GENETICALLY ENGINEERED T CELLS WITH REGNASE-1 AND/OR TGFBRII DISRUPTION HAVE IMPROVED FUNCTIONALITY AND PERSISTENCE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Application No. 63/082,357, filed September 23, 2020, U.S. Provisional Application No. 63/124,429, filed December 11, 2020, and U.S. Provisional Application No. 63/225,673, filed July 26, 2021. The entire contents of each of the prior applications are incorporated by reference herein.

BACKGROUND OF THE INVENTION

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

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

SUMMARY OF THE INVENTION

The present disclosure is based, at least in part, on the development of genetically edited T cells carrying a disrupted *Regnase 1* (*Reg1*) gene (*e.g.*, "Reg1 Knockout T cells"), a disrupted *TGFBRII* gene (*e.g.*, "TGFBRII Knockout T cells", or genetically edited T cells carrying both a disrupted Reg1 gene and a disrupted *TGFBRII* gene, and effective methods of producing such genetically edited T cells *via* CRISPR/Cas-mediated gene editing using guide RNAs, for example, those targeting specific sites within the *Reg1* gene with high on-target editing efficiency and low off-target editing efficiency, and/or those targeting specific sites within the *TGFBRII* gene with high on-target editing efficiency and low off-target editing efficiency.

Such genetically engineered T cells exhibits at least one of the following advantageous features: (a) improved cell growth activity; (b) enhanced persistence; (c) reduced T cell exhaustion; (d) resistant to inhibitory effects induced by TGF-β; (e) enhanced cell killing capacity; and (f) resistant to inhibitory effects by fibroblasts and/or inhibitory factors secreted

thereby.

The *Reg1* disrupted T cells, the TGFBRII disrupted T cells, or the Reg1/TGFBRII double disrupted T cells disclosed herein can further be genetically engineered to express a chimeric antigen receptor (CAR) targeting an antigen of interest, *e.g.*, an antigen associated with an undesired cell such as a cancer cell, and to comprise one or more additional disrupted genes, for example, *TRAC*, *β2M*, *CD70*, or a combination thereof. The resultant CAR-expressing, Reg1 disrupted T cells exhibit enhanced cytotoxic activity against target cells and anti-tumor activity as compared with CAR-T cells having a wild-type *Reg1* gene.

In some aspects, the current disclosure is related to the development of genetically engineered CAR T cells that comprise a disrupted *Reg1* gene. The genetically engineered CAR T cells, in certain aspects, are further genetically engineered to comprise a disrupted cluster of differentiation 70 (CD70) gene. In some aspects, the CAR T cells described herein may express anti-CD70 CAR, anti-cluster of differentiation 19 (CD19) CAR or anti-B-cell maturation antigen (anti-BCMA) CAR.

The genetically edited T cells disclosed herein showed enhanced cell expansion, longevity and proliferation capacity in culture, enhanced potency (*e.g.*, enhanced cytotoxicity), and enhanced *CAR-T* efficacy in animal models (via, *e.g.*, longer persistence). Further, the genetically edited T cells showed cytokine-dependent growth, indicating safety. In addition, disrupting both the *Reg1* and *TGFBRII* genes exhibited synergistic effects in anti-tumor activity and CAR-T cell expansion and persistence as observed in animal models.

Accordingly, the present disclosure provides, in some aspects, a population of genetically engineered T cells, comprising: (i) a disrupted *Regnase-1* (*Reg1*) gene; and/or (ii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene. In some embodiments, the population of genetically engineered T cells comprises (i). In some embodiments, population of genetically engineered T cells comprises (ii). In other embodiments, the population of genetically engineered T cells comprises both (i) and (ii). Any of the genetically engineered T cells may be further engineered to express a chimeric antigen receptor (CAR).

The population of genetically engineered T cells disclosed herein, as compared to non-engineered T cell counterparts, has one or more of the following features: (a) improved cell growth activity; (b) enhanced persistence; (c) reduced T cell exhaustion; (d) resistant to inhibitory effects induced by TGF-β; (e) enhanced cell killing capacity; and (f) resistant to inhibitory effects by fibroblasts and/or inhibitory factors secreted thereby.

In some embodiments, the disrupted *Reg1* gene is genetically edited in exon 1, exone 2, exon 3, or exon 4. In some examples, the disrupted *Reg1* gene is genetically edited in exon 2 and/or exon 4. Alternatively or in addition, the disrupted *TGFBRII* gene is genetically edited in exon 1, exon 2, exon 3, exon 4, or exon 5. In some examples, the disrupted *TGFBRII* gene is genetically edited in exon 4. In other examples, the disrupted *TGFBRII* gene is genetically edited in exon 5. The disrupted *Reg1* gene, the disrupted *TGFBRII* gene, or both can be genetically edited by a CRISPR/Cas-mediated gene editing system.

In some instances, the CRISPR/Cas-mediated gene editing comprises a guide RNA (gRNA) targeting a site in the *Reg1* gene that comprises a nucleotide sequence listed in **Table 22** (with or without PAM) (*e.g.*, SEQ ID NO: 320, 322, 323, or 327, or the corresponding ones with PAM). For example, the gRNA targeting the *Reg1* gene comprises a spacer that comprises the nucleotide sequence of listed in **Table 22** (*e.g.*, SEQ ID NO: 24, 32, 36, or 52). In some examples, the disrupted *Reg1* gene comprises a nucleotide sequence selected from those listed in **Tables 29-38** (e.g., **Table 31**, **33**, **34**, or **38**).

In some instances, the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *TGFBRII* gene that comprises a nucleotide sequence listed in **Table 39** (with or without PAM). For example, the gRNA targeting the *TGFBRII* gene comprises a spacer listed in **Table 39**, for example, having a nucleotide sequence of any one of SEQ ID NOs: 270, 302, 308, and 312. In some examples, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** (*e.g.*, **Table 43**).

Any of the gRNAs disclosed herein may further comprise a scaffold sequence. For example, the gRNA targeting the *Reg1* gene comprises any of the nucleotide sequences listed in **Table 22**. Examples include 22, 23, 30, 31, 34, 35, 50, and 51. Alternatively or in addition, the gRNA targeting the *TGFBRII* gene may comprise any of the nucleotide sequences provided in **Table 39**. Examples include SEQ ID NOs: 270, 271, 300, 301, 306, 307, 312, and 313.

Any of the populations of genetically engineered T cells disclosed herein may further comprise: (iii) a disrupted T cell receptor alpha chain constant region (*TRAC*) gene, (iv) a disrupted beta-2-microglobulin (*β2M*) gene, (v) a disrupted *CD70* gene, or (vi) a combination of any of (iii)-(v). In some embodiments, the T cells comprise a disrupted T cell receptor alpha chain constant region (*TRAC*) gene. Alternatively or in addition, the T cells comprise a disrupted beta-2-microglobulin (*β2M*) gene. Any of the T cells disclosed herein may also comprise a disrupted *CD70* gene. In some examples, the disrupted *TRAC* gene, the disrupted *β2M* gene, and/or the disrupted CD70 gene is genetically edited by one or more CRISPR/Cas-

mediated gene editing system

In some embodiments, the genetically engineered T cells may comprise a nucleic acid encoding the CAR, and wherein the nucleic acid is inserted in the genome of the T cells. In some instances, the nucleic acid encoding the CAR is inserted in the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, the disrupted *β2M*, or the disrupted *CD70* gene. In some examples, the nucleic acid encoding the CAR is inserted in the disrupted *TRAC* gene. In specific examples, the nucleic acid encoding the CAR may replace the deleted fragment comprising SEQ ID NO: 69 in the *TRAC* gene. In some examples, the disrupted *Reg1* gene may comprise a nucleotide sequence listed in **Tables 29-38** (*e.g.*, **Table 31**, **33**, **34**, or **38**). In some examples, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** (*e.g.*, **Table 43**). In some examples, the disrupted *TRAC* gene may comprise a nucleotide sequence of any one of SEQ ID NOs: 75-82 (see **Table 24**). In some examples, the disrupted *β2M* may comprise a nucleotide sequence of any one of SEQ ID NOs: 83-88 (see **Table 25**). In some examples, the disrupted *CD70* gene may comprise a nucleotide sequence of any one of SEQ ID NOs: 89-94 (see **Table 26**).

Any of the CAR constructs disclosed herein may comprise an extracellular antigen binding domain specific to a tumor antigen, a co-stimulatory signaling domain of 4-1BB or CD28, and a cytoplasmic signaling domain of CD3ζ. In some examples, the tumor antigen is CD19. In some examples, the tumor antigen is BCMA. In some examples, the tumor antigen is CD70. In some examples, the tumor antigen is CD33. In some examples, the tumor antigen is PTK7.

In some embodiments, the CAR binds CD19 (anti-CD19 CAR). The extracellular antigen binding domain in the anti-CD19 CAR can be a single chain variable fragment (scFv) that binds CD19 (anti-CD19 scFv). In some instances, the anti-CD19 scFv may comprise (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 124; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 125. In some examples, the V_H comprises the amino acid sequence of SEQ ID NO: 124 and the V_L comprises the amino acid sequence of SEQ ID NO: 125. In one example, the anti-CD19 scFv comprises the amino acid sequence of SEQ ID NO: 120. In one specific example, the anti-CD19 CAR comprises the amino acid sequence of SEQ ID NO: 118 (with an N-terminal signal peptide) or SEQ ID NO:353 (without N-terminal signal peptide).

In some embodiments, the CAR binds CD70 (anti-CD70 CAR). The extracellular

antigen binding domain in the anti-CD70 CAR can be a single chain variable fragment (scFv) that binds CD70 (anti-CD70 scFv). In some instances, the anti-CD70 scFv comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 143; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 144. In some examples, the V_H comprises the amino acid sequence of SEQ ID NO: 143 and the V_L comprises the amino acid sequence of SEQ ID NO: 144. In one example, the anti-CD70 scFv comprises the amino acid sequence of SEQ ID NO: 140 or 142. In one specific example, the anti-CD70 CAR comprises the amino acid sequence of SEQ ID NO: 138 (with an N-terminal signal peptide) or SEQ ID NO:354 (without N-terminal signal peptide)..

In some embodiments, the CAR binds BCMA (anti-BCMA CAR). The extracellular antigen binding domain in the anti-BCMA CAR can be a single chain variable fragment (scFv) that binds BCMA (anti-BCMA CAR). In some instances, the anti-BCMA scFv comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 149; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 150. In some examples, the V_H comprises the amino acid sequence of SEQ ID NO: 149 and the V_L comprises the amino acid sequence of SEQ ID NO: 150. In one example, the anti-BCMA scFv comprises the amino acid sequence of SEQ ID NO: 148. In one specific example, the anti-BCMA CAR comprises the amino acid sequence of SEQ ID NO: 146 (with an N-terminal signal peptide) or SEQ ID NO:355 (without N-terminal signal peptide).

The genetically engineered T cells disclosed herein may be derived from primary T cells of one or more human donors. In some instances, the genetically engineered T cells show cytokine-dependent growth.

In other aspects, the present disclosure provides a method for preparing any of the populations of genetically engineered T cells disclosed herein. In some instances, the method may comprise: (a) providing a plurality of cells, which are T cells or precursor cells thereof; (b) genetically editing the *Reg1* gene and/or the *TGFBRII* gene; and (c) producing the population of genetically engineered T cells having disrupted *Reg1* gene and/or the *TGFBRII* gene. In some examples, the T cells of step (a) are derived from primary T cells of one or more human donors. In some examples, step (b) comprises genetically editing the *Reg1* gene. In some examples, step (b) comprises genetically editing the *TGFBRII* gene. In some examples, step (b) comprises genetically editing both the *Reg1* gene and the *TGFBRII* gene.

In some embodiments, step (b) is performed by one or more CRISPR/Cas-mediated gene editing systems. For example, step (b) can be performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *Reg1* gene. In some instances, the gRNA targeting the *Reg1* gene may be specific to an exon of the *Reg1* gene, e.g., exon 2 or exon 4. In some examples, the gRNA targeting the *Reg1* gene comprises a spacer that comprises a nucleotide sequence listed in **Table 22** (*e.g.*, SEQ ID NO: 24, 32, 36, or 52).

Alternatively or in addition, step (b) can be performed, *inter alia*, by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *TGFBRII* gene. For example, the gRNA targeting the *TGFBRII* gene may be specific to an exon of the *TGFBRII* gene, e.g., exon 1, exon 2, exon 3, exon 4, and exon 5. In one example, the gRNA targeting the *TGFBRII* gene is specific to exon 4. In another example, the gRNA targeting the *TGFBRII* gene is specific to exon 5. In some instances, the gRNA targeting the *TGFBRII* gene comprises a spacer listed in **Table 39**. Examples include SEQ ID NOs: 272, 302, 308, and 314.

Any of the gRNAs disclosed herein may further comprise a scaffold sequence. For example, the gRNA targeting the *Reg1* gene may comprise any of the nucleotide sequences listed in **Table 22**. Examples include SEQ ID NO: 22, 23, 30, 31, 34, 35, 50, and 51. Alternatively or in addition, the gRNA targeting the *TGFBRII* gene may comprise any of the nucleotide sequences provided in **Table 39**. Examples include SEQ ID NOs: 270, 271, 300, 301, 306, 307, 312, and 313.

In any of the methods disclosed herein, the plurality of T cells in step (a) comprises one or more of the following genetic modifications: (i) engineered to express a chimeric antigen receptor (CAR); (ii) has a disrupted T cell receptor alpha chain constant region (*TRAC*) gene; (iii) has a disrupted *β2M* gene; and (iv) has a disrupted *CD70* gene.

Alternatively, any of the methods disclosed herein may further comprise:

(i) delivering to the T cells a nucleic acid encoding a chimeric antigen receptor (CAR);

(ii) genetically editing a *TRAC* gene to disrupt its expression;

(iii) genetically editing a *β2M* gene to disrupt its expression;

(iv) genetically editing a *CD70* gene to disrupt its expression; or

(v) a combination thereof.

In some embodiments, one or more of (i)-(iv) are performed by one or more CRISPR/Cas-mediated gene editing system comprising one or more RNA-guided nucleases and one or more gRNAs targeting the *TRAC* gene, the *β2M* gene, and/or the *CD70* gene. In

some examples, the gRNA targeting the *TRAC* gene comprises a spacer that comprises the nucleotide sequence of SEQ ID NO: 61. In some examples, the gRNA targeting the *β2M* gene comprises a spacer that comprises the nucleotide sequence of SEQ ID NO: 65. In some examples, the gRNA targeting the *CD70* gene comprises a spacer that comprises the nucleotide sequence of SEQ ID NO: 57. See **Table 23**.

In some embodiments, the method disclosed herein may comprise delivering to the T cells one or more ribonucleoprotein particles (RNP), which may comprise the RNA-guided nuclease, one or more of the gRNAs, and the nucleic acid encoding the CAR. In some examples, the RNA-guided nuclease is a Cas9 nuclease, for example, a *S. pyogenes* Cas9 nuclease.

In some embodiments, the nucleic acid encoding the CAR is in an AAV vector. In some instances, the nucleic acid encoding the CAR comprises a left homology arm and a right homology arm flanking the nucleotide sequence encoding the CAR. The left homology arm and the right homology arm are homologous to a genomic locus in the T cells, allowing for insertion of the nucleic acid into the genomic locus. In some examples, the genomic locus is in the *Reg1* gene. In some examples, the genomic locus is in the *TGFBRII* gene. In some examples, the genomic locus is in the *TRAC* gene. In some examples, the genomic locus is in the *β2M* gene. In some examples, the genomic locus is in the *CD70* gene.

In some examples, the method comprising disrupting the *TRAC* gene by a CRISPR/Cas-mediated gene editing system comprising a gRNA comprising the nucleotide sequence of SEQ ID NO: 59 and the nucleic acid encoding the CAR is inserted at the site targeted by the gRNA. Alternatively or in addition, the method may comprise delivering to the T cells a nucleic acid encoding a CAR, which is specific to CD70, and genetically editing the *CD70* gene to disrupt its expression.

Any population of the genetically engineered T cells prepared by a method disclosed herein is also within the scope of the present disclosure.

Further, the present disclosure provides a method for eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof any of the populations of genetically engineered T cells disclosed herein. In some embodiments, the undesired cells are cancer cells, for example, hematopoietic cancer cells or solid tumor cells. In some embodiments, the undesired cells are $CD19^+$. In some embodiments, the undesired cells are $BCMA^+$. In some embodiments, the undesired cells are $CD70^+$. In some embodiments, the undesired cells are $CD33^+$. In some embodiments, the undesired cells are $PTK7^+$.

In yet other aspects, provided herein is a guide RNA (gRNA) targeting a *Reg1* gene, comprising a nucleotide sequence specific to a fragment in exon 2 or exon 4 of the *Reg1* gene. In some embodiments, the gRNA comprises a spacer listed in **Table 22** (*e.g.*, SEQ ID NO: 24, 32, 36 or 52). Such a gRNA may further comprise a scaffold sequence. Alternatively or in addition, the gRNA comprises one or more modified nucleotides. For example, the gRNA comprises one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA. Examples of gRNAs targeting *Reg1* include any of those listed in **Table 22** (*e.g.*, SEQ ID NO: 22, 23, 30, 31, 34, 35, 50, or 51; see also disclosures herein).

In still other aspects, provided herein is a guide RNA (gRNA) targeting a *TGFBRII* gene, comprising a nucleotide sequence specific to a fragment in exon 1, exon 2, exon 3, exon 4, or exon5 of the *TGFBRII* gene. In some examples, the gRNA comprises a nucleotide sequence specific to exon 4 of the *TGFBRII* gene. In other examples, the gRNA comprises a nucleotide sequence specific to exon 5 of the *TGFBRII* gene. In some instances, the gRNA comprises a spacer having the nucleotide sequence listed in **Table 39** (*e.g.*, SEQ ID NOs: 272, 302, 308, and 314). Such a gRNA may further comprise a scaffold sequence. Alternatively or in addition, the gRNA comprises one or more modified nucleotides. For example, the gRNA comprises one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA. Examples of gRNAs targeting the *TGFBRII* gene include any of those listed in **Table 39** (*e.g.*, SEQ ID NOs: 270, 271, 300, 301, 306, 307, 312, and 313).

Also within the scope of the present disclosure are any of the genetically engineered T cells, gRNAs targeting *Reg1*, or gRNAs targeting *TGFBRII* for use in treating a target disease as disclosed herein (e.g., cancer such as those disclosed herein), or uses of such for manufacturing a medicament for the intended therapeutic purposes

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A and **1B** include diagrams showing that exemplary CAR T cells (anti-CD70 CAR T cells) with Reg1 KO exhibit superior **in vitro** expansion **FIG. 1A**: Proliferation of anti-CD70 CAR T cells (CAR T) with Reg1 KO using one of the 10 guides (Z01-Z10) targeting Reg1 as indicated. CAR T indicates anti-CD70 CAR T cells with an unedited (wild-type) *Reg1* gene. **FIG. 1B**: Proliferation of anti-CD70 CAR T cells with Reg1 KO using guide REG1-Z10

(Z10) up to 52 days post HDR. Anti-CD70 CAR T cells with an unedited *Reg1* gene are also shown (CAR T). (A) and (B) refer to duplicative assays.

FIGs. 2A-2E include diagrams showing that exemplary CAR T cells (anti-CD70 CAR T cells) with Reg1 KO (+reg1 KO) exhibit superior *in vitro* potency against tumor cell lines relative to CAR T cells with an unedited *Reg1* gene (CAR T). **FIG. 2A**: Cell lysis of ACHN cells by anti-CD70 CAR T cells with Reg1 KO, using Regnase guides Z03 or Z10, relative to CAR T cells with an unedited (wild-type) *Reg1* gene (CAR T). Cell lysis was measured after 24h co-culture at day 12 post HDR. **FIG. 2B**: Cell lysis of ACHN cells by anti-CD70 CAR T cells with Regnase 1 KO using Regnase guides Z05 or Z06 relative to CAR T cells with an unedited *Reg1* gene. Cell lysis was measured after 24h co-culture at day 12 post HDR. **FIG. 2C**: Cell lysis of ACHN cells by anti-CD70 CAR T cells with Regnase 1 KO, using Regnase guides Z03, Z05, Z06 or Z10 relative to CAR T cells with an unedited *Reg1* gene. Cell lysis was measured after 24h co-culture at day 27 post HDR. **FIG. 2D**: Cell lysis of caki-1 cells by anti-CD70 CAR T cells with Regnase 1 KO using Regnase guides Z03, Z05, Z06 or Z10 relative to CAR T cells with an unedited *Reg1* gene. Cell lysis was measured after 24h co-culture at day 27 post HDR. **FIG. 2E**: Cell lysis of 769P cells by anti-CD70 CAR T cells with Regnase 1 KO using Regnase guides Z03, Z05, Z06 or Z10 relative to CAR T cells with an unedited *Reg1* gene. Cell lysis was measured after 24h co-culture at day 27 post HDR.

FIGs. 3A-3D include diagrams showing that exemplary CAR T cells (anti-CD70 CAR T cells) with Reg1 KO (CAR T + Reg KO, using Z10 guide as an example) express lower levels of T cell exhaustion markers *in vitro* relative to Reg1 wild-type counterparts (CAR T). **FIG. 3A**: Day 13 post HDR PD1 expression in CD4+ and CD8+ anti-CD70 CAR T cells with Reg1 KO (+Reg KO) relative to wild-type counterparts. **FIG. 3B**: Day 26 post HDR PD1 expression in CD4+ and CD8+ anti-CD70 CAR T cells with Reg1 KO (+Reg KO) relative to wild-type counterparts. **FIG. 3C**: Day 13 post HDR Tim3 expression in CD4+ and CD8+ anti-CD70 CAR T cells with Reg1 KO (+Reg KO) relative to wild-type counterparts. **FIG. 3D**: Day 26 post HDR Tim3 expression in CD4+ and CD8+ anti-CD70 CAR T cells with Reg1 KO (+Reg KO) relative to wild-type counterparts.

FIG. 4 is a diagram showing that exemplary CAR T cells (anti-CD19 CAR T cells) with Reg1 KO showed enhanced expansion in the presence of cytokines *in vitro* and continue to depend on cytokines for *in vitro* expansion. Anti-CD19 CAR T cells with a Reg1 KO (Anti-CD19 CAR T/Reg KO) and anti-CD19 CAR T cells with a wild-type *Reg1* gene (Anti-CD19 CAR T) were cultured in the presence and absence (No cytokines) of cytokines for 40 days.

FIGs. 5A-5D include diagrams showing that exemplary CAR T cells (anti-CD19 CAR T cells) with Reg1 KO (Anti-CD19 CAR T/Reg KO) provide superior *in vivo* survival and decreased tumor burden relative to Reg1 wild-type counterparts (Anti-CD19 CAR T) in the intravenous disseminated Nalm-6 human acute lymphoblastic leukemia tumor xenograft mouse model. **FIG. 5A**: Probability of survival of untreated mice, mice dosed with 4e6 anti-CD19 CAR T cells, and 4e6 anti-CD19 CAR T/Reg KO cells. **FIG. 5B**: Probability of survival of untreated mice, mice dosed with 8e6 anti-CD19 CAR T cells, and 8e6 anti-CD19 CAR T/Reg KO cells. **FIG. 5C**: Bioluminescence signal from bioluminescent model leukemia cells in mice treated with 4e6 anti-CD19 CAR T cells or 4e6 anti-*CD19* CAR T/Reg KO cells. **FIG. 5D**: Bioluminescence signal from bioluminescent model leukemia cells in mice treated with 8e6 anti-*CD19* CAR T cells or 8e6 anti-CD19 CAR T/Reg KO cells.

FIGs. 6A-6B include diagrams showing that exemplary CAR T cells (anti-CD70 CAR T cells) with Reg1 KO (CAR T + Reg KO) exhibit superior *in vitro* potency against tumor cell lines relative to Reg1 wild-type counterparts (CAR T). **FIG. 6A**: Cell lysis of ACHN cells by anti-CD70 CAR T cells with Reg1 KO using guide REG1-Z10 (CAR T + Reg KO) relative to Reg1 wild-type counterparts (CAR T). Cell lysis was measured after 24h co-culture at day 19 and 26 post HDR. **FIG. 6B**: Cell lysis of caki-1 cells by anti-CD70 CAR T cells with Reg1 KO using guide REG1-Z10 (CAR T + Reg KO) relative to Reg1 wild-type counterparts (CAR T). Cell lysis was measured after 24h co-culture at day 13, 19 and 26 post HDR.

FIGs. 7A and **7B** include diagrams showing knock out of *TGFBRII* using various guide RNAs as indicated. **FIG. 7A**: Indel rates of edited *TGFBRII* gene by eight gRNAs that target different TGFBRII gene exons as indicated. From left to right, EX1_T1, EX1_T3, EX2_T1, EX3_T1, EX3_T2, EX4_T1, EX4_T2, and EX5_T1, the nucleotide sequence of each of which is provided in **Table 32**. **FIG. 7B**: immunoblot of TGFBRII expression in gene-edited T cells. GAPDH was used as a loading control. The mock sample is unedited T cells with wild-type *TGFBRII*.

FIGs. 8A-8K include diagrams showing the effect of TGF-β on CAR T cell expansion. Anti-CD70 CAR T cells were exposed to different concentrations of recombinant human TGF-β (10, 20, 50, 100 ng/ml) and cell number was recorded at different time points (**FIG. 8A**). T cells with or without TGFBRII knock-out, generated using different TGFBRII gRNAs as indicated, were incubated with 0 or 50 ng/ml of TGFB-β and cell expansion was recorded over time (**FIGs. 8B-8K**).

FIG. 9 is a diagram showing the effect of *TGFBRII* KO on CAR T cell killing ability

against A498 cells at various E:T ratios as indicated. *TGFBRII* KO improves cytotoxicity of CAR-T cells.

FIGs. 10A-10E include diagrams showing the effect of *TGFBRII* KO on CAR T cell kill ability against multiple tumor cell lines. The cell kill capacity of anti-CD70 CAR T cells was compared to anti-CD70 CAR T cells with *TGFBRII* KO. Cell killing activity of the CAR T cells was assessed against CAKI-1 (**FIG. 10A**) H1975 (**FIG. 10B**), Hs-766T (**FIG. 10C**), 786-O (**FIG. 10D**) and SK-OV3 (**FIG. 10E**). *TGFBRII* KO improves cytotoxicity of CAR-T cells.

FIG. 11 is a graph showing the effect of *TGFBRII* KO on CAR T cell phenotype. Anti-CD70 CAR T cells with or without *TGFBRII* KO were exposed to 50 ng/ml recombinant human TGF-β and the expression of CD25 was assessed by flow cytometry. *TGFBRII* KO protects CAR T from TGF-β inhibitory effect on cell phenotype.

FIG. 12 is a graph showing that *TGFBRII* KO protects CAR T cells against TGF-β inhibitory effect on cytotoxicity. Anti-CD70 CAR T cells was co-cultured with target tumor cells (A498) in the presences or absence of TGF-β (0, 1, 10, 50 ng/ml) The ability of anti-CD70 CAR T cells with unedited *TGFBRII* to kill target cells, were compared to anti-CD70 CAR T with *TGFBRII* KO using an exemplary guide RNA as indicated.

FIGs. 13A-13C include diagrams showing that *TGFBRII* KO anti-CD70 CAR T cells are resistant to TGF-β inhibitory effects on effector function. Anti-CD70 CAR T cells were co-cultured with target cells (A498) with TGF-β (50 ng/ml) or without TGF-β and compared to anti-CD70 CAR T with *TGFBRII* KO (e.g.: anti-CD70 CAR+TGFBRII_EX4_T1) in their ability to kill target cells. T cell proliferation (**FIG. 13A**) and effector cytokine secretion was assessed by Luminx (**FIGs. 13B** and **13C**).

FIG. 14 is a graph showing fibroblasts reduce CAR-T cell cytolytic activity. Anti-CD70 CAR T was co-cultured with target cells (A498) with or without fibroblast (CCL-190) placed in a transwell plate at 0.25:1, fibroblast: anti-CD70 CAR T.

FIGs. 15A-15C include graphs showing that *TGFBRII* KO protects CAR-T cells against the inhibitory effect of fibroblasts. Anti-CD70 CAR T was co-cultured with target cells (A498) at 0.1:1 (E:T) in presence of different volumes of conditioned media from CCL-190 (2.5, 5, 10 µL) and the cell kill capacity was evaluated and compared to cells with *TGFBRII* KO. The ability of anti-CD70 CAR T cells (with or without *TGFBRII* KO) to kill target cells is shown in Hs-766T pancreatic tumor cells (**FIG. 15A**), A498 kidney tumor cells (**FIG. 15B**), and H1975 lung tumor cells (**FIG. 15C**).

FIG. 16A-16B include diagrams showing synergistic effects of TGFBRII and Regnase

double disruptions with *in vitro* rechallenge of CAR T Cells with ACHN. **FIG. 16A:** improved potency. **FIG. 16B:** improved CAR expansion.

FIG. 17A-17B include diagrams showing synergistic effects of disrupting both TGFBRII and Regnase genes in cancer xenograph models. **FIG. 17A:** CAKI-1 renal cell carcinoma xenograph model with anti-CD70 CAR T cells. **FIG. 17B:** H1975 lung cancer xenograph model with anti-CD70 CAR T cells.

FIG. 18A-18B include diagrams showing synergistic effects of disrupting both TGFBRII and Regnase genes in an RCC rechallenge xenograph model. **FIG. 18A:** reduction in RCC (A498) tumor size. **FIG. 18B:** inhibition of RCC tumor cell growth following rechallenge with ACHN cells.

Fig. 19A-19B include diagrams showing impact of *Reg1* and/or *TGFBRII* disruption on CAR-T cell differentiation and expansion *in vivo*. **FIG. 19A**: CAR-T cell differentiation. **FIG. 19B**: CAR-T cell expansion.

FIG. 20A-20B include diagrams showing synergistic effects of TGFBRII and Regnase double knock-out in a Nalm6-leukemia (B-ALL) mouse model. **FIG. 20A**: reduction in tumor size. **FIG. 20B**: Survival rates.

FIG. 21 is a diagram showing survival advantage arising from TGFBRII and Regnase double disruptions in a NOG Mantle cell lymphoma (MCL) tumor xenograft mouse model.

FIGs. 22A-22B include diagrams showing increased *in vivo* expansion of CAR-T cells having TGFBRII and/or Regnase knock-out. **FIG. 22A** shows CAR T cell expansion in the Jeko-1 xenograph model. **FIG. 22B** shows CAR T cell expansion in the nalm-6 xenograph model.

FIGs. 23A-23D include diagrams showing consistent rates of CRISPR/Cas editing in anti-BCMA CAR-T cells with *Reg-1* and/or *TGFBRII* disruption as determined by flowcytometry. **FIG. 23A**: levels of TCR^- cells. **FIG. 23B**: levels of $\beta2M^-$ cells. **FIG. 23C**: levels of CAR^+ cells. **FIG. 23D**: ratio of $CD4^+/CD8^+$ cells.

FIGs. 24A-24B include diagrams showing consistent edit editing rates in anti-BCMA CAR-T cells with *Reg-1* and/or *TGFBRII* disruptions. **FIG. 24A**: *TGFBRII* disruption efficiency. **FIG. 24B**: *Reg-1* disruption efficiency.

FIGs. 25A-25D include diagrams showing superior cell cytotoxicity of TRAC-/β2M-/Reg-1- TGFBRII- anti-BCMA CAR+ T-cells. **FIGs. 25A-25B**: cytotoxicity against MM1s (multiple myeloma cell line) cells (**25A**) relative to K562 cells (**25B**). **FIGs. 25C-25D**: cytotoxicity against JeKo-1 cells (mantle cell lymphoma cell line) (**25C**) relative to K562 cells

(**25D**).

FIGs. 26A-26C include diagrams showing that the combined disruption of Regnase-1 and TGFBRII improved anti-BCMA CAR-T activity against murine multiple myeloma in an animal model. **FIG. 26A:** tumor volume reduction. **FIG. 26B**: survival rate. **FIG. 26C**: CAR-T cell expansion in peripheral blood.

FIGs. 27A-27F include diagrams showing that the combined disruption of Regnase and TGFBRII improves anti-BCMA CAR-T activity against murine mantle cell lymphoma in an animal model. FIG. 27A: tumor volume reduction. **FIG. 27B**: survival rate. **FIG. 27C**: CAR-T cell expansion in peripheral blood. **FIG. 27D**: PD-1 and LAG-3 levels in CAR T cells. **FIG. 27E**: levels of circulating T cells at three weeks post CAR-T injection. **FIG. 27F**: levels of exhaustion markers (LAG-3 and PD-1) on circulating T cells at three weeks post CAR-T injection.

FIG. 28 is a diagram showing disruption of *TGFBRII* and *Reg-1* genes increases proliferation of anti-PTK7 CAR T cells.

FIGs. 29A-29D include diagrams showing impact of *TGFBRII* disruption, optionally in combination with *Reg-1* disruption, in long-term *in vitro* rechallenge assays. **FIG. 29A**: *TGFBRII* disruption alone improves anti-PTK7 CAR T-cell potency in a long-term *in vitro* rechallenge assay. **FIG. 29B**: *TGFBRII* disruption improves anti-PTK7 CAR T-cell persistence and expansion in a long-term *in vitro* rechallenge assay as measured by humCD45+ cells. **FIG. 29C**: *TGFBRII* disruption enhances cytotoxic CD8+ T cells expressing the anti-PTK7 CAR. **FIG. 29D**: CD4+ cells expressing the anti-PTK7 CAR remains consistent regardless of *TGFBRII* disruption.

FIGs. 30A-30B include diagrams showing anti-tumor activity of anti-PTK7 CAR T-cells with or without *TGFBRII* disruption. **FIG. 30A**: effect of treatment on tumor volume. **FIG. 30B**: effect of treatment on body weight. • Group 1: no treatment. ○: Group 2: anti-PTK7 CAR-T cells, $5x10^6$ cells/mouse (iv) Day 1. □: Group 3: anti-PTK7 CAR/TGFBRII KO T cells, $5x10^6$ cells/mouse (iv) Day 1. UTA chow was administered 9 days prior to CAR-T cell treatment to applicable groups.

FIGs. 31A-31B include diagrams showing T cell fractions in a pancreatic cell carcinoma (Hs766T) tumor xenograft animal model treated with anti-PTK7 CAR T cells with or without TGFBRII disruption. **FIG. 31A**: number of humCD45+ cells/ul in murine blood at Day 47 post dose. **FIG. 31B**: CAR-T cell differentiation at Day 47 post dose.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure aims at establishing genetically engineered T cells having improved growth activity, persistence, reduced T cell exhaustion, and enhanced potency, a long-felt need in CAR-T therapy. Such a T cell may use *bona fide* T cells as the starting material, for example, non-transformed T cells, terminally differentiated T cells, T cells having stable genome, and/or T cells that depend on cytokines and growth factors for proliferation and expansion. Alternatively, such a T cell may use T cells generated from precursor cells such as hematopoietic stem cells (*e.g.*, iPSCs), *e.g.*, *in vitro* culture. The T cells disclosed herein may confer one or more benefits in both CAR-T cell manufacturing and clinical applications.

Conventional allogenic CAR T cells are produced wherein a single donor leukopak is edited in most cases so that the cells can avoid components of the patient immune system and thus do not cause GvHD. The process of expanding these CAR T cells can yield 10s to 100s of vialed drug product. Patients may receive a single dose or multiple doses. During the manufacturing process, these CAR T cells lose potential due to various mechanisms, for example, apoptosis, exhaustion, replicative senescence, and other processes where the cells become less fit.

The genetically engineered T cells having a disrupted *TGFBRII* gene, a disrupted Reg1 gene, or a combination thereof, and optionally one or more additional genetic edits, for example, a disrupted *TRAC* gene, a disrupted *β2M* gene, a disrupted CD70 gene, and/or an inserted nucleic acid coding for a chimeric antigen receptor (CAR), or a combination thereof.

Unexpectedly, the present disclosure reports that knocking out Reg1 in T cells led to various advantageous features in T cell-mediated cell therapy such as CAR-T therapy. Examples include, but are not limited to: improved cell culture growth and *in vitro* expansion including faster expansion, longer viability, faster proliferation and/or increased resistance to apoptosis, which are beneficial for manufacturing and production of therapeutic T-cell based products such as CAR-T cells; T cell potency advantages related to maintaining therapeutic T cells (*e.g.*, CAR-T cells) *in vitro* and *in vivo* potency and activity (target cell killing) for a more effective and persistent T-cell based therapeutic products; production and/or retention of more central memory cells; lower expression of T cell exhaustion markers (such as, PD-1, Tim-3); improved efficacy of T cell therapeutics *in vivo*, related to decreasing tumor burden and increasing survival of CAR T treated subjects.

Further, unexpectedly, T cells having a disrupted *TGFBRII* gene showed advantageous features, including improved cell growth and expansion, enhanced cytotoxicity activity,

resistant to the inhibitory effect mediated by TGFβ, and/or mediated by fibroblasts. Given such advantageous features, the genetically engineered T cells (*e.g.*, CAR-T cells) disclosed herein, having a disrupted *TGFBRII* gene and optionally other genetic edits as disclosed herein, would be expected to exhibit superior therapeutic effects, for example, superior anti-tumor effects, *e.g.*, in TME of a solid tumor.

Moreover, CAR-T cells having both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene showed much higher anti-tumor activities, as well as CAR-T cell expansion in animal models as relative to CAR-T cells having a disrupted *Reg1* gene or a disrupted *TGFBRII* gene.

Other unlimited advantageous features of the T cells provided herein include:

(a) Improved quality and consistency of CAR-T cell-based therapeutics.

(b) Greater potency and longer-lived potency of CAR-T cells produced from the T cells in human patients.

(c) Reduced dosage requirement. Because the T cells disclosed herein have enhanced proliferation and expansion capacities, they can live longer *in vivo*. As such, a lower dose relative to standard CAR-T therapy may be used to achieve substantially similar therapeutic effects relative to a high dose of conventional CAR-T cell therapy.

(d) Increased efficacy resulting from enhanced proliferation and expansion of the CAR-T cells disclosed herein, enhanced cytotoxicity, and prolonged persistence *in vivo*. Further, the T cells would provide the benefit of titratable dosing in patients to optimize safety and efficacy as noted above.

(e) Extended therapeutic effects due to reduced exhaustion and/or replicative senescence and prolonged persistence of the T cells both *in vitro* and *in vivo*.

(f) Enhanced anti-tumor activity, e.g., reduction of tumor size and/or elongated survival rates.

Accordingly, provided herein are T cells having improved persistence in culture, methods of producing such T cells, and methods of using such T cells for producing therapeutic T cells such as CAR-T cells. Components and processes (*e.g.*, the CRISPR approach for gene editing and components used therein) for making the T cells disclosed herein are also within the scope of the present disclosure.

I. Genetically Engineered T Cells Having Enhanced Features

The T cells disclosed herein comprises genetically engineered T cells having enhanced persistence in culture. Such genetically engineered T cells may have genetic editing of the *Reg1*

gene or genetic editing of the *TGFBRII* gene. In some instances, such genetically engineered T cells may have genetic editing of both the *Reg1* gene and the *TGFBRII* gene.

In some embodiments, the genetically engineered T cells may have genetic editing in one or more additional genes involved in T cell exhaustion, such as CD70. As shown by the studies disclosed herein, such genetically engineered T cells show one or more of the following superior features as relative to the T cell counterparts having a wild-type *Regnase 1* gene: enhanced expansion capacity in culture (*e.g.*, expandable in culture for at least 4 weeks, *e.g.*, at least 6 weeks; and/or at least 10-fold expandable, for example, at least 15-fold expandable, relative to the non-edited counterpart), enhanced longevity, enhanced proliferation capacity, greater T cell activation, enhanced potency, enhanced expression of central memory T cell markers, and reduced expression of T cell exhaustion markers.

The genetically engineered T cells may be derived from parent T cells (*e.g.*, non-edited wild-type T cells) obtained from a suitable source, for example, one or more mammal donors. In some examples, the parent T cells are primary T cells (*e.g.*, non-transformed and terminally differentiated T cells) obtained from one or more human donors. Alternatively, the parent T cells may be differentiated from precursor T cells obtained from one or more suitable donor or stem cells such as hematopoietic stem cells or inducible pluripotent stem cells (iPSC), which may be cultured *in vitro*.

In some embodiments, the genetically engineered T cells carry a disrupted *Reg1* gene, and optionally, one or more disrupted genes involved in cell exhaustion (*e.g.*, *CD70*). Such genetically engineered T cells may further comprise one or more disrupted genes, for example, *TRAC* or *β2M*. Such genetically engineered T cells may further express a chimeric antigen receptor (CAR), which may be capable of binding to an antigen of interest, for example, a tumor associated antigen (e.g., CD19, BCMA, CD70, CD33, or PTK7).

In some embodiments, the genetically engineered T cells carry a disrupted *TGFBRII* gene, and optionally, one or more disrupted genes involved in cell exhaustion (*e.g.*, *CD70*). Such genetically engineered T cells may further comprise one or more disrupted genes, for example, *TRAC* or *β2M*. Such genetically engineered T cells may further express a chimeric antigen receptor (CAR), which may be capable of binding to an antigen of interest, for example, a tumor associated antigen (e.g., CD19, BCMA, CD70, CD33, or PTK7). In some examples, the genetically engineered T cells may express an anti-PTK7 CAR such as those disclosed herein. In some instances, such genetically engineered T cells may have a wild-type endogenous *Reg-1* gene.

In some embodiments, the genetically engineered T cells carry a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, and optionally, one or more disrupted genes involved in cell exhaustion (*e.g.*, *CD70*). Such genetically engineered T cells may further comprise one or more disrupted genes, for example, *TRAC* or *β2M*. Such genetically engineered T cells may further express a chimeric antigen receptor (CAR), which may be capable of binding to an antigen of interest, for example, a tumor associated antigen (e.g., CD19, BCMA, CD70, CD33, or PTK7).

Any of the genetically engineered T cells may be generated *via* gene editing (including genomic editing), a type of genetic engineering in which nucleotide(s)/nucleic acid(s) is/are inserted, deleted, and/or substituted in a DNA sequence, such as in the genome of a targeted cell. Targeted gene editing enables insertion, deletion, and/or substitution at pre-selected sites in the genome of a targeted cell (*e.g.*, in a targeted gene or targeted DNA sequence). When a sequence of an endogenous gene is edited, for example by deletion, insertion or substitution of nucleotide(s)/nucleic acid(s), the endogenous gene comprising the affected sequence may be knocked-out due to the sequence alteration. Therefore, targeted editing may be used to disrupt endogenous gene expression. "Targeted integration" refers to a process involving insertion of one or more exogenous sequences, with or without deletion of an endogenous sequence at the insertion site. Targeted integration can result from targeted gene editing when a donor template containing an exogenous sequence is present.

(a) Genetically Edited Genes

In some aspects, the present disclosure provides genetically engineered T cells that may comprise a disrupted *Reg1* gene, a disrupted *TGFBRII* gene, or a combination thereof. In some embodiments, the genetically engineered T cells provided herein comprise both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene. In some instances, the genetically engineered T cells disclosed herein may further comprise a disrupted *CD70* gene, a disrupted *β2M* gene, a disrupted *TRAC* gene, or a combination thereof.

As used herein, a "disrupted gene" refers to a gene comprising an insertion, deletion or substitution relative to an endogenous gene such that expression of a functional protein from the endogenous gene is reduced or inhibited. As used herein, "disrupting a gene" refers to a method of inserting, deleting or substituting at least one nucleotide/nucleic acid in an endogenous gene such that expression of a functional protein from the endogenous gene is reduced or inhibited. Methods of disrupting a gene are known to those of skill in the art and described herein.

In some embodiments, a cell that comprises a disrupted gene does not express (*e.g.*, at

the cell surface) a detectable level (*e.g.*, in an immune assay using an antibody binding to the encoded protein or by flow cytometry) of the protein encoded by the gene. A cell that does not express a detectable level of the protein may be referred to as a knockout cell.

Reg1 Gene Editing

In some embodiments, the genetically engineered T cells may comprise a disrupted gene involved in mRNA decay. Such a gene may be Reg1. Reg1 contains a zinc finger motif, binds RNA and exhibits ribonuclease activity. Reg1 plays roles in both immune and non-immune cells and its expression can be rapidly induced under diverse conditions including microbial infections, treatment with inflammatory cytokines and chemical or mechanical stimulation. Human *Reg1* gene is located on chromosome 1p34.3. Additional information can be found in GenBank under Gene ID: 80149.

In some examples, the genetically engineered T cells may comprise a disrupted *Reg1* gene such that the expression of Reg1 in the T cells is substantially reduced or eliminated completely. The disrupted *Reg1* gene may comprise one or more genetic edits at one or more suitable target sites (e.g., in coding regions or in non-coding regulatory regions such as promoter regions) that disrupt expression of the *Reg1* gene. Such target sites may be identified based on the gene editing approach for use in making the genetically engineered T cells. Exemplary target sites for the genetic edits may include exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, or a combination thereof. In some examples, one or more genetic editing may occur in exon 2 or exon 4. Such genetic editing may be induced by the CRISPR/Cas technology using a suitable guide RNA, for example, those listed in **Table 22**. The resultant edited *Reg1* gene using a gRNA listed in **Table 22** may comprise one or more edited sequences provided in **Tables 29-38** below.

TGFBRII Gene Editing

In some embodiments, the genetically engineered T cells may comprise a disrupted *TGFBRII* gene, which encodes Transforming Growth Factor Receptor Type II (TGFBRII). TGFBRII receptors are a family of serine/threonine kinase receptors involved in the TGFβ signaling pathway. These receptors bind growth factor and cytokine signaling proteins in the TGFβ family, for example, TGFβs (TGFβ1, TGFβ2, and TGFβ3), bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activin and inhibin, myostatin, anti-Müllerian hormone (AMH), and NODAL.

In some examples, the genetically engineered T cells may comprise a disrupted *TGFBRII* gene such that the expression of TGFBRII in the T cells is substantially reduced or

eliminated completely. The disrupted *TGFBRII* gene may comprise one or more genetic edits at one or more suitable target sites (*e.g.*, in coding regions or in non-coding regulatory regions such as promoter regions) that disrupt expression of the *TGFBRII* gene. Such target sites may be identified based on the gene editing approach for use in making the genetically engineered T cells. Exemplary target sites for the genetic edits may include exon 1, exon 2, exon 3, exon 4, exon 5, or a combination thereof. In some examples, one or more genetic editing may occur in exon 4 and/or exon 5. Such genetic editing may be induced by a gene editing technology, (*e.g.*, the CRISPR/Cas technology) using a suitable guide RNA, for example, those listed in **Table 39**. The resultant edited *TGFBRII* gene using a gRNA listed in **Table 39** may comprise one or more edited sequences provided in **Tables 40-48** below.

CD70 Gene Editing

T cell exhaustion is a process of stepwise and progressive loss of T cell functions, which may be induced by prolonged antigen stimulation or other factors. Genes involved in T cell exhaustion refer to those that either positively regulate or negatively regulate this biological process. The genetically engineered T cells disclosed herein may comprise genetic editing of a gene that positively regulates T cell exhaustion to disrupt its expression. Alternatively or in addition, the genetically engineered T cells may comprise genetic editing of a gene that negatively regulates T cell exhaustion to enhance its expression and/or biologic activity of the gene product.

In some embodiments, the genetically engineered T cells may comprise an edited gene involved in T cell exhaustion, *e.g.*, disruption of a gene that positively regulates T cell exhaustion. Such a gene may be a Cluster of Differentiation 70 (*CD70*) gene. CD70 is a member of the tumor necrosis factor superfamily and its expression is restricted to activated T and B lymphocytes and mature dendritic cells. CD70 is implicated in tumor cell and regulatory T cell survival through interaction with its ligand, CD27. CD70 and its receptor CD27 have multiple roles in immune function in multiple cell types including T cells (activated and T_{reg} cells), and B cells.

It was also found that disrupting the *CD70* gene in immune cells engineered to express an antigen targeting moiety enhanced anti-tumor efficacy against large tumors and induced a durable anti-cancer memory response. Specifically, the anti-cancer memory response prevented tumor growth upon re-challenge. Further, it has been demonstrated disrupting the *CD70* gene results in enhanced cytotoxicity of immune cells engineered to express an antigen targeting moiety at lower ratios of engineered immune cells to target cells, indicating the potential

efficacy of low doses of engineered immune cells. See, *e.g.*, WO2019/215500, the relevant disclosures of which are incorporated by reference for the purpose and subject matter referenced herein.

Structures of *CD70* genes are known in the art. For example, human *CD70* gene is located on chromosome 19p13.3. The gene contains four protein encoding exons. Additional information can be found in GenBank under Gene ID: 970.

In some examples, the genetically engineered T cells may comprise a disrupted *CD70* gene such that the expression of *CD70* in the T cells is substantially reduced or eliminated completely. The disrupted *CD70* gene may comprise one or more genetic edits at one or more suitable target sites (*e.g.*, in coding regions or in non-coding regulatory regions such as promoter regions) that disrupt expression of the *CD70* gene. Such target sites may be identified based on the gene editing approach for use in making the genetically engineered T cells. Exemplary target sites for the genetic edits may include exon 1, exon 2, exon 3, exon 4, or a combination thereof. See also WO2019/215500, the relevant disclosures of which are incorporated by reference for the purpose and subject matter referenced herein.

In some embodiments, the gRNA targeting CD70 listed in **Table 23** (CD70-7) may be used for disrupting the CD70 gene via CRISPR/Cas9 gene editing. In some examples, an edited *CD70* gene may comprise a nucleotide sequence selected from the following sequences in **Table 26**.

β2M Gene Edit

In some embodiments, the genetically engineered T cells disclosed herein may further comprise a disrupted *β2M* gene. β2M is a common (invariant) component of MHC I complexes. Disrupting its expression by gene editing will prevent host versus therapeutic allogeneic T cells responses leading to increased allogeneic T cell persistence. In some embodiments, expression of the endogenous *β2M* gene is eliminated to prevent a host-versus-graft response.

In some embodiments, an edited *β2M* gene may comprise a nucleotide sequence selected from the following sequences in **Table 25**. It is known to those skilled in the art that different nucleotide sequences in an edited gene such as an edited *β2M* gene (*e.g.*, those in **Table 25**) may be generated by a single gRNA such as the one listed in **Table 23** (β2M-1). See also WO2019097305, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

The genetically engineered T cells disclosed herein may further comprise one or more additional gene edits (*e.g.*, gene knock-in or knock-out) to improve T cell function. Examples include knock-in or knock-out genes to improve target cell lysis, knock-in or knock-out genes to enhance performance of therapeutic T cells such as CAR-T cells prepared from the genetically engineered T cells.

TRAC Gene Edit

In some embodiments, the genetically engineered T cells as disclosed herein may further comprise a disrupted *TRAC* gene. This disruption leads to loss of function of the TCR and renders the engineered T cell non-alloreactive and suitable for allogeneic transplantation, minimizing the risk of graft versus host disease. In some embodiments, expression of the endogenous *TRAC* gene is eliminated to prevent a graft-versus-host response. See also WO2019097305, the relevant disclosures of which are incorporated by reference herein for the purpose and subject matter referenced herein.

In some embodiments, an edited *TRAC* gene may comprise a nucleotide sequence selected from the following sequences in **Table 24**. It is known to those skilled in the art that different nucleotide sequences in an edited gene such as an edited *TRAC* gene (*e.g.*, those in **Table 24**) may be generated by a single gRNA such as the one listed in **Table 23** (TA-1).

It should be understood that more than one suitable target site/gRNA can be used for each target gene disclosed herein, for example, those known in the art or disclosed herein. Additional examples can be found in, *e.g.*, WO2019097305, the relevant disclosures of which are incorporated by reference herein for the purpose and subject matter referenced herein.

(b) Exemplary Improved Features of Genetically Engineered T Cells Disclosed Herein

Any of the genetically engineered T cell having a disrupted *Reg1* gene, and optionally one or more additional genetic edits, for example, a disrupted *CD70* gene, a disrupted *TRAC* gene, a disrupted *β2M* gene, a CAR-coding nucleic acid insertion, or a combination thereof, may be expandable in culture for greater than 4 weeks, for example, greater than 5 weeks, greater than 6 weeks, greater than 8 weeks, and greater than 10 weeks. In some examples, the genetically engineered T cells comprise a disrupted Reg1 (optionally, disruptions in CD70) and are expandable after 6 weeks (e.g., after 7 weeks, after 8 weeks, after 9 weeks, or after 10 weeks) in culture. Such genetically engineered T cells may maintain the ability to be activated after 6 weeks (*e.g.*, after 7 weeks, after 8 weeks, after 9 weeks, or after 10 weeks) in culture. Further, such genetically engineered T cells have an increased expansion capacity, which can

be at least 10-fold (*e.g.*, at least 15-fold) higher than the non-engineered counterparts, *i.e.*, T cells having the same genetic background as the engineered T cells disclosed herein except that the counterpart T cells have a wild-type *Reg1* gene.

Further, the genetically engineered T cells disclosed herein may exhibit enhanced T cell persistence. "T cell persistence" as used herein refers to the tendency of T cells to continue to grow, proliferate, self-renew, expand, and maintain healthy activity in culture. In some instances, T cell persistence can be represented by the longevity that T cells can grow and expand *in vitro*, which can be measured by conventional methods and/or assays described herein. In other instances, T cell persistence can be represented by the reduction of cell death (*e.g.*, apoptosis) or reduction in cell states characterized by exhaustion or replicative senescence. In yet other instances, T cell persistence can be presented by the maintenance of T cell activation capacity in culture.

Alternatively or in addition, the genetically engineered T cells disclosed may grow faster and longer than the non-engineered T cells, for example, as observed *in vitro* cell culture. In some instances, the genetically engineered T cells may grow at least 50% (*e.g.*, at least 1-fold, at least 2-fold, at least 5-fold, or more) than the non-engineered T cells in a conventional in vitro T cell culture (*e.g.*, as described in Examples below). In other instances, the genetically engineered T cells may maintain a high growth rate (*e.g.*, having substantially the same growth rate or with only a slight reduction) *in vitro* for at least 20 days (*e.g.*, at least 25 days, at least 30 days, at least 35 days, at least 40 days, at least 45 days, at least 50 days, or longer).

In addition, the genetically engineered T cells may exhibit a reduced level of cell exhaustion as relative to the non-engineered T cell counterpart. In some instances, a reduced level of cell exhaustion is reflected by a higher level of central memory T cells in the whole T cell population. The population of genetically engineered T cells disclosed may comprise a higher number of central memory T cells as compared to non-engineered T cell counterparts. For example, in some instances the population of genetically engineered T cells include a higher number of central memory T cells that are characterized by enhanced expression of CD27 and/or CD45RO as compared to non-engineered T cell counterparts. In some instances, the population of genetically engineered T cells disclosed exhibit reduced T cell exhaustion, which is characterized, for example, by reduced expression of PD-1 and/or TIM3 as compared to non-engineered T cell counterparts.

Any of the genetically engineered T cell having a disrupted *TGFBRII* gene, and optionally one or more additional genetic edits, for example, a disrupted *CD70* gene, a

disrupted *TRAC* gene, a disrupted *β2M* gene, a CAR-coding nucleic acid insertion, or a combination thereof, may have improved growth and expansion activities, both *in vitro* and *in vivo*, as compared with the non-engineered counterpart, which refers to T cells having the same genetic background except for an undisrupted *TGFBRII* gene. Further, such genetically engineered T cells (*e.g.*, CAR-T cells) may exhibit enhanced cytotoxicity activity, for example, against undesired cells (*e.g.*, tumor cells) expressing an antigen targeted by the CAR expressed in the CAR-T cells, as compared with the non-engineered counterpart. Such genetically engineered T cells (*e.g.*, CAR-T cells) may also be resistant to inhibitory effects mediated by the TGFβ signaling and/or by fibroblast (*e.g.*, in TME). For example, the genetically engineered T cells with a disrupted *TGFBRII* gene may be resistant to inhibitory factors secreted by fibroblasts.

In some embodiments, the genetically engineered T cells may further comprise one or more disrupted genes (*e.g.*, *CD70*, *Reg1*, or a combination thereof) to improve T cell persistency. "T cell persistence" as used herein refers to the tendency of T cells to continue to grow, proliferate, self-renew, expand, and maintain healthy activity in culture. In some instances, T cell persistence can be represented by the longevity that T cells can grow and expand *in vitro*, which can be measured by conventional methods and/or assays described herein. In other instances, T cell persistence can be represented by the reduction of cell death (*e.g.*, apoptosis) or reduction in cell states characterized by exhaustion or replicative senescence. In yet other instances, T cell persistence can be presented by the maintenance of T cell activation capacity in culture.

For example, such genetically engineered T cells may be expandable in culture for greater than 4 weeks, for example, greater than 5 weeks, greater than 6 weeks, greater than 8 weeks, and greater than 10 weeks. In some examples, the genetically engineered T cells comprise a disrupted *TGFBRII* gene, and a disrupted *CD70* gene, *Reg1* gene, or both may be expandable after 6 weeks (e.g., after 7 weeks, after 8 weeks, after 9 weeks, or after 10 weeks) in culture. Such genetically engineered T cells may maintain the ability to be activated after 6 weeks (*e.g.*, after 7 weeks, after 8 weeks, after 9 weeks, or after 10 weeks) in culture. Such genetically engineered T cells may exhibit more improved growth and expansion capacity relative to the T cells having the same genetic background except for an undisrupted *TGFBRII* gene, and an undisrupted *CD70* gene and/or *Reg1* gene.

In addition, any of the genetically engineered T cell having a disrupted *TGFBRII* gene and a disrupted *Reg1* gene, and optionally one or more additional genetic edits, for example, a

disrupted *CD70* gene, a disrupted *TRAC* gene, a disrupted *β2M* gene, a CAR-coding nucleic acid insertion, or a combination thereof, may have expansion advantage (e.g., *in vivo*) over counterpart T cells, i.e., having the disrupted *TGFBRII* gene or the disrupted *Reg1* gene (but not both), as well as the other additional genetic edits. CAR-T cells having disruptions of both the *TGFBRII* gene and the *Reg1* gene were found to be more potent in cancer treatment than the counterpart T cells as observed in xenograft mouse models. Accordingly, CAR-T cells having disruptions of both the *TGFBRII* gene and the *Reg1* gene would be expected to show superior cancer treatment efficacy.

(c) Methods of Making Genetically Engineered T cells

The genetically engineered T cells disclosed herein can be prepared by genetic editing of parent T cells or precursor cells thereof *via* a conventional gene editing method or those described herein.

(a) T cells

In some embodiments, T cells can be derived from one or more suitable mammals, for example, one or more human donors. T cells can be obtained from a number of sources, including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, *e.g.*, FICOLL™ separation.

In some examples, T cells can be isolated from a mixture of immune cells (*e.g.*, those described herein) to produce an isolated T cell population. For example, after isolation of peripheral blood mononuclear cells (PBMC), both cytotoxic and helper T lymphocytes can be sorted into naive, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

A specific subpopulation of T cells, expressing one or more of the following cell surface markers: TCRab, CD3, CD4, CD8, CD27 CD28, CD38 CD45RA, CD45RO, CD62L, CD127, CD122, CD95, CD197, CCR7, KLRG1, MCH-I proteins and/or MCH-II proteins, can be further isolated by positive or negative selection techniques. In some embodiments, a specific subpopulation of T cells, expressing one or more of the markers selected from the group consisting of TCRab, CD4 and/or CD8, is further isolated by positive or negative selection techniques. In some embodiments, subpopulations of T cells may be isolated by

positive or negative selection prior to genetic engineering and/or post genetic engineering.

An isolated population of T cells may express one or more of the T cell markers, including, but not limited to a CD3+, CD4+, CD8+, or a combination thereof. In some embodiments, the T cells are isolated from a donor, or subject, and first activated and stimulated to proliferate in vitro prior to undergoing gene editing.

In some instances, the T cell population comprises primary T cells isolated from one or more human donors. Such T cells are terminally differentiated, not transformed, depend on cytokines and/or growth factors for growth, and/or have stable genomes.

Alternatively, the T cells may be derived from stem cells (*e.g.*, HSCs or iPSCs) *via in vitro* differentiation.

T cells from a suitable source can be subjected to one or more rounds of stimulation, activation and/or expansion. T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; and 6,867,041. In some embodiments, T cells can be activated and expanded for about 1 day to about 4 days, about 1 day to about 3 days, about 1 day to about 2 days, about 2 days to about 3 days, about 2 days to about 4 days, about 3 days to about 4 days, or about 1 day, about 2 days, about 3 days, or about 4 days prior to introduction of the genome editing compositions into the T cells.

In some embodiments, T cells are activated and expanded for about 4 hours, about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, or about 72 hours prior to introduction of the gene editing compositions into the T cells. In some embodiments, T cells are activated at the same time that genome editing compositions are introduced into the T cells. In some instances, the T cell population can be expanded and/or activated after the genetic editing as disclosed herein. T cell populations or isolated T cells generated by any of the gene editing methods described herein are also within the scope of the present disclosure.

(b) Gene Editing Methods

Any of the genetically engineered T cells can be prepared using conventional gene editing methods or those described herein to edit one or more of the target genes disclosed herein (targeted editing). Targeted editing can be achieved either through a nuclease-independent approach, or through a nuclease-dependent approach. In the nuclease-independent targeted editing approach, homologous recombination is guided by homologous sequences

flanking an exogenous polynucleotide to be introduced into an endogenous sequence through the enzymatic machinery of the host cell. The exogenous polynucleotide may introduce deletions, insertions or replacement of nucleotides in the endogenous sequence.

Alternatively, the nuclease-dependent approach can achieve targeted editing with higher frequency through the specific introduction of double strand breaks (DSBs) by specific rare-cutting nucleases (*e.g.*, endonucleases). Such nuclease-dependent targeted editing also utilizes DNA repair mechanisms, for example, non-homologous end joining (NHEJ), which occurs in response to DSBs. DNA repair by NHEJ often leads to random insertions or deletions (indels) of a small number of endogenous nucleotides. In contrast to NHEJ mediated repair, repair can also occur by a homology directed repair (HDR). When a donor template containing exogenous genetic material flanked by a pair of homology arms is present, the exogenous genetic material can be introduced into the genome by HDR, which results in targeted integration of the exogenous genetic material.

In some embodiments, gene disruption may occur by deletion of a genomic sequence using two guide RNAs. Methods of using CRISPR-Cas gene editing technology to create a genomic deletion in a cell (*e.g.*, to knock out a gene in a cell) are known (Bauer DE et al. Vis. Exp. 2015; 95:e52118).

Available endonucleases capable of introducing specific and targeted DSBs include, but not limited to, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and RNA-guided CRISPR-Cas9 nuclease (CRISPR/Cas9; Clustered Regular Interspaced Short Palindromic Repeats Associated 9). Additionally, DICE (dual integrase cassette exchange) system utilizing phiC31 and Bxb1 integrases may also be used for targeted integration. Some exemplary approaches are disclosed in detail below.

CRISPR-Cas9 Gene Editing System

The CRISPR-Cas9 system is a naturally-occurring defense mechanism in prokaryotes that has been repurposed as an RNA-guided DNA-targeting platform used for gene editing. It relies on the DNA nuclease Cas9, and two noncoding RNAs, crisprRNA (crRNA) and trans-activating RNA (tracrRNA), to target the cleavage of DNA. CRISPR is an abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats, a family of DNA sequences found in the genomes of bacteria and archaea that contain fragments of DNA (spacer DNA) with similarity to foreign DNA previously exposed to the cell, for example, by viruses that have infected or attacked the prokaryote. These fragments of DNA are used by the prokaryote to detect and destroy similar foreign DNA upon re-introduction, for example, from similar viruses

during subsequent attacks. Transcription of the CRISPR locus results in the formation of an RNA molecule comprising the spacer sequence, which associates with and targets Cas (CRISPR-associated) proteins able to recognize and cut the foreign, exogenous DNA. Numerous types and classes of CRISPR/Cas systems have been described (see, *e.g.*, Koonin et al., (2017) Curr Opin Microbiol 37:67-78).

crRNA drives sequence recognition and specificity of the CRISPR-Cas9 complex through Watson-Crick base pairing typically with a 20 nucleotide (nt) sequence in the target DNA. Changing the sequence of the 5' 20nt in the crRNA allows targeting of the CRISPR-Cas9 complex to specific loci. The CRISPR-Cas9 complex only binds DNA sequences that contain a sequence match to the first 20 nt of the crRNA, if the target sequence is followed by a specific short DNA motif (with the sequence NGG) referred to as a protospacer adjacent motif (PAM).

tracrRNA hybridizes with the 3' end of crRNA to form an RNA-duplex structure that is bound by the Cas9 endonuclease to form the catalytically active CRISPR-Cas9 complex, which can then cleave the target DNA.

Once the CRISPR-Cas9 complex is bound to DNA at a target site, two independent nuclease domains within the Cas9 enzyme each cleave one of the DNA strands upstream of the PAM site, leaving a double-strand break (DSB) where both strands of the DNA terminate in a base pair (a blunt end).

After binding of CRISPR-Cas9 complex to DNA at a specific target site and formation of the site-specific DSB, the next key step is repair of the DSB. Cells use two main DNA repair pathways to repair the DSB: non-homologous end joining (NHEJ) and homology-directed repair (HDR).

NHEJ is a robust repair mechanism that appears highly active in the majority of cell types, including non-dividing cells. NHEJ is error-prone and can often result in the removal or addition of between one and several hundred nucleotides at the site of the DSB, though such modifications are typically < 20 nt. The resulting insertions and deletions (indels) can disrupt coding or noncoding regions of genes. Alternatively, HDR uses a long stretch of homologous donor DNA, provided endogenously or exogenously, to repair the DSB with high fidelity. HDR is active only in dividing cells, and occurs at a relatively low frequency in most cell types. In many embodiments of the present disclosure, NHEJ is utilized as the repair operant.

Endonuclease for use in CRISPR

In some embodiments, the Cas9 (CRISPR associated protein 9) endonuclease is used in

a CRISPR method for making the genetically engineered T cells as disclosed herein. The Cas9 enzyme may be one from *Streptococcus pyogenes*, although other Cas9 homologs may also be used. It should be understood, that wild-type Cas9 may be used or modified versions of Cas9 may be used (*e.g.*, evolved versions of Cas9, or Cas9 orthologues or variants), as provided herein. In some embodiments, Cas9 may be substituted with another RNA-guided endonuclease, such as Cpf1 (of a class II CRISPR/Cas system).

In some embodiments, the CRISPR/Cas system comprises components derived from a Type-I, Type-II, or Type-III system. Updated classification schemes for CRISPR/Cas loci define Class 1 and Class 2 CRISPR/Cas systems, having Types I to V or VI (Makarova et al., (2015) Nat Rev Microbiol, 13(11):722-36; Shmakov et al., (2015) Mol Cell, 60:385-397). Class 2 CRISPR/Cas systems have single protein effectors. Cas proteins of Types II, V, and VI are single-protein, RNA-guided endonucleases, herein called "Class 2 Cas nucleases." Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins. The Cpf1 nuclease (Zetsche et al., (2015) Cell 163:1-13) is homologous to Cas9, and contains a RuvC-like nuclease domain.

In some embodiments, the Cas nuclease is from a Type-II CRISPR/Cas system (*e.g.*, a Cas9 protein from a CRISPR/Cas9 system). In some embodiments, the Cas nuclease is from a Class 2 CRISPR/Cas system (a single-protein Cas nuclease such as a Cas9 protein or a Cpf1 protein). The Cas9 and Cpf1 family of proteins are enzymes with DNA endonuclease activity, and they can be directed to cleave a desired nucleic acid target by designing an appropriate guide RNA, as described further herein.

In some embodiments, a Cas nuclease may comprise more than one nuclease domain. For example, a Cas9 nuclease may comprise at least one RuvC-like nuclease domain (*e.g.*, Cpf1) and at least one HNH-like nuclease domain (*e.g.*, Cas9). In some embodiments, the Cas9 nuclease introduces a DSB in the target sequence. In some embodiments, the Cas9 nuclease is modified to contain only one functional nuclease domain. For example, the Cas9 nuclease is modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, the Cas9 nuclease is modified to contain no functional RuvC-like nuclease domain. In other embodiments, the Cas9 nuclease is modified to contain no functional HNH-like nuclease domain. In some embodiments in which only one of the nuclease domains is functional, the Cas9 nuclease is a nickase that is capable of introducing a single-stranded break (a "nick") into the target sequence. In some embodiments, a conserved amino acid within a Cas9 nuclease domain is substituted to reduce

or alter a nuclease activity. In some embodiments, the Cas nuclease nickase comprises an amino acid substitution in the RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 nuclease). In some embodiments, the nickase comprises an amino acid substitution in the HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the S. pyogenes Cas9 nuclease).

Amino acid sequence of Cas9 nuclease (SEQ ID NO: 1):

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR
RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHL
RKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVD
AKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLD
NLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE
KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQI
HLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVD
KGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLL
FKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVL
TLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFAN
RNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENI
VIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKF
DNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDF
RKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAK
YFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG
FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS
FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHY
EKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIH
LFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

In some embodiments, the Cas nuclease is from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease is a component of the Cascade complex of a Type-I CRISPR/Cas system. For example, the Cas nuclease is a Cas3 nuclease. In some embodiments, the Cas nuclease is derived from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease is derived from Type-IV CRISPR/Cas system. In some embodiments, the Cas nuclease is derived from a Type-V CRISPR/Cas system. In some embodiments, the Cas nuclease is derived from a Type-VI CRISPR/Cas system.

Guide RNAs (gRNAs)

The CRISPR technology involves the use of a genome-targeting nucleic acid that can direct the endonuclease to a specific target sequence within a target gene for gene editing at the specific target sequence. The genome-targeting nucleic acid can be a RNA. A genome-targeting RNA is referred to as a "guide RNA" or "gRNA" herein. A guide RNA comprises at least a spacer sequence that hybridizes to a target nucleic acid sequence within a target gene for

editing, and a CRISPR repeat sequence.

In Type II systems, the gRNA also comprises a second RNA called the tracrRNA sequence. In the Type II gRNA, the CRISPR repeat sequence and tracrRNA sequence hybridize to each other to form a duplex. In the Type V gRNA, the crRNA forms a duplex. In both systems, the duplex binds a site-directed polypeptide, such that the guide RNA and site-direct polypeptide form a complex. In some embodiments, the genome-targeting nucleic acid provides target specificity to the complex by virtue of its association with the site-directed polypeptide. The genome-targeting nucleic acid thus directs the activity of the site-directed polypeptide.

As is understood by the person of ordinary skill in the art, each guide RNA is designed to include a spacer sequence complementary to its genomic target sequence. See Jinek et al., Science, 337, 816-821 (2012) and Deltcheva et al., Nature, 471, 602-607 (2011).

In some embodiments, the genome-targeting nucleic acid (*e.g.*, gRNA) is a double-molecule guide RNA. In some embodiments, the genome-targeting nucleic acid (*e.g.*, gRNA) is a single-molecule guide RNA.

A double-molecule guide RNA comprises two strands of RNA molecules. The first strand comprises in the 5' to 3' direction, an optional spacer extension sequence, a spacer sequence and a minimum CRISPR repeat sequence. The second strand comprises a minimum tracrRNA sequence (complementary to the minimum CRISPR repeat sequence), a 3' tracrRNA sequence and an optional tracrRNA extension sequence.

A single-molecule guide RNA (referred to as a "sgRNA") in a Type II system comprises, in the 5' to 3' direction, an optional spacer extension sequence, a spacer sequence, a minimum CRISPR repeat sequence, a single-molecule guide linker, a minimum tracrRNA sequence, a 3' tracrRNA sequence and an optional tracrRNA extension sequence. The optional tracrRNA extension may comprise elements that contribute additional functionality (*e.g.*, stability) to the guide RNA. The single-molecule guide linker links the minimum CRISPR repeat and the minimum tracrRNA sequence to form a hairpin structure. The optional tracrRNA extension comprises one or more hairpins. A single-molecule guide RNA in a Type V system comprises, in the 5' to 3' direction, a minimum CRISPR repeat sequence and a spacer sequence.

A spacer sequence in a gRNA is a sequence (*e.g.*, a 20 nucleotide sequence) that defines the target sequence (*e.g.*, a DNA target sequences, such as a genomic target sequence) of a target gene of interest. In some embodiments, the spacer sequence range from 15 to 30

nucleotides. For example, the spacer sequence may contain 15, 16, 17, 18, 19, 29, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides. In some embodiments, a spacer sequence contains 20 nucleotides.

The "target sequence" is in a target gene that is adjacent to a PAM sequence and is the sequence to be modified by an RNA-guided nuclease (*e.g.*, Cas9). The "target sequence" is on the so-called PAM-strand in a "target nucleic acid," which is a double-stranded molecule containing the PAM-strand and a complementary non-PAM strand. One of skill in the art recognizes that the gRNA spacer sequence hybridizes to the complementary sequence located in the non-PAM strand of the target nucleic acid of interest. Thus, the gRNA spacer sequence is the RNA equivalent of the target sequence. For example, if the target sequence is 5′-AGAGCAACAGTGCTGTGGCC**-3′ (SEQ ID NO: 69), then the gRNA spacer sequence is 5′-AGAGCAACAGUGCUGUGGCC**-3′ (SEQ ID NO: 61). The spacer of a gRNA interacts with a target nucleic acid of interest in a sequence-specific manner *via* hybridization (*i.e.*, base pairing). The nucleotide sequence of the spacer thus varies depending on the target sequence of the target nucleic acid of interest.

In a CRISPR/Cas system herein, the spacer sequence is designed to hybridize to a region of the target nucleic acid that is located 5' of a PAM recognizable by a Cas9 enzyme used in the system. The spacer may perfectly match the target sequence or may have mismatches. Each Cas9 enzyme has a particular PAM sequence that it recognizes in a target DNA. For example, *S. pyogenes* recognizes in a target nucleic acid a PAM that comprises the sequence 5'-NRG-3', where R comprises either A or G, where N is any nucleotide and N is immediately 3' of the target nucleic acid sequence targeted by the spacer sequence.

In some embodiments, the target nucleic acid sequence has 20 nucleotides in length. In some embodiments, the target nucleic acid has less than 20 nucleotides in length. In some embodiments, the target nucleic acid has more than 20 nucleotides in length. In some embodiments, the target nucleic acid has at least: 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 or more nucleotides in length. In some embodiments, the target nucleic acid has at most: 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 or more nucleotides in length. In some embodiments, the target nucleic acid sequence has 20 bases immediately 5' of the first nucleotide of the PAM. For example, in a sequence comprising 5'-NNNNNNNNNNNNNNNNNNNNNRG-3', the target nucleic acid can be the sequence that corresponds to the Ns, wherein N can be any nucleotide, and the underlined NRG sequence is the *S. pyogenes* PAM.

The guide RNA disclosed herein may target any sequence of interest *via* the spacer sequence in the crRNA. In some embodiments, the degree of complementarity between the spacer sequence of the guide RNA and the target sequence in the target gene can be about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%. In some embodiments, the spacer sequence of the guide RNA and the target sequence in the target gene is 100% complementary. In other embodiments, the spacer sequence of the guide RNA and the target sequence in the target gene may contain up to 10 mismatches, *e.g.*, up to 9, up to 8, up to 7, up to 6, up to 5, up to 4, up to 3, up to 2, or up to 1 mismatch.

For any of the gRNA sequences provided herein, those that do not explicitly indicate modifications are meant to encompass both unmodified sequences and sequences having any suitable modifications.

The length of the spacer sequence in any of the gRNAs disclosed herein may depend on the CRISPR/Cas9 system and components used for editing any of the target genes also disclosed herein. For example, different Cas9 proteins from different bacterial species have varying optimal spacer sequence lengths. Accordingly, the spacer sequence may have 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more than 50 nucleotides in length. In some embodiments, the spacer sequence may have 18-24 nucleotides in length. In some embodiments, the targeting sequence may have 19-21 nucleotides in length. In some embodiments, the spacer sequence may comprise 20 nucleotides in length.

In some embodiments, the gRNA can be an sgRNA, which may comprise a 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. In some embodiments, the sgRNA may comprise a less than 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. In some embodiments, the sgRNA may comprise a more than 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. In some embodiments, the sgRNA comprises a variable length spacer sequence with 17-30 nucleotides at the 5' end of the sgRNA sequence. Examples are provided in **Table 23** below. In these exemplary sequences, the fragment of "n" refers to the spacer sequence at the 5' end.

In some embodiments, the sgRNA comprises comprise no uracil at the 3' end of the sgRNA sequence. In other embodiments, the sgRNA may comprise one or more uracil at the 3' end of the sgRNA sequence. For example, the sgRNA can comprise 1-8 uracil residues, at the 3' end of the sgRNA sequence, *e.g.*, 1, 2, 3, 4, 5, 6, 7, or 8 uracil residues at the 3' end of the sgRNA sequence.

Any of the gRNAs disclosed herein, including any of the sgRNAs, may be unmodified. Alternatively, it may contain one or more modified nucleotides and/or modified backbones. For example, a modified gRNA such as an sgRNA can comprise one or more 2'-O-methyl phosphorothioate nucleotides, which may be located at either the 5' end, the 3' end, or both.

In certain embodiments, more than one guide RNAs can be used with a CRISPR/Cas nuclease system. Each guide RNA may contain a different targeting sequence, such that the CRISPR/Cas system cleaves more than one target nucleic acid. In some embodiments, one or more guide RNAs may have the same or differing properties such as activity or stability within the Cas9 RNP complex. Where more than one guide RNA is used, each guide RNA can be encoded on the same or on different vectors. The promoters used to drive expression of the more than one guide RNA is the same or different.

In some embodiments, the gRNAs disclosed herein target a *Reg1* gene, for example, target a site within exon 1, exon 2, exon 3, exon 4, exon 5, or exon 6 of the *Reg1* gene. Such a gRNA may comprise a spacer sequence complementary (complete or partially) to the target sequences in exon 2 or exon 4 of a *Reg1* gene, or a fragment thereof. Exemplary target sequences of Reg1 and exemplary gRNA sequences are provided in **Table 22** below.

In some embodiments, the gRNAs disclosed herein target a *TGFBRII* gene, for example, target a site within exon 1, exon 2, exon 3, exon 4, exon 5, or exon 6 of the *TGFBRII* gene. Such a gRNA may comprise a spacer sequence complementary (complete or partially) to the target sequences in exon 4 or exon 5 of a *TGFBRII* gene, or a fragment thereof. Exemplary target sequences of TGFBRII and exemplary gRNA sequences are provided in **Table 39** below.

In some embodiments, the gRNAs disclosed herein target a *CD70* gene, for example, target a site within exon 1 or exon 3 of a *CD70* gene. Such a gRNA may comprise a spacer sequence complementary (complete or partially) to the target sequences in exon 1 or exon 3 of a *CD70* gene, or a fragment thereof. Exemplary target sequences in a *CD70* gene and exemplary gRNAs specific to the *CD70* gene are provided in **Table 23** below.

In some embodiments, the gRNAs disclosed herein target a *β2M* gene, for example, target a suitable site within a *β2M* gene. See also WO2019097305, the relevant disclosures of which are incorporated by reference herein for the purpose and subject matter referenced herein. Other gRNA sequences may be designed using the *β2M* gene sequence located on Chromosome 15 (GRCh38 coordinates: Chromosome 15: 44,711,477-44,718,877; Ensembl: ENSG00000166710). In some embodiments, gRNAs targeting the *β2M* genomic region and RNA-guided nuclease create breaks in the *β2M* genomic region resulting in Indels in the *β2M*

gene disrupting expression of the mRNA or protein.

In some embodiments, the gRNAs disclosed herein target a *TRAC* gene. See also WO2019097305, the relevant disclosures of which are incorporated by reference herein for the subject matter and purpose referenced herein. Other gRNA sequences may be designed using the *TRAC* gene sequence located on chromosome 14 (GRCh38: chromosome 14: 22,547,506-22,552,154;. Ensembl; ENSG00000277734). In some embodiments, gRNAs targeting the *TRAC* genomic region and RNA-guided nuclease create breaks in the *TRAC* genomic region resulting Indels in the *TRAC* gene disrupting expression of the mRNA or protein.

Exemplary spacer sequences and gRNAs targeting a *β2M* gene or *TRAC* gene are provided in **Table 23** below.

By way of illustration, guide RNAs used in the CRISPR/Cas/Cpf1 system, or other smaller RNAs can be readily synthesized by chemical means, as illustrated below and described in the art. While chemical synthetic procedures are continually expanding, purifications of such RNAs by procedures such as high performance liquid chromatography (HPLC, which avoids the use of gels such as PAGE) tends to become more challenging as polynucleotide lengths increase significantly beyond a hundred or so nucleotides. One approach used for generating RNAs of greater length is to produce two or more molecules that are ligated together. Much longer RNAs, such as those encoding a Cas9 or Cpf1 endonuclease, are more readily generated enzymatically. Various types of RNA modifications can be introduced during or after chemical synthesis and/or enzymatic generation of RNAs, *e.g.*, modifications that enhance stability, reduce the likelihood or degree of innate immune response, and/or enhance other attributes, as described in the art.

In some examples, the gRNAs of the present disclosure can be are produced *in vitro* transcription (IVT), synthetic and/or chemical synthesis methods, or a combination thereof. Enzymatic (IVT), solid-phase, liquid-phase, combined synthetic methods, small region synthesis, and ligation methods are utilized. In one embodiment, the gRNAs are made using IVT enzymatic synthesis methods. Methods of making polynucleotides by IVT are known in the art and are described in WO2013/151666. Accordingly, the present disclosure also includes polynucleotides, *e.g.*, DNA, constructs and vectors are used to *in vitro* transcribe a gRNA described herein.

Various types of RNA modifications can be introduced during or after chemical synthesis and/or enzymatic generation of RNAs, *e.g.*, modifications that enhance stability, reduce the likelihood or degree of innate immune response, and/or enhance other attributes, as

described in the art. In some embodiments, non-natural modified nucleobases can be introduced into any of the gRNAs disclosed herein during synthesis or post-synthesis. In certain embodiments, modifications are on internucleoside linkages, purine or pyrimidine bases, or sugar. In some embodiments, a modification is introduced at the terminal of a gRNA with chemical synthesis or with a polymerase enzyme. Examples of modified nucleic acids and their synthesis are disclosed in WO2013/052523. Synthesis of modified polynucleotides is also described in Verma and Eckstein, Annual Review of Biochemistry, vol. 76, 99-134 (1998).

In some embodiments, enzymatic or chemical ligation methods can be used to conjugate polynucleotides or their regions with different functional moieties, such as targeting or delivery agents, fluorescent labels, liquids, nanoparticles, etc. Conjugates of polynucleotides and modified polynucleotides are reviewed in Goodchild, Bioconjugate Chemistry, vol. 1(3), 165-187 (1990).

In some embodiments of the present disclosure, a CRISPR/Cas nuclease system for use in genetically editing any of the target genes disclosed here may include at least one guide RNA. In some examples, the CRISPR/Cas nuclease system may contain multiple gRNAs, for example, 2, 3, or 4 gRNAs. Such multiple gRNAs may target different sites in a same target gene. Alternatively, the multiple gRNAs may target different genes. In some embodiments, the guide RNA(s) and the Cas protein may form a ribonucleoprotein (RNP), *e.g.*, a CRISPR/Cas complex. The guide RNA(s) may guide the Cas protein to a target sequence(s) on one or more target genes as those disclosed herein, where the Cas protein cleaves the target gene at the target site. In some embodiments, the CRISPR/Cas complex is a Cpf1/guide RNA complex. In some embodiments, the CRISPR complex is a Type-II CRISPR/Cas9 complex. In some embodiments, the Cas protein is a Cas9 protein. In some embodiments, the CRISPR/Cas9 complex is a Cas9/guide RNA complex.

In some embodiments, the indel frequency (editing frequency) of a particular CRISPR/Cas nuclease system, comprising one or more specific gRNAs, may be determined using a TIDE analysis, which can be used to identify highly efficient gRNA molecules for editing a target gene. In some embodiments, a highly efficient gRNA yields a gene editing frequency of higher than 80%. For example, a gRNA is considered to be highly efficient if it yields a gene editing frequency of at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

Delivery of Guide RNAs and Nucleases to T Cells

The CRISPR/Cas nuclease system disclosed herein, comprising one or more gRNAs

and at least one RNA-guided nuclease, optionally a donor template as disclosed below, can be delivered to a target cell (*e.g.*, a T cell) for genetic editing of a target gene, via a conventional method. In some embodiments, components of a CRISPR/Cas nuclease system as disclosed herein may be delivered to a target cell separately, either simultaneously or sequentially. In other embodiments, the components of the CRISPR/Cas nuclease system may be delivered into a target together, for example, as a complex. In some instances, gRNA and a RNA-guided nuclease can be pre-complexed together to form a ribonucleoprotein (RNP), which can be delivered into a target cell.

RNPs are useful for gene editing, at least because they minimize the risk of promiscuous interactions in a nucleic acid-rich cellular environment and protect the RNA from degradation. Methods for forming RNPs are known in the art. In some embodiments, an RNP containing an RNA-guided nuclease (*e.g.*, a Cas nuclease, such as a Cas9 nuclease) and one or more gRNAs targeting one or more genes of interest can be delivered a cell (*e.g.*, a T cell). In some embodiments, an RNP can be delivered to a T cell by electroporation.

In some embodiments, an RNA-guided nuclease can be delivered to a cell in a DNA vector that expresses the RNA-guided nuclease in the cell. In other examples, an RNA-guided nuclease can be delivered to a cell in an RNA that encodes the RNA-guided nuclease and expresses the nuclease in the cell. Alternatively or in addition, a gRNA targeting a gene can be delivered to a cell as a RNA, or a DNA vector that expresses the gRNA in the cell.

Delivery of an RNA-guided nuclease, gRNA, and/or an RNP may be through direct injection or cell transfection using known methods, for example, electroporation or chemical transfection. Other cell transfection methods may be used.

Other Gene Editing Methods

Besides the CRISPR method disclosed herein, additional gene editing methods as known in the art can also be used in making the genetically engineered T cells disclosed herein. Some examples include gene editing approaching involve zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN), restriction endonucleases, meganucleases homing endonucleases, and the like.

ZFNs are targeted nucleases comprising a nuclease fused to a zinc finger DNA binding domain (ZFBD), which is a polypeptide domain that binds DNA in a sequence-specific manner through one or more zinc fingers. A zinc finger is a domain of about 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include, but not limited to, C2H2 zinc fingers, C3H zinc fingers, and

C4 zinc fingers. A designed zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, e.g., application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. See, for example, U.S. Pat. Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536 and WO 03/016496. A selected zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. ZFNs are described in greater detail in U.S. Pat. No. 7,888,121 and U.S. Pat. No. 7,972,854. The most recognized example of a ZFN is a fusion of the FokI nuclease with a zinc finger DNA binding domain.

A TALEN is a targeted nuclease comprising a nuclease fused to a TAL effector DNA binding domain. A "transcription activator-like effector DNA binding domain", "TAL effector DNA binding domain", or "TALE DNA binding domain" is a polypeptide domain of TAL effector proteins that is responsible for binding of the TAL effector protein to DNA. TAL effector proteins are secreted by plant pathogens of the genus Xanthomonas during infection. These proteins enter the nucleus of the plant cell, bind effector-specific DNA sequences via their DNA binding domain, and activate gene transcription at these sequences via their transactivation domains. TAL effector DNA binding domain specificity depends on an effector-variable number of imperfect 34 amino acid repeats, which comprise polymorphisms at select repeat positions called repeat variable-diresidues (RVD). TALENs are described in greater detail in US Patent Application No. 2011/0145940. The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

Additional examples of targeted nucleases suitable for use as provided herein include, but are not limited to, Bxb1, phiC31, R4, PhiBT1, and Wβ/SPBc/TP901-1, whether used individually or in combination.

Any of the nucleases disclosed herein may be delivered using a vector system, including, but not limited to, plasmid vectors, DNA minicircles, retroviral vectors, lentiviral vectors, adenovirus vectors, poxvirus vectors; herpesvirus vectors and adeno-associated virus vectors, and combinations thereof.

Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids encoding nucleases and donor templates in cells (*e.g.*, T cells). Non-viral vector delivery systems include DNA plasmids, DNA minicircles, naked nucleic acid, and nucleic

acid complexed with a delivery vehicle such as a liposome or poloxamer. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.

Methods of non-viral delivery of nucleic acids include electroporation, lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, naked RNA, capped RNA, artificial virions, and agent-enhanced uptake of DNA. Sonoporation using, *e.g.*, the Sonitron 2000 system (Rich-Mar) can also be used for delivery of nucleic acids. Some specific examples are provided below.

II. Genetically Engineered T Cells Expression a Chimeric Antigen Receptor (CAR)

The genetically engineered T cells having a disrupted *Reg1* gene, a disrupted *TGFBRII* gene, or a combination of disrupted *Reg1* gene and disrupted *TGFBRII* gene. Optionally, such genetically engineered T cells may comprise one or more of additional disrupted genes, *e.g.*, *β2M*, *TRAC*, *CD70*, or a combination thereof as disclosed herein, may further express a chimeric antigen receptor (CAR) targeting an antigen of interest or cells expressing such an antigen.

(a) Chimeric Antigen Receptor (CAR)

A chimeric antigen receptor (CAR) refers to an artificial immune cell receptor that is engineered to recognize and bind to an antigen expressed by undesired cells, for example, disease cells such as cancer cells. A T cell that expresses a CAR polypeptide is referred to as a CAR T cell. CARs have the ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner. The non-MHC-restricted antigen recognition gives CAR-T cells the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed on T-cells, CARs advantageously do not dimerize with endogenous T-cell receptor (TCR) alpha and beta chains.

There are various generations of CARs, each of which contains different components. First generation CARs join an antibody-derived scFv to the CD3zeta (ζ or z) intracellular signaling domain of the T-cell receptor through hinge and transmembrane domains. Second generation CARs incorporate an additional co-stimulatory domain, *e.g.,* CD28, 4-1BB (41BB), or ICOS, to supply a costimulatory signal. Third-generation CARs contain two costimulatory domains (*e.g.*, a combination of CD27, CD28, 4-1BB, ICOS, or OX40) fused with the TCR CD3ζ chain. Maude et al., *Blood.* 2015; 125(26):4017-4023; Kakarla and Gottschalk, *Cancer J.* 2014; 20(2):151-155). Any of the various generations of CAR constructs is within the scope of

the present disclosure.

Generally, a CAR is a fusion polypeptide comprising an extracellular domain that recognizes a target antigen (*e.g.*, a single chain fragment (scFv) of an antibody or other antibody fragment) and an intracellular domain comprising a signaling domain of the T-cell receptor (TCR) complex (*e.g.*, CD3ζ) and, in most cases, a co-stimulatory domain. (Enblad et al., Human Gene Therapy. 2015; 26(8):498-505). A CAR construct may further comprise a hinge and transmembrane domain between the extracellular domain and the intracellular domain, as well as a signal peptide at the N-terminus for surface expression. Examples of signal peptides include SEQ ID NO: 95 and SEQ ID NO: 96 as provided in **Table 27** below. Other signal peptides may be used.

(i) Antigen Binding Extracellular Domain

The antigen-binding extracellular domain is the region of a CAR polypeptide that is exposed to the extracellular fluid when the CAR is expressed on cell surface. In some instances, a signal peptide may be located at the N-terminus to facilitate cell surface expression. In some embodiments, the antigen binding domain can be a single-chain variable fragment (scFv, which may include an antibody heavy chain variable region (V_H) and an antibody light chain variable region (V_L) (in either orientation). In some instances, the V_H and V_L fragment may be linked via a peptide linker. The linker, in some embodiments, includes hydrophilic residues with stretches of glycine and serine for flexibility as well as stretches of glutamate and lysine for added solubility. The scFv fragment retains the antigen-binding specificity of the parent antibody, from which the scFv fragment is derived. In some embodiments, the scFv may comprise humanized V_H and/or V_L domains. In other embodiments, the V_H and/or V_L domains of the scFv are fully human.

The antigen-binding extracellular domain may be specific to a target antigen of interest, for example, a pathologic antigen such as a tumor antigen. In some embodiments, a tumor antigen is a "tumor associated antigen," referring to an immunogenic molecule, such as a protein, that is generally expressed at a higher level in tumor cells than in non-tumor cells, in which it may not be expressed at all, or only at low levels. In some embodiments, tumor-associated structures, which are recognized by the immune system of the tumor-harboring host, are referred to as tumor-associated antigens. In some embodiments, a tumor-associated antigen is a universal tumor antigen, if it is broadly expressed by most types of tumors. In some embodiments, tumor-associated antigens are differentiation antigens, mutational antigens, overexpressed cellular antigens or viral antigens. In some embodiments, a tumor antigen is a

"tumor specific antigen" or "TSA," referring to an immunogenic molecule, such as a protein, that is unique to a tumor cell. Tumor specific antigens are exclusively expressed in tumor cells, for example, in a specific type of tumor cells.

In some embodiments, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds a tumor antigen as disclosed herein. The scFv may comprise an antibody heavy chain variable region (V_H) and an antibody light chain variable region (V_L), which optionally may be connected via a flexible peptide linker. In some instances, the scFv may have the V_H to V_L orientation (from N-terminus to C-terminus). Alternatively the scFv may have the V_L to V_H orientation (from N-terminus to C-terminus).

Exemplary tumor antigens include, but are not limited to, CD19, BCMA, CD70, CD33, and PTK7. Any known antibodies specific to such tumor antigens, for example, those approved for marketing and those in clinical trials, can be used for making the CAR constructs disclosed herein. Non-limiting examples of CAR constructs are provided in WO2019097305 and WO2019215500, WO2020/095107, and International Patent Application No. PCT/IB2021/053849, the relevant disclosures of which are herein incorporated by reference for the purposes and subject matter referenced herein.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human CD19. In some instances, the anti-CD19 scFv may comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 124; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 125. In some specific examples, the anti-CD19 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 108-110, respectively as determined by the Kabat method. Alternatively or in addition, the anti-CD19 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:105-107 as determined by the Kabat method. Alternatively, the anti-CD19 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 114-116, respectively as determined by the Chothia method. Alternatively or in addition, the anti-CD19 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:111-113 as determined by the Chothia method. In one specific example, the anti-CD19 scFv may comprise a V_H comprising the amino acid sequence of SEQ ID NO: 124 and a V_L comprises the amino acid sequence of SEQ ID NO: 125. See Sequence

Table 27 below.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human CD70. In some instances, the anti-CD70 scFv may comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 143; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 144. In some specific examples, the anti-CD70 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 132, 134, and 136, respectively as determined by the Kabat method. Alternatively or in addition, the anti-CD70 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:127, 129, and 130, respectively as determined by the Kabat method. Alternatively, the anti-CD70 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 133, 135, and 137, respectively as determined by the Chothia method. Alternatively or in addition, the anti-CD70 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NO:128, LAS, and SEQ ID NO:131, respetively as determined by the Chothia method. In one specific example, the anti-CD70 scFv may comprise a V_H comprising the amino acid sequence of SEQ ID NO: 143 and a V_L comprises the amino acid sequence of SEQ ID NO: 144. See Sequence **Table 27** below.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human BCMA. In some instances, the anti-BCMA scFv may comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 149; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 150. In some specific examples, the anti-BCMA antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 155, 157, and 159, respectively as determined by the Kabat method. Alternatively or in addition, the anti-BCMA antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:151, 152, and 153, respectively as determined by the Kabat method. Alternatively, the anti-BCMA antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 156, 158, and 160, respectively as determined by the Chothia method. Alternatively or in addition, the anti-BCMA antibody discloses herein may comprise the light chain CDR1, light

chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:151, 152, and 154, respectively as determined by the Chothia method. In one specific example, the anti-BCMA scFv may comprise a V_H comprising the amino acid sequence of SEQ ID NO: 149 and a V_L comprises the amino acid sequence of SEQ ID NO: 150. See Sequence **Table 27** below.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human CD33. Exemplary anti-CD33 scFv and anti-CD33 CAR constructs can be found, for example, in Sequence **Table 27** below and in WO2020/095107, the relevant disclosures of which are incorporated by reference for the subject matter and purpose noted herein.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human CD33. In some instances, the anti-CD33 scFv may comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 334; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 335. In some specific examples, the anti-CD33 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 328-330, respectively as determined by the Kabat method. Alternatively or in addition, the anti-CD33 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:331-333, respectively as determined by the Kabat method. In one specific example, the anti-BCMA scFv may comprise a V_H comprising the amino acid sequence of SEQ ID NO: 149 and a V_L comprises the amino acid sequence of SEQ ID NO: 150. See Sequence **Table 27** below.

In some examples, the antigen-binding extracellular domain can be a single-chain variable fragment (scFv) that binds human PTK7. In some instances, the anti-PTK7 scFv may comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 346; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 347. In some specific examples, the anti-PTK7 antibody discloses herein may comprise the heavy chain CDR1, heavy chain CDR2, and heavy chain CDR3 set forth as SEQ ID NOs: 340-342, respectively as determined by the Kabat method. Alternatively or in addition, the anti-PTK7 antibody discloses herein may comprise the light chain CDR1, light chain CDR2, and light chain CDR3 set forth as SEQ ID NOs:343-345, respectively as determined by the Kabat method. In one specific example, the anti-BCMA scFv may comprise a V_H comprising the

amino acid sequence of SEQ ID NO: 346 and a V_L comprises the amino acid sequence of SEQ ID NO: 347. See Sequence **Table 27** below.

Two antibodies having the same V_H and/or V_L CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, the Kabat approach, the Chothia approach, the AbM approach, the Contact approach, or the IMGT approach as known in the art. See, *e.g.*, bioinf.org.uk/abs/ or abysis.org/abysis/sequence_input).

(ii) Transmembrane Domain

The CAR polypeptide disclosed herein may contain a transmembrane domain, which can be a hydrophobic alpha helix that spans the membrane. As used herein, a "transmembrane domain" refers to any protein structure that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane. The transmembrane domain can provide stability of the CAR containing such.

In some embodiments, the transmembrane domain of a CAR as provided herein can be a CD8 transmembrane domain. In other embodiments, the transmembrane domain can be a CD28 transmembrane domain. In yet other embodiments, the transmembrane domain is a chimera of a CD8 and CD28 transmembrane domain. Other transmembrane domains may be used as provided herein. In some embodiments, the transmembrane domain is a CD8a transmembrane domain containing the sequence of SEQ ID NO: 97 as provided below in **Table 27**. Other transmembrane domains may be used.

(iii) Hinge Domain

In some embodiments, a hinge domain may be located between an extracellular domain (comprising the antigen binding domain) and a transmembrane domain of a CAR, or between a cytoplasmic domain and a transmembrane domain of the CAR. A hinge domain can be any oligopeptide or polypeptide that functions to link the transmembrane domain to the extracellular domain and/or the cytoplasmic domain in the polypeptide chain. A hinge domain may function to provide flexibility to the CAR, or domains thereof, or to prevent steric hindrance of the CAR, or domains thereof.

In some embodiments, a hinge domain may comprise up to 300 amino acids (*e.g.*, 10 to 100 amino acids, or 5 to 20 amino acids). In some embodiments, one or more hinge domain(s) may be included in other regions of a CAR. In some embodiments, the hinge domain may be a CD8 hinge domain. Other hinge domains may be used.

(iv) Intracellular Signaling Domains

Any of the CAR constructs contain one or more intracellular signaling domains (*e.g.*, CD3ζ, and optionally one or more co-stimulatory domains), which are the functional end of the receptor. Following antigen recognition, receptors cluster and a signal is transmitted to the cell.

CD3ζ is the cytoplasmic signaling domain of the T cell receptor complex. CD3ζ contains three (3) immunoreceptor tyrosine-based activation motif (ITAM)s, which transmit an activation signal to the T cell after the T cell is engaged with a cognate antigen. In many cases, CD3ζ provides a primary T cell activation signal but not a fully competent activation signal, which requires a co-stimulatory signaling.

In some embodiments, the CAR polypeptides disclosed herein may further comprise one or more co-stimulatory signaling domains. For example, the co-stimulatory domains of CD28 and/or 4-1BB may be used to transmit a full proliferative/survival signal, together with the primary signaling mediated by CD3ζ. In some examples, the CAR disclosed herein comprises a CD28 co-stimulatory molecule. In other examples, the CAR disclosed herein comprises a 4-1BB co-stimulatory molecule. In some embodiments, a CAR includes a CD3ζ signaling domain and a CD28 co-stimulatory domain. In other embodiments, a CAR includes a CD3ζ signaling domain and 4-1BB co-stimulatory domain. In still other embodiments, a CAR includes a CD3ζ signaling domain, a CD28 co-stimulatory domain, and a 4-1BB co-stimulatory domain.

Table 27 provides examples of signaling domains derived from 4-1BB, CD28 and CD3-zeta that may be used herein.

In specific examples, the anti-CD19 CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 118, which may be encoded by the nucleotide sequence of SEQ ID NO: 117. Alternatively, the anti-CD19 CAR may be a mature form without the N-terminal signal peptide, e.g., comprising the amino acid sequence of SEQ ID NO:353.

In other examples, the anti-BCMA CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 146, which may be encoded by the nucleotide sequence of SEQ ID NO: 145. Alternatively, the anti-CDBCMA CAR may be a mature form without the N-terminal signal peptide, e.g., comprising the amino acid sequence of SEQ ID NO:355.

In other examples, the anti-CD70 CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 138, which may be encoded by the nucleotide sequence of SEQ ID NO: 141. Alternatively, the anti-CD70 CAR may be a mature form without the N-terminal signal peptide, e.g., comprising the amino acid sequence of SEQ ID NO:354.

In some examples, the anti-CD33 CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 338 or 339. Alternatively, the anti-CD33 CAR may be a mature form without the N-terminal signal peptide, e.g., comprising the amino acid sequence of SEQ ID NO:356 or 357.

In some examples, the anti-PTK7 CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 349 or 350. Alternatively, the anti-PTK7 CAR may be a mature form without the N-terminal signal peptide, e.g., comprising the amino acid sequence of SEQ ID NO:358 or 359.

See sequence **Table 27** provided below.

(b) Delivery of CAR Construct to T Cells

In some embodiments, a nucleic acid encoding a CAR can be introduced into any of the genetically engineered T cells disclosed herein by methods known to those of skill in the art. For example, a coding sequence of the CAR may be cloned into a vector, which may be introduced into the genetically engineered T cells for expression of the CAR. A variety of different methods known in the art can be used to introduce any of the nucleic acids or expression vectors disclosed herein into an immune effector cell. Non-limiting examples of methods for introducing nucleic acid into a cell include: lipofection, transfection (*e.g.*, calcium phosphate transfection, transfection using highly branched organic compounds, transfection using cationic polymers, dendrimer-based transfection, optical transfection, particle-based transfection (e.g., nanoparticle transfection), or transfection using liposomes (*e.g.*, cationic liposomes)), microinjection, electroporation, cell squeezing, sonoporation, protoplast fusion, impalefection, hydrodynamic delivery, gene gun, magnetofection, viral transfection, and nucleofection.

In specific examples, a nucleic acid encoding a CAR construct can be delivered to a cell using an adeno-associated virus (AAV). AAVs are small viruses which integrate site-specifically into the host genome and can therefore deliver a transgene, such as CAR. Inverted terminal repeats (ITRs) are present flanking the AAV genome and/or the transgene of interest and serve as origins of replication. Also present in the AAV genome are rep and cap proteins which, when transcribed, form capsids which encapsulate the AAV genome for delivery into target cells. Surface receptors on these capsids which confer AAV serotype, which determines which target organs the capsids will primarily bind and thus what cells the AAV will most efficiently infect. There are twelve currently known human AAV serotypes. In some embodiments, the AAV for use in delivering the CAR-coding nucleic acid is AAV serotype 6

(AAV6).

Adeno-associated viruses are among the most frequently used viruses for gene therapy for several reasons. First, AAVs do not provoke an immune response upon administration to mammals, including humans. Second, AAVs are effectively delivered to target cells, particularly when consideration is given to selecting the appropriate AAV serotype. Finally, AAVs have the ability to infect both dividing and non-dividing cells because the genome can persist in the host cell without integration. This trait makes them an ideal candidate for gene therapy.

A nucleic acid encoding a CAR can be designed to insert into a genomic site of interest in the host T cells. In some embodiments, the target genomic site can be in a safe harbor locus.

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template, which can be carried by a viral vector such as an adeno-associated viral (AAV) vector) can be designed such that it can insert into a location within a *TRAC* gene to disrupt the *TRAC* gene in the genetically engineered T cells and express the CAR polypeptide. Disruption of *TRAC* leads to loss of function of the endogenous TCR. For example, a disruption in the *TRAC* gene can be created with an endonuclease such as those described herein and one or more gRNAs targeting one or more *TRAC* genomic regions. Any of the gRNAs specific to a *TRAC* gene and the target regions disclosed herein can be used for this purpose.

In some examples, a genomic deletion in the *TRAC* gene and replacement by a CAR coding segment can be created by homology directed repair or HDR (*e.g.*, using a donor template, which may be part of a viral vector such as an adeno-associated viral (AAV) vector). In some embodiments, a disruption in the *TRAC* gene can be created with an endonuclease as those disclosed herein and one or more gRNAs targeting one or more *TRAC* genomic regions, and inserting a CAR coding segment into the *TRAC* gene.

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template, which can be carried by a viral vector such as an adeno-associated viral (AAV) vector) can be designed such that it can insert into a location within a *β2M* gene to disrupt the *β2M* gene in the genetically engineered T cells and express the CAR polypeptide. Disruption of *β2M* leads to loss of function of the endogenous MHC Class I complexes. For example, a disruption in the *β2M* gene can be created with an endonuclease such as those described herein and one or more gRNAs targeting one or more *β2M* genomic regions. Any of the gRNAs specific to a *β2M* gene and the target regions disclosed herein can be used for this purpose.

In some examples, a genomic deletion in the *β2M* gene and replacement by a CAR

coding segment can be created by homology directed repair or HDR (*e.g.*, using a donor template, which may be part of a viral vector such as an adeno-associated viral (AAV) vector). In some embodiments, a disruption in the *β2M* gene can be created with an endonuclease as those disclosed herein and one or more gRNAs targeting one or more *β2M* genomic regions, and inserting a CAR coding segment into the *β2M* gene.

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template, which can be carried by a viral vector such as an adeno-associated viral (AAV) vector) can be designed such that it can insert into a location within a *CD70* gene to disrupt the *CD70* gene in the genetically engineered T cells and express the CAR polypeptide. Disruption of *CD70* leads to loss of function of the endogenous CD70 protein. For example, a disruption in the *CD70* gene can be created with an endonuclease such as those described herein and one or more gRNAs targeting one or more *CD70* genomic regions. Any of the gRNAs specific to a *CD70* gene and the target regions disclosed herein can be used for this purpose.

In some examples, a genomic deletion in the *CD70* gene and replacement by a CAR coding segment can be created by homology directed repair or HDR (*e.g.*, using a donor template, which may be part of a viral vector such as an adeno-associated viral (AAV) vector). In some embodiments, a disruption in the *CD70* gene can be created with an endonuclease as those disclosed herein and one or more gRNAs targeting one or more *CD70* genomic regions, and inserting a CAR coding segment into the *CD70* gene.

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template, which can be carried by a viral vector such as an adeno-associated viral (AAV) vector) can be designed such that it can insert into a location within a *Reg1* gene to disrupt the *Reg1* gene in the genetically engineered T cells and express the CAR polypeptide. Disruption of *Reg1* leads to loss of function of the endogenous Reg1 protein. For example, a disruption in the *Reg1* gene can be created with an endonuclease such as those described herein and one or more gRNAs targeting one or more *Reg1* genomic regions. Any of the gRNAs specific to a *Reg1* gene and the target regions disclosed herein can be used for this purpose.

In some examples, a genomic deletion in the *Reg1* gene and replacement by a CAR coding segment can be created by homology directed repair or HDR (*e.g.*, using a donor template, which may be part of a viral vector such as an adeno-associated viral (AAV) vector). In some embodiments, a disruption in the *Reg1* gene can be created with an endonuclease as those disclosed herein and one or more gRNAs targeting one or more *Reg1* genomic regions, and inserting a CAR coding segment into the *Reg1* gene.

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template, which can be carried by a viral vector such as an adeno-associated viral (AAV) vector) can be designed such that it can insert into a location within a *TGFBRII* gene to disrupt the *TGFBRII* gene in the genetically engineered T cells and express the CAR polypeptide. Disruption of *Reg1* leads to loss of function of the endogenous TGFBRII receptor. For example, a disruption in the *TGFBRII* gene can be created with an endonuclease such as those described herein and one or more gRNAs targeting one or more *TGFBRII* genomic regions. Any of the gRNAs specific to a *TGFBRII* gene and the target regions disclosed herein can be used for this purpose.

In some examples, a genomic deletion in the *TGFBRII* gene and replacement by a CAR coding segment can be created by homology directed repair or HDR (*e.g.*, using a donor template, which may be part of a viral vector such as an adeno-associated viral (AAV) vector). In some embodiments, a disruption in the *TGFBRII* gene can be created with an endonuclease as those disclosed herein and one or more gRNAs targeting one or more *TGFBRII* genomic regions, and inserting a CAR coding segment into the *TGFBRII* gene.

A donor template as disclosed herein can contain a coding sequence for a CAR. In some examples, the CAR-coding sequence may be flanked by two regions of homology to allow for efficient HDR at a genomic location of interest, for example, at a *TRAC* gene using a gene editing method known in the art. In some examples, a CRISPR-based method can be used. In this case, both strands of the DNA at the target locus can be cut by a CRISPR Cas9 enzyme guided by gRNAs specific to the target locus. HDR then occurs to repair the double-strand break (DSB) and insert the donor DNA coding for the CAR. For this to occur correctly, the donor sequence is designed with flanking residues which are complementary to the sequence surrounding the DSB site in the target gene (hereinafter "homology arms"), such as the *TRAC* gene. These homology arms serve as the template for DSB repair and allow HDR to be an essentially error-free mechanism. The rate of homology directed repair (HDR) is a function of the distance between the mutation and the cut site so choosing overlapping or nearby target sites is important. Templates can include extra sequences flanked by the homologous regions or can contain a sequence that differs from the genomic sequence, thus allowing sequence editing.

Alternatively, a donor template may have no regions of homology to the targeted location in the DNA and may be integrated by NHEJ-dependent end joining following cleavage at the target site.

A donor template can be DNA or RNA, single-stranded and/or double-stranded, and can be introduced into a cell in linear or circular form. If introduced in linear form, the ends of

the donor sequence can be protected (*e.g.*, from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al., (1987) Proc. Natl. Acad. Sci. USA 84:4959-4963; Nehls et al., (1996) Science 272:886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

A donor template can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, a donor template can be introduced into a cell as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (*e.g.*, adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)).

A donor template, in some embodiments, can be inserted at a site nearby an endogenous prompter (e.g., downstream or upstream) so that its expression can be driven by the endogenous promoter. In other embodiments, the donor template may comprise an exogenous promoter and/or enhancer, for example, a constitutive promoter, an inducible promoter, or tissue-specific promoter to control the expression of the CAR gene. In some embodiments, the exogenous promoter is an EF1α promoter, *see*, *e.g.*, SEQ ID NO: 167 provided in **Table 28** below. Other promoters may be used.

Furthermore, exogenous sequences may also include transcriptional or translational regulatory sequences, for example, promoters, enhancers, insulators, internal ribosome entry sites, sequences encoding 2A peptides and/or polyadenylation signals.

When needed, additional gene editing (*e.g.*, gene knock-in or knock-out) can be introduced into therapeutic T cells as disclosed herein to improve T cell function and therapeutic efficacy. For example, if *β2M* disruption can be performed to reduce the risk of or prevent a host-versus-graft response. Other examples include knock-in or knock-out genes to improve target cell lysis, knock-in or knock-out genes to enhance performance of therapeutic T cells such as CAR-T cells.

In some embodiments, a donor template for delivering an anti-CD19 CAR may be an AAV vector inserted with a nucleic acid fragment comprising the coding sequence of the anti-CD19 CAR, and optionally regulatory sequences for expression of the anti-CD19 CAR (*e.g.*, a

promoter such as the EF1a promoter provided in the sequence Table), which can be flanked by homologous arms for inserting the coding sequence and the regulatory sequences into a genomic locus of interest. In some examples, the nucleic acid fragment is inserted in the endogenous *TRAC* gene locus, thereby disrupting expression of the *TRAC* gene. In specific examples, the nucleic acid may replace a fragment in the *TRAC* gene, for example, a fragment comprising the nucleotide sequence of SEQ ID NO: 69. In some specific examples, the donor template for delivering the anti-CD19 CAR may comprise a nucleotide sequence of SEQ ID NO: 117, which can be inserted into a disrupted *TRAC* gene, for example, replacing the fragment of SEQ ID NO: 69.

In some embodiments, a donor template for delivering an anti-BCMA CAR may be an AAV vector inserted with a nucleic acid fragment comprising the coding sequence of the anti-BCMA CAR, and optionally regulatory sequences for expression of the anti- BCMA CAR (*e.g.*, a promoter such as the EF1a promoter provided in the sequence Table), which can be flanked by homologous arms for inserting the coding sequence and the regulatory sequences into a genomic locus of interest. In some examples, the nucleic acid fragment is inserted in the endogenous *TRAC* gene locus, thereby disrupting expression of the *TRAC* gene. In specific examples, the nucleic acid may replace a fragment in the *TRAC* gene, for example, a fragment comprising the nucleotide sequence of SEQ ID NO: 69. In some specific examples, the donor template for delivering the anti- BCMA CAR may comprise a nucleotide sequence of SEQ ID NO: 145, which can be inserted into a disrupted *TRAC* gene, for example, replacing the fragment of SEQ ID NO: 69.

In some embodiments, a donor template for delivering an anti-CD70 CAR may be an AAV vector inserted with a nucleic acid fragment comprising the coding sequence of the anti-CD70 CAR, and optionally regulatory sequences for expression of the anti-CD70 CAR (*e.g.*, a promoter such as the EF1a promoter provided in the sequence Table), which can be flanked by homologous arms for inserting the coding sequence and the regulatory sequences into a genomic locus of interest. In some examples, the nucleic acid fragment is inserted in the endogenous *TRAC* gene locus, thereby disrupting expression of the *TRAC* gene. In specific examples, the nucleic acid may replace a fragment in the *TRAC* gene, for example, a fragment comprising the nucleotide sequence of SEQ ID NO: 69. In some specific examples, the donor template for delivering the anti-CD70 CAR may comprise a nucleotide sequence of SEQ ID NO: 139, which can be inserted into a disrupted *TRAC* gene, for example, replacing the fragment of SEQ ID NO: 69.

The genetically engineered T cells having a disrupted *Reg1* gene, additional disrupted genes, *e.g.*, *β2M*, *TRAC*, *CD70*, and further expressing a chimeric antigen receptor (CAR) can be produced by sequential targeting of the genes of interest. For example, in some embodiments, the *Reg1* gene may be disrupted first, followed by disruption of *TRAC* and *β2M* genes and CAR insertion. In other embodiments, *TRAC* and *β2M* genes may be disrupted first, followed by CAR insertion and disruption of the *Reg1* gene. Accordingly, in some embodiments, the genetically engineered T cells disclosed herein may be produced by multiple, sequential electroporation events with multiple RNPs targeting the genes of interest, e.g., *Reg1*, *β2M*, *TRAC*, *CD70,* etc.

In other embodiments, the genetically engineered CAR T cells disclosed herein may be produced by a single electroporation event with an RNP complex comprising an RNA-guided nuclease and multiple gRNAs targeting the genes of interest, *e.g.*, *Reg1*, *β2M*, *TRAC*, *CD70,* etc.

(c) Exemplary Genetically Engineered T Cells Expression a Chimeric Antigen Receptor

It should be understood that gene disruption encompasses gene modification through gene editing (*e.g.*, using CRISPR/Cas gene editing to insert or delete one or more nucleotides). A disrupted gene may contain one or more mutations (*e.g.*, insertion, deletion, or nucleotide substitution, *etc.*) relative to the wild-type counterpart so as to substantially reduce or completely eliminate the activity of the encoded gene product. The one or more mutations may be located in a non-coding region, for example, a promoter region, a regulatory region that regulates transcription or translation; or an intron region. Alternatively, the one or more mutations may be located in a coding region (*e.g.*, in an exon). In some instances, the disrupted gene does not express or expresses a substantially reduced level of the encoded protein. In other instances, the disrupted gene expresses the encoded protein in a mutated form, which is either not functional or has substantially reduced activity. In some embodiments, a disrupted gene is a gene that does not encode functional protein. In some embodiments, a cell that comprises a disrupted gene does not express (*e.g.*, at the cell surface) a detectable level (*e.g.* by antibody, *e.g.*, by flow cytometry) of the protein encoded by the gene. A cell that does not express a detectable level of the protein may be referred to as a knockout cell. For example, a cell having a *β2M* gene edit may be considered a *β2M* knockout cell if β2M protein cannot be detected at the cell surface using an antibody that specifically binds β2M protein.

In some embodiments, a population of genetically engineered T cells disclosed herein

express a CAR (*e.g.*, anti-CD19, anti-BCMA, or anti-CD70 CAR), a disrupted *Reg1* gene, a disrupted *TGFBRII* gene, a disrupted *TRAC* gene, and optionally a disrupted *β2M* gene, and optionally a disrupted *CD70* gene. The nucleotide sequence encoding the CAR may be inserted in the disrupted *TRAC* gene (*e.g.*, replacing the site targeted by a sgRNA such as TA-1). In some examples, such a population of genetically engineered T cells may comprise about 70-99% Reg1$^-$ cells, for example about 90-97% Reg1$^-$ cells, about 70-99% TGFBRII$^-$ cells, *e.g.*, for example about 80-89% TGFBRII$^-$ cells, about 70-99% TCR$^-$ cells, for example about 90-99% TCR$^-$ cells, and/or optionally about 60-99% β2M$^-$ cells, for example about 60-82% β2M$^-$ cells, and/or optionally about 70-99% CD70$^-$ cells, for example about 90-99% CD70$^-$ cells,. The cell population may also contain at least about 30%-50% (*e.g.*, at least 60%) cells expressing the CAR.

i. Anti-CD19 CAR T Cells Having *Reg1* and/or *TGFBRII* Gene Disruption

Also provided herein is population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising a disrupted *Reg1* gene, a disrupted *TGFBRII* gene, or a combination thereof, and expressing an anti-CD19 CAR, *e.g.*, those disclosed herein. In some instances, the population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, and expressing an anti-CD19 CAR, *e.g.*, those disclosed herein. In some examples, the anti-CD19 CAR-T cells disclosed herein, which express any of the anti-CD19 CAR disclosed herein (*e.g.*, the anti-CD19 CAR comprising the amino acid sequence of SEQ ID NO: 106), may also comprise a disrupted *TRAC* gene and/or a disrupted *β2M* gene as also disclosed herein.

In some examples, the population of genetically engineered T cells are anti-CD19 CAR cells that further comprise a disrupted Regnanse-1 gene. In some examples, anti-CD19 CAR cells are CD19-directed T cells having disrupted *TRAC* gene and *β2M* gene. The nucleic acid encoding the anti-CD19 CAR can be inserted in the disrupted *TRAC* gene at the site of SEQ ID NO: 69, which is replaced by the nucleic acid encoding the anti-CD19 CAR, thereby disrupting expression of the *TRAC* gene. The disrupted *TRAC* gene in the anti-CD19 CAR cells may comprise the nucleotide sequence of SEQ ID NO: 119.

Anti-CD19 CAR T cells that comprise a disrupted *Reg1* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*Reg1*, optionally *TRAC* and/or *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD19 CAR construct. CRISPR-Cas9-mediated gene editing involves at least a sgRNA

targeting Reg1 (e.g., REG1-Z03 (SEQ ID NO: 22), REG1-Z05 (SEQ ID NO: 30), REG1-Z06 (SEQ ID NO: 34) or REG1-Z10 (SEQ ID NO: 50)), and optionally TA-1 sgRNA (SEQ ID NO: 59), which targets the *TRAC* locus, and β2M-1 sgRNA (SEQ ID NO: 63), which targets the *β2M* locus. For any of the gRNA sequences provided herein, those that do not explicitly indicate modifications are meant to encompass both unmodified sequences and sequences having any suitable modifications.

Anti-CD19 CAR T cells that comprise a disrupted *TGFBRII* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII*, optionally *TRAC* and/or *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD19 CAR construct. CRISPR-Cas9-mediated gene editing involves at least a sgRNA targeting *TGFBRII* (*e.g.*, those listed in **Table 39,** for example, TGFBRII_EX1_T2, TGFBRII_EX4_T1, TGFBRII_EX4_T2, TGFBRII_EX5_T1), and optionally TA-1 sgRNA (SEQ ID NO: 59), which targets the *TRAC* locus, and β2M-1 sgRNA (SEQ ID NO: 63), which targets the *β2M* locus.

Anti-CD19 CAR T cells that comprise both a disrupted *TGFBRII* gene and a disrupted *Reg1* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII and Reg1*, optionally *TRAC* and/or *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD19 CAR construct. CRISPR-Cas9-mediated gene editing involves at least a sgRNA targeting *TGFBRII* (*e.g.*, those listed in **Table 39**) and a sgRNA targeting *Reg1* (*e.g.*, those listed in **Table 22**), optionally TA-1 sgRNA (SEQ ID NO: 59), which targets the *TRAC* locus, and β2M-1 sgRNA (SEQ ID NO: 63), which targets the *β2M* locus.

The anti-CD19 CAR T cells are composed of an anti-CD19 single-chain antibody fragment (scFv, which may comprise the amino acid sequence of SEQ ID NO: 120), followed by a CD8 hinge and transmembrane domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 97) that is fused to an intracellular co-signaling domain of CD28 (*e.g.*, SEQ ID NO: 101) and a CD3ζ signaling domain (*e.g.*, SEQ ID NO: 103). In specific examples, the anti-CD19 CAR T cells comprises the amino acid sequence of SEQ ID NO: 118.

In some embodiments, at least 30% of a population of anti-CD19 CAR T cells express a detectable level of the anti-CD19 CAR. For example, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-

CD19 CAR T cells express a detectable level of the anti-CD19 CAR.

In some embodiments, at least 50% of a population of anti-CD19 CAR T cells may not express a detectable level of β2M surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-CD19 CAR T cells may not express a detectable level of β2M surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of β2M surface protein.

Alternatively or in addition, at least 50% of a population of anti-CD19 CAR T cells may not express a detectable level of TRAC surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-CD19 CAR T cells may not express a detectable level of TRAC surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of TRAC surface protein. In specific examples, more than 90% (*e.g.*, more than 99.5%) of the anti-CD19 CAR T cells do not express a detectable TRAC surface protein.

In some embodiments, a substantial percentage of the population of anti-CD19 CAR T cells may comprise more than one gene edit, which results in a certain percentage of cells not expressing more than one gene and/or protein.

For example, at least 50% of a population of anti-CD19 CAR T cells may not express a detectable level of two surface proteins, *e.g.*, does not express a detectable level of β2M and *TRAC* proteins. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the anti-CD19 CAR T cells do not express a detectable level of TRAC and β2M surface proteins. In another example, at least 50% of a population of the anti-CD19 CAR T cells do not express a detectable level of TRAC and β2M surface proteins.

In some embodiments, the population of anti-CD19 CAR T cells may comprise more than one gene edit (*e.g.*, in more than one gene), which may be an edit described herein. For example, the population of anti-CD19 CAR T cells may comprise a disrupted *TRAC* gene *via* the CRISPR/Cas technology using the TA-1 *TRAC* gRNA. In some examples, the anti-CD19

CAR T cells may comprise a deletion in the *TRAC* gene relative to unmodified T cells. For example, the anti-CD19 CAR T cells may comprise a deletion of the fragment AGAGCAACAGTGCTGTGGCC (SEQ ID NO: 69) in the *TRAC* gene. This fragment can be replaced by the nucleic acid encoding the anti-CD19 CAR (*e.g.*, SEQ ID NO: 117).

Alternatively or in addition, the population of anti-CD19 CAR T cells may comprise a disrupted *β2M* gene *via* CRISPR/Cas9 technology using the gRNA of β2M-1. Such anti-CD19 CAR T cells may comprise Indels in the *β2M* gene, which comprise one or more of the nucleotide sequences of SEQ ID NOs: 83-88. In specific examples, anti-CD19 CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 50% $\beta2M^+$ cells, and ≤ 30% $TCR\alpha\beta^+$ cells. In additional specific examples, anti-CD19 CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 30% $\beta2M^+$ cells, and ≤ 0.5% $TCR\alpha\beta^+$ cells.

See also WO 2019/097305A2, and WO2019215500, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein.

In specific examples, the genetically engineered T cell population may be the anti-CD19 CAR T cells disclosed herein that further comprise a disrupted *Reg1* gene. The disrupted *Reg 1* gene may comprise any of the sequences provided in **Tables 29-38** below. In some examples, the anti-CD19 CAR T cells may comprise at least 80% $Reg1^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $Reg1^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-CD19 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene. In some examples, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** below. In some examples, the anti-CD19 CAR T cells may comprise at least 80% $TGFBRII^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $TGFBRII^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-CD19 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene and a disrupted *Reg1* gene. The disrupted *Reg 1* gene may comprise any of the sequences provided in **Tables 29-38** below. Alternatively or in addition, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** below. In some examples, the anti-CD19 CAR T cells may comprise at least 80% $TGFBRII^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $TGFBRII^-$ cells. Alternatively or in addition, the anti-CD19 CAR T cells may comprise at least 80% $Reg1^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above Reg^- cells. In some examples, the anti-CD19

CAR T cells may comprise at least 60% Reg1$^-$/TGFBRII$^-$ cells, for example, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or above Reg1$^-$/TGFBRII$^-$ cells.

In some examples, such a population of genetically engineered T cells may comprise about 90-97% Reg1$^-$ cells, about 80-89% TGFBRII$^-$ cells, about 90-99% TCR$^-$ cells, and/or about 60-82% β2M$^-$ cells. The cell population may also contain at least 50% (*e.g.*, at least 60%) cells expressing the anti-CD19 CAR.

ii Anti-BCMA CAR-T Cells Having *Reg1* and/or *TGFBRII* Gene Disruption

Also provided herein is population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising a disrupted *Reg1* gene and expressing an anti-BCMA CAR, e.g., those disclosed herein. In some examples, the anti-BCMA CAR T cells disclosed herein, which express any of the anti-BCMA CAR disclosed herein (*e.g.*, the anti-BCMA CAR comprising the amino acid sequence of SEQ ID NO: 146), may also comprise a disrupted *TRAC* gene and/or a disrupted *β2M* gene as also disclosed herein.

In some examples, the population of genetically engineered T cells are anti-BCMA CAR T cells that further comprise a disrupted *Reg1* gene, a disrupted *TGFBRII* gene, or a combination thereof. In some instances, the population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, and expressing an anti-BCMA CAR, *e.g.*, those disclosed herein. In some examples anti-BCMA CAR T cells are anti-BCMA CAR T cells having disrupted *TRAC* gene and *β2M* gene. The nucleic acid encoding the anti-BCMA CAR can be inserted in the disrupted *TRAC* gene at the site of SEQ ID NO: 69, which is replaced by the nucleic acid encoding the anti-BCMA CAR, thereby disrupting expression of the *TRAC* gene. The disrupted *TRAC* gene in the anti-BCMA CAR T cells may comprise the nucleotide sequence of SEQ ID NO: 145.

Anti-BCMA CAR T cells that comprise a disrupted *Reg1* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (Reg1, and optionally *TRAC* and *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-BCMA CAR construct. CRISPR-Cas9-mediated gene editing involves at least three guide RNAs (sgRNAs), as described above for anti-CD19 CAR T cells.

Anti-BCMA CAR T cells that comprise a disrupted *TGFBRII* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short

Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII*, and optionally *TRAC* and *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-BCMA CAR construct. CRISPR-Cas9-mediated gene editing involves at least three guide RNAs (sgRNAs), as described above for anti-BCMA CAR T cells.

Anti-BCMA CAR T cells that comprise a disrupted *Reg1* gene and a disrupted *TGFBRII* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII and Reg1*, and optionally *TRAC* and *β2M* genes), and adeno-associated virus (AAV) transduction to deliver the anti-BCMA CAR construct. CRISPR-Cas9-mediated gene editing involves at least three guide RNAs (sgRNAs), as described above for anti-BCMA CAR T cells.

The anti-BCMA CAR T cells are composed of an anti-BCMA single-chain antibody fragment (scFv, which may comprise the amino acid sequence of SEQ ID NO: 148), followed by a CD8 hinge and transmembrane domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 97) that is fused to an intracellular co-signaling domain of CD28 (*e.g.*, SEQ ID NO: 101) and a CD3ζ signaling domain (*e.g.*, SEQ ID NO: 103). In specific examples, the anti-BCMA CAR T cells comprises the amino acid sequence of SEQ ID NO: 146.

In some embodiments, at least 30% of a population of anti-BCMA CAR T cells express a detectable level of the anti-BCMA CAR. For example, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-BCMA CAR T cells express a detectable level of the anti-BCMA CAR.

In some embodiments, at least 50% of a population of anti-BCMA CAR T cells may not express a detectable level of β2M surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-BCMA CAR T cells may not express a detectable level of β2M surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of β2M surface protein.

Alternatively or in addition, at least 50% of a population of anti-BCMA CAR T cells may not express a detectable level of TRAC surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-BCMA CAR T cells may not express a detectable level of TRAC surface protein. In some

embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of TRAC surface protein. In specific examples, more than 90% (*e.g.*, more than 99.5%) of the anti-BCMA CAR T cells do not express a detectable TRAC surface protein.

In some embodiments, a substantial percentage of the population of anti-BCMA CAR T cells may comprise more than one gene edit, which results in a certain percentage of cells not expressing more than one gene and/or protein.

For example, at least 50% of a population of anti-BCMA CAR T cells may not express a detectable level of two surface proteins, *e.g.*, does not express a detectable level of β2M and *TRAC* proteins. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the anti-BCMA CAR T cells do not express a detectable level of TRAC and β2M surface proteins. In another example, at least 50% of a population of anti-BCMA CAR T cells do not express a detectable level of TRAC and β2M surface proteins.

In some embodiments, the population of anti-BCMA CAR T cells may comprise more than one gene edit (*e.g.*, in more than one gene), which may be an edit described herein. For example, the population of anti-BCMA CAR T cells may comprise a disrupted *TRAC* gene *via* the CRISPR/Cas technology using the TA-1 *TRAC* gRNA. In some examples, the anti-BCMA CAR T cells may comprise a deletion in the *TRAC* gene relative to unmodified T cells. For example, the anti-CD19 CAR T cells may comprise a deletion of the fragment AGAGCAACAGTGCTGTGGCC (SEQ ID NO: 69) in the *TRAC* gene. This fragment can be replaced by the nucleic acid encoding the anti-BCMA CAR (*e.g.*, SEQ ID NO: 145). Alternatively or in addition, the population of anti-BCMA CAR T cells may comprise a disrupted *β2M* gene *via* CRISPR/Cas9 technology using the gRNA of β2M-1. Such anti-BCMA CAR T cells may comprise Indels in the *β2M* gene, which comprise one or more of the nucleotide sequences of SEQ ID NOs: 83-88. In specific examples, anti-BCMA CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 50% $\beta2M^+$ cells, and ≤ 30% $TCR\alpha\beta^+$ cells. In additional specific examples, anti-BCMA CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 30% $\beta2M^+$ cells, and ≤ 0.5% $TCR\alpha\beta^+$ cells.

See also WO 2019/097305A2, and WO2019215500, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein.

In specific examples, the genetically engineered T cell population may be the anti-

BCMA CAR T cells disclosed herein that further comprise a disrupted *Reg1* gene. The disrupted *Regnase 1* (*Reg1*) gene may comprise any of the sequences provided in **Tables 29-38** below. In some examples, the anti-BCMA CAR T cells may comprise at least 80% Reg1$^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above Reg1$^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-BCMA CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene. In some examples, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** below. In some examples, the anti-BCMA CAR T cells may comprise at least 80% TGFBRII$^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above TGFBRII $^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-BCMA CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene and a disrupted *Reg1* gene. The disrupted *Reg 1* gene may comprise any of the sequences provided in **Tables 29-38** below. Alternatively or in addition, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** below. In some examples, the anti-BCMA CAR T cells may comprise at least 80% TGFBRII$^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above TGFBRII $^-$ cells. Alternatively or in addition, the anti-BCMA CAR T cells may comprise at least 80% Reg1$^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above Reg $^-$ cells. In some examples, the anti-BCMA CAR T cells may comprise at least 60% Reg1$^-$/TGFBRII$^-$ cells, for example, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or above Reg1$^-$/TGFBRII$^-$ cells.

iii. Anti-CD70 CAR-T Cells Having *Reg1* and/or *TGFBRII* Gene Disruption

Also provided herein is population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising a disrupted *Reg1* gene, a disrupted *TRFBRII* gene, or a combination thereof, and expressing anti-CD70 CAR, e.g., those disclosed herein. In some instances, the population of genetically engineered immune cells (*e.g.*, T cells such as human T cells) comprising both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, and expressing an anti-CD70 CAR, *e.g.*, those disclosed herein. In some examples, the anti-CD70 CAR T cells disclosed herein, which express any of the anti-CD70 CAR disclosed herein (*e.g.*, the anti-CD70 CAR comprising the amino acid sequence of SEQ ID NO: 138), may also comprise a disrupted *TRAC* gene, a disrupted *β2M* gene, and/or a disrupted CD70 gene as also disclosed herein.

In some examples anti-CD70 CAR T cells are anti-CD70 CAR T cells having disrupted *TRAC* gene, a disrupted *β2M* gene, and a disrupted *CD70* gene. The nucleic acid encoding the anti-CD70 CAR can be inserted in the disrupted *TRAC* gene at the site of SEQ ID NO: 69, which is replaced by the nucleic acid encoding the anti-CD70 CAR, thereby disrupting expression of the *TRAC* gene. The disrupted *TRAC* gene in the anti-CD70 CAR T cells may comprise the nucleotide sequence of SEQ ID NO: 139.

Anti-CD70 CAR T cells that comprise a disrupted *Reg1* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (Reg1, and optionally *TRAC*, *β2M* and/or *CD70* genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD70 CAR construct. CRISPR-Cas9-mediated gene editing involves at least an sgRNA targeting the *Reg 1* gene as those disclosed herein (see, *e.g.*, **Table 22**), and optionally an sgRNA (SEQ ID NO: 55) which targets the CD70 locus, TA-1 sgRNA (SEQ ID NO: 59) which targets the TRAC locus, and β2M-1 sgRNA (SEQ ID NO: 63) which targets the *β2M* locus.

Anti-CD70 CAR T cells that comprise a disrupted *TGFBRII* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII*, and optionally, *TRAC*, *β2M*, and/or CD70 genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD70 CAR construct. CRISPR-Cas9-mediated gene editing involves at least an sgRNA targeting the *TGFBRII* gene as those disclosed herein (see, *e.g.*, **Table 39**), and optionally an sgRNA (SEQ ID NO: 43) which targets the CD70 locus, TA-1 sgRNA (SEQ ID NO: 59) which targets the *TRAC* locus, and β2M-1 sgRNA (SEQ ID NO: 63) which targets the *β2M* locus.

Anti-CD70 CAR T cells that comprise a disrupted *TGFBRII* gene and a disrupted *Reg1* gene can be produced *via ex vivo* genetic modification using the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9) technology to disrupt targeted genes (*TGFBRII* and *Reg1*, and optionally, *TRAC*, *β2M*, and/or CD70 genes), and adeno-associated virus (AAV) transduction to deliver the anti-CD70 CAR construct. CRISPR-Cas9-mediated gene editing involves at least an sgRNA targeting the *TGFBRII* gene as those disclosed herein (see, *e.g.*, **Table 39**), and an sgRNA targeting the *Reg1* gene as those disclosed herein (see, *e.g.*, **Table 22**), and optionally an sgRNA (SEQ ID NO: 55) which targets the CD70 locus, TA-1 sgRNA (SEQ ID NO: 59) which targets the *TRAC* locus, and

β2M-1 sgRNA (SEQ ID NO: 63) which targets the *β2M* locus.

The anti-CD70 CAR T cells are composed of an anti-CD70 CAR single-chain antibody fragment (scFv, which may comprise the amino acid sequence of SEQ ID NO: 138), followed by a CD8 hinge and transmembrane domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 97) that is fused to an intracellular co-signaling domain of CD28 (*e.g.*, SEQ ID NO: 101) and a CD3ζ signaling domain (*e.g.*, SEQ ID NO: 103). In specific examples, the anti-CD70 CAR T cells comprise the amino acid sequence of SEQ ID NO: 138.

In some embodiments, at least 30% of a population of anti-CD70 CAR T cells express a detectable level of the anti-CD70 CAR. For example, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-CD70 CAR T cells express a detectable level of the anti-CD70 CAR.

In some embodiments, at least 50% of a population of anti-CD70 CAR T cells may not express a detectable level of *β2M* surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-CD70 CAR T cells may not express a detectable level of *β2M* surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of β2M surface protein.

Alternatively or in addition, at least 50% of a population of anti-CD70 CAR T cells may not express a detectable level of TRAC surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the anti-CD70 CAR T cells may not express a detectable level of TRAC surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of TRAC surface protein. In specific examples, more than 90% (*e.g.*, more than 99.5%) of the anti-CD70 CAR T cells do not express a detectable TRAC surface protein.

In some embodiments, at least 50% of a population of the anti-CD70 CAR T cells may not express a detectable level of CD70 surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% of the engineered T cells of a population may not express a detectable level of CD70 surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-

100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, 90%-100%, or 95%-100% of the engineered T cells of a population does not express a detectable level of CD70 surface protein.

In some embodiments, a substantial percentage of the population of anti-CD70 CAR T cells may comprise more than one gene edit, which results in a certain percentage of cells not expressing more than one gene and/or protein.

For example, at least 50% of a population of anti-CD70 CAR T cells may not express a detectable level of two surface proteins, *e.g.*, does not express a detectable level of β2M and TRAC proteins, β2M and CD70 proteins, or TRAC and CD70 proteins. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of two surface proteins. In another example, at least 50% of a population of the CTX130 cells may not express a detectable level of all of the three target surface proteins β2M, TRAC, and CD70 proteins. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of β2M, TRAC, and CD70 surface proteins.

In some embodiments, the population of anti-CD70 CAR T cells may comprise more than one gene edit (*e.g.*, in more than one gene), which may be an edit described herein. For example, the population of anti-CD70 CAR T cells may comprise a disrupted *TRAC* gene *via* the CRISPR/Cas technology using the TA-1 *TRAC* gRNA. In some examples, the anti-CD70 CAR T cells may comprise a deletion in the *TRAC* gene relative to unmodified T cells. For example, the anti-CD70 CAR T cells may comprise a deletion of the fragment AGAGCAACAGTGCTGTGGCC (SEQ ID NO: 69) in the *TRAC* gene. This fragment can be replaced by the nucleic acid encoding the anti-CD70 CAR (*e.g.*, SEQ ID NO: 139). Alternatively or in addition, the population of anti-CD70 CAR T cells may comprise a disrupted *β2M* gene *via* CRISPR/Cas9 technology using the gRNA of β2M-1. Such anti-CD70 CAR T cells may comprise indels in the *β2M* gene, which comprise one or more of the nucleotide sequences of SEQ ID NOs: 83-88. In specific examples, anti-CD70 CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 50% $\beta2M^+$ cells, and ≤ 30% $TCR\alpha\beta^+$ cells. In additional specific examples, anti-CD70 CAR T cells comprise ≥ 30% CAR^+ T cells, ≤ 30% $\beta2M^+$ cells, and ≤ 0.5% $TCR\alpha\beta^+$ cells.

See also WO 2019/097305A2, and WO2019215500, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein.

In specific examples, the genetically engineered T cell population may be the anti-CD70 CAR T cells disclosed herein that further comprise a disrupted *Reg1* gene. The disrupted *Regnase 1* gene may comprise any of the sequences provided in **Tables 22-31** below. Such a genetically engineered T cells may have ≥ 30% CAR+ T cells, ≤ 0.4% TCR^+ T cells, ≤ 30% $\beta2M^+$ T cells, and ≤ 2% $CD70^+$ T cells. In some examples, the anti-CD70 CAR T cells may comprise at least 80% $Reg1^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $Reg1^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-CD70 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene. Such a genetically engineered T cells may have ≥ 30% CAR+ T cells, ≤ 0.4% TCR^+ T cells, ≤ 30% $\beta2M^+$ T cells, and ≤ 2% $CD70^+$ T cells. In some examples, the anti-CD70 CAR T cells may comprise at least 80% $TGFBRII^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $TGFBRII^-$ cells.

In specific examples, the genetically engineered T cell population may be the anti-CD70 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene and a disrupted *Reg1* gene. The disrupted *Regnase 1* gene may comprise any of the sequences provided in **Tables 29-38** below. Alternatively or in addition, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Tables 40-48** below. Such a genetically engineered T cells may have ≥ 30% CAR+ T cells, ≤ 0.4% TCR^+ T cells, ≤ 30% $\beta2M^+$ T cells, and ≤ 2% $CD70^+$ T cells. In some examples, the anti-CD70 CAR T cells may comprise at least 80% $TGFBRII^-$ cells, for example, at least 85%, at least 90%, at least 95%, at least 98% or above $TGFBRII^-$ cells. In some examples, the anti-CD70 CAR T cells may comprise at least 60% $Reg1^-/TGFBRII^-$ cells, for example, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or above $Reg1^-/TGFBRII^-$ cells.

III. Therapeutic Applications

The therapeutic T cells generated using the genetically engineered T cells disclosed herein would be expected to maintain T cell health enabled by the disruption of the *Reg1* gene, the disruption of the *TGFBRII* gene, the disruption of the *CD70* gene, or a combination thereof. For example, maintaining T cell health may extend expansion during manufacturing, thereby

increasing yield and consistency. In another example, maintaining T cell health may rescue exhausted/unhealthy T cells, thereby enabling potentially lower doses in patients and more robust responses. Further, the disruption of the *Reg1* gene and the *TGFBRII* gene showed synergistic effects in enhancing CAR-T cell potency and *in vivo* expansion.

The therapeutic T cells disclosed herein can be administered to a subject for therapeutic purposes, for example, treatment of a solid tumor targeted by the CAR construct expressed by the therapeutic T cells.

The step of administering may include the placement (*e.g.*, transplantation) of the therapeutic T cells into a subject by a method or route that results in at least partial localization of the therapeutic T cells at a desired site, such as a tumor site, such that a desired effect(s) can be produced. Therapeutic T cells can be administered by any appropriate route that results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable. The period of viability of the cells after administration to a subject can be as short as a few hours, *e.g.*, twenty-four hours, to a few days, to as long as several years, or even the life time of the subject, *i.e.*, long-term engraftment. For example, in some aspects described herein, an effective amount of the therapeutic T cells can be administered *via* a systemic route of administration, such as an intraperitoneal or intravenous route.

In some embodiments, the therapeutic T cells are administered systemically, which refers to the administration of a population of cells other than directly into a target site, tissue, or organ, such that it enters, instead, the subject's circulatory system and, thus, is subject to metabolism and other like processes. Suitable modes of administration include injection, infusion, instillation, or ingestion. Injection includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. In some embodiments, the route is intravenous.

A subject may be any subject for whom diagnosis, treatment, or therapy is desired. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

In some instances, the therapeutic T cells may be autologous ("self") to the subject, i.e., the cells are from the same subject. Alternatively, the therapeutic T cells can be non-autologous ("non-self," *e.g.*, allogeneic, syngeneic or xenogeneic) to the subject. "Allogeneic" means that the therapeutic T cells are not derived from the subject who receives the treatment but from

different individuals (donors) of the same species as the subject. A donor is an individual who is not the subject being treated. A donor is an individual who is not the patient. In some embodiments, a donor is an individual who does not have or is not suspected of having the cancer being treated. In some embodiments, multiple donors, *e.g.*, two or more donors, are used.

In some embodiments, an engineered T cell population being administered according to the methods described herein comprises allogeneic T cells obtained from one or more donors. Allogeneic refers to a cell, cell population, or biological samples comprising cells, obtained from one or more different donors of the same species, where the genes at one or more loci are not identical to the recipient (*e.g.*, subject). For example, an engineered T cell population, being administered to a subject can be derived from one or more unrelated donors, or from one or more non-identical siblings. In some embodiments, syngeneic cell populations may be used, such as those obtained from genetically identical donors, (*e.g.*, identical twins). In some embodiments, the cells are autologous cells; that is, the engineered T cells are obtained or isolated from a subject and administered to the same subject, *i.e.*, the donor and recipient are the same.

An effective amount refers to the amount of a population of engineered T cells needed to prevent or alleviate at least one or more signs or symptoms of a medical condition (*e.g.*, cancer), and relates to a sufficient amount of a composition to provide the desired effect, *e.g.*, to treat a subject having a medical condition. An effective amount also includes an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate effective amount can be determined by one of ordinary skill in the art using routine experimentation.

Because of the enhanced persistence and efficacy of the therapeutic T cells disclosed herein, the dose of the therapeutic T cells provided herein would be lower than the standard dose of CAR-T cells prepared by conventional approaches (*e.g.*, using T cells that do not have one or more of the genetic editing events disclosed herein, including a disrupted *Reg1* gene and/or a disrupted *CD70* gene). In some examples, the effective amount of the therapeutic T cells disclosed herein may be at least 2-fold lower, at least 5-fold lower, at least 10-fold lower, at least 20-fold lower, at least 50-fold lower, or at least 100-fold lower than a standard dose of a CAR-T therapy. In some examples, an effective amount of the therapeutic T cells disclosed

herein may be less than 10^6 cells, *e.g.*, 10^5 cells, 5 x10^4 cells, 10^4 cells, 5x 10^3 cells, or 10^3 cells. In some examples described herein, the cells are expanded in culture prior to administration to a subject in need thereof.

The efficacy of a treatment using the therapeutic T cells disclosed herein can be determined by the skilled clinician. A treatment is considered "effective", if any one or all of the signs or symptoms of, as but one example, levels of functional target are altered in a beneficial manner (*e.g.*, increased by at least 10%), or other clinically accepted symptoms or markers of disease (*e.g.*, cancer) are improved or ameliorated. Efficacy can also be measured by failure of a subject to worsen as assessed by hospitalization or need for medical interventions (*e.g.*, progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in subject and includes: (1) inhibiting the disease, *e.g.*, arresting, or slowing the progression of symptoms; or (2) relieving the disease, *e.g.*, causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of symptoms.

Combination therapies are also encompassed by the present disclosure. For example, the therapeutic T cells disclosed herein may be co-used with other therapeutic agents, for treating the same indication, or for enhancing efficacy of the therapeutic T cells and/or reducing side effects of the therapeutic T cells.

IV. KITS

The present disclosure also provides kits for use in producing the genetically engineered T cells, the therapeutic T cells, and for therapeutic uses,

In some embodiments, a kit provided herein may comprise components for performing genetic edit of one or more of *Reg1* gene, *TGFBRII* gene, and *CD70* gene, and optionally a population of immune cells to which the genetic editing will be performed (*e.g.*, a leukopak). A leukopak sample may be an enriched leukapheresis product collected from peripheral blood. It typically contains a variety of blood cells including monocytes, lymphocytes, platelets, plasma, and red cells. The components for genetically editing one or more of the target genes may comprise a suitable endonuclease such as an RNA-guided endonuclease and one or more nucleic acid guides, which direct cleavage of one or more suitable genomic sites by the endonuclease. For example, the kit may comprise a Cas enzyme such as Cas 9 and one or more gRNAs targeting a *Reg1* gene, a *TGFBRII* gene, and/or a *CD70* gene. Any of the gRNAs specific to these target genes can be included in the kit. Such a kit may further comprise

components for further gene editing, for example, gRNAs and optionally additional endonucleases for editing other target genes such as *β2M* and/or *TRAC*.

In some embodiments, a kit provided herein may comprise a population of genetically engineered T cells as disclosed herein, and one or more components for producing the therapeutic T cells as also disclosed herein. Such components may comprise an endonuclease suitable for gene editing and a nucleic acid coding for a CAR construct of interest. The CAR-coding nucleic acid may be part of a donor template as disclosed herein, which may contain homologous arms flanking the CAR-coding sequence. In some instances, the donor template may be carried by a viral vector such as an AAV vector.

The kit may further comprise gRNAs specific to a *TRAC* gene for inserting the CAR-coding sequence into the *TRAC* gene. In other examples, the kit may further comprise gRNAs specific to a *β2M* gene for inserting the CAR-coding sequence into the *β2M* gene. In other examples, the kit may further comprise gRNAs specific to a *CD70* gene for inserting the CAR-coding sequence into the *CD70* gene. In yet other examples, the kit may further comprise gRNAs specific to a *Reg1* gene for inserting the CAR-coding sequence into the *Reg1* gene. In still other examples, the kit may further comprise gRNAs specific to a *TGFBRII* gene for inserting the CAR-coding sequence into the *TGFBRII* gene.

In yet other embodiments, the kit disclosed herein may comprise a population of therapeutic T cells as disclosed for the intended therapeutic purposes.

Any of the kit disclosed herein may further comprise instructions for making the therapeutic T cells, or therapeutic applications of the therapeutic T cells. In some examples, the included instructions may comprise a description of using the gene editing components to genetically engineer one or more of the target genes (*e.g.*, *Reg1*, *TGFBRII*, *CD70*, or a combination thereof). In other examples, the included instructions may comprise a description of how to introduce a nucleic acid encoding a CAR construction into the T cells for making therapeutic T cells.

Alternatively, the kit may further comprise instructions for administration of the therapeutic T cells as disclosed herein to achieve the intended activity, *e.g.*, eliminating disease cells targeted by the CAR expressed on the therapeutic T cells. The kit may further comprise a description of selecting a subject suitable for treatment based on identifying whether the subject is in need of the treatment. The instructions relating to the use of the therapeutic T cells described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages

(*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert. The label or package insert indicates that the therapeutic T cells are used for treating, delaying the onset, and/or alleviating a disease or disorder in a subject.

The kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as an infusion device for administration of the therapeutic T cells. A kit may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port.

Kits optionally may provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiment, the disclosure provides articles of manufacture comprising contents of the kits described above.

General techniques

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual,* second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P. E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D. M. Weir and C. C. Blackwell, eds.): Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos, eds., 1987); Current Protocols in Molecular Biology (F. M. Ausubel, et al. eds. 1987); PCR: The Polymerase Chain Reaction, (Mullis, et al., eds. 1994); Current Protocols in Immunology (J. E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C. A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practice approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press,

2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach,* Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.(1985»; *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984»; *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (lRL Press, (1986»; and B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

Example 1: Screening of Reg1 Targeting Site by CRISPR/Cas-Mediated Gene Editing

(A) Efficient Disruption of Reg1 by Cas9:sgRNA RNPs in T cells

The *Reg1* gene was efficiently edited in primary human T cells *ex vivo* using CRISPR/Cas9 gene editing. Genomic segments of the *Reg1* gene containing the six (6) protein coding exons were used as input in gRNA design software. Desired gRNAs were those that would lead to insertions or deletions in the coding sequence, disrupting the amino acid sequence of Reg1, leading to out of frame/loss of function allele(s) (referred to as "Reg1 knockout (KO)" alleles or "Reg1 disrupted alleles"). All ten (10) in silico-identified gRNA spacer sequences targeting the *Reg1* gene were synthesized, and the gRNAs were specifically modified, as indicated in **Table 1**. While the gRNAs used in this example were modified with 2'-O-methyl phosphorothioate modifications, unmodified gRNAs, or gRNAs with other modifications, may be used. The target sequences and gRNA sequences of the Reg1 guides Z01-Z10 are provided in **Table 22** below.

Table 1. Indel Rate of *Reg1* Gene by Ten gRNAs

Guide Name	Indel Efficiency (TIDE)
REG1-Z01	98.3%
REG1-Z02	97.2%

REG1-Z03	96.8%
REG1-Z04	92.7%
REG1-Z05	98.5%
REG1-Z06	95%
REG1-Z07	94.8%
REG1-Z08	71%
REG1-Z09	88.2%
REG1-Z10	94.9%

Primary human T cells were transfected (electroporated) with a ribonucleoprotein particle (RNP) containing Cas9 nuclease and a synthetic modified sgRNA targeting the *Reg1* gene (sequences in **Table 22**) or controls (no Cas9, no gRNA). Four (4) days post transfection, cells were subjected to a TIDE analysis to assess indel frequency.

Ten (10) gRNAs yielded measurable data by TIDE analysis, as indicated in **Table 1**. Eight (8) gRNA sequences yielded indel percentages (editing frequencies) above 90%, indicating highly efficient gene editing.

Four gRNAs which target either exon 2 or 4 were selected for subsequent studies (REG1-Z03, REG1-Z05, REG1-Z06 and REG1-Z10, which showed 96.8%, 98.5%, 95% and 94.9% editing rate of Reg1, respectively as shown in (**Table 1**).

(B) On-Target and Off-Target Editing of REG1 Guide RNAs

On-target and off-target editing efficiencies of various REG1-targeting gRNAs noted above were examined following the method disclosed in the above section. Briefly, activated T cells were transfected (electroporated) with a ribonucleoprotein particle (RNP) containing Cas9 nuclease and a synthetic modified sgRNA targeting the *Reg1* gene (sequences in **Table 22** below) or controls (no Cas9, no gRNA).

For genomic on- and off-target assessment, these electroporation methods were used to generate two cell populations of edited cells from two different donor T cells (termed 1 and 2). Cells were gene edited with each of the ten guides noted above, and then collected ten (10) days post transfection. These samples were analyzed with hybrid capture, a homology-dependent method to enrich on- and off-target sites, combined with next-generation sequencing. Briefly, on- and off-target sites with homology to each gRNA target site were identified computationally, single-stranded RNA probes were used to enrich these sites from

bulk genomic DNA, these enriched sites were sequenced with next-generation sequencing, and the data were analyzed for insertions and deletions (indels) indicating repair following CRISPR editing.

(i) Analysis of on-target indel profiles in T cells

The data used to quantify off-target editing were also used to quantify and summarize the most frequent on-target indels for all Reg1 guides listed in **Table 22**. This data was generated from hybrid capture of the Reg1 locus combined with next-generation sequencing in two donors (termed Donor 1 and Donor 2).

Following gene editing, hybrid capture analysis of the Reg1 locus in a population of T cells following CRISPR/Cas9 gene editing to produce Reg1 KO T cells results in specific indel frequencies and edited gene sequences at the *Reg1* locus (**Tables 29-38**; deletions as dashes and insertions in bold).

For the purposes of individual sequence quantification from hybrid capture data, sequence reads aligning across the Regnase 1 on-target site, 20 bp upstream and downstream of the cut site, were selected and considered for indel sequence quantification. From the selected reads, the sequence within 10 bp upstream and downstream of each putative cut site (~3bp upstream of the PAM (Jinek, et al., Science 2012) was quantified as a representative region of on-target non-homologous end joining (NHEJ) editing.

Table 2 below shows the on and off target editing results (from two donors) of exemplary Reg1 gRNAs obtained by the hybrid capture assay disclosed herein.

Table 2. On and Off Target Results by Hybrid Capture

Guide	Number of predicted off target sites tested	On-target mean editing hyb cap	Detected off-targets
REG1-Z01	35	97.0%	1 0.75% off-target; 1 0.25% off-target
REG1-Z02	27	97.7%	No off-target editing detected
REG1-Z03	52	99.0%	1 5.0% off-target; 1 0.6% off-target; 1 0.4% off-target; 1 0.3% off-target; 1 0.2% off-target
REG1-Z04	6	97.0%	No off-target editing detected
REG1-Z05	14	98.6%	No off-target editing detected

Guide	Number of predicted off target sites tested	On-target mean editing hyb cap	Detected off-targets
REG1-Z06	1	94.2%	No off-target editing detected
REG1-Z07	16	94.2%	1 0.2% off-target
REG1-Z08	6	53.8%	No off-target editing detected
REG1-Z)9	6	86.2%	No off-target editing detected
REG1-Z10	14	98.2%	No off-target editing detected

On-target gene edited sequences by the exemplary Reg1 gRNAs are presented in **Tables 29-38** below, with the frequencies of these sequences representing the percent of all sequences spanning the on-target site within 20 bp upstream and downstream of each cut site. The indels for each guide are shown relative to an on-target reference sequence in **Tables 29-38**. The reference sequence is centered on the cleavage site with 10 bp in either direction, ending 4 bp 3′ of the PAM.

Example 2: *Regnase 1* Disruption Improves CAR-T Cell Expansion

Using T cells expressing an anti-CD70 CAR disclosed herein as an example, this study demonstrated that knocking out Reg1 in the CAR-T cells resulted in enhanced *in vitro* CAR-T cell culture expansion.

Allogeneic human T cells that lack expression of the *TRAC* gene, β2M gene, CD70 gene, and Regnase-1 gene, and express a chimeric antigen receptor (CAR) targeting CD70 were produced. Briefly, activated human T cells were first isolated and then Cas9:sgRNA RNPs (1 µM Cas9, 5 µM gRNA) were delivered to the activated human T cells by electroporation, followed by incubation with recombinant adeno-associated adenoviral vectors (AAVs), serotype 6 (AAV6) (MOI 50,000). The nucleofection mix contained the Nucleofector™ Solution, 5x10^6 cells, 1 µM Cas9, and 5 µM gRNA (as described in Hendel *et al.*, *Nat Biotechnol.* 2015; 33(9):985-989, PMID: 26121415). The RNP complex comprised Cas9 and sgRNA targeting the *TRAC*, *B2M*, and *CD70* (shown in **Table 23**) and optionally *Regnase-1* genes (using the REG1-Z01 to REG1-Z10 sgRNAs shown in **Table 22**). The rAAV vector included the nucleotide sequence encoding an anti-CD70 CAR (the donor template in SEQ ID NO: 169, encoding an anti-CD70 CAR amino acid sequence of SEQ ID NO: 138).

To assess the ability of anti-CD70 CAR T cells to expand in cytokine containing media (IL-2+IL-7), anti-CD70 CAR T cells were utilized. Specifically, 2.5 to 3.8 x10^6 total anti-CD70

CAR T cells comprising a quadruple disruption (TRAC-/β2M-/CD70-/Reg1-) were generated and compared to anti-CD70 CAR T cells with unedited Reg1 (TRAC-/β2M-/CD70-).

Cells were plated and allowed to grow in flasks with cytokine containing media. Every 3-4 days the total number of cells were enumerated and re-plated as needed. This process was repeated each week for a total of 21 days. Allogeneic anti-CD70 CAR-T cells containing a disruption in the *Reg1* gene show an increase in cell expansion after 21 days (**FIG. 1A**). Reg1 guides REG1-Z01, REG1-Z03, REG1-Z07, REG1-Z09, and REG1-Z10 appear to have a greater effect on cell expansion than cells made using Reg1 guides REG1-Z02 or REG1-Z08.

In a second experiment, Reg1 guide REG1-Z10 was used in CAR T cells made from a different T cell donor in replicates by two operators (labelled A and B). The effect of increased cell culture expansion was demonstrated again. The increase in cell expansion can be seen as early as day 13 and continues throughout the experiment to day 52 (**FIG. 1B**). Furthermore, anti-CD70 CAR-T cells containing a Reg1 disruption are maintained over a longer time in culture (at least up to day 52) as compared to anti-CD70 CAR-T cells with an unedited *Regnase 1* gene, one of which was no longer viable on day 26. Collectively, these data show that disruption of the *Reg1* gene results in greater cell culture yields and longer cell maintenance in culture as compared to CAR T cells with an unedited *Reg1* gene.

Example 3: Cell Killing Function of Anti-CD70 CAR T Cells with *Reg1* Disruption.

Allogeneic human T cells that lack expression of the *TRAC* gene, *β2M* gene and *CD70* gene, and express a chimeric antigen receptor (CAR) targeting CD70 were produced. The edited CAR T cells further comprised knock out of *Reg1* gene. As in the examples above, activated human T cells we electroporated with Cas9:sgRNA RNPs (1 μM Cas9, 5 μM gRNA), followed by incubation with a recombinant adeno-associated adenoviral vectors, serotype 6 (AAV6) (MOI 50, 000).

Recombinant AAV comprised the nucleotide sequence of SEQ ID NO: 169 (encoding anti-CD70 CAR comprising the amino acid sequence of SEQ ID NO: 138). The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *CD70* (SEQ ID NO: 54), and optionally *Reg1* (e.g., REG1-Z03, Z05, Z06, and Z10; see **Table 22** and **FIGs. 2A** to **2E**).

At time points of one week and one month post-electroporation, T cells were checked for CAR expression by flow cytometry. Both anti-CD70 CAR T cells and anti-CD70 CAR T cells that lack *Reg1* (using four gRNAs REG1-Z03, Z05, Z06, Z10) expressed nearly equivalent amount of CAR on their surface at day 7 (85.6% and 81.8%, 80%, 84.4%, 85.6%)

and day 32 (97.6% and 90.7%, 91.5%, 92.6%, 93.2%) post HDR.

Cell killing function of anti-CD70 CAR T cells with Regnase-1 (Reg1) disruption

A cell killing assay was used to assess the ability of the TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+ cells to kill CD70+ adherent renal cell carcinoma (RCC)-derived cell lines (ACHN, Caki-1, and/or 769P cell lines). Adherent cells were seeded in 96-well plates at 50,000 cells per well and incubated overnight at 37 °C. The next day edited anti-CD70 CAR T cells (cultured until day 12 post HDR or day 27 post HDR) were added to the wells containing target cells at 1:1, 2:1 or 1.5:1 CAR T:Target cell ratios. After 24 hours co-culture, CAR T cells were removed from the culture by aspiration and 100 µL Cell titer-Glo (Promega) was added to each well of the plate to assess the number of remaining viable target cells. The amount of light emitted per well was then quantified using a plate reader.

Cells with *Reg1* disruption exhibited a more potent cell killing of RCC-derived cells following 24-hour co-incubation. The anti-CD70 CAR T cells at day 12 post HDR (**FIG. 2A** and **2B**) demonstrated slightly higher potency when Reg1 was knocked out, and much higher potency at day 27 post HDR (**FIGs. 2C, 2D**, and **2E**). This suggests that knocking-out the *Reg1* gene gives a maintained/persistent higher cell kill potency to anti-CD70 CAR+ T cells over time post HDR. This finding was consistent across the three tumor lines from Renal cell carcinoma tumor lines. CD70 CAR+ T cells with Reg1 disruption using gRNAs REG1-Z03, REG1-Z05, REG1- Z10 gave a higher persistent potency than when using gRNA REG1-Z06. CAR-T cells with Reg1 disruption demonstrated a visible increased in potency after 24h co-culture with caki-1 (**FIGs. 2A**, **2B**, and **2C**) and ACHN (**FIG. 2D**), and after 6 hours co-culture with 769P (difference not visible anymore after 24h) (**FIG. 2E**).

While CAR-T cells with or without the Regnase KO show similar efficacy at Day 13 post HDR, efficacy appears to be diminished in older cells (Day 19 and Day 26) without the Regnase KO. Surprisingly, TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+ cells still retain the ability to kill with similar activity ACHN and Caki-1 cells in culture (**FIGs. 6A** and **6B**).

This suggests that disrupting the *Reg1* gene gives a persistent activity and higher cell kill potency to CAR+ T cells over a longer period of time post HDR editing.

Example 4: Effect of Regnase-1 (Reg1) Disruption on Exhaustion Marker Expression

The levels of the T cell exhaustion markers were assessed on TRAC-/β2M-/CD70-/anti-CD70 CAR+ and TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+ cells. CD4+ and CD8+ T cells were assessed for PD-1 expression (**FIGs. 3A** and **3B**) and TIM3 expression (**FIGs. 3C** and

3D) by flow cytometry at Day 13 (**FIGs. 3A** and **3C**) and Day 26 (**FIGs. 3B** and **3D**) post HDR.

The data demonstrate that Reg1 KO (using Z10 guide as an example) reduces exhaustion marker expression in CAR T cells at all time points measured. The data demonstrate that knocking out Reg1 could reduce the potential exhaustion of CD8+ and CD4+ gene edited populations of CAR+ T cells leading to better therapeutics.

Example 5: *Regnase-1* (*Reg1*) Disruption Increases the Proportion of Central Memory Cells in CAR T Cells Population

Upon activation of antigen peptides presented by antigen-presenting cells, native T cells differentiate to various types of T cells in the order of T stem cell memory (T_{SCM}), T central memory cell (T_{CM}), T effector memory cell ($_{TEM}$), and T effector cell (T_{EFF}). Exemplary surface markers of T cells at different differentiation stages are provided below. T_{CM} cells have been associated with T cell long term persistence in vivo: CD8+ clones isolated from T_{CM} cells were shown to persist long term in vivo during adoptive T cell transfer in non-human primates while clones isolated from effector cells did not. (Berger et al., J. Clin. Investig. (2008) 118:294-305). Representative cell surface markers of the various types of T cells are provided in **Table 3** below.

Table 3. Representative Cell Surface Markers of Various Types of T Cells

	Naïve	Stem Central Memory	Central Memory	Effector Memory
CD27	+	+	+	-
CD45RO	-	-	+	+
CD45RA	+	+	-	-
CD62L	+	+	+	-
CD95	-	+	+	+

The levels of CD27 and CD45 RO T central memory T cell markers were assessed on TRAC-/β2M-/CD70-/anti-CD70 CAR+ and TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+ cells. Cells were stained using commercial antibodies for CD27 (Biolegend, clone M-T271) and CD45 RO (Biolegend, clone UCHL1) and analyzed by flow cytometry.

CAR-T cells with Reg1 knock out were more likely to exhibit central memory T cell identity (double positive for CD27 and CD45 RO) and less likely to exhibit effector memory cell identity (identified as CD27- and CD45 RO+), as shown in **Table 4**.

Table 4. Central memory and effector memory T cell markers in cells with and without Reg1 KO

Experiment	Cells	CD27+ / CD45 RO+ Central memory cells	CD27-/ CD45 RO+ Effector memory cells
1	TRAC-/β2M-/anti-CD70 CAR+	62.3%	30%
	TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+	82.3%	15.1%
2	TRAC-/β2M-/anti-CD70 CAR+	61.8%	27.5%
	TRAC-/β2M-/CD70-/Reg1-/anti-CD70 CAR+	74.3%	22.2%

The results obtained from this study indicate that Reg1 disruption led to an enhanced level of T_{CM} cells in the total T cell population compared to the Reg1 WT counterparts, indicating that Reg1 disruption could increase T cell long term persistence in vivo, which would benefit CAR-T therapy.

Example 6: *Reg1* Disruption Does Not Affect Cytokine Dependency of CAR T cells

To determine whether the gene editing resulted in unwanted off-target editing that could generate cells with adverse properties, such as uncontrolled cell growth, the ability of TRAC-/β2M-/anti-CD19 CAR+ and TRAC-/β2M-/Reg1-/anti-CD19 CAR+ cells to grow in the absence of cytokines and/or serum was assessed. $5x10^6$ cells were plated approximately 2 weeks post cell production (Day 0) in 10 mL of full media containing IL2, IL7 and human serum, or in serum-containing media lacking cytokines (IL-2 and IL-7). Fresh full media or media lacking cytokines were added to the respective cultures once per week. The volume of media added allowed for the cultures to maintain a density of approximately 1-2 million cells/mL. If the cell density was below 1 million cells/mL, media was not added to the cultures. The number of viable cells were enumerated twice weekly until 40 days post plating. TRAC-/β2M-/anti-CD19 CAR+ or TRAC-/β2M-/Reg1-/anti-CD19 CAR+ were no longer detectable at 40 days in the cultures that lacked cytokines, indicating that any potential off-target effects due to genome editing did not induce growth factor independent growth/proliferation to the cells (**FIG. 4**). The cells only proliferated in the presence of cytokines (full media that contains cytokines) and did not proliferate in the presence of serum alone. Thus, genome editing did not induce any adverse events that allow the cells to grow in the absence of cytokine, growth factor or antigen stimulation.

Example 7: *In vivo* effect of Reg1 KO on allogeneic CAR T Cells in the Intravenous Disseminated Nalm-6 Human Acute Lymphoblastic Leukemia Tumor Xenograft Model

A disseminated mouse model was utilized to further assess the *in vivo* efficacy of allogeneic CAR T cells lacking *β2M* and *TRAC*, as well as *Reg1*. The intravenous disseminated model (disseminated model) utilized CD19+ B-ALL derived Nalm-6 Human Acute Lymphoblastic Leukemia tumor cell line in NOG mice to demonstrate the efficacy of TRAC-/β2M-/anti-CD19 CAR+ T cells (anti-CD19 CAR T cells) with or without editing of the *Reg1* locus. The *Reg1* gene was edited *via* CRISPR/Cas-mediated gene editing using REG1-Z10 guide RNA (see **Table 22**). The anti-CD19 CAR T cells express an anti-CD19 CAR comprising the amino acid sequence of SEQ ID NO: 118. See also the sequence **Tables 27** and **28** below, and WO2019/097305, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

Efficacy of the anti-CD19 CAR T cells was evaluated in the disseminated model using methods employed by Translations Drug Development, LLC (Scottsdale, AZ) and described herein. In brief, 25, 5-8 week old female CIEA NOG (NOD.Cg-PrkdcscidI12rgtm1Sug/ JicTac) mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. At the start of the study, the mice were divided into 5 treatment groups as shown in **Table 5**. The mice were inoculated with Nalm6-Fluc-GFP (Nalm6-Fluc-Neo/eGFP--Puro) cells intravenously to model disseminated disease. On Day 1, all mice received an intravenous injection of $0.5x10^6$ Nalm6 cells/mouse. On Day 4, Groups 2-5 received an intravenous injection of CAR T cells ($4x10^6$ CAR+ cells/mouse) as indicated in **Table 5**.

Table 5. Treatment groups for intravenous disseminated disease study

Group	Nalm6 tumor cells $0.5x10^6$ cells/mouse	CAR T cells (i.v.) $4x10^6$ cells/mouse	N
1	X	NA	5
2	X	anti-CD19 CAR/TRAC-/β2M- (4e6 CAR+)	5
3	X	anti-CD19 CAR/TRAC-/β2M- (8e6 CAR+)	5
4	X	anti-CD19 CAR/TRAC-/β2M-/Reg1- (4e6 CAR+)	5
5	X	anti-CD19 CAR/TRAC-/β2M-/Reg1- (8e6 CAR+)	5

During the course of the study, the mice were monitored daily and body weight was measured two times weekly. Bioluminescence (BLI; total ROI, photon/s) was measured twice weekly beginning on Day 4 of the study. A significant endpoint was the time to peri-morbidity and the effect of T-cell engraftment was also assessed. The percentage of animal mortality and

time to death were recorded for every group in the study. Mice were euthanized prior to reaching a moribund state. Mice may be defined as moribund and sacrificed if one or more of the following criteria were met:

Loss of body weight of 20% or greater sustained for a period of greater than 1 week;

Tumors that inhibit normal physiological function such as eating, drinking, mobility and ability to urinate and or defecate;

Prolonged, excessive diarrhea leading to excessive weight loss (>20%); or

Persistent wheezing and respiratory distress.

Animals were also considered moribund if there was prolonged or excessive pain or distress as defined by clinical observations such as: prostration, hunched posture, paralysis/paresis, distended abdomen, ulcerations, abscesses, seizures and/or hemorrhages.

In vivo Survival Rate

Mice in groups receiving TRAC-/β2M-/anti-CD19 CAR+ T cells with or without an additional Reg1 disruption exhibited an increase in survival relative to mice in the untreated group (Group 1). Mice receiving either dose of TRAC-/β2M-/Reg1-/anti-CD19 CAR+ T cells exhibited increased survival in comparison to TRAC-/β2M-/anti-CD19 CAR+ T cells at each respective dose (**FIGs. 5A** and **5B**). In addition, mice receiving either dose of TRAC-/β2M-/Reg1-/anti-CD19 CAR+ T cells had reduced leukemia burdens as indicated by diminished bioluminescence signal in comparison to TRAC-/β2M-/anti-CD19 CAR+ T cells at each respective dose (**FIGs. 5C** and **5D**).

These data demonstrate that the Reg1 disruption in CAR T cells increases efficacy of CAR T cells *in vivo*, decreasing tumor burden and increasing survival.

Example 8: Efficient Disruption of *TGFBRII* by Cas9:sgRNA RNPs in T Cells

This example describes efficient editing of the *TGFBRII* gene in primary human T cells *ex vivo* using CRISPR/Cas9 gene editing. Genomic segments of the *TGFBRII* gene containing the first five (5) protein coding exons were used as input in gRNA design software. The genomic segments also included flanking splice site acceptor/donor sequences. Desired gRNAs were those that would lead to insertions or deletions in the coding sequence, disrupting the amino acid sequence of TFBRII, leading to out of frame/loss of function allele(s) (referred to as "TGFBRII knockout alleles" or "TGFBRII disrupted alleles"). Eight (8) in silico-identified gRNA spacer sequences targeting the CD70 gene were synthesized, and the gRNAs were specifically modified, as indicated in **Table 39** and **FIGs. 7A** and **7B.** While the modified

gRNAs in **Table 39** were modified with 2'-O-methyl phosphorothioate modifications, unmodified gRNAs, or gRNAs with other modifications, can be used.

Primary human T cells were transfected (electroporated) with a ribonucleoprotein particle (RNP) containing Cas9 nuclease and a synthetic modified sgRNA targeting the *TGFBRII* gene (sequences in **Table 39**) or controls (no Cas9, no gRNA). Four to six (4-6) days post transfection, cells were: (1) subjected to a TIDE analysis to assess indel frequency, and (2) processed by western blot (primary antibody: anti-human TGFBRII antibody, clone# 16H2L4) to assess TGFBRII expression levels at the cell surface (**Figure 7B**).

Eight (8) gRNAs yielded measurable data by TIDE analysis, as indicated in **FIG. 7A**. Seven (7) gRNA sequences yielded indel percentages (editing frequencies) above 80% indicating highly efficient gene editing (**FIG. 7A**). The level of TGFBRII protein expression was assessed by western blot to confirm the TIDE analysis data and GAPDH was used as a loading control. Seven (7) of the gRNAs showed nearly complete knock out of TGFBRII on the T cells **(FIG. 7B)**.

On-Target and Off-Target Editing of TGFBRII Guide RNAs

On-target and off-target editing efficiencies of various TGFBRII-targeting gRNAs noted above were examined following the method disclosed in the above section. Briefly, activated T cells were transfected (electroporated) with a ribonucleoprotein particle (RNP) containing Cas9 nuclease and a synthetic modified sgRNA targeting the *TGFBRII* gene (sequences in **Table 39** below) or controls (no Cas9, no gRNA).

For genomic on- and off-target assessment, these electroporation methods were used to generate two cell populations of edited cells from two different donor T cells. Cells were gene edited with each of the nine guides noted in **Table 39** and then collected ten (10) days post transfection. These samples were analyzed with hybrid capture, a homology-dependent method to enrich on- and off-target sites, combined with next-generation sequencing. Briefly, on- and off-target sites with homology to each gRNA target site were identified computationally, single-stranded RNA probes were used to enrich these sites from bulk genomic DNA, these enriched sites were sequenced with next-generation sequencing, and the data were analyzed for insertions and deletions (indels) indicating repair following CRISPR editing.

Five (5) gRNAs showed no off-target effect with an on-target editing rate greater than 85%, which includes TGFBRII_Ex1_T1, TGFBRII-Ex1-T2, TGFBRII_Ex1_T3, TGFBRII_Ex2_T1 and TGFBRII_Ex5_T1 as shown in **Table 6** below.

Table 6. On-Targeting Editing Efficiency and Off-Target Effects of Anti-TGFBRII gRNAs

Guide	gRNA target sequence + (PAM)	Number of predicted off-target sites tested	On-target mean editing hyb cap	Detected off-targets
TGFBRII-Ex1-T1	CCGACTTCTGAACGTGCGGT (GGG) (SEQ ID NO: 2)	7	86.80%	None
TGFBRII-Ex1-T2	TGCTGGCGATACGCGTCCAC (AGG) (SEQ ID NO: 3)	8	98.30%	None
TGFBRII-Ex1-T3	TCGGTCTATGACGAGCAGCG (GGG) (SEQ ID NO: 4)	7	99.60%	None
TGFBRII-Ex2-T1	ATGGGCAGTCCTATTACAGC (TGG) (SEQ ID NO: 5)	82	96.00%	None
TGFBRII-Ex3-T1	ATTGTTCACTTGTTAGCCCC (AGG) (SEQ ID NO: 6)	83	98.50%	One < 1% off-target
TGFBRII-Ex3-T2	GCTGAAGAACTGCCTCTATA (TGG) (SEQ ID NO: 7)	133	98.10%	One 1-10% off-target
TGFBRII-Ex4-T1	GCAGGATTTCTGGTTGTCAC (AGG) (SEQ ID NO: 8)	222	98.80%	One < 1% off-target
TGFBRII-Ex4-T2	CTCCATCTGTGAGAAGCCAC (AGG) (SEQ ID NO: 9)	255	99.40%	Four < 1% off-targets
TGFBRII-Ex5-T1	CCCCTACCATGACTTTATTC (TGG) (SEQ ID NO: 10)	85	94.20%	None

Tables 29-38 list potential indel sequences that may be generated by the gRNAs disclosed herein (deletions as dashes and insertions in bold).

Example 9: Generation of Genetically Modified T Cells That Lack TGFBRII Expression and Are Resistant to TGF-β

This example describes the production of CAR T cells that lack expression of TGFBRII and the assessment of the effect of TGF-β on CAR T cell expansion with TGFBRII KO cells grown in complete media (X-Vvivo 15 supplemented with IL-2 and IL-7).

Briefly, human T cells were first isolated and Cas9:sgRNA RNPs (1 µM Cas9, 5 µM gRNA) were delivered to activated human T cells by electroporation, followed by incubation with the recombinant adeno-associated adenoviral vectors (AAVs), serotype 6 (AAV6) (MOI 50,000). The nucleofection mix contained the Nucleofector™ Solution, $5x10^6$ cells, 1 µM Cas9, and 5 µM gRNA (as described in Hendel *et al.*, *Nat Biotechnol.* 2015; 33(9):985-989, PMID: 26121415). The RNP complex comprised Cas9 and sgRNA targeting the *TRAC*, *B2M*,

CD70, and optionally *TGFBRII* genes (sgRNA sequences are shown in **Table 23** and **Tables 39**, SEQ ID NOs: 58, 62, 54, and 301, respectively). The rAAV vector included the nucleotide sequence encoding an anti-CD70 CAR (the donor template in SEQ ID NO: 169 and the anti-CD70 CAR amino acid sequence of SEQ ID NO: 138.

About one week post-electroporation, CAR T cells with an intact (i.e.: wild-type or non-engineered counterpart) *TGFBRII* gene were exposed to varying amounts recombinant human TGF-β (10, 20, 50 and 100 ng/ml) and cell expansion was recorded over time. TGF-β significantly inhibited CAR T expansion, a concentration as low as 10 ng/ml was sufficient to reduce CAR T expansion in cells with an intact *TGFBRII* gene (**FIG. 8A**).

In another study, anti-CD70 CAR T cells with TGFBRII disruption were incubated with or without 50 ng/ml of recombinant human TGF-β, and the T cell expansion was monitored at day 2 and day 8 post-incubation with TGF-β and compared to mock cells. Mock cells (**FIG. 8B**) were anti-CD70 CAR T cells that did not have a disrupted TGFBRII gene. As shown in **FIGs. 8C-8K**, T cells with *TGFBRII* knocked-out were protected against the inhibitory effect of TGF-β on T cell expansion. The extent of protection varied with the sgRNA used to disrupt the TGFBRII gene. T cells that were transfected with gRNA targeting exon 1, 4 and 5 (TGFBRII_EX1_T2, TGFBRII_EX4_T1, TGFBRII_EX4_T2, TGFBRII_EX5_T1) showed the most resistance against a TGF-β inhibitory effect. Sequences of these gRNAs are provided in **Table 39** below.

Example 10: Cell Killing Function of Anti-CD70 CAR T Cells with *TGFBRII* Disruption.

This example describes the production of allogeneic human T cells that lack expression of the *TRAC* gene, *β2M* gene and *CD70* gene, and express a chimeric antigen receptor (CAR) targeting CD70. The edited CAR T cells further comprised knock out of the *TGFBRII* gene. As in the examples above, activated human T cells were electroporated with Cas9:sgRNA RNPs (1 μM Cas9, 5 μM gRNA), followed by incubation with a recombinant adeno-associated adenoviral vectors, serotype 6 (AAV6) (MOI 50, 000).

Recombinant AAV comprised the nucleotide sequence of SEQ ID NO: 169 (encoding anti-CD70 CAR comprising the amino acid sequence of SEQ ID NO: 138). The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *CD70* (SEQ ID NO: 54) and *TGFBRII* (SEQ ID NO: 301).

About one week post-electroporation, T cells were checked for CAR expression by flow cytometry. Both anti-CD70 CAR T cells and anti-CD70 CAR T cells lacking TGFBRII expressed nearly equivalent amount of CAR on their surface (71.5% CAR^+ cells versus 73.7%

CAR^+ cells).

A cell killing assay was used to assess the ability of the TRAC-/β2M-/CD70-/TGFBRII-/anti-CD70 CAR+ cells to kill a CD70+ adherent renal cell carcinoma (RCC)-derived cell line (A498 cells). Adherent cells were seeded in 96-well plates at 50,000 cells per well and left overnight at 37 °C. The next day edited anti-CD70 CAR T cells were added to the wells containing target cells at 0.05:1 or 0.1:1 CAR T:T cell (E:T) ratios. After the indicated incubation period, CAR T cells were removed from the culture by aspiration and 100 µL Cell titer-Glo (Promega) was added to each well of the plate to assess the number of remaining viable cells. The amount of light emitted per well was then quantified using a plate reader. Cells with TGFBRII knock out exhibited a more potent cell killing of RCC-derived cells following 24-hour co-incubation. The anti-CD70 CAR T cells demonstrated higher potency when TGFBRII was knocked out, which is clearly visible at two T cell: A498 ratios (0.05:1 and 0.1:1) (**FIG. 9**). This suggests that knocking-out the TGFBRII gene gives a higher cell kill potency to anti-CD70 CAR+ T cells. This finding was consistent across a wide panel of tumor lines from different tissues as shown in **FIGs. 10A-10E**. Knocking-out the TGFBRII gene enhances the cell killing capacity of anti-CD70 CAR T cells against 786-O and CAKI-1 (Renal cell carcinoma tumor lines), H1975 (Non-small cell lung cancer), Hs-766T (Pancreatic carcinoma) and SK-OV3 (Ovarian cancer) (**FIGs. 10A-10E**).

In another study, anti-CD70 CAR T was incubated with 50 ng/ml of recombinant human TGF-β for 24 hours and the expression of CD25 (IL-2R) on cell surface was assessed by flow cytometry. As shown in **FIG. 11**, anti-CD70 CAR T cells are susceptible to the inhibitory effect of TGF-β that causes downregulation of CD25. CD25 is an activation marker and involved in T cell proliferation. When the *TGFBRII* gene was knocked out, these cells become resistant to TGF-β and the CAR T cells retain activity and CD25 expression.

Also, when the cell kill of target cells (A498) was repeated in presence of 1, 10 and 50 ng/ml of recombinant human TGF-β. Anti-CD70 CAR T cells were adversely affected by presence of TGF-β as demonstrated by reduction in the cell kill capacity by CAR T cells with an intact TGFBRII gene (**FIG. 12**). However, anti-CD70 CAR T cells with a *TGFBRII* KO (anti-CD70 CAR+TGFBRII_EX4_T1) did not exhibit reduced cell killing ability in the presence of TGF-β (**FIG. 12**). In addition, T cell proliferation upon exposure to target antigen and effector cytokines production (IFN-γ and IL-2) were reduced in the presence of TGF-β (**FIGs. 13A- 13C**). However, when the cells lacked the expression of TGFBRII, they we were completely protected against TGF-β inhibitory effects, also shown in **FIGs. 13A-13C**. This

suggests that knocking out *TGFBRII* on the surface of CAR T cells protects the CAR T cells from the adverse effect of TGF-β in the tumor microenvironment.

Example 11: Generation of Anti-CD70 CAR T Cells That Lack TGFBRII Expression and Are Resistant to the Inhibitory Effect of Fibroblasts

This example describes the production of allogeneic human T cells that lack expression of the *TRAC* gene, *β2M* gene and *CD70* gene, and express a chimeric antigen receptor (CAR) targeting CD70 and how they are susceptible to the inhibitory effect of fibroblasts, which are a major component of solid tumor microenvironment (TME). The edited CAR T cells further comprised knock out of the *TGFBRII* gene. As in the examples above, activated human T cells we electroporated with a recombinant adeno-associated adenoviral vectors, serotype 6 (AAV6) (MOI 50, 000), and Cas9:sgRNA RNPs (1 µM Cas9, 5 µM gRNA).

Recombinant AAV comprised the nucleotide sequence of SEQ ID NO: 169 (encoding anti-CD70 CAR comprising the amino acid sequence of SEQ ID NO: 138). The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *CD70* (SEQ ID NO: 54) and *TGFBRII* (SEQ ID NO: 301).

A cell killing assay was used to assess the inhibitory effect of fibroblast on anti-CD70 CAR T cells to kill CD70+ adherent tumor cell lines: H1975 (Non-small cell lung cancer), Hs-766T (Pancreatic carcinoma), or SK-OV3 (Ovarian cancer). The cell kill assay was performed as described in example 3. Briefly, Adherent cells were seeded in 96-well plates at 50,000 cells per well and left overnight at 37 °C and the fibroblast cells (LL 86 (LeSa) ATCC® CCL-190™) were added to the top chamber of a transwell plate without direct contact with target cells. The next day edited anti-CD70 CAR T cells were added to the wells containing target cells. After the indicated incubation period, CAR T cells were removed from the culture by aspiration and 100 µL Cell titer-Glo (Promega) was added to each well of the plate to assess the number of remaining viable cells. The amount of light emitted per well was then quantified using a plate reader. As shown in **FIG. 14**, the presence of the fibroblast cells on the top chamber led to a decrease of the cell kill capacity of anti-CD70 CAR T cells against the target cells which might suggest that these fibroblast secreted a factor that decrease anti-CD70 CAR T killing effect.

This finding was confirmed when this experiment was repeated with the presence of conditioned media from the fibroblast instead on the cells and similar inhibition was observed. Briefly, $1x10^6$ CCL-190 fibroblast cells we seeded/0.5ml in a 24 well plate and incubated overnight and supernatants were collected. A cell kill assay as previously described was carried

out with anti-CD70 CAR T cells and tumors cells at a ratio of 0.1:1 effector to target cell ratio, in the presence or absence of fibroblast supernatant and incubated overnight. Cell kill was measured using the CellTiter-Glo® Luminescent Cell Viability Assay. This experiment confirms that fibroblasts secrete a factor that causes a reduction in the killing capacity of anti-CD70 CAR T cells. Disruption of the *TGFBRII* gene on the surface of anti-CD70 CAR T protected these cells against this inhibitory effect. The *TGFBRII* KO improved the cell killing ability of anti-CD70 CAR T cells against pancreatic tumor cells, Hs-766T (**FIG. 15A**), kidney tumor cells, A498 (**FIG. 15B**), and lung tumor cells, H1975 (**FIG. 15C**) in the presence of fibroblasts. These data suggest that fibroblasts are contributing to the TGF-β production in TME and reduce the cell kill capacity of anti-CD70 CAR T cells and this could be avoided by disrupting *TGFBRII* on the surface of the CAR T cell.

Example 12: Generation of CAR T cells with Disrupted *TGFBRII* and *Regnase-1* Genes.

Allogeneic human T cells that lack expression of the TRAC gene, β2M gene, CD70 gene, TGFBRII gene and Regnase-1 gene, and express a chimeric antigen receptor (CAR) targeting CD70 were produced. Activated human T cells were electroporated with Cas9:sgRNA RNPs (1 µM Cas9, 5 µM gRNA), followed by incubation with a recombinant adeno-associated adenoviral vectors, serotype 6 (AAV6) (MOI 50, 000).

Recombinant AAV comprised the nucleotide sequence of SEQ ID NO: 169 (encoding anti-CD70 CAR comprising the amino acid sequence of SEQ ID NO: 138). The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *CD70* (SEQ ID NO:54), *TGFBRII* (SEQ ID NO: 313) and *REG-1* (SEQ ID NO: 51). The sgRNAs, which form RNPs with the Cas9 enzyme, can be introduced into the T cells in a single electroporation event to produce the resulting modified cell populations shown in **Table 7** below. Alternatively, they can be introduced into the T cells in two sequential electroporation events to produce the resulting cell populations. After the electroporation, the cells were transduced with the recombinant AAVs to introduce the donor template encoding for the anti-CD70 CAR.

Table 7. Genetically Engineered CAR-T Cell Populations

Population	Edits
Anti-CD70 CAR T cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-
Anti-CD70 CAR T + Reg KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-
Anti-CD70 CAR T + TGFBRII KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-

Anti-CD70 CAR T + Reg KO + TGFBRII KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-

At 7 days post-electroporation, T cells were checked for CAR expression by flow cytometry. Both anti-CD70 CAR T cells and anti-CD70 CAR T cells that lack Regnase expressed nearly equivalent amount of CAR on their surface at day 7 post HDR. The results are provided in **Table 7A** below.

Table 7A. CAR Expression Levels in Genetically Engineered Anti-CD70 CAR T Cells

Population	Edits	CAR%
Anti-CD70 CAR T cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-	82.2
Anti-CD70 CAR T + Reg KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-	83.1
Anti-CD70 CAR T + TGFBRII KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-	79.7
Anti-CD70 CAR T + Reg KO + TGFBRII KO cells	anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-	81.8

Example 13: Disruption of *Regnase-1* and *TGFBRII* increases CAR T Cell Killing Upon Serial Rechallenge *in vitro*.

The anti-CD70 CAR$^+$ T cells generated above were serially rechallenged with CD70+ kidney cancer cell line, ACHN, and evaluated for their ability to kill the CD70+ kidney cancer cell line ACHN.

The anti-CD70 CAR$^+$ T cells used in this experiment contained the following edits:

- Anti-CD70 CAR T cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-
- Anti-CD70 CAR T + Reg KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-
- Anti-CD70 CAR T + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-
- Anti-CD70 CAR T + Reg KO + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-

In a 96-well plate format, CAR T cells were first co-cultured with ACHN cells (4,000 CAR T cells, 16,000 tumor cells) on D0 and re-challenged with tumor cells as follows: 16,000 tumor cells on D2 and D4; 40,000 cells on D7; 50,000 cells on D9; 50,000 cells on D11).

Analysis of tumor cell and CAR T cell number was performed at D1, D3, D6, D8, D10

and D12 using flow cytometry (method adapted from Wang et al., JoVE 2019). The following antibodies in **Table 8** were used at 1:100 dilution.

Table 8. Antibody Information

Antibody	Flour	cat #	Dilution	Vendor
CD4	BV510	344718	1:100	Biolegend
CD8	PacBlue	300546	1:100	Biolegend
CD70	FITC	355106	1:100	Biolegend
CD62L	BV605	304833	1:100	Biolegend
human CD45	BV785	304048	1:100	Biolegend
PD1	APC/Cy7	329922	1:100	Biolegend
CD45RO	PE/Cy7	304230	1:100	Biolegend
Streptavidin	APC	405207	1:100	Biolegend
Tim3	PE	345006	1:100	Biolegend
Live/Dead	7AAD	BDB559925	1:500	BD

The results demonstrate that disrupting both the TGFBRII gene and the Regnase gene improved potency (**FIG. 16A**) and CAR+ T cell expansion (**FIG. 16B**) when CAR T cells are repeatedly challenged with CD70+ positive target cells. Potency and expansion is improved compared to CAR T cells that have neither, or only one (*i.e.*: TGFBRII or Regnase), of the genes disrupted.

Example 14: Treatment Efficacy of Anti-CD70 CART Cells with Multiple Gene Disruptions in the Subcutaneous Renal Cell Carcinoma Tumor Xenograft Model

Treatment in the Renal Cell Carcinoma Tumor Model

The ability of T cells expressing a CD70 CAR with TGFBRII and/or Regnase gene edits to eliminate renal cell carcinoma cells that express medium levels of CD70 was evaluated *in vivo* using a subcutaneous renal cell carcinoma (CAKI-1) tumor xenograft mouse model. Anti-CD70 CAR+ T cells were produced as described above. See, e.g., Example 13.

The ability of these anti-CD70 CAR+ T cells to ameliorate disease caused by a CD70+ renal carcinoma cell line was evaluated in NSG mice using methods employed by Translational Drug Development, LLC (Scottsdale, AZ). In brief, 20, 5-8 week old female, NSG mice were

individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. Mice received a subcutaneous inoculation of $5x10^6$ Caki-1 renal cell carcinoma cells/mouse in the right hind flank. When mean tumor size reached target of ~70 mm^3, the mice were further divided into 5 treatment groups as shown in **Table 9**. On Day 1, treatment four groups received a single 200 µl intravenous dose of $1x10^7$ anti-CD70 CAR+ T cells according to **Table 9**.

Table 9. Treatment groups

Group	CAR-T	Caki-1 cells	CAR-T cell treatment (*i.v.*)	N
1	None	$5x10^6$ cells/mouse	None	4
2	Anti-CD70 CAR T cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
3	Anti-CD70 CAR T + Reg KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
4	Anti-CD70 CAR T + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
5	Anti-CD70 CAR T + Reg KO + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4

Tumor volume was measured 2 times weekly (~every 3-4 days) from day of treatment initiation. By day 11 post-injection, anti-CD70 CAR T cells with both TGFBRII and Regnase genes KO began to show a significant effect on reducing tumor volume compared to other treatment groups. Approximately one month later the anti-CD70 CAR T + Reg KO + TGFBRII KO cells had completely eliminated tumor growth in the subcutaneous CAKI-1 model (**FIG. 17A**).

These results demonstrated that disrupting both the TGFBRII and Regnase genes in CAR T cells increased the potency of the CAR T Cells and effectively cleared tumors in the subcutaneous CAKI-1 renal cell carcinoma tumor xenograft model.

Treatment in the Non-Small Cell Lung Carcinoma (NSCLC) Tumor Model

The ability of T cells expressing a CD70 CAR with TGFBRII and/or Regnase gene edits to eliminate lung adenocarcinoma cells that express moderate levels of CD70 was evaluated *in vivo* using a subcutaneous lung carcinoma (NCI-H1975) tumor xenograft mouse

model. Anti-CD70 CAR+ T cells were produced as described herein. See, *e.g.*, Example 13.

The ability of these anti-CD70 CAR+ T cells to ameliorate disease caused by a CD70+ lung carcinoma cell line was evaluated in NSG mice using methods employed by Translational Drug Development, LLC (Scottsdale, AZ). In brief, 20, 5-8 week old female, NSG mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. Mice received a subcutaneous inoculation of $5x10^6$ NCI-H1975 lung carcinoma cells/mouse in the right hind flank. When mean tumor size reached target of ~85 mm^3, the mice were further divided into 5 treatment groups as shown in **Table 10**. On Day 1, treatment four groups received a single 200 µl intravenous dose of $1x10^7$ anti-CD70 CAR+ T cells according to **Table 10**.

Table 10. Treatment groups

Group	CAR-T	NCI-H1975 cells	CAR+ T cell treatment (*i.v.*)	N
1	None	$5x10^6$ cells/mouse	None	4
2	Anti-CD70 CAR T cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
3	Anti-CD70 CAR T + Reg KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
4	Anti-CD70 CAR T + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4
5	Anti-CD70 CAR T + Reg KO + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-	$5x10^6$ cells/mouse	$1x10^7$ cells/mouse	4

Tumor volume was measured 2 times weekly from day of treatment initiation. By day 12 post-injection, animal treated with anti-CD70 CAR T cells having the TGFBRII edit exhibited attenuated tumor growth. Tumors treated with anti-CAR T cells with both TGFBRII and Regnase genes disrupted began to show a decrease in tumor volume by day 8 post-injection and cleared tumors by day 29 in 4 mice out of 4. This complete regression of tumors in treated animals continued through day 53 post injection. Treatment with anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII- T cells resulted in potent activity against established H1975 lung cancer xenografts through 53 days post injection (**FIG. 17B**). These data demonstrate that disrupting *TGFBRII* alone or *TGFBRII* and *Regnase-1* in CAR T cells

have potent activity against human CD70+ lung cancer tumors *in vivo*.

Example 15: Tumor re-challenge model Renal Cell Carcinoma Large Tumor Xenograft Model

The efficacy of anti-CD70 CAR T cells having *TGFBRII* and/or *Regnase-1* genes disrupted (see, e.g., Example 10) were tested in a subcutaneous A498 xenograft model with an ACHN re-challenge. In brief, five million A498 cells were injected subcutaneously in the right flank of NSG mice. Tumors were allowed to grow to an average size of approximately 425 mm^3 after which the tumor-bearing mice were randomized in five groups (N=5/group). Group 1 was left untreated (no treatment) while Groups 2-5 received one of the anti-CD70 CAR T cell treatments shown **Table 11**.

Table 11. Treatment Conditions

Group	CAR-T	A498 cells	CAR+ T cell treatment (*i.v.*)	N
2	Anti-CD70 CAR T cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-	$5x10^6$ cells/mouse	$8x10^6$ cells/mouse	5
3	Anti-CD70 CAR T + Reg KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-	$5x10^6$ cells/mouse	$8x10^6$ cells/mouse	5
4	Anti-CD70 CAR T + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/TGFBRII-	$5x10^6$ cells/mouse	$8x10^6$ cells/mouse	5
5	Anti-CD70 CAR T + Reg KO + TGFBRII KO cells: anti-CD70 CAR+/TRAC-/B2M-/CD70-/Reg-/TGFBRII-	$5x10^6$ cells/mouse	$8x10^6$ cells/mouse	5

On Day 56, a tumor re-challenge was initiated whereby $1x10^7$ ACHN cells were injected into the left flank of treated mice and into a new control group (no treatment).

As shown in **FIG. 18A**, all mice treated with all CAR T cell populations having a disrupted TGFBRII and/or Regnase gene showed complete clearance of the A498 tumor by day 50. However, when mice were rechallenged with a new RCC tumor cell (ACHN) only CAR T Cells with both Regnase and TGFBRII edits were able to clear the tumor compared to cells with either *Regnase-1* or *TGFBRII* disruptions alone (**FIG. 18B**).

Example 16: Analysis of T cell Fraction in Renal Cell Carcinoma (CAKI-1) Tumor Xenograft Model

Blood samples were taken from mice with CAKI-1 RCC tumors, 44 days after CAR T

administration. Briefly, 100ul of mouse whole blood was collected via submandibular vein. Red blood cell lysis buffer was used to achieve optimal lysis of erythrocytes with minimal effect on lymphocytes. Human CD45 and mouse CD45 were used as a biomarker to separate human and mouse cells by FACS. The blood samples were evaluated by flow cytometry looking for absolute CAR T counts as well as memory T cell subsets. An anti-CD70 CAR anti-idiotype antibody was used to detect CAR T cells and CD45RO+CD27+ to define central memory T cells. See U.S. Patent Application No. 63/069,889, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

The results demonstrate that the addition of the *TGFBRII* and *Regnase-1* gene edit significantly enhanced the population of central memory T cells compared to the edit of either *TFGBRII* or *Regnase-1* alone, which correlates with massive expansion of CAR T cells (**FIG 19A**) seen in these animals. And the *TGFBRII* edit further promoted the potential of CAR T cell proliferation *in vivo*, suggesting a robust synergistic effect along with the *Regnase* edit (**FIG. 19B**).

Example 17: Assessment of Anti-CD19 CAR-T Cells Having *TGFBRII* and/or *Regnase-1* Gene Disruptions in an Intravenous Disseminated Models in NOG Mice

Intravenous Disseminated Nalm-6 Human Acute Lymphoblastic Leukemia Tumor Xenograft Model

The Intravenous Disseminated Model (Disseminated Model) using the Nalm-6 Human Acute Lymphoblastic Leukemia tumor cell line in NOG mice was used to further demonstrate the efficacy of anti-CD19 CAR T cells with *TGFBRII* and/or *Regnase-1* gene edits. Efficacy of various anti-CD19 CAR T populations were evaluated in the Disseminated Model using methods employed by Translations Drug Development, LLC (Scottsdale, AZ) and described herein. In brief, 24, 5-8 week old female CIEA NOG (NOD.Cg-Prkdc^{scid}I12rg^{tm1Sug}/ JicTac) mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. At the start of the study, the mice were divided into 5 treatment groups as shown in **Table 12**. On Day 1 mice in Groups 2-4 received an intravenous injection of 0.5x10^6 Nalm6 cells/mouse. The mice were inoculated intravenously to model disseminated disease. On Day 4 (3 days post injection with the Nalm6 cells), treatment Groups 2-4 received a single 200 µl intravenous dose of CAR+ T cells per **Table 12**.

Table 12. Treatment groups.

Group	CAR T	Nalm6 Cells (i.v.)	anti-CD19 CAR T Treatment (i.v.)	N
1	Untreated	$0.5x10^6$ cells/mouse	None	5
2	Anti-CD19 CAR T cells: anti-CD19 CAR+/TRAC-/B2M-	$0.5x10^6$ cells/mouse	$4x10^6$ CAR-T positive cells/mouse	5
3	Anti-CD19 CAR T + Reg KO cells: anti-CD19 CAR+/TRAC-/B2M-/Reg-	$0.5x10^6$ cells/mouse	$4x10^6$ CAR-T positive cells/mouse	5
4	Anti-CD19 CAR T + TGFBRII KO cells: anti-CD19 CAR+/TRAC-/B2M-/TGFBRII-	$0.5x10^6$ cells/mouse	$4x10^6$ CAR-T positive cells/mouse	5
5	Anti-CD19 CAR T + Reg KO + TGFBRII KO cells: anti-CD19 CAR+/TRAC-/B2M-/Reg-	$0.5x10^6$ cells/mouse	$4x10^6$ CAR-T positive cells/mouse	5

During the course of the study mice were monitored daily and body weight was measured two times weekly as described above.

TGFBRII gene editing combined with Regnase editing induced a maintained NALM6 tumor regression at an early time point (day 18) post tumor inoculation, compared to either edit alone. This reduction in tumor size was maintain (**FIG. 20A**). The sharp decline in tumor size in the TGFBRII KO group at day 74 post tumor inoculation represents only 5 of 15 mince. Ten of the 15 mice in TGFBRIIKO group had already reached the tumor BLI endpoint.

While disruption of either *TGFBRII* or *Regnase* showed some survival advantage in the Nalm6 Model mice treated with anti-CD19 CAR+ cells, having both *TGFBRII* and *Regnase* gene disruptions exhibited the greatest survival advantage (**FIG. 20B**).

Intravenous Disseminated JeKo-1 Tumor Xenograft Model

The Intravenous Disseminated Model (Disseminated Model) using the JeKo-1 Human Mantle cell lymphoma (MCL) tumor cell line in NOG mice was used to further demonstrate the efficacy of anti-CD19 CAR T cells with TGFBRII and/or Regnase gene edits. Efficacy of various anti-CD19 CAR T populations were evaluated in the Disseminated Model using methods employed by Translations Drug Development, LLC (Scottsdale, AZ) and described herein. In brief, 24, 5-8 week old female CIEA NOG (NOD.Cg-Prkdc^{scid}I12rg^{tm1Sug}/ JicTac) mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. At the start of the study,

the mice were divided into 5 treatment groups as shown in **Table 13**. On Day 1 mice in Groups 2-4 received an intravenous injection of $0.5x10^6$ JeKo-1 cells/mouse. The mice were inoculated intravenously to model disseminated disease. On Day 4 (3 days post injection with the JeKo-1 cells), treatment Groups 2-4 received a single 200 µl intravenous dose of CAR T cells per **Table 13**.

Table 13. Treatment groups.

Group	CAR T	JeKo-1 Cells (i.v.)	anti-CD19 CAR+ T cell Treatment (i.v.)	N
1	Untreated	$5x10^6$ cells/mouse	None	5
2	Anti-CD19 CAR T cells: anti-CD19 CAR+/TRAC-/B2M-	$5x10^6$ cells/mouse	$4x10^6$ cells/mouse*	5
3	Anti-CD19 CAR T + Reg KO cells: anti-CD19 CAR+/TRAC-/B2M-/Reg-	$5x10^6$ cells/mouse	$4x10^6$ cells/mouse*	5
4	Anti-CD19 CAR T + TGFBRII KO cells: anti-CD19 CAR+/TRAC-/B2M-/TGFBRII-	$5x10^6$ cells/mouse	$4x10^6$ cells/mouse*	5
5	Anti-CD19 CAR T + Reg KO + TGFBRII KO cells: anti-CD19 CAR+/TRAC-/B2M-/Reg-/TGFBRII-	$5x10^6$ cells/mouse	$4x10^6$ cells/mouse*	5

During the course of the study mice were monitored daily and body weight was measured two times weekly as described above.

While either TGFBRII or Regnase showed some survival advantage in the JeKo-1 Model, mice treated with anti-CD19 CAR+ cells having both TGFBRII and Regnase gene edits exhibited the greatest survival advantage **FIG. 21**.

CAR T cell expansion in vivo

CAR T cell expansion was assessed by measuring the CAR copy number by ddPCR of DNA isolated from blood samples collected throughout the Jeko-1 and Nalm-6 studies as described above.

DNA was isolated from mouse tissue using the Qiagen Dneasy blood and tissue kit (Qiagen, Venlo, Netherlands). Total mass of nucleic acid from RBC-lysed samples was quantitated using either Nanodrop (Thermo Fisher Scientific) or DropSense96 (trinean, Gentbrugge, Belgium) machines. Primers and 6-carboxyfluorescein (FAM)-labeled probe sets (provided in **Table 14** below) were designed to quantitate the levels of the integrated CAR

construct into the human TRAC locus by droplet digital PCR (ddPCR). ddPCR was performed using the Bio-Rad Automated Droplet Generator, Bio-Rad T100 Thermal Cycler, and Bio-Rad QX200 Droplet Reader machine(s) (Bio-rad Laboratories, Hercules, CA). QuantaSoft Version 1.7.4.0917 (Bio-rad Laboratories) software was used to calculate the absolute number of integrated CAR copies per sample. Finally, the number of detected CAR alleles was divided by the input total DNA amount to compute the absolute number of CAR copies per mass of input sample. The ddPCR assay detects the number of copies of integrated CAR transgene per mass of genomic DNA (gDNA) by amplifying an 866 bp amplicon spanning endogenous TRAC sequence and the CAR expression cassette promotor (EF-1α). In brief, qualification of the assay yielded linear data (R^2>0.95) within the range tested (2 to 300,000 copies per ug of gDNA) as well as generated a % relative error (%RE) and % coefficient of variation (% CV) within normal ranges (%RE ≤ 100% and %CV ≤ 20%) for conditions ≥ LLOQ. The LLOD and LLOQ were calculated based on the available data and the LLOD was set to 5 copies per 0.2 µg of gDNA and the LLOQ was set to 40 copies per 0.2 µg.

Table 14. Primers and probes used for ddPCR

CAR primers and probe	
CTX110-20-30_dd_1 Forward	GGCACCATATTCATTTTGCAGGTGAA (SEQ ID NO: 11)
CTX110-20-30_dd_1 Reverse	ATGTGCGCTCTGCCCACTGACGGGC (SEQ ID NO: 12)
CTX110-20-30_dd_1 Probe (FAM)	AGACATGAGGTCTATGGACTTCAGGCTCC (SEQ ID NO: 13)

These analysis demonstrate that the addition of either TGFBRII or Regnase-1 KO to allogeneic CAR T cells ($TRAC^-$/$B2M^-$; Group C-10) allowed the T cells to expand to larger levels in the blood of treated mice (*e.g.*, Groups C10-TG, C10-R, C10-TG/R) compared to groups treated with the allogeneic CAR T cells without those KOs (*e.g.*, Group C10) (**FIG. 22A**). This expansion was apparent at day 14 of the Jeko-1 study. Loss of both *TGFBRII* and *Regnase-1* (**FIG. 22A**, C10-TG/R) led to a more uniform expansion relative to *TGFBRII* (**FIG. 22A**, C10-TG) or *Regnase-1* (**FIG. 22A**, C10-R) single KOs. In the Nalm-6 study, disruption of both *TGFBRII* and *Regnase-1* had a synergistic effect on CAR T cell expansion at day 28 as shown in **Fig. 22B**

In sum, all groups with loss of either *TGFBRII* or *Regnase-1* had expanded CAR-T cells in the peripheral blood.

Example 18: Generation of CAR T Cells with Multiple Gene Editing and Verification of Gene Edits

Activated primary human T cells were electroporated with Cas9/sgRNA RNP complexes (200 pmol Cas9, 1000 pmol gRNA) to generate cells edited for TRAC-/β2M-, TRAC-/β2M-/Regnase-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Regnase-1-/TGFBRII-. Sequence encoding anti-BCMA CAR was inserted into the TRAC locus using recombinant AAV6 carrying the DNA sequence for anti-BCMA CAR (SEQ ID NO: 170). The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *Reg-1* (SEQ ID NO: 51; REG1-Z10) and TGFBRII (SEQ ID NO: 313).

Flowcytometry was used to verify the editing for *TRAC*, *β2M* and the insertion and expression of anti-BCMA CAR. Briefly, about one week post electroporation, cells were stained with anti-human TCR, anti-human *β2M* and recombinant biotinylated human BCMA/streptavidin-APC to assess the levels of editing for *TRAC* and *β2M*, and insertion of the nucleotide sequence encoding anti-BCMA CAR.

TRAC-/β2M-, TRAC-/β2M-/Reg-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells show consistent rates of *TCR* and *β2M* disruptions at >90% and >60% rates, respectively as determined by flow cytometry (**FIGs. 23A** and **23B**). Anti-BCMA CAR expression was measured flow cytometrically by determining the percentage of cells that bound recombinant biotinylated BCMA/streptavidin-APC conjugate. All the conditions including TRAC-/β2M-, TRAC-/β2M-/Reg-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells show consistent rates of CAR insertion (>70%), while the unedited RNP- T-cells have no detectable staining for anti-BCMA CAR (**FIG. 23C**). The ratio of CD4/CD8 T cells as assessed by flow cytometry in the TRAC-/β2M-, TRAC-/β2M-/Reg-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells were found to be consistent in the range of 55-60%/40-45% across all the samples (**FIG. 23D**).

TIDE analysis was performed for the verification of editing rates for *Reg-1* and *TGFBRII* genes. Briefly, about one week post electroporation, two million cells from TRAC-/β2M-, TRAC-/β2M-/Reg-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells and two million unedited T-cells from the same donor were removed from culture and transferred to 1.5mL microcentrifuge tubes. Cells were spun down in a tabletop microcentrifuge at 300g for 10 minutes and the resulting supernatant was discarded. The cells were washed twice with 1000 uL 1X PBS and the cell pellets were frozen at -80 °C. The frozen cell pellets were then used for the extraction of genomic DNA using

QIAamp DNA Blood Mini Kit (Qiagen, catalog # 51106). Gene-specific primers were used to amplify the region flanking the cut sites of *Reg-1* and *TGFBRII* (Invitrogen™ Platinum™ SuperFi™ II Green PCR Master Mix; catalog # 12369050) and the PCR amplicons derived were subsequently sequenced and analyzed by TIDE to determine the indel patterns/frequencies (editing frequencies).

The analyzed indel frequencies were found to be within the expected range of 65-80% for TGF sgRNA and >80% for the Regnase-1 sgRNA, respectively (**FIGs. 24A** and **24B**).

Example 19: Cytotoxicity of Anti-BCMA CAR T Cells with Multiple Gene Edits

A cytotoxicity (cell kill) assay was used to assess the ability of the TRAC-/β2M-, TRAC-/β2M-/Reg-1-, TRAC-/β2M-/TGFBRII- and TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells (produced by the methods disclosed herein, see, *e.g.*, Example 18) to cause cell lysis in two target cell lines, MM.1S (multiple myeloma cell line) and JeKo-1 (mantle cell lymphoma cell line). Unedited RNP- cells without CAR were used as a negative control to determine the specific lysis by CAR+ T cells. Briefly, the target cell lines were stained with eBioscience™ Cell Proliferation Dye eFluor™ 670 (Thermofisher Scientific; catalog# 65-0840-85) per manufacturer's instructions and seeded into 96-well plates at 50,000 cells per well. Next, CAR T-cells or RNP- T cells were added to the wells containing target cells at ratios of 0, 0.5:1, 1:1, 2:1, or 4:1 (T cell: target cell) and incubated further for approximately 4 hours for MM.1S and 24 hours for JeKo-1. After the respective incubation period, the 96-well plates were spun down at 300g for 10 minutes and 100 µL of supernatant was removed for cytokine quantification. Cells were then washed once with 1X PBS and stained with 150 ul of 1XPBS supplemented with 0.5% BSA and 5µg/mL DAPI (Invitrogen; catalog# D3571) and incubated for 15 minutes in dark. Post-incubation, cells were washed-off DAPI, resuspended in 150 µl of 1XPBS supplemented with 0.5% BSA, and acquired and analyzed using a flow cytometer. Target cells were identified via eFluor-based fluorescence and then divided into live and dead cells based on their DAPI fluorescence.

The TRAC-/β2M-/Reg-1-/TGFBRII- anti-BCMA CAR+ T-cells exhibited greater cytotoxicity towards the MM.1S (**FIG. 25A**) and JeKo-1 cell lines (**FIG. 25C**) compared to TRAC-/β2M-, TRAC-/β2M-/Regnase-1- or TRAC-/β2M-/TGFBRII- anti-BCMA CAR+ T-cells. Comparative data from K562 cells (as controls) are provided in **FIG. 25B** and **FIG. 25D**.

Example 20: *In Vivo* Effects of TGFBRII+Regnase-1 Disruption on Allogeneic CAR T Cells in the Subcutaneous RPMI-8226 Xenograft Tumor Model

A subcutaneous tumor mouse model was utilized to assess the *in vivo* efficacy of allogeneic anti-BCMA CARs with the following gene disruptions: 1) *β2M* and *TRAC*, 2) *β2M*, *TRAC*, and *TGFBRII*, 3) *β2M*, *TRAC*, and *Reg-1*, and 4) *β2M*, *TRAC*, *TGFBRII*, and *Reg-1*. The subcutaneous tumor mouse model utilized the BCMA+ multiple myeloma derived RPMI-8226 tumor cell line in NSG mice. The TGFBRII gene was edited via CRISPR/Cas-mediated gene editing using the TGFBRII Ex5_T1 guide (SEQ ID NO. 313). The Reg-1 gene was edited via CRISPR/Cas-mediated gene editing using the Z10 guide (SEQ ID NO. 51). The anti-BCMA CAR T cells express an anti-BCMA CAR comprising the amino acid sequence of SEQ ID NO: 146). See also the sequence **Tables 22, 23, 27**, and **39** below.

Efficacy of the anti-BCMA CAR T cells was evaluated in the subcutaneous xenograft model using methods employed by Translations Drug Development, LLC (Scottsdale, AZ) and described herein. In brief, 25 5-8 week old female NSG mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. On day 1, mice received a subcutaneous inoculation of $1x10^7$ RPMI-8226 cells/mouse in the right hind flank. Nine days later (Day 10), the tumor inoculation sites were inspected to determine if the tumors were palpable. After confirming palpability, the mice were further divided into 5 treatment groups as shown in Table 1. All treatment groups received a single 200 ul intravenous dose of 1e6 anti-BCMA CAR+ T cells.

Table 15. Treatment Groups for the RMPI-8226 Xenograft Study

Group	CAR T cells (i.v.)	N
1	NA	5
2	anti-BCMA CAR/TRAC-/β2M-	5
3	anti-BCMA CAR/TRAC-/β2M-/TGFBRII-	5
4	anti-BCMA CAR/TRAC-/β2M-/TGFBRII-/Regnase-	5
5	anti-BCMA CAR/TRAC-/β2M-/TGFBRII-/Regnase-	5

Throughout the course of the study, the mice were subjected to gross observations daily, while tumor volume and body weight were measured twice weekly (~every 3-4 days) starting on Day 10. A significant endpoint was the time to peri-morbidity and the effect of T-cell engraftment was also assessed. The percentage of animal mortality and time to death were recorded for every group in the study. Mice were euthanized prior to reaching a moribund state. Mice may be defined as moribund and sacrificed if one or more of the following criteria

were met:

- Loss of body weight of 20% or greater sustained for a period of greater than 1 week;
- Tumors that inhibit normal physiological function such as eating, drinking, mobility and ability to urinate and or defecate;
- Prolonged, excessive diarrhea leading to excessive weight loss (>20%); or
- Persistent wheezing and respiratory distress.

Animals were also considered moribund if there was prolonged or excessive pain or distress as defined by clinical observations such as: prostration, hunched posture, paralysis/paresis, distended abdomen, ulcerations, abscesses, seizures and/or hemorrhages.

Mice in groups receiving TRAC-/β2M-/TGFBRII-/Reg-1- anti-BCMA CAR+ T-cells saw an increase in survival relative to untreated mice; mice treated TRAC-/β2M- anti-BCMA CAR+ T-cells, TRAC-/β2M-/TGFBRII anti-BCMA CAR+ T-cells, or TRAC-/β2M-/Reg-1- anti-BCMA CAR+ T-cells (**FIG. 26B**). Mice receiving TRAC-/B2M-/TGFBRII-/Regnase- anti-BCMA CAR+ T cells showed significant tumor regression, while none of the other conditions tested showed significant inhibition of tumor growth (**FIG. 26A**). These data demonstrate that disruption of *TGFBRII* and *Reg-1* in CAR T cells increases efficacy of CAR T cells in a mouse xenograft tumor model.

Next, small amounts of blood were taken from each mouse for FACS analysis to characterize circulating CAR-T cells and determine drug pharmacokinetics. Approximately 75uL of blood was drawn 2 weeks post CAR-T dosing via submandibular bleeds. The blood was then transferred into K2 EDTA tubes and shipped overnight to CRISPR Therapeutics on 4C cold packs. The following day, blood samples were processed with RBC (Red Blood Cell) Lysis Buffer (BioLegend®, catalog # 420301) per manufacturer's instructions. The samples then underwent anti-mouse CD16/32 blocking *via* anti-mouse Trustain FcX™ (BioLegend®, catalog # 101320) per manufacturer's instructions. The samples were then processed via flow cytometry to determine prevalence of human CD45 expressing cells, which would represent the circulating CAR-T cells. Blood from mice that had received TRAC-/β2M-/TGFBRII-/Regnase- anti-BCMA CAR+ T-cells showed a high amount of circulating human CD45+ cells, which was not seen in any other treatment groups (**FIG. 26C**). This indicates that the *TGFBRII* and *Reg-1* knockouts confer superior expansion of CAR-T cells in a multiple myeloma mouse xenograft model.

Example 21: In Vivo Synergistic Effects of TGFBRII+Regnase-1 Disruptions on Allogeneic CAR T Cells in the Subcutaneous JeKo-1 Xenograft Tumor Model

A subcutaneous tumor mouse model was utilized to further assess the *in vivo* efficacy of TRAC-/β2M- anti-BCMA CAR+ T-cells and TRAC-/β2M-/TGFBRII-/Reg-1/anti-BCMA CAR+ T-cells. The subcutaneous tumor mouse model utilized the low BCMA expressing mantle cell lymphoma derived JeKo-1 tumor cell line in NSG mice. The *TGFBRII* gene was edited via CRISPR/Cas-mediated gene editing using TGFBRII Ex5_T1 guide (SEQ ID NO: 313). The *Reg*-1 gene was edited via CRISPR/Cas-mediated gene editing using the Z10 guide (SEQ ID NO: 51). The anti-BCMA CAR T cells express an anti-BCMA CAR comprising the amino acid sequence of SEQ ID NO: 146. See also the sequence **Tables 22, 23, 27**, and **39** below.

Efficacy of the anti-BCMA CAR T cells was evaluated in the subcutaneous xenograft model using methods employed by Translations Drug Development, LLC (Scottsdale, AZ) and described herein. In brief, 15 5-8 week old female NSG mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. On day 1, mice received a subcutaneous inoculation of $5x10^6$ JeKo-1 cells/mouse in the right hind flank. Tumors were then periodically sized in via calipers. Once average tumor size reached an average of $150mm^3$ (with an acceptable range of $125\text{-}175mm^3$), the mice were further divided into 3 treatment groups as shown in Table 1. All treatment groups received a single 200 ul intravenous dose of 10e6 anti-BCMA CAR+ T cells. The day of T-cell injection was marked as Day 1.

Table 16. Treatment Groups for the RMPI-8226 Xenograft Study

Group	CAR T cells (i.v.)	N
1	NA	5
2	anti-BCMA CAR/TRAC-/β2M-	5
5	anti-BCMA CAR/TRAC-/β2M-/TGFBRII-/Regnase-	5

Throughout the course of the study, the mice were subjected to gross observations daily, while tumor volume and body weight were measured twice weekly (~every 3-4 days) starting on Day 1. A significant endpoint was the time to peri-morbidity and the effect of T-cell engraftment was also assessed. The percentage of animal mortality and time to death were recorded for every group in the study. Mice were euthanized prior to reaching a moribund

state. Mice may be defined as moribund and sacrificed if one or more of the following criteria were met:

- Loss of body weight of 20% or greater sustained for a period of greater than 1 week;
- Tumors that inhibit normal physiological function such as eating, drinking, mobility and ability to urinate and or defecate;
- Prolonged, excessive diarrhea leading to excessive weight loss (>20%); or
- Persistent wheezing and respiratory distress.

Animals were also considered moribund if there was prolonged or excessive pain or distress as defined by clinical observations such as: prostration, hunched posture, paralysis/paresis, distended abdomen, ulcerations, abscesses, seizures and/or hemorrhages.

Mice in groups receiving TRAC-/β2M-/TGFBRII-/Reg-1- anti-BCMA CAR+ T-cells saw a significant increase in survival relative to both untreated mice and mice treated TRAC-/β2M- anti-BCMA CAR+ T-cells (**FIG. 27B**). Mice receiving TRAC-/B2M-/TGFBRII-/Reg-1- anti-BCMA CAR+ T cells arrested tumor growth, while TRAC-/β2M- anti-BCMA CAR+ T-cells did not significant inhibit tumor growth (**FIG. 27A**). These data demonstrate that disruption of *TGFBRII* and *Reg-1* genes in CAR T cells increases efficacy of CAR-T cells in a mouse xenograft tumor model.

Next, small amounts of blood were taken from each mouse for FACS analysis to characterize circulating CAR-T cells and determine drug pharmacokinetics. Approximately 75uL of blood was drawn 2 and 3 weeks post CAR-T dosing via submandibular bleeds. The blood was then transferred into K2 EDTA tubes and shipped overnight to CRISPR Therapeutics on 4C cold packs. The following day, blood samples were processed with RBC (Red Blood Cell) Lysis Buffer (BioLegend®, catalog # 420301) per manufacturer's instructions. The samples then underwent anti-mouse CD16/32 blocking via anti-mouse Trustain FcX™ (BioLegend®, catalog # 101320) per manufacturer's instructions. To quantify the number of circulating T-cells, the sum of cells positive for human CD4 and CD8 was determined. At the two week timepoint, blood from mice that had received TRAC-/β2M-/TGFBRII-/Reg-1- anti-BCMA CAR+ T-cells showed significantly higher concentrations of human CD4 and human CD8+ expressing cells relative to blood from mice that received TRAC-/β2M-anti-BCMA CAR+ T-cells (**FIG. 27C**).

Furthermore, the TRAC-/β2M-TGFBRII-/Reg-1- anti-BCMA CAR+ T-cells showed lower expression of the T-cell exhaustion markers Lag3 and PD1 relative to the TRAC-/β2M-

anti-BCMA CAR+ T-cells (**FIG. 27D**). At the three week timepoint, the overall level of hCD45+ cells in circulation had equalized between groups (**FIG. 27E**), but the expression of Lag3 and PD1 remained lower in mice treated with TRAC-/β2M-TGFBRII-/Reg-1- anti-BCMA CAR+ T-cells (**FIG. 27F**). This indicates that CAR-T cells containing the TGFBRII and Regnase knockouts have a superior ability to expand when compared to CAR-T cells lacking those edits while also reducing the expression of T-cell exhaustion markers PD-1 and Lag3.

Example 22: Generation of Anti-PTK7 CAR T Cells with Disrupted *TGFBRII* and *Regnase-1* Genes.

Allogeneic human T cells that lack expression of the *TRAC* gene, *β2M* gene, *TGFBRII* gene and *Reg-1* gene, and express a chimeric antigen receptor (CAR) targeting PTK7 were produced. Activated human T cells were electroporated with Cas9:sgRNA RNPs (1 µM Cas9, 5 µM gRNA), followed by incubation with a recombinant adeno-associated adenoviral vectors, serotype 6 (AAV6) (MOI 50, 000).

Recombinant AAV comprised a nucleotide sequence encoding an anti-PTK7 CAR comprising the amino acid sequence of SEQ ID NO: 349. The following sgRNAs were used: *TRAC* (SEQ ID NO: 58), *β2M* (SEQ ID NO: 62), *TGFBRII* (SEQ ID NO: 313) and *REGNASE-1* (SEQ ID NO: 51). The sgRNAs, which form RNPs with the Cas9 enzyme, can be introduced into the T cells in a single electroporation event to produce the resulting modified cell populations shown in **Table 17** below. After the electroporation, the cells were transduced with the recombinant AAV to introduce the donor template encoding for the anti-PTK7 CAR.

Table 17. Genetically Engineered CAR-T Cell Populations

Population	Edits
Anti-PTK7 CAR T cells	anti-PTK7 CAR+/TRAC-/B2M-
Anti-PTK7 CAR T + TGFBRII KO cells	anti-PTK7 CAR+/TRAC-/B2M-/TGFBRII-
Anti-PTK7 CAR T + TGFBRII KO + Reg KO cells	anti-PTK7 CAR+/TRAC-/B2M-/TGFBRII-/Reg-

At 7 days post-electroporation, T cells were checked for CAR expression by flow cytometry. Both anti-PTK7 CAR T cells and anti-PTK7 CAR T cells that lack TGFBRII and anti-PTK7 CAR T cells that lack TGFBRII and Regnase expressed nearly equivalent amount of CAR on their surface at day 7 post HDR. The results are provided in **Table 18** below.

Table 18. Percentage of CAR, TCR, and b2M Expression on Day 7 Post HDR

Treatment	CAR+%	TCR+%	β2M+%
No RNP	3.33	92	93.7
No AAV	5.16	2.63	3.87
Anti-Ptk7 CAR	82.2	1.24	2.49
Anti-Ptk7 CAR & *TGFBRII* KO	83.2	0.82	2.1
Anti-Ptk7 CAR & *TGFBRII*/*Reg-1* KO	81.7	0.77	2

Efficient editing of TGFBRII and/or Regnase was achieved in the engineered anti-Ptk7 CAR T cell (**Table 19 below**) and show an increase in cell proliferation with *TGFBRII* and *Reg-1* disruption (**FIG. 28**), while cell viability and CD4+/CD8+ T cells ratios remain unchanged.

Table 19. Indel Percentage in TGFBRII and Regnase-1 on Day 7 Post HDR

Treatment	*TGFBRII* Indel%	*Reg-1* Indel%
No RNP	1.8	1.6
No AAV	97.75	88.8
Anti-Ptk7 CAR	1.45	2.2
Anti-Ptk7 CAR & *TGFBRII* KO	97.15	3
Anti-Ptk7 CAR & *TGFBRII*/*Reg-1* KO	97.7	92.2

In summary, the data presented in this example demonstrated that *TGFBRII* and/or *Reg-1* disruption in anti-Ptk7 CAR T cells (*e.g.*, anti-PTK7 CAR+/TRAC-/B2M-/TGFBRII- or anti-PTK7 CAR+/TRAC-/B2M-/TGFBRII-/Reg-1-), can increase cell proliferation, while not affecting cell viability or CD4/CD8 cell ratios.

Example 23: Disruption of *TGFBRII* Alone Increases CAR T Cell Killing Upon Serial Rechallenge *in vitro*.

The anti-PTK7 CAR$^+$ T cells generated above were serially rechallenged with PTK7+ osteosarcoma cancer cell line, Saos2, and evaluated for their ability to kill the PTK7+ osteosarcoma cancer cell line Saos2.

The anti-PTK7 CAR$^+$ T cells used in this experiment contained the following edits:

- Anti-PTK7 CAR T cells: anti-PTK7 CAR+/TRAC-/B2M-

- Anti-PTK7 CAR T + TGFBRII KO cells: anti-PTK7 CAR+/TRAC-/B2M-/ TGFBRII-
- Anti-PTK7 CAR T + TGFBRII KO + Reg KO cells: anti-PTK7 CAR+/TRAC-/B2M-/ TGFBRII-/Reg-

In a 96-well plate format, CAR T cells were first co-cultured with Saos2 cells (6,250 CAR T cells, 50,000 tumor cells) on D0 and re-challenged with 50,000 tumor cells on D2, D4, D6, D8, D10, D12 and D14.

Analysis of tumor cell and CAR T cell number was performed at D1, D3, D5, D7, D9, D11 and D13 using flow cytometry (method adapted from Wang et al., JoVE 2019). The following antibodies in **Table 20** were used at 1:100 dilution.

Table 20. Antibody Information

Antibody	Flour	cat #	Dilution	Vendor
CD4	BV510	300546	1:100	Biolegend
CD8	FITC	344704	1:100	Biolegend
PTK7	PE	130-091-364	1:50	Miltenyi
CD62L	BV605	304833	1:100	Biolegend
human CD45	BV785	304048	1:100	Biolegend
PD1	APC/Cy7	329922	1:100	Biolegend
CD45RO	PE/Cy7	304230	1:100	Biolegend
Streptavidin	APC	405207	1:100	Biolegend
Tim3	BV421	345008	1:100	Biolegend
Live/Dead	7AAD	BDB559925	1:500	BD

The results demonstrate that disrupting the *TGFBRII* gene improved potency (**FIG. 29A**) and CAR T cell expansion (**FIG. 29B**) as measured by hum CD45 staining, when CAR T cells are repeatedly challenged with PTK7+ positive target cells. The addition of Regnase gene disruption does not provide an added advantage in potency over TGFBRII deletion alone. Potency and expansion is improved compared to CAR T cells that have neither, or both (*i.e.*: TGFBRII and Regnase), of the genes disrupted. In addition, the results demonstrate that cytotoxic CD8+ CAR T cells persist longer during serial rechallenge (**FIG. 29C**) with tumor cells if the TGFBRII gene is disrupted compared to anti-PTK7 CAR T cells that have neither

or both (*i.e.*: TGFBRII and Regnase) of the genes disrupted. CD4+ CAR T cells remain consistent regardless of whether TGFBRII and/or Regnase genes are disrupted (**FIG. 29D**).

Example 24: Treatment Efficacy of Anti-PTK7 CART Cells with Multiple Gene Disruptions in the Subcutaneous Pancreatic Cell Carcinoma Tumor Xenograft Model

Treatment in the Pancreatic Cell Carcinoma Tumor Model

The ability of T cells expressing a PTK7 CAR with *TGFBRII* and/or *Reg-1* gene edits to eliminate pancreatic cell carcinoma cells that express medium levels of PTK7 was evaluated *in vivo* using a subcutaneous renal cell carcinoma (Hs766T) tumor xenograft mouse model. Anti-PTK7 CAR+ T cells were produced as described above. See, *e.g.*, Example 22.

The ability of these anti-PTK7 CAR+ T cells to ameliorate disease caused by a PTK7+ pancreatic carcinoma cell line was evaluated in NSG mice using methods employed by Translational Drug Development, LLC (Scottsdale, AZ). In brief, 20, 5-8 week old female, NSG mice were individually housed in ventilated microisolator cages, maintained under pathogen-free conditions, 5-7 days prior to the start of the study. Mice received a subcutaneous inoculation of $5x10^6$ Hs766T pancreatic cell carcinoma cells/mouse in the right hind flank. When mean tumor size reached target of ~50 mm^3, the mice were further divided into 3 treatment groups as shown in **Table 21**. On Day 1, treatment four groups received a single 200 µl intravenous dose of $0.5x10^7$ anti-PTK7 CAR+ T cells according to **Table 21**.

Table 21. Treatment groups

Group	CAR-T	Hs766T cells	CAR-T cell treatment (*i.v.*)	N
1	None	$5x10^6$ cells/mouse	None	5
2	Anti-PTK7 CAR T cells: anti-PTK7 CAR+/TRAC-/B2M-	$5x10^6$ cells/mouse	$0.5x10^7$ cells/mouse	5
3	Anti-PTK7 CAR T + TGFBRII KO cells: anti-PTK7 CAR+/TRAC-/B2M-/TGFBRII-	$5x10^6$ cells/mouse	$0.5x10^7$ cells/mouse	5

Tumor volume was measured 2 times weekly (~every 3-4 days) from day of treatment initiation. By day 11 post-injection, anti-PTK7 CAR T cells with and without TGFBRII gene KO began to show a significant effect on reducing tumor volume compared to no treatment group 1. Approximately one month later the anti-PTK7CAR T with and without TGFBRII KO cells had completely eliminated tumor growth in the subcutaneous Hs766T model (**FIG. 30A**).

These results demonstrated that disrupting the TGFBRII gene in CAR T cells effectively cleared tumors in the subcutaneous Hs766T renal cell carcinoma tumor xenograft model. No clinical signs of GvHD were observed in anti-PTK7 CAR T cells with and without TGFBRII KO cells (**FIG. 30B**).

Example 25: Analysis of T cell Fraction in Pancreatic Cell Carcinoma (Hs766T) Tumor Xenograft Model

Blood samples were taken from mice with Hs766T tumors, 47 days after CAR T administration. Briefly, 100ul of mouse whole blood was collected via submandibular vein. Red blood cell lysis buffer was used to achieve optimal lysis of erythrocytes with minimal effect on lymphocytes. Human CD45 and mouse CD45 were used as a biomarker to separate human and mouse cells by FACS. The blood samples were evaluated by flow cytometry looking for absolute human CD45+ counts as well as memory T cell subsets. Staining for CD45RO+CD27+ was used to define central memory T cells.

The results demonstrate that the addition of the *TGFBRII* gene edit significantly enhanced the population of central memory T cells (**FIG. 31B**) compared to anti-PTK7 CAR T cells without TGFBRII KO which correlates with massive expansion of CAR T cells (**FIG. 31A**) seen in these animals. And the *TGFBRII* edit further promoted the potential of CAR T cell proliferation *in vivo* (**FIG. 31B**).

Sequence Tables

The following tables provide details for the various nucleotide and amino acid sequences disclosed herein.

Table 22. sgRNA Sequences and Target Gene Sequences for Reg1

Name	Unmodified Sequence	Modified Sequence	Target Sequences (PAM)
REG1-Z01 sgRNA (EX2_T1)	GGUCAUCGAUGGGAGCAA CGguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 14)	G*G*U*CAUCGAUGGGAGCAAC Gguuuuagagcuagaaauagca aguuaaaauaaggcuaguccgu uaucaacuugaaaaaguggcac cgagucggugcU*U*U*U (SEQ ID NO: 15)	GGTCATCGATGGGAGCAACG (TGG) (SEQ ID NO: 171) GGTCATCGATGGGAGCAACG (SEQ ID NO: 318)
REG1-Z01 sgRNA (EX2_T1) spacer	GGUCAUCGAUGGGAGCAA CG (SEQ ID NO: 16)	G*G*U*CAUCGAUGGGAGCAAC G (SEQ ID NO: 17)	
REG1-Z02 sgRNA (EX2_T2)	CACCACCCCGCGGGACUA GAguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU	C*A*C*CACCCCGCGGGACUAG Aguuuuagagcuagaaauagca aguuaaaauaaggcuaguccgu uaucaacuugaaaaaguggcac cgagucggugcU*U*U*U (SEQ ID NO: 19)	CACCACCCCGCGGGACTAGA (GGG) (SEQ ID NO: 172) CACCACCCCGCGGGACTAGA (SEQ ID NO: 319)

Name	Unmodified Sequence	Modified Sequence	Target Sequences (PAM)
	(SEQ ID NO: 18)		
REG1-Z02 sgRNA (EX2_T2) spacer	CACCACCCCGCGGGACUAGA (SEQ ID NO: 20)	mC*mA*mC*CACCCCGCGGGACUAGA (SEQ ID NO: 21)	
REG1-Z03 sgRNA (EX2_T3)	GGUCUGGCGCUCCCGCUCGGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 22)	G*G*U*CUGGCGCUCCCGCUCGGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 23)	GGTCTGGCGCTCCCGCTCGG (TGG) (SEQ ID NO: 173) GGTCTGGCGCTCCCGCTCGG (SEQ ID NO: 320)
REG1-Z03 sgRNA (EX2_T3) spacer	GGUCUGGCGCUCCCGCUCGG (SEQ ID NO: 24)	mG*mG*mU*CUGGCGCUCCCGCUCGG (SEQ ID NO: 25)	
REG1-Z04 sgRNA (EX4_T1)	UUCACACCAUCACGACGCGUguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 26)	U*U*C*ACACCAUCACGACGCGUguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 27)	TTCACACCATCACGACGCGT (GGG) (SEQ ID NO: 174) TTCACACCATCACGACGCGT (SEQ ID NO: 321)
REG1-Z04 sgRNA (EX4_T1) spacer	UUCACACCAUCACGACGCGU (SEQ ID NO: 28)	U*U*C*ACACCAUCACGACGCGU (SEQ ID NO: 29)	
REG1-Z05 sgRNA (EX4_T2)	ACACCAUCACGACGCGUGGGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 30)	A*C*A*CCAUCACGACGCGUGGGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 31)	ACACCATCACGACGCGTGGG (TGG) (SEQ ID NO: 175) ACACCATCACGACGCGTGGG (SEQ ID NO: 322)
REG1-Z05 sgRNA (EX4_T2) spacer	ACACCAUCACGACGCGUGGG (SEQ ID NO: 32)	A*C*A*CCAUCACGACGCGUGGG (SEQ ID NO: 33)	
REG1-Z06 sgRNA (EX4_T3)	CUACGAGUCUGACGGGAUCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 34)	C*U*A*CGAGUCUGACGGGAUCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 35)	CTACGAGTCTGACGGGATCG (TGG) (SEQ ID NO: 176) CTACGAGTCTGACGGGATCG (SEQ ID NO: 323)
REG1-Z06 sgRNA (EX4_T3) spacer	CUACGAGUCUGACGGGAUCG (SEQ ID NO: 36)	C*U*A*CGAGUCUGACGGGAUCG (SEQ ID NO: 37)	
REG1-Z07 sgRNA (EX4_T4)	UUGCCACCCACGCGUCGUGAguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 38)	U*U*G*CCACCCACGCGUCGUGAguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U(SEQ ID NO: 39)	TTGCCACCCACGCGTCGTGA (TGG) (SEQ ID NO: 177) TTGCCACCCACGCGTCGTGA (SEQ ID NO: 324)

Name	Unmodified Sequence	Modified Sequence	Target Sequences (PAM)
REG1-Z07 sgRNA (EX4_T4) spacer	UUGCCACCCACGCGUCGUGA (SEQ ID NO: 40)	U*U*G*CCACCCACGCGUCGUGA (SEQ ID NO: 41)	
REG1-Z08 sgRNA (EX4_T5)	GUUCACACCAUCACGACGCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 42)	G*U*U*CACACCAUCACGACGCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 43)	GTTCACACCATCACGACGCG (TGG) (SEQ ID NO: 178) GTTCACACCATCACGACGCG (SEQ ID NO: 325)
REG1-Z08 sgRNA (EX4_T5) spacer	GUUCACACCAUCACGACGCG (SEQ ID NO: 44)	G*U*U*CACACCAUCACGACGCG (SEQ ID NO: 45)	
REG1-Z09 sgRNA (EX4_T6)	CACGAUCCCGUCAGACUCGUguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 46)	C*A*C*GAUCCCGUCAGACUCGUguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 47)	CACGATCCCGTCAGACTCGT (AGG) (SEQ ID NO: 179) CACGATCCCGTCAGACTCGT (SEQ ID NO: 326)
REG1-Z09 sgRNA (EX4_T6) spacer	CACGAUCCCGUCAGACUCGU (SEQ ID NO: 48)	C*A*C*GAUCCCGUCAGACUCGU (SEQ ID NO: 49)	
REG1-Z10 sgRNA (EX4_T7)	ACGACGCGUGGGUGGCAAGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 50)	A*C*G*ACGCGUGGGUGGCAAGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 51)	ACGACGCGTGGGTGGCAAGC (GGG) (SEQ ID NO: 180) ACGACGCGTGGGTGGCAAGC (SEQ ID NO: 327)
REG1-Z10 sgRNA (EX4_T7) spacer	ACGACGCGUGGGTGGCAAGC (SEQ ID NO: 52)	A*C*G*ACGCGUGGGUGGCAAGC (SEQ ID NO: 53)	

* indicates a nucleotide with a 2′-O-methyl phosphorothioate modification.

Table 23. sgRNA Sequences and Target Gene Sequences for *TRAC*, *β2M*, and *CD70*

sgRNA Sequences			SEQ ID NO:
***CD70* sgRNA (CD70-7)**	Modified	G*C*U*UUGGUCCCAUUGGUCGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U	54
	Unmodified	GCUUUGGUCCCAUUGGUCGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU	55
***CD70* sgRNA spacer**	Modified	G*C*U*UUGGUCCCAUUGGUCGC	56
	Unmodified	GCUUUGGUCCCAUUGGUCGC	57
***TRAC* sgRNA (TA-1)**	Modified	A*G*A*GCAACAGUGCUGUGGCCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U	58

	Unmodified	AGAGCAACAGUGCUGUGGCCguuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcUUUU	59
***TRAC* sgRNA spacer**	Modified	A*G*A*GCAACAGUGCUGUGGCC	60
	Unmodified	AGAGCAACAGUGCUGUGGCC	61
***β2M* sgRNA (β2M-1)**	Modified	G*C*U*ACUCUCUCUUUCUGGCCguuuuagagcuagaaauagc aaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggc accgagucggugcU*U*U*U	62
	Unmodified	GCUACUCUCUCUUUCUGGCCguuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuaucaacuugaaaaaguggcacc gagucggugcUUUU	63
***β2M* sgRNA spacer**	Modified	G*C*U*ACUCUCUCUUUCUGGCC	64
	Unmodified	GCUACUCUCUCUUUCUGGCC	65
Target Sequences (PAM)			
***CD70* target sequence with (PAM)**	GCTTTGGTCCCATTGGTCGC (GGG)		66
***CD70* target sequence**	GCTTTGGTCCCATTGGTCGC		67
***TRAC* target sequence with (PAM)**	AGAGCAACAGTGCTGTGGCC (TGG)		68
***TRAC* target sequence**	AGAGCAACAGTGCTGTGGCC		69
***β2M* target sequence with (PAM)**	GCTACTCTCTCTTTCTGGCC (TGG)		70
***β2M* target sequence**	GCTACTCTCTCTTTCTGGCC		71
Exemplary sgRNA Formulas			
sgRNA sequence	nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugcuuuu		72
sgRNA sequence	nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcu aguccguuaucaacuugaaaaaguggcaccgagucggugc		73
sgRNA sequence	n(17-30)guuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguua ucaacuugaaaaaguggcaccgagucggugcu(1-8)		74

* indicates a nucleotide with a 2′-O-methyl phosphorothioate modification.
"n" refers to the spacer sequence at the 5′ end.

Sequence Table 24. Edited TRAC Gene Sequence.

Description	**Sequence** (Deletions indicated by dashes (-); insertions indicated by **bold**)	**SEQ ID NO:**
TRAC gene edit	AA----------GAGCAACAAATCTGACT	75
TRAC gene edit	AAGAGCAACAGTGCTGT-GCCTGGAGCAACAAATCTGACT	76
TRAC gene edit	AAGAGCAACAGTG-------CTGGAGCAACAAATCTGACT	77
TRAC gene edit	AAGAGCAACAGT------GCCTGGAGCAACAAATCTGACT	78
TRAC gene edit	AAGAGCAACAGTG----------CTGACT	79
TRAC gene edit	AAGAGCAACAGTGCTGT**G**GGCCTGGAGCAACAAATCTGACT	80

TRAC gene edit	AAGAGCAACAGTGC--TGGCCTGGAGCAACAAATCTGACT	81
TRAC gene edit	AAGAGCAACAGTGCTGTG**T**GCCTGGAGCAACAAATCTGACT	82

Sequence Table 25. Edited *β2M* Gene Sequence.

Description	**Sequence** (Deletions indicated by dashes (-); insertions indicated by **bold**)	**SEQ ID NO:**
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCT- GCCTGGAGGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	83
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTC-- GCCTGGAGGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	84
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTT----- CTGGAGGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	85
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTG**GATA**GCCTGGAGGC TATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	86
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGC---------- GCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	87
β2M gene-edit	CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTC**TG**TGGCCTGGAGGCTA TCCAGCGTGAGTCTCTCCTACCCTCCCGCT	88

Sequence Table 26. Edited *CD70* Gene Sequence.

Description	**Sequence** (Deletions indicated by dashes (-); insertions indicated by **bold**)	**SEQ ID NO:**
CD70 gene-edit	CACACCACGAGGCAGATCACCAAGCCCGCG-- CAATGGGACCAAAGCAGCCCGCAGGACG	89
CD70 gene-edit	CACACCACGAGGCAGATCACCAAGCCCGCG**A**ACCAATGGGACCAAAGCAGCC CGCAGGACG	90
CD70 gene-edit	CACACCACGAGGCAGATC---------- ACCAATGGGACCAAAGCAGCCCGCAGGACG	91
CD70 gene-edit	CACACCACGAGGCAGATCACCAAGCCCGCG- CCAATGGGACCAAAGCAGCCCGCAGGACG	92
CD70 gene-edit	CACACCACGAGGCAGATCACCAAGCCCGC- ACCAATGGGACCAAAGCAGCCCGCAGGACG	93
CD70 gene-edit	CACACCACGAGGCAGATCACCA---------- AGCCCGCAGGACG	94

Sequence Table 27. Chimeric Antigen Receptor Sequences

SEQ ID NO	**Description**	**Sequence**
95	signal peptide	MLLLVTSLLLCELPHPAFLLIP
96	signal peptide	MALPVTALLLPLALLLHAARP
97	CD8a transmembrane domain	IYIWAPLAGTCGVLLLSLVITLY
98	4-1BB nucleotide sequence	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGA GACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCC AGAAGAAGAAGAAGGAGGATGTGAACTG
99	4-1BB amino acid sequence	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO	Description	Sequence
100	CD28 nucleotide sequence	TCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTCCTC GCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCCCCCCACG AGACTTCGCTGCGTACAGGTCC
101	CD28 amino acid sequence	SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
102	CD3-zeta nucleotide sequence	CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGAC AGAATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGA CGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCC CGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATA AGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACG GGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAA GATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGA
103	CD3-zeta amino acid sequence	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
105	anti-CD19 VL CDR1 (Kabat)	RASQDISKYLN
106	anti-CD19 VL CDR2 (Kabat)	HTSRLHS
107	anti-CD19 VL CDR3 (Kabat)	QQGNTLPYT
108	anti-CD19 VH CDR1 (Kabat)	DYGVS
109	anti-CD19 VH CDR2 (Kabat)	VIWGSETTYYNSALKS
110	anti-CD19 VH CDR3 (Kabat)	HYYYGGSYAMDY
111	anti-CD19 VL CDR1 (Chothia)	RASQDISKYLN
112	anti-CD19 VL CDR2 (Chothia)	HTSRLHS
113	anti-CD19 VL CDR3 (Chothia)	QQGNTLPYT
114	anti-CD19 VH CDR1 (Chothia)	GVSLPDY
115	anti-CD19 VH CDR2 (Chothia)	WGSET
116	anti-CD19 VH CDR3 (Chothia)	HYYYGGSYAMDY
117	Anti-CD19 CAR FMC63-28Z (FMC63-CD8[tm]-CD28[co-	ATGCTTCTTTTGGTTACGTCTCTGTTGCTTTGCGAACTTCCTCATCCAG CGTTCTTGCTGATCCCCGATATTCAGATGACTCAGACCACCAGTAGCTT GTCTGCCTCACTGGGAGACCGAGTAACAATCTCCTGCAGGGCAAGTCAA GACATTAGCAAATACCTCAATTGGTACCAGCAGAAGCCCGACGGAACGG TAAAACTCCTCATCTATCATACGTCAAGGTTGCATTCCGGAGTACCGTC ACGATTTTCAGGTTCTGGGAGCGGAACTGACTATTCCTTGACTATTTCA

SEQ ID NO	Description	Sequence
	stimulatory domain]-CD3z)	AACCTCGAGCAGGAGGACATTGCGACATATTTTTGTCAACAAGGTAATA CCCTCCCTTACACTTTCGGAGGAGGAACCAAACTCGAAATTACCGGGTC CACCAGTGGCTCTGGGAAGCCTGGCAGTGGAGAAGGTTCCACTAAAGGC GAGGTGAAGCTCCAGGAGAGCGGCCCCGGTCTCGTTGCCCCCAGTCAAA GCCTCTCTGTAACGTGCACAGTGAGTGGTGTATCATTGCCTGATTATGG CGTCTCCTGGATAAGGCAGCCCCCGCGAAAGGGTCTTGAATGGCTTGGG GTAATATGGGGCTCAGAGACAACGTATTATAACTCCGCTCTCAAAAGTC GCTTGACGATAATAAAAGATAACTCCAAGAGTCAAGTTTTCCTTAAAAT GAACAGTTTGCAGACTGACGATACCGCTATATATTATTGTGCTAAACAT TATTACTACGGCGGTAGTTACGCGATGGATTATTGGGGGCAGGGGACTT CTGTCACAGTCAGTAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGC CAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACC ATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCG CCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTA CATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTC GTTATTACTTTGTATTGTAATCACAGGAATCGCTCAAAGCGGAGTAGGT TGTTGCATTCCGATTACATGAATATGACTCCTCGCCGGCCTGGGCCGAC AAGAAAACATTACCAACCCTATGCCCCCCCCACGAGACTTCGCTGCGTAC AGGTCCCGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGC AAGGACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGA GTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATGGGGGGT AAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGA AGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACG ACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCA ACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGA
118	Anti-CD19 CAR FMC63-28Z (FMC63-CD8[tm]-CD28[co-stimulatory domain]-CD3z) Amino Acid with signal peptide	*MLLLVTSLLLCELPHPAFLLIP*DIQMTQTTSSLSASLGDRVTISCRASQ DISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTIS NLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH YYYGGSYAMDYWGQGTSVTVSSAAAFVPVFLPAKPTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL VITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
353	Anti-CD19 CAR FMC63-28Z (FMC63-CD8[tm]-CD28[co-stimulatory domain]-CD3z) Amino Acid without signal peptide	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF GGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTC TVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIK DNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDY MNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
119	Anti-CD19 scFv coding sequence	GATATTCAGATGACTCAGACCACCAGTAGCTTGTCTGCCTCACTGGGAG ACCGAGTAACAATCTCCTGCAGGGCAAGTCAAGACATTAGCAAATACCT CAATTGGTACCAGCAGAAGCCCGACGGAACGGTAAAACTCCTCATCTAT CATACGTCAAGGTTGCATTCCGGAGTACCGTCACGATTTTCAGGTTCTG GGAGCGGAACTGACTATTCCTTGACTATTTCAAACCTCGAGCAGGAGGA CATTGCGACATATTTTTGTCAACAAGGTAATACCCTCCCTTACACTTTC GGAGGAGGAACCAAACTCGAAATTACCGGGTCCACCAGTGGCTCTGGGA AGCCTGGCAGTGGAGAAGGTTCCACTAAAGGCGAGGTGAAGCTCCAGGA GAGCGGCCCCGGTCTCGTTGCCCCCAGTCAAAGCCTCTCTGTAACGTGC ACAGTGAGTGGTGTATCATTGCCTGATTATGGCGTCTCCTGGATAAGGC

SEQ ID NO	Description	Sequence
		AGCCCCCGCGAAAGGGTCTTGAATGGCTTGGGGTAATATGGGGCTCAGA GACAACGTATTATAACTCCGCTCTCAAAAGTCGCTTGACGATAATAAAA GATAACTCCAAGAGTCAAGTTTTCCTTAAAATGAACAGTTTGCAGACTG ACGATACCGCTATATATTATTGTGCTAAACATTATTACTACGGCGGTAG TTACGCGATGGATTATTGGGGGCAGGGGACTTCTGTCACAGTCAGTAGT
120	Anti-CD19 scFv amino acid sequence Linker underlined	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF GGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTC TVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIK DNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS
121	CD8a extracellular + CD8a transmembrane + 5' Linker (underlined)	GCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTC CCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCT TAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCAT ACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGG CGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTG TAATCACAGGAATCGC
122	CD8a extracellular + CD8a transmembrane (without linker)	TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGC GCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCG CCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGC TTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGT GCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAG GAATCGC
123	CD8a extracellular + CD8a transmembrane	FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNR
124	Anti-CD19 VH	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLG VIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKH YYYGGSYAMDYWGQGTSVTVSS
125	Anti-CD19 VL	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIY HTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTF GGGTKLEIT
126	CD19 linker	GSTSGSGKPGSGEGSTKG
127	CD70 VL CDR1 (Kabat)	RASKSVSTSGYSFMH
128	CD70 VL CDR1 (Chothia)	SKSVSTSGYSF
129	CD70 VL CDR2 (Kabat)	LASNLES
N/A	CD70 VL CDR2 (Chothia)	LAS
130	CD70 VL CDR3 (Kabat)	QHSREVPWT
131	CD70 VL CDR3 (Chothia)	SREVPW
132	CD70 VH CDR1 (Kabat)	NYGMN
133	CD70 VH CDR1 (Chothia)	GYTFTNYGMN
134	CD70 VH CDR2 (Kabat)	WINTYTGEPTYADAFKG
135	CD70 VH CDR2	NTYTGE

SEQ ID NO	Description	Sequence
	(Chothia)	
136	CD70 VH CDR3 (Kabat)	DYGDYGMDY
137	CD70 VH CDR3 (Chothia)	CARDYGDYGMDYWG
138	CD70 CAR amino acid sequence (CD70B scFv with 41BB) With signal peptide	*MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGYT FTNYGMNWVRQAPGQGLKWMGWINTYTGEPTYADAFKGRVTMTRDTSIS TAYMELSRLRSDDTAVYYCARDYGDYGMDYWGQGTTVTVSSGGGGSGGG GSGGGGSGDIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQ QKPGQPPKLLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVY YCQHSREVPWTFGQGTKVEIKSAAAFVPVFLPAKPTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL VITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR
354	CD70 CAR amino acid sequence (CD70B scFv with 41BB) Without signal peptide	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLKWMG WINTYTGEPTYADAFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR DYGDYGMDYWGQGTTVTVSSGGGGSGGGGSGGGGSGDIVMTQSPDSLAV SLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPKLLIYLASNLESGV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREVPWTFGQGTKVEIK SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
139	Anti-CD70A scFv nucleotide sequence	GATATAGTTATGACCCAATCACCCGATAGTCTTGCGGTAAGCCTGGGGG AGCGAGCAACAATAAACTGTCGGGCATCAAAATCCGTCAGTACAAGCGG GTATTCATTCATGCACTGGTATCAACAGAAACCCGGTCAGCCACCCAAG CTCCTGATTTATCTTGCGTCTAATCTTGAGTCCGGCGTCCCAGACCGGT TTTCCGGCTCCGGGAGCGGCACGGATTTTACTCTTACTATTTCTAGCCT TCAGGCCGAAGATGTGGCGGTATACTACTGCCAGCATTCAAGGGAAGTT CCTTGGACGTTCGGTCAGGGCACGAAAGTGGAAATTAAAGGCGGGGGGG GATCCGGCGGGGGAGGGTCTGGAGGAGGTGGCAGTGGTCAGGTCCAACT GGTGCAGTCCGGGGCAGAGGTAAAAAAACCCGGCGCGTCTGTTAAGGTT TCATGCAAGGCCAGTGGATATACTTTCACCAATTACGGAATGAACTGGG TGAGGCAGGCCCCTGGTCAAGGCCTGAAATGGATGGGATGGATAAACAC GTACACCGGTGAACCTACCTATGCCGATGCCTTTAAGGGTCGGGTTACG ATGACGAGAGACACCTCCATATCAACAGCCTACATGGAGCTCAGCAGAT TGAGGAGTGACGATACGGCAGTCTATTACTGTGCAAGAGACTACGGCGA TTATGGCATGGATTACTGGGGCCAGGGCACTACAGTAACCGTTTCCAGC
140	Anti-CD70A scFv amino acid sequence (linker underlined)	DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPK LLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREV PWTFGQGTKVEIKGGGGSGGGGSGGGGSGQVQLVQSGAEVKKPGASVKV SCKASGYTFTNYGMNWVRQAPGQGLKWMGWINTYTGEPTYADAFKGRVT MTRDTSISTAYMELSRLRSDDTAVYYCARDYGDYGMDYWGQGTTVTVSS
141	Anti-CD70B scFv nucleotide sequence	CAGGTCCAGTTGGTGCAAAGCGGGGCGGAGGTGAAAAAACCCGGCGCTT CCGTGAAGGTGTCCTGTAAGGCGTCCGGTTATACGTTCACGAACTACGG GATGAATTGGGTTCGCCAAGCGCCGGGGCAGGGACTGAAATGGATGGGG TGGATAAATACCTACACCGGCGAACCTACATACGCCGACGCTTTTAAAG GGCGAGTCACTATGACGCGCGATACCAGCATATCCACCGCATACATGGA GCTGTCCCGACTCCGGTCAGACGACACGGCTGTCTACTATTGTGCTCGG GACTATGGCGATTATGGCATGGACTACTGGGGTCAGGGTACGACTGTAA

SEQ ID NO	Description	Sequence
		CAGTTAGTAGTGGTGGAGGCGGCAGTGGCGGGGGGGGAAGCGGAGGAGG GGGTTCTGGTGACATAGTTATGACCCAATCCCCAGATAGTTTGGCGGTT TCTCTGGGCGAGAGGGCAACGATTAATTGTCGCGCATCAAAGAGCGTTT CAACGAGCGGATATTCTTTTATGCATTGGTACCAGCAAAAACCCGGACA ACCGCCGAAGCTGCTGATCTACTTGGCTTCAAATCTTGAGTCTGGGGTG CCGGACCGATTTTCTGGTAGTGGAAGCGGAACTGACTTTACGCTCACGA TCAGTTCACTGCAGGCTGAGGATGTAGCGGTCTATTATTGCCAGCACAG TAGAGAAGTCCCCTGGACCTTCGGTCAAGGCACGAAAGTAGAAATTAAA
142	Anti-CD70B scFv amino acid sequence (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLKWMG WINTYTGEPTYADAFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR DYGDYGMDYWGQGTTVTVSSGGGGSGGGGSGGGGSGDIVMTQSPDSLAV SLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPKLLIYLASNLESGV PDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREVPWTFGQGTKVEIK
143	Anti-CD70 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLKWMG WINTYTGEPTYADAFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR DYGDYGMDYWGQGTTVTVSS
144	Anti-CD70 VL	DIVMTQSPDSLAVSLGERATINCRASKSVSTSGYSFMHWYQQKPGQPPK LLIYLASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSREV PWTFGQGTKVEIK
145	BCMA CAR nucleotide sequence	ATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCC ACGCAGCAAGGCCGCAGGTGCAGCTGGTGCAGAGCGGAGCCGAGCTCAA GAAGCCCGGAGCCTCCGTGAAGGTGAGCTGCAAGGCCAGCGGCAACACC CTGACCAACTACGTGATCCACTGGGTGAGACAAGCCCCCGGCCAAAGGC TGGAGTGGATGGGCTACATCCTGCCCTACAACGACCTGACCAAGTACAG CCAGAAGTTCCAGGGCAGGGTGACCATCACCAGGGATAAGAGCGCCTCC ACCGCCTATATGGAGCTGAGCAGCCTGAGGAGCGAGGACACCGCTGTGT ACTACTGTACAAGGTGGGACTGGGACGGCTTCTTTGACCCCTGGGGCCA GGGCACAACAGTGACCGTCAGCAGCGGCGGCGGAGGCAGCGGCGGCGGC GGCAGCGGCGGAGGCGGAAGCGAAATCGTGATGACCCAGAGCCCCGCCA CACTGAGCGTGAGCCCTGGCGAGAGGGCCAGCATCTCCTGCAGGGCTAG CCAAAGCCTGGTGCACAGCAACGGCAACACCCACCTGCACTGGTACCAG CAGAGACCCGGACAGGCTCCCAGGCTGCTGATCTACAGCGTGAGCAACA GGTTCTCCGAGGTGCCTGCCAGGTTTAGCGGCAGCGGAAGCGGCACCGA CTTTACCCTGACCATCAGCAGCGTGGAGTCCGAGGACTTCGCCGTGTAT TACTGCAGCCAGACCAGCCACATCCCTTACACCTTCGGCGGCGGCACCA AGCTGGAGATCAAAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGC CAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACC ATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCG CCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTA CATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTC GTTATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGA AACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTAC TCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGA GGATGTGAACTGCGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCAT ATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCCG CGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATG GGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAAC TCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGG CGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGT ACGGCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTC CCAGA
146	BCMA CAR amino	*MALPVTALLLPLALLLHAARP*QVQLVQSGAELKKPGASVKVSCKASGNT LTNYVIHWVRQAPGQRLEWMGYILPYNDLTKYSQKFQGRVTITRDKSAS

SEQ ID NO	Description	Sequence
	acid sequence With signal peptide	TAYMELSSLRSEDTAVYYCTRWDWDGFFDPWGQGTTVTVSSGGGGSGGG GSGGGGSEIVMTQSPATLSVSPGERASISCRASQSLVHSNGNTHLHWYQ QRPGQAPRLLIYSVSNRFSEVPARFSGSGSGTDFTLTISSVESEDFAVY YCSQTSHIPYTFGGGTKLEIKSAAAFVPVFLPAKPTTTPAPRPPTPAPT IASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL VITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR
355	BCMA CAR amino acid sequence Without signal peptide	QVQLVQSGAELKKPGASVKVSCKASGNTLTNYVIHWVRQAPGQRLEWMG YILPYNDLTKYSQKFQGRVTITRDKSASTAYMELSSLRSEDTAVYYCTR WDWDGFFDPWGQGTTVTVSSGGGGSGGGGSGGGGSEIVMTQSPATLSVS PGERASISCRASQSLVHSNGNTHLHWYQQRPGQAPRLLIYSVSNRFSEV PARFSGSGSGTDFTLTISSVESEDFAVYYCSQTSHIPYTFGGGTKLEIK SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
147	BCMA scFv nucleotide sequence	CAGGTGCAGCTGGTGCAGAGCGGAGCCGAGCTCAAGAAGCCCGGAGCCT CCGTGAAGGTGAGCTGCAAGGCCAGCGGCAACACCCTGACCAACTACGT GATCCACTGGGTGAGACAAGCCCCCGGCCAAAGGCTGGAGTGGATGGGC TACATCCTGCCCTACAACGACCTGACCAAGTACAGCCAGAAGTTCCAGG GCAGGGTGACCATCACCAGGGATAAGAGCGCCTCCACCGCCTATATGGA GCTGAGCAGCCTGAGGAGCGAGGACACCGCTGTGTACTACTGTACAAGG TGGGACTGGGACGGCTTCTTTGACCCCTGGGGCCAGGGCACAACAGTGA CCGTCAGCAGCGGCGGCGGAGGCAGCGGCGGCGGCGGCAGCGGCGGAGG CGGAAGCGAAATCGTGATGACCCAGAGCCCCGCCACACTGAGCGTGAGC CCTGGCGAGAGGGCCAGCATCTCCTGCAGGGCTAGCCAAAGCCTGGTGC ACAGCAACGGCAACACCCACCTGCACTGGTACCAGCAGAGACCCGGACA GGCTCCCAGGCTGCTGATCTACAGCGTGAGCAACAGGTTCTCCGAGGTG CCTGCCAGGTTTAGCGGCAGCGGAAGCGGCACCGACTTTACCCTGACCA TCAGCAGCGTGGAGTCCGAGGACTTCGCCGTGTATTACTGCAGCCAGAC CAGCCACATCCCTTACACCTTCGGCGGCGGCACCAAGCTGGAGATCAAA
148	BCMA scFv amino acid sequence (linker underlined)	QVQLVQSGAELKKPGASVKVSCKASGNTLTNYVIHWVRQAPGQRLEWMG YILPYNDLTKYSQKFQGRVTITRDKSASTAYMELSSLRSEDTAVYYCTR WDWDGFFDPWGQGTTVTVSSGGGGSGGGGSGGGGSEIVMTQSPATLSVS PGERASISCRASQSLVHSNGNTHLHWYQQRPGQAPRLLIYSVSNRFSEV PARFSGSGSGTDFTLTISSVESEDFAVYYCSQTSHIPYTFGGGTKLEIK
149	BCMA VH	QVQLVQSGAELKKPGASVKVSCKASGNTLTNYVIHWVRQAPGQRLEWMG YILPYNDLTKYSQKFQGRVTITRDKSASTAYMELSSLRSEDTAVYYCTR WDWDGFFDPWGQGTTVTVSS
150	BCMA VL	EIVMTQSPATLSVSPGERASISCRASQSLVHSNGNTHLHWYQQRPGQAP RLLIYSVSNRFSEVPARFSGSGSGTDFTLTISSVESEDFAVYYCSQTSH IPYTFGGGTKLEIK
151	BCMA VL CDR1 (Kabat & Chothia)	RASQSLVHSNGNTHLH
152	BCMA VL CDR2 (Kabat & Chothia)	SVSNRFS
153	BCMA VL CDR3	SQTSHIPYT

SEQ ID NO	Description	Sequence
	(Kabat)	
154	BCMA VL CDR3 (Chothia)	SQTSHIPYT
155	BCMA VH CDR1 (Kabat)	NYVIH
156	BCMA VH CDR1 (Chothia)	GNTLTNY
157	BCMA VH CDR2 (Kabat)	YILPYNDLTKYSQKFQG
158	BCMA VH CDR2 (Chothia)	LPYNDL
159	BCMA VH CDR3 (Kabat)	WDWDGFFDP
160	BCMA VH CDR3 (Chothia)	WDWDGFFDP
328	anti-CD33 antibody VH CDR1 (Kabat)	SYYIH
329	anti-CD33 antibody VH CDR2 (Kabat)	VIYPGNDDISYNQKFQG
330	anti-CD33 antibody VH CDR3 (Kabat)	EVRLRYFDV
331	anti-CD33 antibody VL CDR1 (Kabat)	KSSQSVFFSSSQKNYLA
332	anti-CD33 antibody VL CDR2 (Kabat)	WASTRES
333	anti-CD33 antibody VL CDR3 (Kabat)	HQYLSSRT
334	anti-CD33 antibody VH	QVQLQQPGAEVVKPGASVKMSCKASGYTFTSYYIHWIKQTPGQGLEWVG VIYPGNDDISYNQKFQGKATLTADKSSTTAYMQLSSLTSEDSAVYYCAR EVRLRYFDVWGQGTTVTVSS
335	anti-CD33 antibody VL	EIVLTQSPGSLAVSPGERVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQS PRLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAIYYCHQYL SSRTFGQGTKLEIK
336	Anti-CD33 and anti-CD33b scFv Linker underlined	EIVLTQSPGSLAVSPGERVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQS PRLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAIYYCHQYL SSRTFGQGTKLEIKGGGGGSGGGGSGGGGSQVQLQQPGAEVVKPGASVK MSCKASGYTFTSYYIHWIKQTPGQGLEWVGVIYPGNDDISYNQKFQGKA TLTADKSSTTAYMQLSSLTSEDSAVYYCAREVRLRYFDVWGQGTTVTVS S
337	Anti-CD33 and anti-CD33b scFv	GAAATCGTCCTCACACAATCCCCGGGGAGCCTCGCAGTCAGTCCTGGGG AACGAGTCACTATGAGCTGCAAATCCAGTCAGAGTGTTTTTTTCTCAAG TAGCCAGAAGAACTACCTCGCATGGTACCAACAAATACCGGGGCAATCT CCCCGCTTGCTTATATACTGGGCAAGTACCCGCGAATCCGGCGTACCGG ATCGATTCACGGGATCTGGGTCAGGTACTGATTTCACTTTGACTATCAG CTCTGTTCAGCCTGAAGATTTGGCAATTTACTACTGTCACCAATACTTG AGTAGCCGAACTTTCGGCCAGGGCACGAAGCTCGAAATCAAGGGCGGAG GGGGAGGTTCTGGTGGGGGCGGTTCTGGCGGTGGAGGAAGCCAAGTACA GTTGCAACAGCCAGGGGCGGAGGTCGTAAAACCTGGGGCGTCTGTCAAG ATGAGCTGTAAAGCAAGTGGATACACCTTCACCTCCTACTATATACATT GGATTAAGCAAACTCCGGGTCAGGGGCTGGAATGGGTTGGCGTTATATA

SEQ ID NO	Description	Sequence
		CCCCGGGAACGATGATATATCATACAACCAAAAATTTCAAGGCAAGGCG ACTCTGACTGCCGATAAGAGTAGCACAACAGCTTACATGCAGCTTTCTT CCCTGACCAGCGAAGATTCAGCAGTTTACTACTGCGCTCGGGAAGTGCG CCTGCGATACTTTGATGTCTGGGGTCAAGGAACTACAGTTACTGTATCA AGC
338	Anti-CD33 CAR CD28 costim. With signal peptide	*MALPVTALLLPLALLLHAARP*EIVLTQSPGSLAVSPGERVTMSCKSSQS VFFSSSQKNYLAWYQQIPGQSPRLLIYWASTRESGVPDRFTGSGSGTDF TLTISSVQPEDLAIYYCHQYLSSRTFGQGTKLEIKGGGGGSGGGGSGGG GSQVQLQQPGAEVVKPGASVKMSCKASGYTFTSYYIHWIKQTPGQGLEW VGVIYPGNDDISYNQKFQGKATLTADKSSTTAYMQLSSLTSEDSAVYYC AREVRLRYFDVWGQGTTVTVSSSAAAFVPVFLPAKPTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAA YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR
356	Anti-CD33 CAR CD28 costim. Without signal peptide	EIVLTQSPGSLAVSPGERVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQS PRLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAIYYCHQYL SSRTFGQGTKLEIKGGGGGSGGGGSGGGGSQVQLQQPGAEVVKPGASVK MSCKASGYTFTSYYIHWIKQTPGQGLEWVGVIYPGNDDISYNQKFQGKA TLTADKSSTTAYMQLSSLTSEDSAVYYCAREVRLRYFDVWGQGTTVTVS SSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHS DYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
339	Anti-CD33b CAR 41BB costim. With signal peptide	*MALPVTALLLPLALLLHAARP*EIVLTQSPGSLAVSPGERVTMSCKSSQS VFFSSSQKNYLAWYQQIPGQSPRLLIYWASTRESGVPDRFTGSGSGTDF TLTISSVQPEDLAIYYCHQYLSSRTFGQGTKLEIKGGGGGSGGGGSGGG GSQVQLQQPGAEVVKPGASVKMSCKASGYTFTSYYIHWIKQTPGQGLEW VGVIYPGNDDISYNQKFQGKATLTADKSSTTAYMQLSSLTSEDSAVYYC AREVRLRYFDVWGQGTTVTVSSSAAAFVPVFLPAKPTTTPAPRPPTPAP TIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEE GGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR
357	Anti-CD33b CAR 41BB costim. Without signal peptide	EIVLTQSPGSLAVSPGERVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQS PRLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQPEDLAIYYCHQYL SSRTFGQGTKLEIKGGGGGSGGGGSGGGGSQVQLQQPGAEVVKPGASVK MSCKASGYTFTSYYIHWIKQTPGQGLEWVGVIYPGNDDISYNQKFQGKA TLTADKSSTTAYMQLSSLTSEDSAVYYCAREVRLRYFDVWGQGTTVTVS SSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLY IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
340	Anti-PTK7 VH CDR1	SYGMH
341	Anti-PTK7 VH CDR2	VIWDDGSNKYYVDSVKG
342	Anti-PTK7 VH CDR3	DDYYGSGSFNSYYGTDV
343	Anti-PTK7 VL CDR1	RASQSVSIYLA
344	Anti-PTK7	DASNRAT

SEQ ID NO	Description	Sequence
	VL CDR2	
345	Anti-PTK7 VL CDR3	QQRSNWPPFT
346	Anti-PTK7 V_H	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVIWDDGSNKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARDDYYGSGSFNSYYGTDVWGQGTTVTVSS
347	Anti-PTK7 V_L	EIVLTQSPATLSLSPGERATLSCRASQSVSIYLAWYQQKPGQAPR LLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQ RSNWPPFTFGPGTKVDIK
348	Anti-PTK7 scFv (linker underlined)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVIWDDGSNKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARDDYYGSGSFNSYYGTDVWGQGTTVTVSSGGGGSGGGG SGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSIYLAWYQQK PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFA VYYCQQRSNWPPFTFGPGTKVDIK
349	Anti-PTK7 CAR CD28 co-stim With signal peptide	*MALPVTALLLPLALLLHAARP*QVQLVESGGGVVQPGRSLRLSCAA SGFTFSSYGMHWVRQAPGKGLEWVAVIWDDGSNKYYVDSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARDDYYGSGSFNSYYGTD VWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGER ATLSCRASQSVSIYLAWYQQKPGQAPRLLIYDASNRATGIPARFS GSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIK SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSK RSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
358	Anti-PTK7 CAR CD28 co-stim Without signal peptide	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVIWDDGSNKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCARDDYYGSGSFNSYYGTDVWGQGTTVTVSSGGGGSGGGG SGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSIYLAWYQQK PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFA VYYCQQRSNWPPFTFGPGTKVDIKSAAAFVPVFLPAKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTR KHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
350	Anti-PTK7 CAR 41BB co-stim With signal peptide	*MALPVTALLLPLALLLHAARP*QVQLVESGGGVVQPGRSLRLSCAA SGFTFSSYGMHWVRQAPGKGLEWVAVIWDDGSNKYYVDSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARDDYYGSGSFNSYYGTD VWGQGTTVTVSSGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGER ATLSCRASQSVSIYLAWYQQKPGQAPRLLIYDASNRATGIPARFS GSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGPGTKVDIK SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR
359	Anti-PTK7 CAR 41BB co-stim	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGL EWVAVIWDDGSNKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAED

SEQ ID NO	Description	Sequence
	Without signal peptide	TAVYYCARDDYYGSGSFNSYYGTDVWGQGTTVTVSSGGGGSGGGG SGGGGSEIVLTQSPATLSLSPGERATLSCRASQSVSIYLAWYQQK PGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFA VYYCQQRSNWPPFTFGPGTKVDIKSAAAFVPVFLPAKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPL AGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQ EEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Table 28. AAV Donor Template Sequences

161	Left ITR (5' ITR)	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGAC CAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGA GCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT
162	Left ITR (5' ITR) (alternate)	CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTC GGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA GGGAGTGGCCAACTCCATCACTAGGGGTTCCT
163	Right ITR (3' ITR)	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTC GCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGT CGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA
164	Right ITR (3' ITR) (alternate)	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTC GCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCC CGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG
165	*TRAC*-LHA (800bp)	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCA
166	*TRAC*-RHA (800bp)	TGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCA TTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGG TGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCA GAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCC TTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTT GTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAG GTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTC CTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAG GCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTG TCTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCAC TCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGT GTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCAGAGGAA GCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAA TAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTAC CTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAA

		GATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGA CAGGAGCTCAATGAGAAAGG
167	EF1a	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCG AGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTG GCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTT CCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACG TTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTG TGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCT TGAATTACTTCCACTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCG GGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCT TCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCG TGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTC TCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGG CAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGG TTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATG TTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGG TAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGT GTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAA TGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAA GGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGT ACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCC ACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTA ATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTC AAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGT GA
168	CD19 LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCG GTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCA GTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC AGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTA TGGCCCTTGCGTGCCTTGAATTACTTCCACTGGCTGCAGTACGTGATTC TTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTG CGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCG CTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC GACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTG CACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTG CGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGC TGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGT

		GAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTT CTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGGAGGGGTTTTAT GCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAG CTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGG ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTT CCATTTCAGGTGTCGTGACCACCATGCTTCTTTTGGTTACGTCTCTGTT GCTTTGCGAACTTCCTCATCCAGCGTTCTTGCTGATCCCCGATATTCAG ATGACTCAGACCACCAGTAGCTTGTCTGCCTCACTGGGAGACCGAGTAA CAATCTCCTGCAGGGCAAGTCAAGACATTAGCAAATACCTCAATTGGTA CCAGCAGAAGCCCGACGGAACGGTAAAACTCCTCATCTATCATACGTCA AGGTTGCATTCCGGAGTACCGTCACGATTTTCAGGTTCTGGGAGCGGAA CTGACTATTCCTTGACTATTTCAAACCTCGAGCAGGAGGACATTGCGAC ATATTTTTGTCAACAAGGTAATACCCTCCCTTACACTTTCGGAGGAGGA ACCAAACTCGAAATTACCGGGTCCACCAGTGGCTCTGGGAAGCCTGGCA GTGGAGAAGGTTCCACTAAAGGCGAGGTGAAGCTCCAGGAGAGCGGCCC CGGTCTCGTTGCCCCCAGTCAAAGCCTCTCTGTAACGTGCACAGTGAGT GGTGTATCATTGCCTGATTATGGCGTCTCCTGGATAAGGCAGCCCCCGC GAAAGGGTCTTGAATGGCTTGGGGTAATATGGGGCTCAGAGACAACGTA TTATAACTCCGCTCTCAAAAGTCGCTTGACGATAATAAAAGATAACTCC AAGAGTCAAGTTTTCCTTAAAATGAACAGTTTGCAGACTGACGATACCG CTATATATTATTGTGCTAAACATTATTACTACGGCGGTAGTTACGCGAT GGATTATTGGGGGCAGGGGACTTCTGTCACAGTCAGTAGTGCTGCTGCC TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGC GCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCG CCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGC TTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGT GCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAG GAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATG ACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCC CCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAAG CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAA CTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGG GGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGA AGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCA GAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCC TCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCA TATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAG ATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGC ATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTC CCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTG CTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAA AACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCT CTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGAC ACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCC CAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGA CTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCC AAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGC TCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGAT TGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAG TCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCC CATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTT TAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAG GGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAG GGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGG
169	CD70 LHA to RHA (CD70B scFV with	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT

	41BB)	TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCG GTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCA GTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC AGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTA TGGCCCTTGCGTGCCTTGAATTACTTCCACTGGCTGCAGTACGTGATTC TTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTG CGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCG CTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC GACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTG CACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTG CGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGC TGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGT GAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTT CTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTAT GCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAG CTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGG ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTT CCATTTCAGGTGTCGTGACCACCATGGCGCTTCCGGTGACAGCACTGCT CCTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTCCAGTTG GTGCAAAGCGGGGCGGAGGTGAAAAAACCCGGCGCTTCCGTGAAGGTGT CCTGTAAGGCGTCCGGTTATACGTTCACGAACTACGGGATGAATTGGGT TCGCCAAGCGCCGGGGCAGGGACTGAAATGGATGGGGTGGATAAATACC TACACCGGCGAACCTACATACGCCGACGCTTTTAAAGGGCGAGTCACTA TGACGCGCGATACCAGCATATCCACCGCATACATGGAGCTGTCCCGACT CCGGTCAGACGACACGGCTGTCTACTATTGTGCTCGGGACTATGGCGAT TATGGCATGGACTACTGGGGTCAGGGTACGACTGTAACAGTTAGTAGTG GTGGAGGCGGCAGTGGCGGGGGGGGAAGCGGAGGAGGGGGTTCTGGTGA CATAGTTATGACCCAATCCCCAGATAGTTTGGCGGTTTCTCTGGGCGAG AGGGCAACGATTAATTGTCGCGCATCAAAGAGCGTTTCAACGAGCGGAT ATTCTTTTATGCATTGGTACCAGCAAAAACCCGGACAACCGCCGAAGCT GCTGATCTACTTGGCTTCAAATCTTGAGTCTGGGGTGCCGGACCGATTT TCTGGTAGTGGAAGCGGAACTGACTTTACGCTCACGATCAGTTCACTGC AGGCTGAGGATGTAGCGGTCTATTATTGCCAGCACAGTAGAGAAGTCCC CTGGACCTTCGGTCAAGGCACGAAAGTAGAAATTAAAAGTGCTGCTGCC TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGC GCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCG CCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGC TTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGT GCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAG GAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCA TTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTC CCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTAT AACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAAC

		GCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCC CCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCC TACTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACG ATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGC ACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCTATCCA TCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCTGA CTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACC TTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTT TCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGAT GTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAA AACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAG AATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCAC GTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTG CTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATC ACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATT AAAAAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGG GGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAAT GTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCA GGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAA GGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAA AGG
170	BCMA RHA to LHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACA TCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCG GTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCA GTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC AGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTA TGGCCCTTGCGTGCCTTGAATTACTTCCACTGGCTGCAGTACGTGATTC TTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTG CGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGG CGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCG CTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGC GACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTG CACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTG CGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGC TGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGT GAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTT CTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTAT

		GCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAG CTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGG ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTT CCATTTCAGGTGTCGTGACCACCATGGCGCTTCCGGTGACAGCACTGCT CCTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTGCAGCTG GTGCAGAGCGGAGCCGAGCTCAAGAAGCCCGGAGCCTCCGTGAAGGTGA GCTGCAAGGCCAGCGGCAACACCCTGACCAACTACGTGATCCACTGGGT GAGACAAGCCCCCGGCCAAAGGCTGGAGTGGATGGGCTACATCCTGCCC TACAACGACCTGACCAAGTACAGCCAGAAGTTCCAGGGCAGGGTGACCA TCACCAGGGATAAGAGCGCCTCCACCGCCTATATGGAGCTGAGCAGCCT GAGGAGCGAGGACACCGCTGTGTACTACTGTACAAGGTGGGACTGGGAC GGCTTCTTTGACCCCTGGGGCCAGGGCACAACAGTGACCGTCAGCAGCG GCGGCGGAGGCAGCGGCGGCGGCGGCAGCGGCGGAGGCGGAAGCGAAAT CGTGATGACCCAGAGCCCCGCCACACTGAGCGTGAGCCCTGGCGAGAGG GCCAGCATCTCCTGCAGGGCTAGCCAAAGCCTGGTGCACAGCAACGGCA ACACCCACCTGCACTGGTACCAGCAGAGACCCGGACAGGCTCCCAGGCT GCTGATCTACAGCGTGAGCAACAGGTTCTCCGAGGTGCCTGCCAGGTTT AGCGGCAGCGGAAGCGGCACCGACTTTACCCTGACCATCAGCAGCGTGG AGTCCGAGGACTTCGCCGTGTATTACTGCAGCCAGACCAGCCACATCCC TTACACCTTCGGCGGCGGCACCAAGCTGGAGATCAAAAGTGCTGCTGCC TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGC GCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCG CCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGC TTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGT GCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAG GAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCA TTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTC CCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTAT AACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAAC GCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCC CCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCC TACTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACG ATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGC ACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCTATCCA TCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCTGA CTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACC TTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTT TCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGAT GTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAA AACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAG AATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCAC GTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTG CTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATC ACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATT AAAAAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGG GGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAAT GTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCA GGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAA GGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAA AGG
351	Anti-CD33 CAR Donor LHA to RHA CD28 costim.	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC

		GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgcaca tcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccg gtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgta ctggctccgccttttcccgagggtgggggagaaccgtatataagtgca gtagtcgccgtgaacgttctttttcgcaacgggtttgccgccagaacac aggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggtta tggcccttgcgtgccttgaattacttccactggctgcagtacgtgattc ttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttg cgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcctggg cgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtctcg ctgctttcgataagtctctagccatttaaaatttttgatgacctgctgc gacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctg cacactggtatttcggttttggggccgcgggcggcgacggggcccgtg cgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggccacc gagaatcggacgggggtagtctcaagctggccggcctgctctggtgcct ggcctcgcgccgccgtgtatcgccccgccctgggcggcaaggctggccc ggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgc tgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggt gagtcacccacacaaaggaaaagggcctttccgtcctcagccgtcgctt catgtgactccacggagtaccgggcgccgtccaggcacctcgattagtt ctcgagcttttggagtacgtcgtctttaggttggggggagggggttttat gcgatggagtttccccacactgagtgggtggagactgaagttaggccag cttggcacttgatgtaattctccttggaatttgccctttttgagtttgg atcttggttcattctcaagcctcagacagtggttcaaagttttttttctt ccatttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCT CCTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGAAATCGTCCTC ACACAATCCCCGGGGAGCCTCGCAGTCAGTCCTGGGGAACGAGTCACTA TGAGCTGCAAATCCAGTCAGAGTGTTTTTTTCTCAAGTAGCCAGAAGAA CTACCTCGCATGGTACCAACAAATACCGGGGCAATCTCCCCGCTTGCTT ATATACTGGGCAAGTACCCGCGAATCCGGCGTACCGGATCGATTCACGG GATCTGGGTCAGGTACTGATTTCACTTTGACTATCAGCTCTGTTCAGCC TGAAGATTTGGCAATTTACTACTGTCACCAATACTTGAGTAGCCGAACT TTCGGCCAGGGCACGAAGCTCGAAATCAAGGGCGGAGGGGGAGGTTCTG GTGGGGGCGGTTCTGGCGGTGGAGGAAGCCAAGTACAGTTGCAACAGCC AGGGGCGGAGGTCGTAAAACCTGGGGCGTCTGTCAAGATGAGCTGTAAA GCAAGTGGATACACCTTCACCTCCTACTATATACATTGGATTAAGCAAA CTCCGGGTCAGGGGCTGGAATGGGTTGGCGTTATATACCCCGGGAACGA TGATATATCATACAACCAAAAATTTCAAGGCAAGGCGACTCTGACTGCC GATAAGAGTAGCACAACAGCTTACATGCAGCTTTCTTCCCTGACCAGCG AAGATTCAGCAGTTTACTACTGCGCTCGGGAAGTGCGCCTGCGATACTT TGATGTCTGGGGTCAAGGAACTACAGTTACTGTATCAAGCAGTGCTGCT GCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCC CGCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCT TCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGG GGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTA CGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCA CAGGAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAAT ATGACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATG CCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCG AAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAAC GAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCC GGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCA AGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTAC

		TCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATG GCCTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACT GCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCG AAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTT TGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTC TTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCC TTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTC TAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAAC CCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAAT GACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTG GCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTC AGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTC TCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCC AGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACT GATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAA AAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGA GCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTG TTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGG AAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGG CAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGG
352	Anti-CD33b CAR Donor LHA to RHA 41BB costim.	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAA CGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAAC CTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAAC TTAATGCCAACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAA GTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGG CCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGT TTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAGTATTATTAAGTAG CCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAAC GTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGT CCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTC CCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCC TTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGG AAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAG AACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACA AGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACA AAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATG AGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgcaca tcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccg gtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgta ctggctccgccttttcccgagggtgggggagaaccgtatataagtgca gtagtcgccgtgaacgttcttttcgcaacgggtttgccgccagaacac aggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggtta tggcccttgcgtgccttgaattacttccactggctgcagtacgtgattc ttgatcccgagcttcgggttggaagtgggtgggagagttcgaggccttg cgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcctggg cgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtctcg ctgctttcgataagtctctagccatttaaaatttttgatgacctgctgc gacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctg cacactggtatttcggtttttgggggccgcgggcggcgacggggcccgtg cgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggccacc gagaatcggacgggggtagtctcaagctggccggcctgctctggtgcct ggcctcgcgccgccgtgtatcgccccgccctgggcggcaaggctggccc ggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgc tgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggt gagtcacccacacaaaggaaaagggcctttccgtcctcagccgtcgctt catgtgactccacggagtaccgggcgccgtccaggcacctcgattagtt ctcgagcttttggagtacgtcgtctttaggttgggggagggggttttat gcgatggagtttccccacactgagtgggtggagactgaagttaggccag cttggcacttgatgtaattctccttggaatttgccctttttgagtttgg atcttggttcattctcaagcctcagacagtggttcaaagttttttcctt ccatttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCT

		CCTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGAAATCGTCCTC ACACAATCCCCGGGGAGCCTCGCAGTCAGTCCTGGGGAACGAGTCACTA TGAGCTGCAAATCCAGTCAGAGTGTTTTTTTCTCAAGTAGCCAGAAGAA CTACCTCGCATGGTACCAACAAATACCGGGGCAATCTCCCCGCTTGCTT ATATACTGGGCAAGTACCCGCGAATCCGGCGTACCGGATCGATTCACGG GATCTGGGTCAGGTACTGATTTCACTTTGACTATCAGCTCTGTTCAGCC TGAAGATTTGGCAATTTACTACTGTCACCAATACTTGAGTAGCCGAACT TTCGGCCAGGGCACGAAGCTCGAAATCAAGGGCGGAGGGGGAGGTTCTG GTGGGGGCGGTTCTGGCGGTGGAGGAAGCCAAGTACAGTTGCAACAGCC AGGGGCGGAGGTCGTAAAACCTGGGGCGTCTGTCAAGATGAGCTGTAAA GCAAGTGGATACACCTTCACCTCCTACTATATACATTGGATTAAGCAAA CTCCGGGTCAGGGGCTGGAATGGGTTGGCGTTATATACCCCGGGAACGA TGATATATCATACAACCAAAAATTTCAAGGCAAGGCGACTCTGACTGCC GATAAGAGTAGCACAACAGCTTACATGCAGCTTTCTTCCCTGACCAGCG AAGATTCAGCAGTTTACTACTGCGCTCGGGAAGTGCGCCTGCGATACTT TGATGTCTGGGGTCAAGGAACTACAGTTACTGTATCAAGCAGTGCTGCT GCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCC CGCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCT TCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGG GGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTA CGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCA CAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAA CCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTT TTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTG TATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAA TCCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAG GCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTC ACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGA TGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCTAT CCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATC TGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGAC ACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCT GTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAAT GATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCAC CAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCA GAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGG CACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCT TTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGC CCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATC TTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCA ATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGA ATTAAAAAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTT GGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGG AATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAG TCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTAC CAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGA GAAAGG

Table 29. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z01 gRNA.

Reference on-target sequence [a]: GATGGGAGCAACG(TGG)CCAT (SEQ ID NO: 104)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
181	GATGGGAGCA**A**ACGTGGCCAT	46.1	43.9	45.0	1.6

	----------GTGGCCAT	6.5	4.3	5.4	1.6
182	GATGGGAGC-ACGTGGCCAT	4.1	4.9	4.5	0.6
	GA----------TGGCCAT	3.5	3.9	3.7	0.3
	----------	3.3	3.7	3.5	0.3
183	GATGGG---AACGTGGCCAT	2.6	3.6	3.1	0.7
184	GATGGGA--------GCCAT	3.6	2.1	2.8	1.1
	----------CAT	2.4	1.8	2.1	0.4
	----------CGTGGCCAT	1.4	1.2	1.3	0.1
185	GATG----------GGCCAT	1.1	1.3	1.2	0.1
	GAT----------	0.9	1.1	1.0	0.1
	GATGG----------	0.7	1.2	1.0	0.4
186	----------ACGTGGCCAT	1.1	0.5	0.8	0.4

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.

[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 30. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z02 gRNA.

Reference on-target sequence [a]: CCGCGGGACTAGA(GGG)AGCT (SEQ ID NO: 268)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
187	CCGCGGGACTT**A**AGAGGGAGCT	49.2	39.4	44.3	6.9
188	CCGCGGGA---------GCT	11.9	11.5	11.7	0.3
	----------	2.6	4.6	3.6	1.4
	CCGCGGG----------	2.1	3.4	2.8	0.9
	----------T	2.1	2.0	2.0	0.1
189	CCGCGGGA-TAGAGGGAGCT	1.7	1.8	1.8	0.1
190	CCGCGGGACT----------	1.8	1.3	1.6	0.4
191	CCGCGGG--TAGAGGGAGCT	1.0	1.6	1.3	0.4
192	CCGCGGG--------GAGCT	1.1	1.3	1.2	0.1
193	CCGCGGGAC-AGAGGGAGCT	1.0	1.2	1.1	0.1
194	CCGCGGGACT-GAGGGAGCT	1.3	0.9	1.1	0.3
195	CCG---------AGGGAGCT	1.2	0.9	1.0	0.2
196	CCGCGGGA-----GGGAGCT	0.8	1.1	1.0	0.2
197	CCG------TAGAGGGAGCT	0.3	1.1	0.7	0.6

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.

[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 31. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z03 gRNA.

Reference on-target sequence [a]: CGCTCCCGCTCGG (TGG) CTGT (SEQ ID NO: 274)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
198	CGCTCCCGCTT**C**GGTGGCTGT	41.3	38.6	40.0	1.9
	C----------TGT	7.9	7.8	7.8	0.1
	CGCTCCCG----------	7.9	7.5	7.7	0.3
199	CGCTCCCGC-CGGTGGCTGT	3.3	3.7	3.5	0.3
	----------	2.7	3.7	3.2	0.7
200	CGCTCCCG-TCGGTGGCTGT	2.8	3.7	3.2	0.6
201	CGCTCCCGC--GGTGGCTGT	2.3	2.8	2.6	0.4
	----------T	1.7	3.0	2.4	0.9
202	CGCTCCCGCT-GGTGGCTGT	2.2	2.4	2.3	0.1
	----------GCTGT	2.3	1.7	2.0	0.4
203	CGCTCCC--TCGGTGGCTGT	1.6	1.8	1.7	0.1
204	CGCTCCCGCTT**TC**GGTGGCTGT	1.1	1.4	1.2	0.2
205	CGCTCCCG----GTGGCTGT	1.3	0.8	1.0	0.4

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 32. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell donor for the REG1-Z04 gRNA.

Reference on-target sequence [a]: CATCACGACGCGT (GGG) TGGC (SEQ ID NO: 280)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
206	CATCACGA--CGTGGGTGGC	34.0	32.9	33.4	0.8
207	CATCA-----CGTGGGTGGC	7.7	6.2	7.0	1.1
	----------	2.9	3.8	3.4	0.6
208	CATCACGACGC**C**GTGGGTGGC	2.5	4.2	3.4	1.2
209	CATCACGAC------GTGGC	3.1	3.6	3.4	0.4
210	CATCACGACG**G**CGTGGGTGGC	2.3	3.4	2.8	0.8
	CATCACGA----------	2.3	2.4	2.3	0.1
211	----------CGTGGGTGGC	1.5	1.7	1.6	0.1
212	CATCACGACG---TGGTGGC	1.8	1.2	1.5	0.4
213	CATCACGACGT**C**GTGGGTGGC	1.5	1.2	1.4	0.2
	CATCACGAC----------	1.7	1.1	1.4	0.4
	----------C	1.5	1.2	1.4	0.2
	----------GGTGGC	1.1	1.3	1.2	0.1
	----------TGGC	1.1	1.0	1.0	0.1
214	CATCACGAC----GGGTGGC	0.7	1.3	1.0	0.4

	CATCA----------	0.9	1.1	1.0	0.1
215	CATCACGAC-----GGTGGC	1.1	0.7	0.9	0.3

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 33. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z05 gRNA.

Reference on-target sequence[a]: CACGACGCGTGGG (TGG) CAAG (SEQ ID NO: 286)					
SEQ ID NO:	**Gene Edited Sequence[b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
216	CACGACGCGT**T**GGGTGGCAAG	58.4	50.0	54.2	5.9
	CACGAC----------G	5.5	7.8	6.6	1.6
217	CACGACGC--GGGTGGCAAG	1.7	3.7	2.7	1.4
218	CACGAC---------GCAAG	2.2	2.8	2.5	0.4
219	CACGACGC----GTGGCAAG	2.4	1.5	2.0	0.6
220	CACGACGCG-GGGTGGCAAG	1.6	1.9	1.8	0.2
	----------	1.4	1.5	1.4	0.1
	CACGA----------	1.0	1.4	1.2	0.3
	CACGACGC----------	0.9	1.3	1.1	0.3

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 34. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z06 gRNA.

Reference on-target sequence[a]: TCTGACGGGATCG (TGG) TTTC (SEQ ID NO: 292)					
SEQ ID NO:	**Gene Edited Sequence[b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
221	TCTGACGGGA**A**TCGTGGTTTC	28.1	21.9	25.0	4.4
222	TCTGACG-------GGTTTC	7.0	7.4	7.2	0.3
223	TCTGA------CGTGGTTTC	7.3	7.2	7.2	0.1
224	TCTGACGGGAT**T**CGTGGTTTC	5.4	2.6	4.0	2.0
225	TCTGACGGGA-CGTGGTTTC	4.2	2.8	3.5	1.0
226	TCTG------TCGTGGTTTC	3.5	3.1	3.3	0.3
	TCTG----------	2.3	3.4	2.8	0.8
	----------	2.4	3.1	2.8	0.5
	----------TC	2.9	2.2	2.6	0.5
227	TCTGAC--------GGTTTC	2.0	2.0	2.0	0.0
	TCT----------	1.5	2.3	1.9	0.6

228	TCTGACGGG-TCGTGGTTTC	1.7	2.1	1.9	0.3
229	TCTGACGGGAG**T**CGTGGTTTC	2.4	1.3	1.8	0.8
230	TCTGACGGGAC**T**CGTGGTTTC	1.5	1.8	1.6	0.2
231	----------TCGTGGTTTC	1.3	1.6	1.5	0.2
	--------------------C	1.0	1.5	1.2	0.4
232	TCTGACGG--TCGTGGTTTC	0.6	1.4	1.0	0.6
233	TCTGACGGGA--GTGGTTTC	1.2	0.5	0.8	0.5

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 35. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z07 gRNA.

Reference on-target sequence [a]: CCACGCGTCGTGA (TGG) TGTG (SEQ ID NO: 298)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
234	CCACGCGTCGG**T**GATGGTGTG	15.1	12.9	14.0	1.6
235	CCACGCGTCGTT**G**ATGGTGTG	12.3	8.5	10.4	2.7
	----------	4.4	5.1	4.8	0.5
236	CCACGCGT---------GTG	4.9	4.4	4.6	0.4
	CCACGCGT----------G	3.6	3.0	3.3	0.4
237	CCACGCGTCGA**T**GATGGTGTG	2.9	1.4	2.2	1.1
	CCACGCGTC----------	1.9	2.5	2.2	0.4
238	CCACGCGTCG--ATGGTGTG	2.2	2.1	2.2	0.1
239	CCACGCGTC-TGATGGTGTG	2.0	2.2	2.1	0.1
	CCAC----------	1.9	2.2	2.0	0.2
	C----------	2.2	1.9	2.0	0.2
240	CCACGCGTCGC**T**GATGGTGTG	1.9	1.6	1.8	0.2
241	CCACGCGTCG----------	2.0	1.7	1.8	0.2
242	CCACGCGTCG-----GTGTG	1.7	1.7	1.7	0.0
243	CCACGCGTGG-----GTGTG	1.8	1.5	1.6	0.2
244	CCACGCGT---GATGGTGTG	1.4	1.3	1.4	0.1
	CCA----------	1.1	1.7	1.4	0.4
245	CCACGCGTCGTG------TG	1.4	1.1	1.2	0.2
246	CCACGCGTCGTGA-------	1.2	1.1	1.2	0.1
	CCACGC----------	0.8	1.5	1.2	0.5
	CCACGCG----------	1.1	0.9	1.0	0.1
	CCACG----------TGTG	0.8	1.2	1.0	0.3
247	CCACGCGTGG-------GTG	1.1	0.7	0.9	0.3

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 36. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z08 gRNA.

Reference on-target sequence [a]: CCATCACGACGCG (TGG) GTGG (SEQ ID NO: 304)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
248	CCATCACGAC**C**GCGTGGGTGG	28.0	15.4	21.7	8.9
249	CCATCA-----CGTGGGTGG	8.5	3.4	6.0	3.6
250	CCATC---ACGCGTGGGTGG	4.4	2.4	3.4	1.4
	----------	2.3	1.8	2.0	0.4
	----------GGTGG	1.5	0.7	1.1	0.6
251	CCATCACGACA**G**CGTGGGTGG	1.3	0.2	0.8	0.8

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 37. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z09 gRNA.

Reference on-target sequence [a]: CCGTCAGACTCGT (AGG) CCAG (SEQ ID NO: 310)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
	CCGTCAG----------	13.5	9.9	11.7	2.5
252	CCGTCAGACTT**C**GTAGGCCAG	11.3	8.5	9.9	2.0
253	CCGT---------AGGCCAG	7.5	8.3	7.9	0.6
254	CCGTCAGACT----------	6.9	6.1	6.5	0.6
255	CCGTCAGAC--------CAG	4.2	4.3	4.2	0.1
	----------	3.9	4.2	4.0	0.2
	CCGTCA----------	3.6	2.3	3.0	0.9
256	CCGTCAGAC--GTAGGCCAG	2.5	2.4	2.4	0.1
257	CCGTCAG--------GCCAG	1.9	2.4	2.2	0.4
	CCG----------CCAG	1.2	2.2	1.7	0.7
258	CCGTCAGAC-CGTAGGCCAG	1.7	1.4	1.5	0.2
	----------TAGGCCAG	1.0	1.4	1.2	0.3
259	CCGTCAGACT-GTAGGCCAG	1.5	1.0	1.2	0.4
	CCGTCAGA----------	1.6	0.7	1.2	0.6
	CCGTCAGAC----------	1.2	0.6	0.9	0.4

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 38. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the REG1-Z10 gRNA.

Reference on-target sequence [a]: GTGGGTGGCAAGC (GGG) TGGT (SEQ ID NO: 316)					
SEQ ID NO:	**Gene Edited Sequence [b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
260	GTGGGTGGCA**A**AGCGGGTGGT	23.8	21.7	22.8	1.5
	GT----------GGGTGGT	20.7	22.9	21.8	1.6
	----------GCGGGTGGT	10.4	7.7	9.0	1.9
261	GTGGGTGGC-AGCGGGTGGT	7.0	6.5	6.8	0.4
	----------GTGGT	3.3	4.3	3.8	0.7
	GTG----------GGT	2.8	4.0	3.4	0.8
	----------CGGGTGGT	2.6	3.3	3.0	0.5
	----------	2.0	3.5	2.8	1.1
	GTGGGTGGC----------	2.4	1.8	2.1	0.4
262	GTGGGTGGCAT**A**GCGGGTGGT	1.8	1.8	1.8	0.0
	GTGGGTG----------	1.6	1.5	1.6	0.1
	GTGG----------	1.5	1.8	1.6	0.2
263	GTGGGTGG--AGCGGGTGGT	0.9	1.1	1.0	0.1

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold

Table 39. TGFBRII gRNA Sequences/Target Sequences

Name	Unmodified Sequence	Modified Sequence	Target Sequence (PAM)
TGFBRII sgRNA (EX1_T1)	CCGACUUCUGAACGUGCG GUguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcmUUUU (SEQ ID NO: 264)	C*C*G*ACUUCUGAACGUG CGGUGGGguuuuagagcua gaaauagcaaguuaaaaua aggcuaguccguuaucaac uugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 265)	CCGACTTCTGAACGTGCGGT (GGG) (SEQ ID NO: 2) CCGACTTCTGAACGTGCGGT (SEQ ID NO: 269)
TGFBRII sgRNA (EX1_T1) spacer	CCGACUUCUGAACGUGCG GU (SEQ ID NO: 266)	C*C*C*GACUUCUGAACGU GCGGU (SEQ ID NO: 267)	
TGFBRII sgRNA (EX1_T2)	UGCUGGCGAUACGCGUCC ACguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcmUUUU (SEQ ID NO: 270)	U*G*C*UGGCGAUACGCGU CCACguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcmU*U*U*U (SEQ ID NO: 271)	TGCTGGCGATACGCGTCCAC (AGG) (SEQ ID NO: 3) TGCTGGCGATACGCGTCCAC (SEQ ID NO: 275)
TGFBRII sgRNA (EX1_T2) spacer	UGCUGGCGAUACGCGUCC AC (SEQ ID NO: 272)	U*G*C*UGGCGAUACGCGU CCAC (SEQ ID NO: 273)	
TGFBRII	UCGGUCUAUGACGAGCAG	U*C*G*GUCUAUGACGAGC	TCGGTCTATGACGAGCAGCG(

Name	Unmodified Sequence	Modified Sequence	Target Sequence (PAM)
sgRNA (EX1_T3)	CGguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 276)	AGCGguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 277)	GGG) (SEQ ID NO: 4) TCGGTCTATGACGAGCAGCG (SEQ ID NO: 281)
TGFBRII sgRNA (EX1_T3) spacer	UCGGUCUAUGACGAGCAG CG (SEQ ID NO: 278)	U*C*G*GUCUAUGACGAGC AGCG (SEQ ID NO: 279)	
TGFBRII sgRNA (EX2_T1)	AUGGGCAGUCCUAUUACA GCguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcmUUUU (SEQ ID NO: 282)	A*U*G*GGCAGUCCUAUUA CAGCguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 283)	ATGGGCAGTCCTATTACAGC (TGG) (SEQ ID NO: 5) ATGGGCAGTCCTATTACAGC (SEQ ID NO: 287)
TGFBRII sgRNA (EX2_T1) spacer	AUGGGCAGUCCUAUUACA GC (SEQ ID NO: 284)	A*U*G*GGCAGUCCUAUUA CAGC (SEQ ID NO: 285)	
TGFBRII sgRNA (EX3_T1)	AUUGUUCACUUGUUAGCC CCguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 288)	A*U*U*GUUCACUUGUUAG CCCCAGGguuuuagagcua gaaauagcaaguuaaaaua aggcuaguccguuaucaac uugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 289)	ATTGTTCACTTGTTAGCCCC (AGG) (SEQ ID NO: 6) ATTGTTCACTTGTTAGCCCC (SEQ ID NO: 293)
TGFBRII sgRNA (EX3_T1) spacer	AUUGUUCACUUGUUAGCC CC (SEQ ID NO: 290)	A*U*U*GUUCACUUGUUAG CCCC (SEQ ID NO: 291)	
TGFBRII sgRNA (EX3_T2)	GCUGAAGAACUGCCUCUA UAguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 294)	G*C*U*GAAGAACUGCCUC UAUAguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 295)	GCTGAAGAACTGCCTCTATA (TGG) (SEQ ID NO: 7) GCTGAAGAACTGCCTCTATA (SEQ ID NO: 299)
TGFBRII sgRNA (EX3_T2) spacer	GCUGAAGAACUGCCUCUA UA (SEQ ID NO: 296)	G*C*U*GAAGAACUGCCUC UAUA (SEQ ID NO: 297)	
TGFBRII sgRNA (EX4_T1)	GCAGGAUUUCUGGUUGUC ACguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 300)	G*C*A*GGAUUUCUGGUUG UCACguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 301)	GCAGGATTTCTGGTTGTCAC (AGG) (SEQ ID NO: 8) GCAGGATTTCTGGTTGTCAC (SEQ ID NO: 305)
TGFBRII sgRNA (EX4_T1) spacer	GCAGGAUUUCUGGUUGUC AC (SEQ ID NO: 302)	G*C*A*GGAUUUCUGGUUG UCAC (SEQ ID NO: 303)	
TGFBRII sgRNA (EX4_T2)	CUCCAUCUGUGAGAAGCC ACguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu	C*U*C*CAUCUGUGAGAAG CCACguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug	CTCCATCTGTGAGAAGCCAC (AGG) (SEQ ID NO: 9)

Name	Unmodified Sequence	Modified Sequence	Target Sequence (PAM)
	gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 306)	aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 307)	CTCCATCTGTGAGAAGCCAC (SEQ ID NO: 311)
TGFBRII sgRNA (EX4_T2) spacer	CUCCAUCUGUGAGAAGCC AC (SEQ ID NO: 308)	C*U*C*CAUCUGUGAGAAG CCAC (SEQ ID NO: 309)	
TGFBRII sgRNA (EX5_T1)	CCCCUACCAUGACUUUAU UCguuuuagagcuagaaa uagcaaguuaaaauaagg cuaguccguuaucaacuu gaaaaaguggcaccgagu cggugcUUUU (SEQ ID NO: 312)	C*C*C*CUACCAUGACUUU AUUCguuuuagagcuagaa auagcaaguuaaaauaagg cuaguccguuaucaacuug aaaaaguggcaccgagucg gugcU*U*U*U (SEQ ID NO: 313)	CCCCTACCATGACTTTATTC (TGG) (SEQ ID NO: 10) CCCCTACCATGACTTTATTC (SEQ ID NO: 317)
TGFBRII sgRNA (EX5_T1) spacer	CCCCUACCAUGACUUUAU UCUGG (SEQ ID NO: 314)	C*C*C*CUACCAUGACUUU AUUC (SEQ ID NO: 315)	

*: 2'-O-methyl phosphorothioate residue

Table 40. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex1-T1 gRNA.

Reference on-target sequence[a]: CTGAACGTGCGGT(GGG)ATCG (SEQ ID NO: 360)					
SEQ ID NO:	Gene Edited Sequence[b]	Donor 1 (%)	Donor 2 (%)	Mean (%)	Std. Dev. (%)
361	CTGAACGTGC----------	28.7	29.8	29.2	0.8
362	CTGAACGTG-----GGATCG	10.7	12	11.4	0.9
	CTGA----------TCG	9.8	9.3	9.6	0.4
	----------	3.7	1.3	2.5	1.7
363	CTGAACGTGC**C**GGTGGGATCG	1.2	3.2	2.2	1.4
	CTG----------	2.8	1.1	2	1.2
364	CTGAACGTG-GGTGGGATCG	0.8	2.1	1.5	0.9
365	----------GGTGGGATCG	2.2	0.8	1.5	1
366	CTGAACGTG--GTGGGATCG	1	1.6	1.3	0.4
367	CTGAACG----GTGGGATCG	1.5	0.8	1.2	0.5
	CTGAACG----------	1.3	1	1.2	0.2
368	CTG--------GTGGGATCG	1.3	0.4	0.8	0.6
369	CTGAACGTGC**A**GGTGGGATCG	1.3	0.3	0.8	0.7
370	CTGAACGTGCGT--GGATCG	0	1.1	0.6	0.8
	----------TCG	0	1.1	0.6	0.8

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.

[b] Deletions indicated by dashes (-); insertions indicated by bold

[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 41. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex1-T2 gRNA.

Reference on-target sequence[a]: GATACGCGTCCAC(AGG)ACGA (SEQ ID NO: 371)

SEQ ID NO:	Gene Edited Sequence[b]	Donor 1 (%)	Donor 2 (%)	Mean (%)	Std. Dev. (%)
372	GATACGCGTC-ACAGGACGA	15.2	15.3	15.2	0.1
	GAT----------	8.5	10.3	9.4	1.3
	GATACGC----------	6.7	5.9	6.3	0.6
373	GATACGCGTCC**C**ACAGGACGA	3.7	6.1	4.9	1.7
	GATACG----------A	4.3	5.6	4.9	0.9
	----------	5.4	3.5	4.4	1.3
	----------ACGA	3.4	3.9	3.6	0.4
	----------AGGACGA	3.7	2.2	3	1.1
374	GATACGCGTCCA--GGACGA	2.2	3.2	2.7	0.7
375	GATACGC----ACAGGACGA	2.3	2.8	2.6	0.4
376	GATAC------ACAGGACGA	2.8	1.7	2.2	0.8
	----------ACAGGACGA	1.4	2.5	2	0.8
	GATACGCG----------A	2.5	1.4	2	0.8
377	GATACGCGTCC-------GA	1.9	1.7	1.8	0.1
378	GATACGCGTC--------GA	1.1	2	1.6	0.6
379	GATACGCGTC---AGGACGA	1.9	1.1	1.5	0.6
380	GATAC--------AGGACGA	1.2	1.5	1.4	0.2
381	GATACGC---CACAGGACGA	1.5	0.8	1.2	0.5
382	GATACGCGTC----------	1	1.3	1.2	0.2
383	GATACGCGTC**A**CACAGGACGA	1.4	0.8	1.1	0.4
384	GATACGC-TGCACAGGACGA	1.1	0.8	1	0.2
385	GATACGC------AGGACGA	0.8	1.3	1	0.4
	GATACGCG----------	0.6	1.1	0.8	0.4
	GATACGCGT----------	0.6	1.1	0.8	0.4
	----------A	1.1	0.3	0.7	0.6
386	---ACGC----ACAGGACGA	1.2	0	0.6	0.8

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.

[b] Deletions indicated by dashes (-); insertions indicated by bold

[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 42. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex1-T3 gRNA.

Reference on-target sequence[a]: ATGACGAGCAGCG(GGG)TCTG (SEQ ID NO: 387)					
SEQ ID NO:	Gene Edited Sequence[b]	Donor 1 (%)	Donor 2 (%)	Mean (%)	Std. Dev. (%)
388	ATGACGAGC**A**AGCGGGGTCTG	66.7	65.9	66.3	0.6
389	ATGACG---AGCGGGGTCTG	4.5	5.8	5.2	0.9
	----------GGTCTG	2.2	2.5	2.4	0.2
390	ATGACGA--AGCGGGGTCTG	1.9	1.9	1.9	0
	----------	2.1	1.4	1.8	0.5
	----------GGGGTCTG	1	1.7	1.4	0.5
391	ATG------AGCGGGGTCTG	1.6	1.1	1.4	0.4

392	ATGACGAGC**AA**AGCGGGGTCTG	1.8	0.6	1.2	0.8
393	ATGA-------CGGGGTCTG	0.7	1.5	1.1	0.6
	A----------------TG	1.2	0.5	0.8	0.5

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 43. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex5-T1 gRNA.

Reference on-target sequence[a]: CATGACTTTATTC(TGG)AAGA (SEQ ID NO: 394)					
SEQ ID NO:	**Gene Edited Sequence[b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
395	CATGA-------CTGGAAGA	10.6	12.4	11.5	1.3
396	CATGAC----TTCTGGAAGA	8.8	8.9	8.9	0.1
397	CATGACT---TTCTGGAAGA	7	5.4	6.2	1.1
398	CATGACTTTA**T**TTCTGGAAGA	5	6.2	5.6	0.8
399	CATGACTTT**A**ATTCTGGAAGA	5.1	6.2	5.6	0.8
	CA----------TGGAAGA	3.7	3.8	3.8	0.1
400	CATGACTT--TTCTGGAAGA	3.6	3	3.3	0.4
	CAT----------GAAGA	2.2	3.2	2.7	0.7
	C----------------A	2.5	2.1	2.3	0.3
	----------	2.5	1.9	2.2	0.4
	CATGA----------	2.6	1.8	2.2	0.6
	CAT----------GA	2	2	2	0
401	CA---------TCTGGAAGA	2	2.1	2	0.1
402	CATGACTTT-TTCTGGAAGA	1.6	2.3	2	0.5
403	CATGACTTTA-TCTGGAAGA	2.1	1.4	1.8	0.5
404	CATGACTTT-------AAGA	1.1	1	1	0.1
405	----------TTCTGGAAGA	1.2	0.9	1	0.2
406	CATGACTTTA--CTGGAAGA	1.1	0.9	1	0.1

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 44. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex2-T1 gRNA.

Reference on-target sequence[a]: GTCCTATTACAGC(TGG)GGCA (SEQ ID NO: 407)					
SEQ ID NO:	**Gene Edited Sequence[b]**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
	G----------	18.4	17.4	17.9	0.7
408	GTCCTATTA--GCTGGGGCA	6.4	13	9.7	4.7
	----------GCA	9.2	5.7	7.4	2.5
409	GTCCTATTA-AGCTGGGGCA	7.5	7.1	7.3	0.3
410	GTCCTAT---AGCTGGGGCA	6.8	7.5	7.2	0.5

411	GTCCTA----AGCTGGGGCA	7.3	4.6	5.9	1.9
412	GTCCTA-----GCTGGGGCA	7.5	4.2	5.8	2.3
	--------------------	2.8	2.2	2.5	0.4
413	GTCCTATTAC---TGGGGCA	2	1.7	1.8	0.2
	G----------CTGGGGCA	1	2	1.5	0.7
414	GTCC------AGCTGGGGCA	1	1.7	1.4	0.5
415	GTCCTATTAC**C**AGCTGGGGCA	1.2	1.3	1.2	0.1
	GTCCTAT-------------	1.4	0.8	1.1	0.4
416	GTCCTATT---GCTGGGGCA	1.1	1.1	1.1	0
417	GTCCTATTAC-GCTGGGGCA	0.7	1.2	1	0.4
418	GTCCT---------GGGGCA	1.6	0.3	1	0.9
	GT------------------	1.1	0.1	0.6	0.7

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 45. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex3-T1 gRNA.

Reference on-target sequence[a]: ACTTGTTAGCCCC(AGG)GCCA (SEQ ID NO: 419)					
SEQ ID NO:	**Gene Edited Sequence**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
420	ACTTGTTAG--CCAGGGCCA	26.7	22.6	24.6	2.9
421	ACTTGTTAG-CCCAGGGCCA	5.1	9.1	7.1	2.8
	----------------------	6	4.1	5	1.3
422	ACTTGTTAG---CAGGGCCA	4.9	3.7	4.3	0.8
423	ACTTGTTA--------GCCA	4.6	3.1	3.8	1.1
	------------CAGGGCCA	4.1	2.7	3.4	1
424	ACTTGTT------AGGGCCA	2.1	3.3	2.7	0.8
	------------------CA	3.6	1.6	2.6	1.4
425	ACTTGTTAG**C**CCCCAGGGCCA	2	3.3	2.6	0.9
426	ACTTGTT---CCCAGGGCCA	1.3	3	2.2	1.2
427	----------CCCAGGGCCA	2.3	1.7	2	0.4
428	ACTTGTTA--CCCAGGGCCA	2	1.8	1.9	0.1
429	ACTTG-----CCCAGGGCCA	2	1.7	1.8	0.2
	ACT-----------------	1.3	1.3	1.3	0
430	ACTTGT----CCCAGGGCCA	0.8	1.5	1.2	0.5
431	A----------CCAGGGCCA	1.6	0.7	1.2	0.6
	----------------GGCCA	1.1	1.1	1.1	0
	A-----------CAGGGCCA	0.5	1.1	0.8	0.4
432	ACTTG-------CAGGGCCA	0.2	1.2	0.7	0.7
433	ACTTGTTAGC-------CCA	0.3	1.1	0.7	0.6

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 46. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex3-T2 gRNA.

Reference on-target sequence[a]: AACTGCCTCTATA(TGG)TGTG (SEQ ID NO: 434)					
SEQ ID NO:	**Gene Edited Sequence**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
435	AACTGCCTCT**T**ATATGGTGTG	37.1	41.7	39.4	3.3
	AAC----------	7	6	6.5	0.7
	----------	7.2	5	6.1	1.6
436	AACTGCCT--ATATGGTGTG	2.9	4.1	3.5	0.8
437	AACTGCCTCTAT--GGTGTG	3	3	3	0
	AACTG----------	2.7	2.3	2.5	0.3
438	AACTGCCTC-ATATGGTGTG	2	2.4	2.2	0.3
439	AACTG----TATATGGTGTG	1.6	2.4	2	0.6
440	AACTGC---TATATGGTGTG	1.6	1.8	1.7	0.1
441	AACT------ATATGGTGTG	1.1	1.8	1.5	0.5
	AACTGCC----------	1.2	1.5	1.4	0.2
	A----------	1.8	0.9	1.4	0.6
442	AACTGCCT-TATATGGTGTG	1.1	1.3	1.2	0.1
443	AACTGCCTCT----------	1.5	1	1.2	0.4
444	---------TATATGGTGTG	1.1	0.9	1	0.1
	AACTG----------TG	0.8	1.1	1	0.2
	AACTGCCTC----------	0.6	1.4	1	0.6
	AACT----------	1.1	1	1	0.1
445	AACTGCCTCTA---------	1.1	0.7	0.9	0.3
446	AACTGCCTCT-TATGGTGTG	0.7	1.1	0.9	0.3
447	AACTG----------GTGTG	1.1	0.7	0.9	0.3

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.

[b] Deletions indicated by dashes (-); insertions indicated by bold

[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 47. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex4-T1 gRNA.

Reference on-target sequence[a]: TTCTGGTTGTCAC(AGG)TGGA (SEQ ID NO: 448)					
SEQ ID NO:	**Gene Edited Sequence**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
449	TTCTGGTTGT**T**CACAGGTGGA	31.3	33.1	32.2	1.3
450	TTCTGGT----------GGA	11.2	11.5	11.4	0.2
451	TTC----------AGGTGGA	5.2	4	4.6	0.8
	----------TGGA	4.2	3.7	4	0.4
452	TTCTGGTT--CACAGGTGGA	3.5	3.5	3.5	0
453	TTCTGGTTGT**TT**CACAGGTGGA	2.1	2.7	2.4	0.4
454	TTCTGGTTG---------GA	2.3	2.2	2.2	0.1
	TTCTGG----------A	1.9	1.6	1.8	0.2
455	TTCTGGTTGTC**C**ACAGGTGGA	1.6	1.9	1.8	0.2

456	TTC-------CACAGGTGGA	1.4	2.1	1.8	0.5
457	TTCTGGTT-TCACAGGTGGA	1.4	2	1.7	0.4
	--------------------	2	1.1	1.6	0.6
458	TTCTGGTTG-CACAGGTGGA	1.1	1.4	1.2	0.2
459	TTCTGGTTGT**A**CACAGGTGGA	1.1	1.2	1.2	0.1
	TTCT----------------	1.4	0.7	1	0.5
460	TTCTGGTTG----------A	1.1	1	1	0.1
461	TTCTGGTTGT-ACAGGTGGA	0.7	1.2	1	0.4

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

Table 48. On-Target Gene Edited Sequences > 1% Frequency in At Least One Gene Edited T Cell Donor for the TGFBRII-Ex4-T2 gRNA.

Reference on-target sequence[a]: TGTGAGAAGCCAC(AGG)AAGT (SEQ ID NO: 462)					
SEQ ID NO:	**Gene Edited Sequence**	**Donor 1 (%)**	**Donor 2 (%)**	**Mean (%)**	**Std. Dev. (%)**
463	TGTGA----------GAAGT	22.3	17.3	19.8	3.5
464	TGTGAGAAG-CACAGGAAGT	9.9	12.7	11.3	2
	--------------------T	11.8	8.2	10	2.5
465	TGTGAGAAG**C**CCACAGGAAGT	4.8	8.1	6.4	2.3
466	TGTG---------AGGAAGT	3.1	3.5	3.3	0.3
467	TGTGAGAAGC--CAGGAAGT	3	3.1	3	0.1
468	TGTGAGA------AGGAAGT	3	2.8	2.9	0.1
469	----------CACAGGAAGT	2.5	2.7	2.6	0.1
470	TGTGAGAAGC**A**CACAGGAAGT	1.2	2.3	1.8	0.8
471	TGTGAGAAG---CAGGAAGT	1.6	1.6	1.6	0
	------------CAGGAAGT	1.3	1.8	1.6	0.4
472	TGTG------CACAGGAAGT	1.2	1.8	1.5	0.4
	--------------------	1.7	1	1.4	0.5
473	---------CCACAGGAAGT	1.5	1.4	1.4	0.1
474	TGTGAGA---CACAGGAAGT	0.7	1.4	1	0.5
475	TGTGAG-----ACAGGAAGT	1.2	0.8	1	0.3
	TGT------------GAAGT	1.2	0.7	1	0.4
476	TGTGAGAA--CACAGGAAGT	0.6	1.4	1	0.6
477	TGTGAGAAGC----------	0.8	1.1	1	0.2
478	TGTGAGAAGC**CA**CACAGGAAGT	1.4	0.7	1	0.5

[a] On-target sequence centered on cleavage site, with 10 bp in either direction. For comparison, the portion of the gRNA target sequence aligning with the Reference on-target sequence is underlined and the PAM is indicated by parenthesis.
[b] Deletions indicated by dashes (-); insertions indicated by bold
[c] Positions of inserted bases in the gene edited sequence indicated by dashes (-) in the Reference Sequence

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

The term "about" as used herein means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within an acceptable standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to ± 20 %, preferably up to ± 10 %, more preferably up to ± 5 %, and more preferably still up to ± 1 % of a given value. Where particular

values are described in the application and claims, unless otherwise stated, the term "about" is implicit and in this context means within an acceptable error range for the particular value.

As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

What Is Claimed Is:

1. A population of genetically engineered T cells, comprising:

(i) a disrupted *Regnase-1* (*Reg1*) gene; and/or

(ii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene.

2. The population of genetically engineered T cells of claim 1, which comprises both (i) and (ii).

3. The population of genetically engineered T cells of claim 1 or claim 2, wherein the population of genetically engineered T cells, as compared to non-engineered T cell counterparts, has one or more of the following features:

(a) improved cell growth activity;

(b) enhanced persistence;

(c) reduced T cell exhaustion;

(d) resistant to inhibitory effects induced by TGF-β;

(e) enhanced cell killing capacity; and

(f) resistant to inhibitory effects by fibroblasts and/or inhibitory factors secreted thereby.

4. The population of genetically engineered T cells of any one of claims 1-3, wherein the T cells are further engineered to express a chimeric antigen receptor (CAR).

5. The population of genetically engineered T cells of any one of claims 1-4, wherein the disrupted *Reg1* gene is genetically edited in exon 2 and/or exon 4.

6. The population of genetically engineered T cells of any one of claims 1-5, wherein the disrupted *TGFBRII* gene is genetically edited in exon 1, exon 2, exon 3, exon 4, or exon 5, optionally wherein the disrupted *TGFBRII* gene is genetically edited in exon 4 or exon 5.

7. The population of genetically engineered T cells of any one of claims 1-6, wherein the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, or both are genetically edited

by a CRISPR/Cas-mediated gene editing system.

8. The population of genetically engineered T cells of claim 7, wherein the CRISPR/Cas-mediated gene editing comprises a guide RNA (gRNA) targeting a site in the *Reg1* gene that comprises a nucleotide sequence listed in Table 22, which optionally is selected from the group consisting of SEQ ID NO: SEQ ID NO: 320, 322, 323, and 327.

9. The population of genetically engineered T cells of claim 8, wherein the gRNA targeting the *Reg1* gene comprises a nucleotide sequence listed in Table 22, which optionally is selected from the group consisting of SEQ ID NO: 24, 32, 36, or 52.

10. The population of genetically engineered T cells of claim 9, wherein the disrupted *Reg1* gene comprises a nucleotide sequence selected from those listed in Table 31, 33, 34, or 38.

11. The population of genetically engineered T cells of any one of claims 7-10, wherein the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *TGFBRII* gene that comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 269, 275, 281, 287, 293, 299, 305, 311, and 317.

12. The population of genetically engineered T cells of claim 11, wherein the gRNA targeting the *TGFBRII* gene comprises a spacer having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 266, 272, 278, 284, 290, 296, 302, 308, and 314.

13. The population of genetically engineered T cells of any one of claims 7-12, wherein the gRNA further comprises a scaffold sequence.

14. The population of genetically engineered T cells of claim 13, wherein:

the gRNA targeting the *Reg1* gene comprises the nucleotide sequence of any of SEQ ID NO: 22, 30, 34, or 50; and/or

the gRNA targeting the *TGFBRII* gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 270, 300, 306, or 312.

15. The population of genetically engineered T cells of any one of claims 1-14, which further comprises:

(iii) a disrupted T cell receptor alpha chain constant region (*TRAC*) gene,

(iv) a disrupted beta-2-microglobulin (*β2M*) gene,

(v) a disrupted *CD70* gene, or

(vi) a combination of any of (iii)-(v).

16. The population of genetically engineered T cells of claim 15, wherein the T cells comprise a disrupted T cell receptor alpha chain constant region (*TRAC*) gene.

17. The population of genetically engineered T cells of claim 15 or claim 16, wherein the T cells comprise a disrupted beta-2-microglobulin (*β2M*) gene.

18. The population of genetically engineered T cells of any one of claims 15-17, wherein the T cells comprise a disrupted *CD70* gene.

19. The population of genetically engineered T cells of any one of claims 15-18, wherein the disrupted *TRAC* gene, the disrupted *β2M* gene, and/or the disrupted CD70 gene is genetically edited by one or more CRISPR/Cas-mediated gene editing system

20. The population of genetically engineered T cells of any one of claims 4-19, wherein the T cells comprise a nucleic acid encoding the CAR, and wherein the nucleic acid is inserted in the genome of the T cells.

21. The population of genetically engineered T cells of claim 20, wherein the nucleic acid encoding the CAR is inserted in the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, the disrupted *β2M*, or the disrupted *CD70* gene.

22. The population of genetically engineered T cells of claim 21, wherein the nucleic acid encoding the CAR is inserted in the disrupted *TRAC* gene, and optionally wherein the nucleic acid encoding the CAR replaces the deleted fragment comprising SEQ ID NO: 69 in the *TRAC* gene.

23. The population of genetically engineered T cells of claim 15, wherein the disrupted *Reg1* gene comprises a nucleotide sequence listed in Table 31, 33 34, or 38, the disrupted *TRAC* gene comprises a nucleotide sequence listed in Table 24; the disrupted *β2M* comprises a nucleotide sequence listed in Table 25 and/or the disrupted *CD70* gene comprises a nucleotide sequence listed in Table 26.

24. The population of genetically engineered T cells of claims 4-23, wherein the CAR comprises an extracellular antigen binding domain specific to a tumor antigen, a co-stimulatory signaling domain of 4-1BB or CD28, and a cytoplasmic signaling domain of CD3ζ.

25. The population of genetically engineered T cells of claim 24, wherein the tumor antigen is CD19, BCMA, CD70, CD33, or PTK7.

26. The population of genetically engineered T cells of claim 24, wherein the CAR binds CD19 (anti-CD19 CAR) and wherein the extracellular antigen binding domain in the anti-CD19 CAR is a single chain variable fragment (scFv) that binds CD19 (anti-CD19 scFv).

27. The population of genetically engineered T cells of claim 24, wherein the anti-CD19 scFv comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 124; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 125; optionally wherein the V_H comprises the amino acid sequence of SEQ ID NO: 124 and the V_L comprises the amino acid sequence of SEQ ID NO: 125.

28. The population of genetically engineered T cells of claim 27, wherein the anti-CD19 scFv comprises the amino acid sequence of SEQ ID NO: 120.

29. The population of genetically engineered T cells of claim 28, wherein the anti-CD19 CAR comprises the amino acid sequence of SEQ ID NO: 117 or SEQ ID NO:353.

30. The population of genetically engineered T cells of claim 24, wherein the

CAR binds CD70 (anti-CD70 CAR) and wherein extracellular antigen binding domain in the anti-CD70 CAR is a single chain variable fragment (scFv) that binds CD70 (anti-CD70 scFv).

31. The population of genetically engineered T cells of claim 30, wherein the anti-CD70 scFv comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 143; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 144; optionally wherein the V_H comprises the amino acid sequence of SEQ ID NO: 143 and the V_L comprises the amino acid sequence of SEQ ID NO: 144.

32. The population of genetically engineered T cells of claim 31, wherein the anti-CD70 scFv comprises the amino acid sequence of SEQ ID NO: 140 or 142.

33. The population of genetically engineered T cells of claim 32, wherein the anti-CD70 CAR comprises the amino acid sequence of SEQ ID NO: 138 or SEQ ID NO:354.

34. The population of genetically engineered T cells of claim 24, wherein the CAR binds BCMA (anti-BCMA CAR) and wherein the extracellular antigen binding domain in the anti-BCMA CAR is a single chain variable fragment (scFv) that binds BCMA (anti-BCMA CAR).

35. The population of genetically engineered T cells of claim 34, wherein the anti-BCMA scFv comprises (i) a heavy chain variable region (V_H) that comprises the same heavy chain complementary determining regions (CDRs) as those in SEQ ID NO: 149; and (ii) a light chain variable region (V_L) that comprises the same light chain CDRs as those in SEQ ID NO: 150; optionally wherein the V_H comprises the amino acid sequence of SEQ ID NO: 149 and the V_L comprises the amino acid sequence of SEQ ID NO: 150.

36. The population of genetically engineered T cells of claim 35, wherein the anti-BCMA scFv comprises the amino acid sequence of SEQ ID NO: 148.

37. The population of genetically engineered T cells of claim 36, wherein the anti-

BCMA CAR comprises the amino acid sequence of SEQ ID NO: 146 or SEQ ID NO:355.

38. The population of genetically engineered T cells of any one of claims 1-37, wherein the genetically engineered T cells are derived from primary T cells of one or more human donors.

39. The population of genetically engineered T cells of any one of claims 1-38, wherein the genetically engineered T cells show cytokine-dependent growth.

40. A method for preparing the population of genetically engineered T cells of claim 1, the method comprising:

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

(b) genetically editing the *Reg1* gene and/or the *TGFBRII* gene; and

(c) producing the population of genetically engineered T cells having disrupted *Reg1* gene and/or the *TGFBRII* gene.

41. The method of claim 40, wherein step (b) comprises genetically editing both the *Reg1* gene and the *TGFBRII* gene.

42. The method of claim 40 or claim 41, wherein step (b) is performed by one or more CRISPR/Cas-mediated gene editing systems.

43. The method of any one of claims 40-42, wherein step (b) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *Reg1* gene.

44. The method of claim 43, wherein the gRNA targeting the *Reg1* gene is specific to an exon of the *Reg1* gene selected from the group consisting of exon 2 and exon 4.

45. The method of claim 44, wherein the gRNA targeting the *Reg1* gene comprises a spacer having a nucleotide sequence of SEQ ID NO: 24, 32, 36, or 52.

46. The method of any one of claims 40-45, wherein step (b) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *TGFBRII* gene.

47. The method of claim 46, wherein the gRNA targeting the *TGFBRII* gene is specific to an exon of the *TGFBRII* gene selected from the group consisting of exon 1, exon 2, exon 3, exon 4, and exon 5, preferably wherein the gRNA targeting the *TGFBRII* gene is specific to exon 4 or exon 5.

48. The method of claim 47, wherein the gRNA targeting the *TGFBRII* gene comprises a spacer having a nucleotide sequence selected from the group consisting of SEQ ID NOs: 272, 300, 308, and 314.

49. The method of any one of claims 40-48, wherein the gRNA targeting the *Reg1* gene and the gRNA targeting the *TGFBRII* gene further comprises a scaffold sequence.

50. The method of claim 49, wherein the gRNA targeting the *Reg1* gene comprises a nucleotide sequence of SEQ ID NO: 22, 30, 34, or 50; and/or wherein the gRNA targeting the *TGFBRII* gene comprises a nucleotide sequence of SEQ ID NOs: 270, 300, 306, or 312.

51. The method of any one of claims 40-50, wherein the plurality of T cells in step (a) comprises one or more of the following genetic modifications:

(i) engineered to express a chimeric antigen receptor (CAR);

(ii) has a disrupted T cell receptor alpha chain constant region (*TRAC*) gene;

(iii) has a disrupted *β2M* gene; and

(iv) has a disrupted *CD70* gene.

52. The method of any one of claims 40-51, wherein the method further comprises:

(i) delivering to the T cells a nucleic acid encoding a chimeric antigen receptor (CAR);

(ii) genetically editing a *TRAC* gene to disrupt its expression;

(iii) genetically editing a *β2M* gene to disrupt its expression;

(iv) genetically editing a *CD70* gene to disrupt its expression; or
(v) a combination thereof.

53. The method of claim 52, wherein one or more of (i)-(iv) are performed by one or more CRISPR/Cas-mediated gene editing system comprising one or more RNA-guided nucleases and one or more gRNAs targeting the *TRAC* gene, the *β2M* gene, and/or the *CD70* gene.

54. The method of claim 53, wherein the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 59.

55. The method of claim 53 or claim 54, wherein the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 63.

56. The method of any one of claims 53-55, wherein the gRNA targeting the *CD70* gene comprises the nucleotide sequence of SEQ ID NO: 55.

57. The method of any one of claims 50-56, wherein the method comprises delivering to the T cells one or more ribonucleoprotein particles (RNP), comprising the RNA-guided nuclease, one or more of the gRNAs, and the nucleic acid encoding the CAR.

58. The method of any one of claims 50-57, wherein the RNA-guided nuclease is a Cas9 nuclease, which optionally is a *S. pyogenes* Cas9 nuclease.

59. The method of any one of claims 50-58, wherein the nucleic acid encoding the CAR is in an AAV vector.

60. The method of any one of claims 50-59, wherein the nucleic acid encoding the CAR comprises a left homology arm and a right homology arm flanking the nucleotide sequence encoding the CAR; and wherein the left homology arm and the right homology arm are homologous to a genomic locus in the T cells, allowing for insertion of the nucleic acid into the genomic locus.

61. The method of claim 60, wherein the genomic locus is in the *Reg1* gene, the *TGFBRII* gene, the *TRAC* gene, the *β2M* gene, or the *CD70* gene; optionally wherein the genomic locus is the *TRAC* gene.

62. The method of any one of claims 50-61, wherein the method comprising disrupting the *TRAC* gene by a CRISPR/Cas-mediated gene editing system comprising a gRNA comprising nucleotide sequence of SEQ ID NO: 47 and the nucleic acid encoding the CAR is inserted at the site targeted by the gRNA.

63. The method of any one of claims 40-62, wherein the method comprising delivering to the T cells a nucleic acid encoding a CAR, which is specific to CD70, and genetically editing the *CD70* gene to disrupt its expression.

64. The method of any one of claims 40-63, wherein the T cells of step (a) are derived from primary T cells of one or more human donors.

65. A population of genetically engineered T cells, which is prepared by a method of any one of claims 40-64.

66. A method for eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof a population of genetically engineered T cells set forth in any one of claims 4-39 and 65.

67. The method of claim 66, wherein the undesired cells are cancer cells, which optionally are hematopoietic cancer cells or solid tumor cells.

68. The method of claim 66 or claim 67, wherein the undesired cells are $CD19^+$, $BCMA^+$, $CD70^+$, $CD33^+$, or $PTK7^+$.

69. A guide RNA (gRNA) targeting a *Reg1* gene, comprising a nucleotide sequence specific to a fragment in exon 1, exon2, exon3, or exon4, optionally in exon 2 or exon 4 of the *Reg1* gene.

70. The gRNA of claim 69, wherein the gRNA comprises a spacer listed in Table 22, which optionally is selected from the group consisting of SEQ ID NO: 24, 32, 36, and 52.

71. The gRNA of claim 69 or claim 70, wherein the gRNA further comprises a scaffold sequence.

72. The gRNA of any one of claims 69-71, wherein the gRNA comprises one or more modified nucleotides.

73. The gRNA of claim 72, wherein the gRNA comprises one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA.

74. The gRNA of claim 73, which comprises the nucleotide sequence listed in Table 22, which optionally is any of SEQ ID NOs: 22, 23, 30, 31, 34, 35, 50, or 51.

75. A guide RNA (gRNA) targeting a *TGFBRII* gene, comprising a nucleotide sequence specific to a fragment in exon 1, exon 2, exon 3, exon 4, or exon5 of the *TGFBRII* gene, preferably wherein the gRNA comprises a nucleotide sequence specific to exon 4 or exon 5 of the *TGFBRII* gene.

76. The gRNA of claim 75, wherein the gRNA comprises a spacer listed in Table 32, which optionally has the nucleotide sequence selected from the group consisting of SEQ ID NOs: 272, 302, 308, and 314.

77. The gRNA of claim 76, wherein the gRNA further comprises a scaffold sequence.

78. The gRNA of any one of claims 75-77, wherein the gRNA comprises one or more modified nucleotides.

79. The gRNA of claim 78, wherein the gRNA comprises one or more 2'-O-methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA.

80. The gRNA of claim 79, which comprises the nucleotide sequence listed in Table 39, optionally wherein the gRNA comprises the nucleotide sequence of any one of SEQ ID NO: SEQ ID NOs: 270, 271, 300, 301, 306, 307, 312, and 313.

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