

METHODS AND COMPOSITIONS FOR MODULATING CAR-T ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Nos. 63/133,171 filed December 31, 2020; 63/136,172 filed January 11, 2021; 63/175,003 filed April 14, 2021; 63/255,795 filed October 14, 2021; and 63/288,477 filed December 10, 2021, the disclosures of which are herein incorporated by reference in their entireties.

SUMMARY

[0002] Off-the-shelf CAR-T cells and other therapeutic cells can offer advantages over autologous cell-based strategies, including ease of manufacturing, quality control and avoidance of malignant contamination and T cell dysfunction. However, the vigorous host-versus-graft immune response against histoincompatible T cells prevents expansion and persistence of allogeneic CAR-T cells and mitigates the efficacy of this approach.

[0003] There is substantial evidence in both animal models and human patients that hypoinmunogenic cell transplantation is a scientifically feasible and clinically promising approach to the treatment of numerous disorders, conditions, and diseases.

[0004] There remains a need for novel approaches, compositions and methods for producing cell-based therapies that avoid detection by the recipient's immune system.

[0005] In one aspect, the present disclosure refers to an engineered T cell comprising (a) reduced expression of B2M, CIITA, and/or T cell receptor (TCR)-alpha relative to a control T cell, (b) increased expression of CD47 encoded by a first exogenous polynucleotide relative to the control T cell, and (c) expression of a CD19-specific chimeric antigen receptor (CAR) encoded by a second exogenous polynucleotide, wherein the engineered T cell is derived from a primary T cell, wherein wherein the CAR comprises an antigen binding domain comprising: (i) a light chain amino acid sequence comprising a light chain CDR1, a light chain CDR2, and a light chain CDR3 amino acid, wherein the light chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 21, the light chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 22, and the light chain CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 23, and (ii) a heavy chain amino acid sequence comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 amino acid, wherein the heavy chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO:

26, the heavy chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 27, and the heavy chain CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 28, wherein the first exogenous polynucleotide is inserted into a locus of at least one allele of the engineered T cell, and wherein the second exogenous polynucleotide is inserted into a locus of at least one allele of the engineered T cell.

[0005a] In some examples, described herein is an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type cell or a control cell, the engineered cell further comprising a set of exogenous polynucleotides comprising a first exogenous polynucleotide encoding CD47 and a second exogenous polynucleotide encoding a chimeric antigen receptor (CAR), wherein the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the cell. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis virus glycoprotein (VSV-G) envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous

polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0006] In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In many examples, the first exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the second exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0007] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into different loci. In many examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the same locus. In several examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus. In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *CIITA* locus. In many examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *TRAC* locus. In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *TRB* locus. In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the safe harbor or target locus. In some examples, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus (*e.g.*,

ROSA26 gene locus), an F3 gene locus (also known as CD142), a MICA gene locus, a MICB gene locus, a LRP1 gene locus (also known as a CD91 gene locus), a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus. In various examples, the safe harbor or target locus is selected from the group consisting of the *AAVS1* locus, the *CCR5* locus, and the *ROSA26* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0008] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR. In some examples, the CAR is a bispecific CAR. In some examples, the CAR is a CD19-specific CAR. In some examples, the CAR is a CD22-specific CAR. In some examples, the CAR is a bispecific CAR. In some examples, the CAR is a CD19/CD22-bispecific CAR.

[0009] In many examples, the engineered cell does not express HLA-A, HLA-B, and/or HLA-C antigens. In some examples, the engineered cell does not express B2M. In other examples, the engineered cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens. In some examples, the engineered cell does not express CIITA. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-alpha and/or TCR-beta.

[0010] In many examples, the engineered cell is a pluripotent stem cell. In some examples, the engineered cell is an induced pluripotent stem cell.

[0011] In some examples, the engineered cell is a differentiated cell derived from an induced pluripotent stem cell. In various examples, the differentiated cell is selected from the group consisting of an NK cell and a T cell.

[0012] In some examples, the engineered cell is a cell derived from a primary T cell. In many examples, the cell derived from the primary T cell is derived from a pool of T

cells comprising primary T cells from one or more donor subjects who are different from a recipient subject.

[0013] In some examples, the engineered cell is a cell derived from a primary NK cell. In many examples, the cell derived from the primary NK cell is derived from a pool of NK cells comprising primary NK cells from one or more donor subjects who are different from a recipient subject.

[0014] In some examples, the engineered cell retains pluripotency and/or retains differentiation potential.

[0015] In many examples, following transfer into a first subject, the engineered cell exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject. In some instances, the first subject and the second subject are different subjects. In some instances, the macrophage response is engulfment. In various examples, following transfer into a subject the engineered cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject. In many examples, following transfer into a subject the engineered cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[0016] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRAC^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR. In some examples, the first and/or second exogenous polynucleotides are inserted into at least one allele of the T cell using viral transduction. In some examples, the first and/or second exogenous polynucleotides are inserted into at least one allele of the T cell using a lentivirus based viral vector. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or

TRAC^{indel/indel} cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In numerous examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *B2M* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *B2M* locus. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a *B2M*^{indel/indel}, *CIITA*^{indel/indel}, and/or *TRAC*^{indel/indel} cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide

encoding CAR inserted into the *CIITA* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRAC^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *CIITA* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0017] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising the first

exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In numerous examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *B2M* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *B2M* locus. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *CIITA* locus. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *CIITA* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0018] In some examples, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, and/or $TRB^{indel/indel}$ cell comprising first exogenous polynucleotide encoding

CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus. In many examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In some examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus. In numerous examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *B2M* locus. In many examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *B2M* locus. In some examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *CIITA* locus. In many examples, the engineered cell is a *B2M^{indel/indel}*, *CIITA^{indel/indel}*, *TRAC^{indel/indel}*, and/or *TRB^{indel/indel}* cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *CIITA* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0019] In some examples, described is an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type cell or a control cell.

[0020] In some examples, the engineered cell does not express HLA-A, HLA-B and/or HLA-C antigens. In many examples, the engineered cell does not express CIITA.

[0021] In many examples, the engineered cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens. In some examples, the engineered cell does not express B2M.

[0022] In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-alpha. In many examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-beta.

[0023] In various examples, the engineered cell overexpresses CD47 relative to a wild-type cell or a control cell.

[0024] In some examples, the engineered cell is a pluripotent stem cell. In many examples, the engineered cell is an induced pluripotent stem cell.

[0025] In many examples, the engineered cell is a differentiated cell derived from an induced pluripotent stem cell. In some examples, the differentiated cell is selected from the group consisting of an NK cell and a T cell.

[0026] In many examples, the engineered cell is a cell derived from a primary T cell. In several examples, the cell derived from the primary T cell is derived from a pool of T cells comprising primary T cells from one or more donor subjects who are different from a recipient subject.

[0027] In various examples, the engineered cell retains pluripotency and/or retains differentiation potential.

[0028] In some examples, following transfer into a subject the engineered cell elicits one or more response selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject. In some instances, the first subject and the second subject are different subjects. In some instances, the macrophage response is engulfment.

[0029] In various examples, following transfer into a subject the engineered cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject. In many

examples, following transfer into a subject the engineered cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[0030] In some examples, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, and/or $TRB^{indel/indel}$ cell. In some instances, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, and/or $TRB^{indel/indel}$ primary T cell. In some instances, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$, and/or $TRB^{indel/indel}$ T cell differentiated from a hypoinmunogenic induced pluripotent stem cell. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell. In some instances, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ primary T cell. In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRB^{indel/indel}$ cell. In some instances, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRB^{indel/indel}$ primary T cell. In some instances, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRAC^{indel/indel}$ T cell differentiated from a hypoinmunogenic induced pluripotent stem cell. In some examples, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ cell. In some instances, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ primary T cell. In some instances, the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, and/or $TRB^{indel/indel}$ T cell differentiated from a hypoinmunogenic induced pluripotent stem cell.

[0031] In some examples, the engineered cell is a hypoinmunogenic cell.

[0032] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[0033] In some examples, the pharmaceutically acceptable additive, carrier, diluent or excipient comprises one or more selected from the group consisting of Plasma-Lyte A®, dextrose, dextran, sodium chloride, human serum albumin (HSA), dimethylsulfoxide (DMSO), and a combination thereof. In some examples, the pharmaceutical composition further comprises a pharmaceutically acceptable buffer. In some examples, the pharmaceutically acceptable buffer is neutral buffer saline or phosphate buffered saline.

[0034] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, a base solution of CryoStor® CSB at a concentration of about 70-80% w/w, and one or more of about 20-30% w/w PlasmaLyte-A™, about 0.3-5.3% w/v human serum albumin (HSA), about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[0035] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, a base solution of PlasmaLyte-A™ at a concentration of about 20-30% w/w, and one or more of about 70-80% w/w CryoStor® CSB, about 0.3-5.3% w/v human serum albumin (HSA), about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[0036] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, about 0.3-5.3% w/v human serum albumin (HSA), and one or more of about 70-80% w/w CryoStor® CSB, about 20-30% w/w PlasmaLyte-A™, about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[0037] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, about 0-20% v/v dimethylsulfoxide (DMSO), and one or more of about 70-80% w/w CryoStor® CSB, about 20-30% w/w PlasmaLyte-A™, about 0.3-5.3% w/v human serum albumin (HSA), and about 100-400 mM trehalose.

[0038] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, about 100-400 mM trehalose, and one or more of about 70-80% w/w CryoStor® CSB, about 20-30% w/w PlasmaLyte-A™, about 0.3-5.3% w/v human serum albumin (HSA), and about 0-20% v/v dimethylsulfoxide (DMSO).

[0039] In some examples, the pharmaceutical composition comprises about 75% w/w of CryoStor® CSB. In some examples, the pharmaceutical composition comprises about 25% w/w of PlasmaLyte-A™. In some examples, the pharmaceutical composition comprises about 0.3% w/v of HSA. In some examples, the pharmaceutical composition comprises about 7.5% v/v of DMSO.

[0040] In some examples, described is a pharmaceutical composition comprising a population of any of the engineered cells described herein, a base solution of CryoStor® CSB at a concentration of about 75% w/w, about 25% w/w PlasmaLyte-A™, about 0.3% w/v human serum albumin (HSA), and about 7.5% v/v dimethylsulfoxide (DMSO).

[0041] In some examples, the population of the engineered cells is up to about 8.0×10^8 cells. In many examples, the population of the engineered cells is up to about 6.0×10^8 cells. In other examples, the population of the engineered cells is from about 1.0×10^6 to about 2.5×10^8 cells. In some examples, the population of the engineered cells is from about 2.0×10^6 to about 2.0×10^8 cells.

[0042] In various examples, the population of the engineered cells ranges from about 5 ml to about 80 ml. In many examples, the population of the engineered cells ranges from about 10 ml to about 70 ml. In some examples, the population of the engineered cells ranges from about 10 ml to about 50 ml.

[0043] In some examples, the composition is formulated for administration in a single dose. In many examples, the composition is formulated for administration in up to three doses.

[0044] In some examples, the composition is formulated for administration of a single dose to a subject takes a duration of time of about 60 minutes or less. In many examples, the composition is formulated for administration of a single dose to a subject takes a duration of time of about 30 minutes or less.

[0045] In some examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 40% survival in a subject after 10 days following administration. In various examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 80% survival in a subject after about 2 weeks following administration. In several examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 100% survival in a subject after about 3 weeks following administration. In many examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 150% survival in a subject after about 4 weeks following administration.

[0046] In another embodiment, described is a dosage regimen for treating a disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of any of the engineered cells described herein and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 doses.

[0047] In some examples, the pharmaceutical composition administered is up to about 6.0×10^8 cells in about 1-3 doses. In some examples, the pharmaceutical composition administered is from about 0.6×10^6 to about 6.0×10^8 cells in about 1-3 doses. In some examples, the pharmaceutical composition administered is from about 0.2×10^6 to about 5.0×10^6 cells per kg of the subject's body weight in about 1-3 doses, if the subject has a body weight of 50 kg or less. In some examples, the pharmaceutical composition administered is from about 0.1×10^8 to about 2.5×10^8 cells in about 1-3 doses, if the subject has a body weight greater than 50 kg. In some examples, the pharmaceutical composition administered is from about 2.0×10^6 cells per kg of the subject's body weight and up to about 2×10^8 cells in about 1-3 doses.

[0048] In some examples, the administration of a single dose to the subject takes a duration of time of about 60 minutes or less. In some examples, the administration of a single dose to the subject takes a duration of time of about 30 minutes or less.

[0049] In some examples, the pharmaceutically acceptable additive, carrier, diluent or excipient comprises one or more selected from the group consisting of Plasma-Lyte A[®], dextrose, dextran, sodium chloride, human serum albumin (HSA), dimethylsulfoxide (DMSO), and a combination thereof.

[0050] In some examples, the pharmaceutical composition further comprises a pharmaceutically acceptable buffer. In some examples, the pharmaceutically acceptable buffer is neutral buffer saline or phosphate buffered saline.

[0051] In some examples, after the administration of the pharmaceutical composition, the population of cells or progeny thereof are present in the subject up to 9 months. In some examples, after the administration of the pharmaceutical composition, the population of cells or progeny thereof are present in the subject at least 2 years or more.

[0052] In some examples, after the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 40% survival in a subject after about 10 days following administration. In some examples, after the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 80% survival in a subject after about 2 weeks following

administration. In some examples, after the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 100% survival in a subject after about 3 weeks following administration. In some examples, after the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 150% survival in a subject after about 4 weeks following administration.

[0053] In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 24 hours apart. In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 28 days apart. In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 6 weeks apart. In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 12 months or more apart.

[0054] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR). In some examples, the set of exogenous polynucleotides are inserted into at least one allele of the T cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the set of exogenous polynucleotides. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the set of exogenous polynucleotides. In some examples, set of exogenous polynucleotides are inserted into at least one allele of the T cell using a lentivirus based viral vector. In some examples, the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition comprises up to about 6.0×10^8 cells. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least

one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0055] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR). In some examples, the set of exogenous polynucleotides are inserted into at least one allele of the T cell using viral transduction. In some examples, set of exogenous polynucleotides are inserted into at least one allele of the T cell using a lentivirus based viral vector. In some examples, the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in 1-3 doses. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0056] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR), wherein the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein a dose of the pharmaceutical composition is administered for a duration of time of about 60 minutes or less. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating

lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0057] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition comprises up to about 6.0×10^8 cells.

[0058] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in 1-3 doses.

[0059] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein a dose of the pharmaceutical composition is administered for a duration of 60 minutes or less.

[0060] In some examples, described is a method of treating a cancer in a subject comprising administration of any of the engineered cells described herein or any of the pharmaceutical compositions described herein or any of the dosage regimens described herein to the subject. In some examples, the cancer is a CD19⁺ cancer.

[0061] In some examples, described is a method of preventing T cell exhaustion or treating a disease in a subject comprising administration of any of the engineered cells described herein to the subject, wherein the CAR is a CD19/CD22-bispecific CAR.

[0062] In some examples, described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising: (i) administration of a first dosage regimen comprising a first population of any of the engineered cells described herein to the subject at a first timepoint, and (ii) administration of a second dosage regimen comprising a second

population of any of the engineered cells described herein to the subject at a second timepoint, wherein the first dosage regimen and the second dosage regimen are different.

[0063] In some examples, described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising: (i) administration of a first dosage regimen comprising a first population of any of the engineered cells described herein to the subject at a first timepoint, and (ii) administration of a second dosage regimen comprising a second population of any of the engineered cells described herein to the subject at a second timepoint, wherein the first population of engineered cells and the second population of engineered cells both comprise the same chimeric antigen receptor.

[0064] In some examples, described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising: (i) administration of a first dosage regimen comprising a first population of any of the engineered cells described herein to the subject at a first timepoint, and (ii) administration of a second dosage regimen comprising a second population of any of the engineered cells described herein to the subject at a second timepoint, wherein the first population of engineered cells and the second population of engineered cells both comprise different chimeric antigen receptors.

[0065] In some examples, described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising: (i) administration of a first dosage regimen comprising a first population of any of the engineered cells described herein to the subject at a first timepoint, and (ii) administration of a second dosage regimen comprising a second population of any of the engineered cells described herein to the subject at a second timepoint, wherein the engineered cells of the first population comprise a first chimeric antigen receptor that binds a first antigen and the engineered cells of the second population comprise a second chimeric antigen receptor that binds a second antigen, and wherein the first antigen and the second antigen are the same.

[0066] In some examples, described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising: (i) administration of a first dosage regimen comprising a first population of any of the engineered cells described herein to the subject at a first timepoint, and (ii) administration of a second dosage regimen comprising a second population of any of the engineered cells described herein to the subject at a second timepoint, wherein the engineered cells of the first population comprise a first chimeric antigen receptor that binds a first antigen and the engineered cells of the second population comprise a second chimeric antigen receptor that binds a second antigen, and wherein the first antigen and the second antigen are different.

[0067] Described herein are non-activated T cells comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, and a first exogenous polynucleotide encoding a chimeric antigen receptor (CAR). In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0068] In some examples, the non-activated T cell is a primary T cell. In other examples, the non-activated T cell is differentiated from the engineered cells of the present technology.

[0069] In some examples, the T cell is a CD8⁺ T cell.

[0070] In some examples, the non-activated T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory molecule.

[0071] In some examples, the anti-CD3 antibody is OKT3. In some examples, the anti-CD28 antibody is CD28.2. In some examples, the T cell activating cytokine is selected from the group of T cell activating cytokines consisting of IL-2, IL-7, IL-15, and IL-21. In some examples, the soluble T cell costimulatory molecule is selected from the group of soluble T cell costimulatory molecules consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody.

[0072] In some examples, the non-activated T cell does not express activation markers.

[0073] In some examples, the non-activated T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[0074] In some examples, the first exogenous polynucleotide is carried by a lentiviral vector comprising a CD8 binding agent.

[0075] In some examples, the non-activated T cell further comprises a second exogenous polynucleotide encoding CD47.

[0076] In some examples, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell. In some examples, the first and/or second exogenous polynucleotides are inserted into at least one allele of the T cell using viral transduction. In some examples, the first and/or second exogenous polynucleotides are inserted into at least one allele of the T cell using a lentivirus based viral vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the first and/or second exogenous polynucleotides. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the first and/or second exogenous polynucleotides. In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus. In some examples, the second exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the first exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into different loci. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the same locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *CIITA* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *TRAC* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *TRB* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the safe harbor or target locus. In some examples, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C (also known as AAVS1) gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus (*e.g.*, ROSA26 gene locus), an F3 gene locus (also known as CD142), a MICA gene locus, a MICB gene locus, a LRP1 gene locus (also

known as a CD91 gene locus), a HMGB1 gene locus, an ABO gene locus, ad RHD gene locus, a FUT1 locus, and a KDM5D gene locus. In some examples, the safe harbor or target locus is selected from the group consisting of the *AAVS1* locus, the *CCR5* locus, and the *ROSA26* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0077] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR. In some examples, the CAR is a bispecific CAR. In some examples, the bispecific CAR is a CD19/CD22-bispecific CAR.

[0078] In some examples, the non-activated T cell does not express HLA-A, HLA-B, and/or HLA-C antigens. In some examples, the non-activated T cell does not express B2M. In some examples, the non-activated T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens. In some examples, the non-activated T cell does not express CIITA. In some examples, the non-activated T cell does not express TCR-alpha and TCR-beta.

[0079] In some examples, the non-activated T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.

[0080] In some examples, the non-activated T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.

[0081] In some examples, the non-activated T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[0082] In some examples, the non-activated T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[0083] In some examples, the non-activated T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *B2M* locus.

[0084] In some examples, the non-activated T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into a *B2M* locus.

[0085] In some examples, the non-activated T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *CIITA* locus.

[0086] In some examples, the non-activated T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into a *CIITA* locus.

[0087] In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0088] Described herein are engineered T cells comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, *B2M*, *CIITA*, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, wherein the engineered T cell further comprises a first exogenous polynucleotide encoding a chimeric antigen receptor (CAR) carried by a lentiviral vector. Described herein are engineered T cells comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, *B2M*, *CIITA*, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, wherein the engineered T cell further comprises a first exogenous polynucleotide encoding a chimeric antigen receptor (CAR) carried by a lentiviral vector that comprises a CD8 binding agent. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G

envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0089] In some examples, the engineered T cell is a primary T cell. In other examples, the engineered T cell is differentiated from the engineered cell of the present technology. In some examples, the T cell is a CD8⁺ T cell.

[0090] In some examples, the engineered T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory molecule. In some examples, the anti-CD3 antibody is OKT3, wherein the anti-CD28 antibody is CD28.2, wherein the T cell activating cytokine is selected from the group of T cell activating cytokines consisting of IL-2, IL-7, IL-15, and IL-21, and wherein soluble T cell costimulatory molecule is selected from the group of soluble T cell costimulatory molecules consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody.

[0091] In some examples, the engineered T cell does not express activation markers. In some examples, the engineered T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[0092] In some examples, the engineered T cell further comprises a second exogenous polynucleotide encoding CD47. In some examples, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell. In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus. In some examples, the second exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the first exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into different loci. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the same locus. In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus, the *CIITA* locus, the *TRAC*

locus, the *TRB* locus, or the safe harbor or target locus. In some examples, the safe harbor or target locus is selected from the group consisting of a *CCR5* gene locus, a *CXCR4* gene locus, a *PPP1R12C* (also known as *AAVS1*) gene locus, an albumin gene locus, a *SHS231* gene locus, a *CLYBL* gene locus, a *Rosa* gene locus (*e.g.*, *ROSA26* gene locus), an *F3* gene locus (also known as *CD142*), a *MICA* gene locus, a *MICB* gene locus, a *LRP1* gene locus (also known as a *CD91* gene locus), a *HMGB1* gene locus, an *ABO* gene locus, an *RHD* gene locus, a *FUT1* locus, and a *KDM5D* gene locus. In some examples, the safe harbor or target locus is selected from the group consisting of the *AAVS1* locus, the *CCR5* locus, and the *ROSA26* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0093] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[0094] In some examples, the engineered T cell does not express HLA-A, HLA-B, and/or HLA-C antigens, wherein the engineered T cell does not express B2M, wherein the engineered T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens, wherein the engineered T cell does not express *CIITA*, and/or wherein the engineered T cell does not express TCR-alpha and TCR-beta.

[0095] In some examples, the engineered T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising the second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus, into the *TRB* locus, into the *B2M* locus, or into the *CIITA* locus. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral

transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[0096] In some examples, the non-activated T cell and/or the engineered T cell of the present technology are in a subject. In other examples, the non-activated T cell and/or the engineered T cell of the present technology are in vitro.

[0097] In some examples, the non-activated T cell and/or the engineered T cell of the present technology express a CD8 binding agent. In some examples, the CD8 binding agent is an anti-CD8 antibody. In some examples, the anti-CD8 antibody is selected from the group consisting of a mouse anti-CD8 antibody, a rabbit anti-CD8 antibody, a human anti-CD8 antibody, a humanized anti-CD8 antibody, a camelid (*e.g.*, llama, alpaca, camel) anti-CD8 antibody, and a fragment thereof. In some examples, the fragment thereof is an scFV or a VHH. In some examples, the CD8 binding agent binds to a CD8 alpha chain and/or a CD8 beta chain.

[0098] In some examples, the CD8 binding agent is fused to a transmembrane domain incorporated in the viral envelope. In some examples, the lentivirus vector is pseudotyped with a viral fusion protein. In some examples, the viral fusion protein comprises one or more modifications to reduce binding to its native receptor.

[0099] In some examples, the viral fusion protein is fused to the CD8 binding agent. In some examples, the viral fusion protein comprises Nipah virus F glycoprotein and Nipah virus G glycoprotein fused to the CD8 binding agent. In some examples, the lentivirus vector does not comprise a T cell activating molecule or a T cell costimulatory molecule. In some examples, the lentivirus vector encodes the first exogenous polynucleotide and/or the second exogenous polynucleotide.

[00100] In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction, for example, with a vector. In some examples, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the exogenous polynucleotide. In some examples, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction. In some examples, the exogenous polynucleotide is inserted into at least one allele of the cell using a lentivirus based viral vector.

[00101] In some examples, following transfer into a first subject, the non-activated T cell or the engineered T cell exhibits one or more responses selected from the group

consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject. In some examples, the first subject and the second subject are different subjects. In some examples, the macrophage response is engulfment.

[00102] In some examples, following transfer into a subject, the non-activated T cell or the engineered T cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.

[00103] In some examples, following transfer into a subject, the non-activated T cell or the engineered T cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[00104] In some examples, the non-activated T cell or the engineered T cell is transduced with a lentivirus vector comprising a CD8 binding agent within the subject. In some examples, the lentivirus vector carries a gene encoding the CAR and/or CD47.

[00105] Described herein are pharmaceutical compositions comprising a population of the non-activated T cells and/or the engineered T cells of the present technology and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[00106] Described herein are methods comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present technology, or one or more the pharmaceutical compositions of the present technology.

[00107] In some examples, the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some examples, the T cell activating treatment comprises lymphodepletion.

[00108] Described herein are methods of treating a subject suffering from cancer, comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present technology, or one or more the pharmaceutical compositions of the present technology, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some examples, the T cell activating treatment comprises lymphodepletion.

[00109] Described herein are methods for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof within the subject, comprising administering to a subject a composition comprising a population of the non-activated T cells and/or the engineered T cells of the present technology, or one or more the pharmaceutical compositions of the present technology, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with administration of the composition. In some examples, the T cell activating treatment comprises lymphodepletion.

[00110] Described herein are dosage regimens for treating a disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of the non-activated T cells and/or the engineered T cells of the present technology, or one or more the pharmaceutical compositions of the present technology, and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 doses.

[00111] Described herein is an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type cell or a control cell, the engineered cell further comprising a set of exogenous polynucleotides comprising a first exogenous polynucleotide encoding CD47 and a second exogenous polynucleotide encoding a chimeric antigen receptor (CAR), wherein the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the cell.

[00112] In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a B2M locus, a CIITA locus, a TRAC locus and a TRB locus.

[00113] In some examples, the first exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00114] In some examples, the second exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00115] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into different loci.

[00116] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the same locus.

[00117] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus.

[00118] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *CIITA* locus.

[00119] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *TRAC* locus.

[00120] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the *TRB* locus.

[00121] In some examples, the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the safe harbor or target locus.

[00122] In some examples, the safe harbor locus is selected from the group consisting of a CCR5 gene locus, a PPP1R12C gene locus, a CLYBL gene locus, and a Rosa gene locus, and the target locus is selected from the group consisting of a CXCR4 gene locus, an albumin gene locus, a SHS231 gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[00123] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[00124] In some examples, the CD19-specific CAR is substantially equivalent to the CD19-specific CAR of any one of the CAR-T cell based therapies selected from the group consisting of axicabtagene ciloleucel, lisocabtagene maraleucel, brexucabtagene autoleucel, and tisagenlecleucel.

[00125] In some examples, the CAR is a bispecific CAR.

[00126] In some examples, the CAR is a CD19/CD22-bispecific CAR.

[00127] In some examples, the engineered cell does not express HLA-A, HLA-B, and/or HLA-C antigens.

[00128] In some examples, the engineered cell does not express B2M.

[00129] In some examples, the engineered cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens.

[00130] In some examples, the engineered cell does not express CIITA.

[00131] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-alpha and/or TCR-beta.

[00132] In some examples, the engineered cell is a pluripotent stem cell.

- [00133] In some examples, the engineered cell is an induced pluripotent stem cell.
- [00134] In some examples, the engineered cell is a differentiated cell derived from an induced pluripotent stem cell.
- [00135] In some examples, the differentiated cell is selected from the group consisting of an NK cell and a T cell.
- [00136] In some examples, the engineered cell is a cell derived from a primary T cell.
- [00137] In some examples, the cell derived from the primary T cell is derived from a pool of T cells comprising primary T cells from one or more donor subjects who are different from a recipient subject.
- [00138] In some examples, the engineered cell retains pluripotency and/or retains differentiation potential.
- [00139] In some examples, following transfer into a first subject, the engineered cell exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject.
- [00140] In some examples, the first subject and the second subject are different subjects.
- [00141] In some examples, the macrophage response is engulfment.
- [00142] In some examples, following transfer into a subject the engineered cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.
- [00143] In some examples, following transfer into a subject the engineered cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.
- [00144] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.
- [00145] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced

pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.

[00146] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[00147] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[00148] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *B2M* locus.

[00149] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *B2M* locus.

[00150] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$, $TRAC^{indel/indel}$ cell comprising first exogenous polynucleotide encoding CD47 and/or the second exogenous polynucleotide encoding CAR inserted into the *CIITA* locus.

[00151] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding CAR inserted into a *CIITA* locus.

[00152] Described herein is an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type cell or a control cell.

[00153] In some examples, the engineered cell does not express HLA-A, HLA-B and/or HLA-C antigens.

[00154] In some examples, the engineered cell does not express CIITA.

[00155] In some examples, the engineered cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens.

[00156] In some examples, the engineered cell does not express B2M.

[00157] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-alpha.

[00158] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell does not express TCR-beta.

[00159] In some examples, the engineered cell overexpresses CD47 relative to a wild-type cell or a control cell.

[00160] In some examples, the engineered cell is a pluripotent stem cell.

[00161] In some examples, the engineered cell is an induced pluripotent stem cell.

[00162] In some examples, the engineered cell is a differentiated cell derived from an induced pluripotent stem cell.

[00163] In some examples, the differentiated cell is selected from the group consisting of an NK cell and a T cell.

[00164] In some examples, the engineered cell is a cell derived from a primary T cell.

[00165] In some examples, the cell derived from the primary T cell is derived from a pool of T cells comprising primary T cells from one or more donor subjects who are different from a recipient subject.

[00166] In some examples, the engineered cell retains pluripotency and/or retains differentiation potential.

[00167] In some examples, following transfer into a first subject, the engineered cell exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject.

[00168] In some examples, the first subject and the second subject are different subjects.

[00169] In some examples, the macrophage response is engulfment.

[00170] In some examples, following transfer into a subject the engineered cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.

[00171] In some examples, following transfer into a subject the engineered cell elicits one or more selected from the group consisting of (a) reduced donor-specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in the subject, as compared to a wild-type cell following transfer into the subject.

[00172] In some examples, the engineered cell is selected from the group consisting a pluripotent stem cell, an induced pluripotent stem cell, a T cell differentiated from an induced pluripotent stem cell, a primary T cell, and a cell derived from a primary T cell, and the engineered cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel}, and/or TRB^{indel/indel} cell.

[00173] In some examples, the engineered cell is a hypoinmunogenic cell.

[00174] In some examples, the wild type cell or the control cell is a starting material.

[00175] In some examples, the first and/or second exogenous polynucleotide is inserted into at least one allele of the cell using viral transduction.

[00176] In some examples, the viral transduction is via a lentivirus based viral vector.

[00177] In some examples, the lentivirus based viral vector is a pseudotyped, self-inactivating lentiviral vector that carries the first and/or second exogenous polynucleotides.

[00178] In some examples, the lentivirus based viral vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the first and/or second exogenous polynucleotides.

[00179] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[00180] In some examples, the pharmaceutically acceptable additive, carrier, diluent or excipient comprises one or more selected from the group consisting of Plasma-Lyte A[®], dextrose, dextran, sodium chloride, human serum albumin (HSA), dimethylsulfoxide (DMSO), and a combination thereof.

[00181] In some examples of the composition, the composition further comprises a pharmaceutically acceptable buffer.

[00182] In some examples, the pharmaceutically acceptable buffer is neutral buffer saline or phosphate buffered saline.

[00183] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, a base solution of CryoStor[®] CSB at a concentration of about 70-80% w/w, and one or more of about 20-30% w/w PlasmaLyte-A[™], about 0.3-5.3% w/v human serum albumin (HSA), about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[00184] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, a base solution of PlasmaLyte-A[™] at a concentration of about 20-30% w/w, and one or more of about 70-80% w/w CryoStor[®] CSB, about 0.3-5.3% w/v human serum albumin (HSA), about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[00185] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, about 0.3-5.3% w/v human serum albumin (HSA), and one or more of about 70-80% w/w CryoStor[®] CSB, about 20-30% w/w PlasmaLyte-A[™], about 0-20% v/v dimethylsulfoxide (DMSO), and about 100-400 mM trehalose.

[00186] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, about 0-20% v/v dimethylsulfoxide (DMSO), and one or more of about 70-80% w/w CryoStor[®] CSB, about 20-30% w/w PlasmaLyte-A[™], about 0.3-5.3% w/v human serum albumin (HSA), and about 100-400 mM trehalose.

[00187] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, about 100-400 mM trehalose, and one or more of about 70-80% w/w CryoStor[®] CSB, about 20-30% w/w PlasmaLyte-A[™], about 0.3-5.3% w/v human serum albumin (HSA), and about 0-20% v/v dimethylsulfoxide (DMSO).

[00188] In some examples, the pharmaceutical composition comprises about 75% w/w of CryoStor® CSB.

[00189] In some examples, the pharmaceutical composition comprises about 25% w/w of PlasmaLyte-A™.

[00190] In some examples, the pharmaceutical composition comprises about 0.3% w/v of HSA.

[00191] In some examples, the pharmaceutical composition comprises about 7.5% v/v of DMSO.

[00192] Described herein is a pharmaceutical composition comprising a population of the engineered cells as described herein, a base solution of CryoStor® CSB at a concentration of about 75% w/w, about 25% w/w PlasmaLyte-A™, about 0.3% w/v human serum albumin (HSA), and about 7.5% v/v dimethylsulfoxide (DMSO).

[00193] In some examples, the population of the engineered cells is up to about 8.0×10^8 cells.

[00194] In some examples, the population of the engineered cells is up to about 6.0×10^8 cells.

[00195] In some examples, the population of the engineered cells is from about 1.0×10^6 to about 2.5×10^8 cells.

[00196] In some examples, the population of the engineered cells is from about 2.0×10^6 to about 2.0×10^8 cells.

[00197] In some examples, the population of the engineered cells ranges from about 5 ml to about 80 ml.

[00198] In some examples, the population of the engineered cells ranges from about 10 ml to about 70 ml.

[00199] In some examples, the population of the engineered cells ranges from about 10 ml to about 50 ml.

[00200] In some examples, the composition is formulated for administration in a single dose.

[00201] In some examples, the composition is formulated for administration in up to three doses.

[00202] In some examples, the composition is formulated for administration of a single dose to a subject takes a duration of time of about 60 minutes or less.

[00203] In some examples, the composition is formulated for administration of a single dose to a subject takes a duration of time of about 30 minutes or less.

[00204] In some examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 40% survival in a subject after 10 days following administration.

[00205] In some examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 80% survival in a subject after about 2 weeks following administration.

[00206] In some examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 100% survival in a subject after about 3 weeks following administration.

[00207] In some examples, the population of engineered cells of the pharmaceutical composition or progeny thereof exhibit at least 150% survival in a subject after about 4 weeks following administration.

[00208] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of engineered cells as described herein and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 doses.

[00209] In some examples, the pharmaceutical composition administered is up to about 6.0×10^8 cells in about 1-3 doses.

[00210] In some examples, the pharmaceutical composition administered is from about 0.6×10^6 to about 6.0×10^8 cells in about 1-3 doses.

[00211] In some examples, the pharmaceutical composition administered is from about 0.2×10^6 to about 5.0×10^6 cells per kg of the subject's body weight in about 1-3 doses, if the subject has a body weight of 50 kg or less.

[00212] In some examples, the pharmaceutical composition administered is from about 0.1×10^8 to about 2.5×10^8 cells in about 1-3 doses, if the subject has a body weight greater than 50 kg.

[00213] In some examples, the pharmaceutical composition administered is from about 2.0×10^6 cells per kg of the subject's body weight and up to about 2×10^8 cells in about 1-3 doses.

[00214] In some examples, the administration of a single dose to the subject takes a duration of time of about 60 minutes or less.

[00215] In some examples, the administration of a single dose to the subject takes a duration of time of about 30 minutes or less.

[00216] In some examples, the pharmaceutically acceptable additive, carrier, diluent or excipient comprises one or more selected from the group consisting of Plasma-Lyte A[®], dextrose, dextran, sodium chloride, human serum albumin (HSA), dimethylsulfoxide (DMSO), and a combination thereof.

[00217] In some examples, the pharmaceutical composition further comprises a pharmaceutically acceptable buffer.

[00218] In some examples, the pharmaceutically acceptable buffer is neutral buffer saline or phosphate buffered saline.

[00219] In some examples, the administration of the pharmaceutical composition, the population of cells or progeny thereof are present in the subject up to 9 months.

[00220] In some examples, the administration of the pharmaceutical composition, the population of cells or progeny thereof are present in the subject at least 2 years or more.

[00221] In some examples, the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 40% survival in a subject after about 10 days following administration.

[00222] In some examples, the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 80% survival in a subject after about 2 weeks following administration.

[00223] In some examples, the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 100% survival in a subject after about 3 weeks following administration.

[00224] In some examples, the administration of the pharmaceutical composition, the population of engineered cells or progeny thereof exhibit at least 150% survival in a subject after about 4 weeks following administration.

[00225] In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 24 hours apart.

[00226] In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 28 days apart.

[00227] In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 6 weeks apart.

[00228] In some examples, the administration of 2-3 doses to the subject occurs such that each dose is administered ranging from 1 to 12 months or more apart.

[00229] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

(i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR), wherein the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and

(ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,

wherein the pharmaceutical composition comprises up to about 6.0×10^8 cells.

[00230] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

(i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR), wherein the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and

(ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,

wherein the pharmaceutical composition is administered in 1-3 doses.

[00231] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

(i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta, the engineered cell further comprising a set of exogenous polynucleotides encoding CD47 and a chimeric antigen receptor (CAR), wherein the set of exogenous polynucleotides are inserted into a safe harbor or target locus of at least one allele of the cell; and

(ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,

wherein a dose of the pharmaceutical composition is administered for a duration of time of about 60 minutes or less.

[00232] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

(i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and

(ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,

wherein the pharmaceutical composition comprises up to about 6.0×10^8 cells.

[00233] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

- (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and
 - (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,
- wherein the pharmaceutical composition is administered in 1-3 doses.

[00234] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administering a pharmaceutical composition comprising

- (i) an engineered cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta; and
 - (ii) a pharmaceutically acceptable additive, carrier, diluent or excipient,
- wherein a dose of the pharmaceutical composition is administered for a duration of 60 minutes or less.

[00235] Described herein is a method of treating a cancer in a subject comprising administration of the engineered cell as described herein, the pharmaceutical composition as described herein, or the dosage regimen as described herein to the subject.

[00236] In some examples, the cancer is a CD19+ cancer.

[00237] Described herein is a method of preventing T cell exhaustion in a subject comprising administration of the engineered cell as described herein to the subject, wherein the CAR is a CD19-specific CAR or a CD22-specific CAR.

[00238] In some examples, the CD19-specific CAR is substantially equivalent to the CD19-specific CAR of any one of the CAR-T cell based therapies selected from the group consisting of axicabtagene ciloleucel, lisocabtagene maraleucel, brexucabtagene autoleucel, and tisagenlecleucel.

[00239] Described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising:

- (i) administration of a first dosage regimen comprising a first population of engineered cells as described herein to the subject at a first timepoint, and
- (ii) administration of a second dosage regimen comprising a second population of engineered cells as described herein to the subject at a second timepoint,

wherein the first dosage regimen and the second dosage regimen are different.

[00240] A method of preventing T cell exhaustion or treating a disease in a subject comprising:

(i) administration of a first dosage regimen comprising a first population of engineered cells as described herein to the subject at a first timepoint, and

(ii) administration of a second dosage regimen comprising a second population of engineered cells as described herein to the subject at a second timepoint,

wherein the first population of engineered cells and the second population of engineered cells both comprise the same chimeric antigen receptor.

[00241] Described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising:

(i) administration of a first dosage regimen comprising a first population of engineered cells as described herein to the subject at a first timepoint, and

(ii) administration of a second dosage regimen comprising a second population of engineered cells as described herein to the subject at a second timepoint,

wherein the first population of engineered cells and the second population of engineered cells both comprise different chimeric antigen receptors.

[00242] Described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising:

(i) administration of a first dosage regimen comprising a first population of engineered cells as described herein to the subject at a first timepoint, and

(ii) administration of a second dosage regimen comprising a second population of engineered cells as described herein to the subject at a second timepoint,

wherein the engineered cells of the first population comprise a first chimeric antigen receptor that binds a first antigen and the engineered cells of the second population comprise a second chimeric antigen receptor that binds a second antigen, and wherein the first antigen and the second antigen are the same.

[00243] Described herein is a method of preventing T cell exhaustion or treating a disease in a subject comprising:

(i) administration of a first dosage regimen comprising a first population of engineered cells as described herein to the subject at a first timepoint, and

(ii) administration of a second dosage regimen comprising a second population of engineered cells as described herein to the subject at a second timepoint,

wherein the engineered cells of the first population comprise a first chimeric antigen receptor that binds a first antigen and the engineered cells of the second population comprise a second chimeric antigen receptor that binds a second antigen, and wherein the first antigen and the second antigen are different.

[00244] In some examples, the engineered cell is not activated.

[00245] Described herein is a non-activated T cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, and a first exogenous polynucleotide encoding a chimeric antigen receptor (CAR).

[00246] In some examples, the non-activated T cell is a primary T cell.

[00247] In some examples, the non-activated T cell is differentiated from the engineered cell as described herein.

[00248] In some examples, the T cell is a CD8⁺ T cell.

[00249] In some examples, the non-activated T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory molecule.

[00250] In some examples, the anti-CD3 antibody is OKT3.

[00251] In some examples, the anti-CD28 antibody is CD28.2.

[00252] In some examples, the T cell activating cytokine is selected from the group consisting of IL-2, IL-7, IL-15, and IL-21.

[00253] In some examples, the soluble T cell costimulatory molecule is selected from the group consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody.

[00254] In some examples, the non-activated T cell does not express activation markers.

[00255] In some examples, the non-activated T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[00256] In some examples, the first exogenous polynucleotide is carried by a lentiviral vector that comprises a CD8 binding agent.

[00257] In some examples, the non-activated T cell further comprises a second exogenous polynucleotide encoding CD47.

[00258] In some examples, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell.

[00259] In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

[00260] In some examples, the second exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00261] In some examples, the first exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00262] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into different loci.

[00263] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the same locus.

[00264] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus.

[00265] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *CIITA* locus.

[00266] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into a TCR gene locus.

[00267] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *TRAC* locus.

[00268] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *TRB* locus.

[00269] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the safe harbor or target locus.

[00270] In some examples, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[00271] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[00272] In some examples, the CD19-specific CAR is substantially equivalent to the CD19-specific CAR of any one of the CAR-T cell based therapies selected from the group consisting of axicabtagene ciloleucel, lisocabtagene maraleucel, brexucabtagene autoleucel, and tisagenlecleucel.

[00273] In some examples, the CAR is a bispecific CAR.

[00274] In some examples, the bispecific CAR is a CD19/CD22 bispecific CAR.

[00275] In some examples, the non-activated T cell does not express HLA-A, HLA-B, and/or HLA-C antigens.

[00276] In some examples, the non-activated T cell does not express B2M.

[00277] In some examples, the non-activated T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens.

[00278] In some examples, the non-activated T cell does not express CIITA.

[00279] In some examples, the non-activated T cell does not express TCR-alpha and/or TCR-beta.

[00280] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.

[00281] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus.

[00282] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[00283] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into the *TRB* locus.

[00284] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *B2M* locus.

[00285] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into a *B2M* locus.

[00286] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *CIITA* locus.

[00287] In some examples, the non-activated T cell is a B2M^{indel/indel}, CIITA^{indel/indel}, TRAC^{indel/indel} cell comprising the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding CAR inserted into a *CIITA* locus.

[00288] Described herein is an engineered T cell comprising reduced expression of HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ, HLA-DR, B2M, CIITA, TCR-alpha, and/or TCR-beta relative to a wild-type T cell, wherein the engineered T cell further comprises a first exogenous polynucleotide encoding a chimeric antigen receptor (CAR) carried by a lentiviral vector comprising a CD8 binding agent.

[00289] In some examples, the engineered T cell is a primary T cell.

[00290] In some examples, the engineered T cell is differentiated from the engineered cell as described herein.

[00291] In some examples, the T cell is a CD8⁺ T cell.

[00292] In some examples, the engineered T cell has not been treated with an anti-CD3 antibody, an anti-CD28 antibody, a T cell activating cytokine, or a soluble T cell costimulatory molecule.

[00293] In some examples, the anti-CD3 antibody is OKT3, wherein the anti-CD28 antibody is CD28.2, wherein the T cell activating cytokine is selected from the group consisting of IL-2, IL-7, IL-15, and IL-21, and wherein the soluble T cell costimulatory molecule is selected from the group consisting of an anti-CD28 antibody, an anti-CD80 antibody, an anti-CD86 antibody, an anti-CD137L antibody, and an anti-ICOS-L antibody.

[00294] In some examples, the engineered T cell does not express activation markers.

[00295] In some examples, the engineered T cell expresses CD3 and CD28, and wherein the CD3 and/or CD28 are inactive.

[00296] In some examples, the engineered T cell further comprises a second exogenous polynucleotide encoding CD47.

[00297] In some examples, the first and/or second exogenous polynucleotides are inserted into a specific locus of at least one allele of the T cell.

[00298] In some examples, the specific locus is selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus, and a *TRB* locus.

[00299] In some examples, the second exogenous polynucleotide encoding CD47 is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00300] In some examples, the first exogenous polynucleotide encoding the CAR is inserted into the specific locus selected from the group consisting of a safe harbor or target locus, a *B2M* locus, a *CIITA* locus, a *TRAC* locus and a *TRB* locus.

[00301] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into different loci.

[00302] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the same locus.

[00303] In some examples, the second exogenous polynucleotide encoding CD47 and the first exogenous polynucleotide encoding the CAR are inserted into the *B2M* locus, the *CIITA* locus, the *TRAC* locus, the *TRB* locus, or the safe harbor or target locus.

[00304] In some examples, the safe harbor or target locus is selected from the group consisting of a CCR5 gene locus, a CXCR4 gene locus, a PPP1R12C gene locus, an albumin gene locus, a SHS231 gene locus, a CLYBL gene locus, a Rosa gene locus, an F3 (CD142) gene locus, a MICA gene locus, a MICB gene locus, a LRP1 (CD91) gene locus, a HMGB1 gene locus, an ABO gene locus, an RHD gene locus, a FUT1 locus, and a KDM5D gene locus.

[00305] In some examples, the CAR is selected from the group consisting of a CD19-specific CAR and a CD22-specific CAR.

[00306] In some examples, the CD19-specific CAR is substantially equivalent to the CD19-specific CAR of any one of the CAR-T cell based therapies selected from the group consisting of axicabtagene ciloleucel, lisocabtagene maraleucel, brexucabtagene autoleucel, and tisagenlecleucel.

[00307] In some examples, the engineered T cell does not express HLA-A, HLA-B, and/or HLA-C antigens, wherein the engineered T cell does not express B2M, wherein the engineered T cell does not express HLA-DP, HLA-DQ, and/or HLA-DR antigens, wherein the engineered T cell does not express CIITA, and/or wherein the engineered T cell does not express TCR-alpha and/or TCR-beta.

[00308] In some examples, the engineered T cell is a $B2M^{\text{indel/indel}}$, $CIITA^{\text{indel/indel}}$, $TRAC^{\text{indel/indel}}$ cell comprising the second exogenous polynucleotide encoding CD47 and/or the first exogenous polynucleotide encoding CAR inserted into the *TRAC* locus, into the *TRB* locus, into the *B2M* locus, or into the *CIITA* locus.

[00309] In some examples, the non-activated T cell is as described herein or the engineered T cell is as described herein, wherein the non-activated T cell or the engineered T cell is in a subject.

[00310] In some examples, the non-activated T cell is as described herein or the engineered T cell is as described herein, wherein the non-activated T cell or the engineered T cell is in vitro.

[00311] In some examples, the non-activated T cell is as described herein or the engineered T cell is as described herein, wherein the CD8 binding agent is an anti-CD8 antibody.

[00312] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the anti-CD8 antibody is selected from the group consisting of a mouse anti-CD8 antibody, a rabbit anti-CD8 antibody, a human anti-CD8 antibody, a humanized anti-CD8 antibody, a camelid anti-CD8 antibody, and a fragment thereof.

[00313] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the fragment thereof is an scFV or a VHH.

[00314] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the CD8 binding agent binds to a CD8 alpha chain and/or a CD8 beta chain.

[00315] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the CD8 binding agent is fused to a transmembrane domain incorporated in a viral envelope.

[00316] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the lentivirus vector is pseudotyped with a viral fusion protein.

[00317] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the viral fusion protein comprises one or more modifications to reduce binding to its native receptor.

[00318] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the viral fusion protein is fused to the CD8 binding agent.

[00319] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the viral fusion protein comprises Nipah virus F glycoprotein and Nipah virus G glycoprotein fused to the CD8 binding agent.

[00320] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the lentivirus vector does not comprise a T cell activating molecule or a T cell costimulatory molecule.

[00321] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the lentivirus vector encodes the first exogenous polynucleotide and/or the second exogenous polynucleotide.

[00322] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein, following transfer into a first subject, the non-activated T cell or the engineered T cell exhibits one or more responses selected from the group consisting of (a) a T cell response, (b) an NK cell response, and (c) a macrophage response, that are reduced as compared to a wild-type cell following transfer into a second subject.

[00323] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the first subject and the second subject are different subjects.

[00324] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the macrophage response is engulfment.

[00325] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein, following transfer into a subject, the non-activated T cell or the engineered T cell exhibits one or more selected from the group consisting of (a) reduced TH1 activation in the subject, (b) reduced NK cell killing in the subject, and (c) reduced killing by whole PBMCs in the subject, as compared to a wild-type cell following transfer into the subject.

[00326] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein, following transfer into a subject, the non-activated T cell or the engineered T cell elicits one or more selected from the group consisting of (a) reduced donor specific antibodies in the subject, (b) reduced IgM or IgG antibodies in the subject, and (c) reduced complement-dependent cytotoxicity (CDC) in a subject, as compared to a wild-type cell following transfer into the subject.

[00327] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the non-activated T cell or the engineered T cell is transduced with a lentivirus vector comprising a CD8 binding agent within the subject.

[00328] In some examples, the non-activated T cell or the engineered T cell is as described herein, wherein the lentivirus vector carries a gene encoding the CAR and/or CD47.

[00329] Described herein is a pharmaceutical composition comprising a population of the non-activated T cell or the engineered T cell is as described herein and a pharmaceutically acceptable additive, carrier, diluent or excipient.

[00330] Described herein is a method comprising administering to a subject a composition comprising the non-activated T cell as described herein, the engineered T cell as described herein, or the pharmaceutical composition as described herein.

[00331] In some examples, the subject is not administered a T cell activating treatment before, after, and/or concurrently with the administration of the composition.

[00332] In some examples, the T cell activating treatment comprises lymphodepletion.

[00333] Described herein is a method of treating a subject suffering from cancer, comprising administering to a subject a composition comprising the non-activated T cell as described herein, the engineered T cell as described herein, or the pharmaceutical composition as described herein, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with the administration of the composition.

[00334] In some examples, the T cell activating treatment comprises lymphodepletion.

[00335] A method for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof within the subject, comprising administering to a subject a composition comprising the non-activated T cell as described herein, the engineered T cell as described herein, or the pharmaceutical composition as described herein, wherein the subject is not administered a T cell activating treatment before, after, and/or concurrently with the administration of the composition.

[00336] In some examples, the T cell activating treatment comprises lymphodepletion.

[00337] Described herein is a dosage regimen for treating a disease or disorder in a subject comprising administration of a pharmaceutical composition comprising a population of subject a composition comprising the non-activated T cell as described herein, and/or the engineered T cell as described herein, and a pharmaceutically acceptable additive, carrier, diluent or excipient, wherein the pharmaceutical composition is administered in about 1-3 doses.

[00338] The present disclosure is related to U.S. Provisional Application filed on December 31, 2020 (Attorney Docket No. 112864-5057-PR) and U.S. Provisional Application filed on January 11, 2021 filed by Morrison and Foerester having Attorney Docket No. 18615-30046.00, the contents of which are hereby incorporated by reference in their entirety. Detailed descriptions of engineered and/or hypoinmunogenic cells, methods of producing thereof, and methods of using thereof are found in U.S. Provisional Application No. 63/065,342 filed on August 13, 2020, WO2016/183041 filed May 9, 2015, WO2018/132783 filed January 14, 2018, WO2020/018615 filed July 17, 2019, WO2020/018620 filed July 17, 2019, WO2020/168317 filed February 16, 2020, the disclosures of which including the

WHAT IS CLAIMED IS:

1. An engineered T cell comprising (a) reduced expression of B2M, CIITA, and/or T cell receptor (TCR)-alpha relative to a control T cell, (b) increased expression of CD47 encoded by a first exogenous polynucleotide relative to the control T cell, and (c) expression of a CD19-specific chimeric antigen receptor (CAR) encoded by a second exogenous polynucleotide, wherein the engineered T cell is derived from a primary T cell, wherein the CAR comprises an antigen binding domain comprising:
 - (i) a light chain amino acid sequence comprising a light chain CDR1, a light chain CDR2, and a light chain CDR3 amino acid, wherein the light chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 21, the light chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 22, and the light chain CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 23, and
 - (ii) a heavy chain amino acid sequence comprising a heavy chain CDR1, a heavy chain CDR2, and a heavy chain CDR3 amino acid, wherein the heavy chain CDR1 comprises the amino acid sequence set forth in SEQ ID NO: 26, the heavy chain CDR2 comprises the amino acid sequence set forth in SEQ ID NO: 27, and the heavy chain CDR3 comprises the amino acid sequence set forth in SEQ ID NO: 28,wherein the first exogenous polynucleotide is inserted into a locus of at least one allele of the engineered T cell, and wherein the second exogenous polynucleotide is inserted into a locus of at least one allele of the engineered T cell.
2. The engineered T cell of claim 1, wherein the CAR further comprises (i) a hinge domain, (ii) a transmembrane domain, (iii) a costimulatory domain, and (iv) an intracellular signaling domain.
3. The engineered T cell of claim 2, wherein
 - (i) the hinge domain is selected from the group of hinge domains consisting of a CD8 α hinge domain, a CD28 hinge domain, an IgG4 hinge domain, and an IgG4 hinge-CH2-CH3 domain;
 - (ii) the transmembrane domain is selected from the group of transmembrane domains consisting of TCR ζ , CD5, CD8, CD9, CD45, CD22, CD32, CD33, CD37, CD40, CD40L/CD154, CD64, CD80, CD86, CD134, CD137, CD154, VEGFR2, FAS, FGFR2B,

CD8 α , CD8 β , 4-1BB/CD137, CD28, CD34, CD4, Fc ϵ RI γ , CD16, OX40/CD134, CD3 ζ , CD3 ϵ , CD3 γ , CD3 β , TCR α , and TCR β ;

(iii) the costimulatory domain is selected from the group of costimulatory domains consisting of B7-H3, CD28, CTLA-4, PD-1, 4-1BB/TNFSF9/CD137, OX40/TNFRSF4, CD200, HLA Class I, HLA-DR, DAP12, CD3 ζ , and an immunoreceptor tyrosine-based activation motif (ITAM), 2B4/CD244/SLAMF4, 4-1BB, 4-1BB ligand/TNFSF9, 4-1BB/CD137, a ligand that specifically binds with CD83, TCR β , B7-2/CD86, B7-H1/PD-L1, B7-H2, B7-H4, B7-H6, B7-H7, BAFF R/TNFRSF13C, BAFF/BLyS/TNFSF13B, BLAME/SLAMF8, BTLA/CD272, CD134, CD134/OX40, CD137, CD154, CD16, CD160, CD2, CD22, CD229/SLAMF3, CD27, CD27/TNFRSF7, CD27-1/SLAMF9, CD30, CD30/TNFRSF8, CD300a/LMIR1, CD32, CD33, CD34, CD37, CD3 γ , CD38, CD4, CD40, CD40/TNFRSF5, CD40/TNFSF5, CD40L/CD154, CD45, CD48/SLAMF2, CD5, CD53, CD58/LFA-3, CD64, CD7, CD8, CD80, CD82/Kai-1, CD84/SLAMF5, CD84/SLAMF8, CD86, CD8a, CD8B, CD9, CD90/Thy1, CD96, CRACC/SLAMF7, CRTAM, Dectin-1/CLEC7A, DPPIV/CD26, DR3/TNFRSF25, EphB6, FAS, Fc ϵ RI γ , FGFR2B, Gi24/VISTA/B7-H5, GITR/TNFRSF18, HVEM/TNFRSF14, ICOS, ICOS/CD278, Ikaros, integrin α 4 β 1, integrin α 4 β 7/LPAM-1, integrin α 4/CD49d, LAG-3, LIGHT, LIGHT/TNFSF14, lymphocyte function-associated antigen-1 (LFA-1), lymphotoxin- α /TNF β , NKG2C, NTB-A/SLAMF6, OX40/CD134, PDCD6, PD-L2/B7-DC, RELT/TNFRSF19L, SLAM/CD150, TACI/TNFRSF13B, TCL1A, TCL1B, TCR α , TCR ζ , TIM-1/KIM-1/HAVCR, TIM-4, TL1A/TNFSF15, TNF RII/TNFRSF1B, TNF α , TSLP, TSLP R, and VEGFR2; and

(iv) the intracellular signaling domain is a CD3 ζ signaling domain.

4. The engineered T cell of claim 3, wherein the hinge domain comprises the amino acid sequence set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 113.

5. The engineered T cell of claim 3, wherein the transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 14, SEQ ID NO: 15, or SEQ ID NO: 114.

6. The engineered T cell of claim 3, wherein the costimulatory domain comprises the amino acid sequence set forth in SEQ ID NO: 16 or SEQ ID NO: 17.

7. The engineered T cell of claim 3, wherein the intracellular signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 115.
8. The engineered T cell of claim 3, wherein
- (i) the hinge domain comprises the amino acid sequence set forth in SEQ ID NO: 9,
 - (ii) the transmembrane domain comprises the amino acid sequence set forth in SEQ ID NO: 14,
 - (iii) the costimulatory domain comprises the amino acid sequence set forth in SEQ ID NO: 16, and
 - (iv) the intracellular signaling domain comprises the amino acid sequence set forth in SEQ ID NO: 18.
9. The engineered T cell of claim 1, wherein the light chain amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 20, and wherein the heavy chain amino acid sequence comprises the amino acid sequence set forth in SEQ ID NO: 25.
10. The engineered T cell of claim 3, wherein the antigen binding domain comprises a linker comprising the amino acid sequence set forth in SEQ ID NO: 24 or SEQ ID NO: 30.
11. The engineered T cell of claim 10, wherein the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO: 19 or SEQ ID NO: 29.
12. The engineered T cell of claim 3, wherein the CAR further comprises a signal peptide.
13. The engineered T cell of claim 12, wherein the signal peptide comprises the amino acid sequence set forth in SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8.
14. The engineered T cell of claim 3, further comprising reduced expression of human leukocyte antigen (HLA) class I antigens and/or HLA class II antigens.
15. The engineered T cell of claim 14, wherein the engineered T cell does not express HLA class I antigens, does not express HLA class II antigens, and/or does not express TCR-alpha.

16. The engineered T cell of claim 14, wherein the reduced expression of HLA class I antigens results from the reduced expression or no expression of B2 microglobulin (B2M), and wherein the reduced expression of HLA class II antigens results from the reduced expression or no expression of class II major histocompatibility complex transactivator (CIITA).

17. The engineered T cell of claim 16, wherein the engineered T cell is a $B2M^{indel/indel}$, $CIITA^{indel/indel}$ cell and/or a $TRAC^{indel/indel}$ cell.

18. The engineered T cell of claim 1, wherein the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into the same locus.

19. The engineered T cell of claim 1, wherein the first exogenous polynucleotide encoding CD47 and the second exogenous polynucleotide encoding the CAR are inserted into different loci.

20. The engineered T cell of claim 3, wherein the first and second exogenous polynucleotides are inserted into at least one allele of the engineered T cell using viral transduction.

21. The engineered T cell of claim 20, wherein the viral transduction includes a lentivirus based viral vector.

22. The engineered T cell of claim 8, wherein the first and second exogenous polynucleotides are inserted into at least one allele of the engineered T cell using viral transduction.

23. The engineered T cell of claim 22, wherein the viral transduction includes a lentivirus based viral vector.

24. The engineered T cell of claim 23, wherein the lentivirus based viral vector is a pseudotyped, self-inactivating lentiviral vector that carries the first and/or the second exogenous polynucleotides.

25. The engineered T cell of claim 24, wherein the lentivirus based viral vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope and carries the first and/or second exogenous polynucleotides.

26. The engineered T cell of claim 8, further comprising reduced expression of HLA class I antigens, HLA class II antigens, TCR-alpha, or a combination thereof.

27. The engineered T cell of claim 26, wherein the reduced expression is reduced surface expression.