood that the disclosures described in this specification are not limited to the examples summarized in this Summary. Various other examples are described and exemplified herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Various aspects of materials and methods for producing genome-edited cells engineered to express a chimeric antigen receptor (CAR) construct on the cell surface, and materials and methods for treating a patient using the genome-edited engineered cells disclosed and described in this specification can be better understood by reference to the accompanying figures, in which:

Figure 1 is a graph depicting a rank ordered list of IVT gRNAs targeting the TRAC gene and their respective activities (% InDel) in 293 cells.

Figures 2A and 2B are a series of graphs depicting a rank ordered list of IVT gRNAs targeting the CD3-epsilon (CD3E) gene and their respective activities (% InDel) in 293 cells.

5 Figure 3 is a graph depicting a rank ordered list of IVT gRNAs targeting the B2M gene and their respective activities (% InDel) in 293 cells.

Figures 4A, 4B, 4C, and 4D are a series of graphs depicting a rank ordered list of IVT gRNAs targeting the CIITA gene and their respective activities (% InDel) in 293 cells.

10 Figures 5A, 5B, and 5C are a series of graphs depicting a rank ordered list of IVT gRNAs targeting the PD1 gene and their respective activities (% InDel) in 293 cells.

Figures 6A and 6B are a series of images of flow cytometry plots depicting lack of reactivity to PHA-L, but normal responses to PMA/ionomycin by TCRA or CD3ε null human T cells as compared to controls. Figure 6A shows levels of the T cell activation marker CD69 (top panel) and levels of CFSE (marking proliferative history) (bottom panel), and Figure 6B depicts levels of degranulation (CD107a) and IFNγ I (left panel) and depicts levels of IL-2 and TNF (right panel) in control and gene edited human T cells.

Figure 7 is a series of graphs depicting the loss of MHC-II surface expression measured by flow cytometry after treatment of primary human T cells with RNPs containing RNPs to the CIITA or RFX-5 genes.

CLAIMS

What is claimed is:

1. A population of cells comprising engineered human primary T cells, wherein the
5 engineered human primary T cells comprise: (a) a disrupted T cell receptor alpha chain
constant region (TRAC) gene comprising a deletion of the nucleotide sequence of SEQ ID
NO: 76; (b) a nucleic acid comprising a nucleotide sequence encoding a chimeric antigen
receptor (CAR), wherein the CAR comprises (i) an ectodomain that comprises an anti-CD19
single-chain variable fragment (scFv), which comprises a variable heavy chain comprising
10 the amino acid sequence of SEQ ID NO: 1595, and a variable light chain comprising the
amino acid sequence of SEQ ID NO: 1596, (ii) a CD8 transmembrane domain, and (iii) an
endodomain that comprises a CD28 co-stimulatory domain and a CD3z co-stimulatory
domain, and wherein the nucleic acid is inserted in the disrupted TRAC gene; and (c) a
disrupted beta-2-microglobulin (B2M) gene.
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2. The population of cells of claim 1, wherein at least 90% of the engineered human T cells
do not express a detectable level of TCR surface protein.
3. The population of cells of claim 1, wherein at least 50% of the engineered human T cells
20 express a detectable level of the CAR.
4. The population of cells of claim 1, wherein co-culture of the engineered human T cells
with CD19+ B cells results in lysis of at least 50% of the CD19+ B cells.
- 25 5. The population of cells of claim 1, wherein the engineered human T cells produce
interferon gamma in the presence of CD19+ cells.
6. The population of cells of claim 1, wherein the engineered human T cells do not proliferate
in the absence of cytokine stimulation, growth factor stimulation, or antigen stimulation.
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7. The population of cells of claim 1, wherein the disrupted B2M gene comprises an insertion
of at least one nucleotide base pair and/or a deletion of at least one nucleotide base pair.

8. The population of cells of claim 7, wherein the disrupted B2M gene comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1560; SEQ ID NO: 1561; SEQ ID NO: 1562; SEQ ID NO: 1563; SEQ ID NO: 1564; and SEQ ID NO: 1565.
- 5 9. The population of cells of claim 1, wherein at least 70% of the engineered human T cells do not express a detectable level of TCR surface protein, wherein at least 50% of the engineered human T cells do not express a detectable level of B2M surface protein, and wherein at least 50% of the engineered human T cells express a detectable level of the CAR.
- 10 10. A composition comprising the population of cells of claim 1, and a pharmaceutically acceptable carrier.
11. The population of cells of claim 1, wherein the human primary T cells are from healthy donors.
- 15 12. A population of cells comprising engineered human primary T cells, wherein the engineered human primary T cells comprise: (a) a disrupted TRAC gene comprising a deletion of the nucleotide sequence of SEQ ID NO: 76; (b) a nucleic acid comprising a nucleotide sequence encoding a CAR, wherein the CAR comprises: (i) an ectodomain that
- 20 comprises an anti-CD19 scFv, which comprises the amino acid sequence of SEQ ID NO: 1334, (ii) a CD8 transmembrane domain, and (iii) an endodomain that comprises a CD28 co-stimulatory domain and a CD3z co-stimulatory domain, and wherein the nucleic acid is inserted in the disrupted TRAC gene; and (c) a disrupted B2M gene.
- 25 13. The population of cells of claim 12, wherein the anti-CD19 scFv is encoded by the nucleotide sequence of SEQ ID NO: 1333.
14. The population of cells of claim 12, wherein at least 90% of the engineered human T cells do not express a detectable level of TCR surface protein.
- 30 15. The population of cells of claim 12, wherein at least 50% of the engineered human T cells express a detectable level of the CAR.

16. The population of cells of claim 12, wherein co-culture of the engineered human T cells with CD19⁺ B cells results in lysis of at least 50% of the CD19⁺ B cells.

5 17. The population of cells of claim 12, wherein the engineered human T cells produce interferon gamma in the presence of CD19⁺ cells.

10 18. The population of cells of claim 12, wherein the engineered human T cells do not proliferate in the absence of cytokine stimulation, growth factor stimulation, or antigen stimulation.

19. The population of cells of claim 12, wherein the disrupted B2M gene comprises an insertion of at least one nucleotide base pair and/or a deletion of at least one nucleotide base pair.

15 20. The population of cells of claim 19, wherein the disrupted B2M gene comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO: 1560; SEQ ID NO: 1561; SEQ ID NO: 1562; SEQ ID NO: 1563; SEQ ID NO: 1564; and SEQ ID NO: 1565.

20 21. The population of cells of claim 12, wherein the disrupted TRAC gene comprises the nucleotide sequence of SEQ ID NO: 1390.

25 22. The population of cells of claim 12, wherein at least 70% of the engineered human T cells do not express a detectable level of TCR surface protein, wherein at least 50% of the engineered human T cells do not express a detectable level of B2M surface protein, and wherein at least 50% of the engineered human T cells express a detectable level of the CAR.

23. A composition comprising the population of cells of claim 12, and a pharmaceutically acceptable carrier.

30 24. The population of cells of claim 12, wherein the human primary T cells are from healthy donors.