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(54) Title: ENGINEERED ANTI-LIV1 CELL WITH REGNASE-1 AND/OR TGFBRII DISRUPTION

(57) Abstract: Provided herein include engineered T cells, and related methods and compositions for producing the engineered T cells. Also disclosed herein include therapeutic uses of the engineered T cells. The engineered T cells can express a chimeric antigen receptor (CAR) that specifically binds to LIV1 and have at least one of a disrupted Regnase-1 (Reg1) gene and a disrupted Transforming Growth Factor Beta Receptor II (TGFBRII) gene.

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# ENGINEERED ANTI-LIV1 CELL WITH REGNASE-1 AND/OR TGFBRII DISRUPTION

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing dates of U.S. Provisional Application No. 63/290,059, filed on December 15, 2021, and U.S. Provisional Application No. 63/290,550, filed on December 16, 2021, the entire contents of each of which are incorporated by reference herein.

## REFERENCE TO SEQUENCE LISTING

The instant application contains a Sequence Listing which has been filed electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on December 12, 2022, is named 095136-0734-074WO1_SEQ.xml and is 555,238 bytes in size.

## FIELD OF INVENTION

The present disclosure relates generally to the field of engineered cells, for example, engineered immune cells.

## BACKGROUND

Chimeric antigen receptor (CAR) T-cell therapy uses genetically engineered T cells to specifically and efficiently target and kill cancer cells. T cells can be engineered to express CARs on their surface by introducing CARs into the T cells using a gene editing technology such as CRISPR/Cas9 gene editing technology. When these engineered CAR T cells are injected into a patient, the receptors enable the T cells to kill cancer cells.

## SUMMARY

Described herein includes an engineered T cell, where the engineered T cell comprises, in some embodiments, a nucleic acid encoding a chimeric antigen receptor (CAR), and at least one of (i) a disrupted *Regnase-1* (*Reg1*) gene, and (ii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene. The CAR (anti-LIV1 CAR) comprises (1) an ectodomain that binds specifically to LIV1, which may be an anti-LIV1 antigen-binding fragment. Also provided herein is a population of engineered T cells, which comprise or collectively comprise the anti-LIV CAR and at least one of (i) a disrupted

*Regnase-1* (*Reg1*) gene, and (ii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene. It is reported herein that disruption of the *Reg1* gene, the *TGFBRII* gene, or both has improved functionality of the anti-LIV1 CAR-T cells disclosed herein.

In some embodiments, the engineered T cell(s) can comprise both (i) and (ii). In some embodiments, the engineered T cell(s) may further comprise a disrupted T cell receptor alpha chain constant region (*TRAC*) gene, a disrupted beta-2-microglobulin (*β2M*) gene, or both. In some examples, the engineered T cell(s) further comprise the disrupted *TRAC* gene. In some examples, the engineered T cell(s) further comprise both the disrupted *TRAC* gene and the disrupted *β2M* gene.

In some embodiments, the ectodomain of the CAR comprises an anti-LIV1 antigen-binding fragment, which may comprise an anti-LIV1 antibody. The anti-LIV1 antibody can be, for example, an anti-LIV1 single-chain variable fragment (scFv). In some embodiments, the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody.

In some instances, the reference antibody comprises (a) a VH comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 533 and a VL comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 534. In one example, the reference antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 533 and (b) a VL comprising the amino acid sequence of SEQ ID NO: 534.

In other instances, the reference antibody comprises (a) a VH comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 568 and a VL comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 566. In one example, the reference antibody comprises (a) a VH comprising the amino acid sequence of SEQ ID NO: 568 and (b) a VL comprising the amino acid sequence of SEQ ID NO: 566.

The anti-LIV1 scFv may comprise any of the scFv sequences provide in Sequence **Tables 29** and **32** below. In some embodiments, the anti-LIV1 scFv comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 532, 548, 561, and 564. In some embodiments, the anti-LIV1 scFv comprises an amino acid sequence having the amino acid sequence of any one of SEQ ID NOs: 532, 548, 561, and 564.

Any of the anti-LIV1 CARs disclosed herein may further comprise a CD28 co-stimulatory domain or a 41BB co-stimulatory domain. In some embodiments, the anti-LIV1 CAR may further comprise a CD3ζ cytoplasmic signaling domain, a CD8 transmembrane domain, or both.

The anti-LIV1 CAR may be any CAR constructs provided in **Tables 28, 29**, **31**, and **32** below. In some examples, the anti-LIV1 CAR may comprise the amino acid sequence of SEQ ID NO: 528 (with signal peptide) or SEQ ID NO: 600 (with no signal peptide). In some examples, the anti-LIV1 CAR may comprise the amino acid sequence of SEQ ID NO: 530 (with signal peptide) or SEQ ID NO: 601 (with no signal peptide). In some examples, the anti-LIV1 CAR may comprise the amino acid sequence of SEQ ID NO: 583 (with signal peptide) or SEQ ID NO: 570 (with no signal peptide). In some examples, the anti-LIV1 CAR may comprise the amino acid sequence of SEQ ID NO: 587 (with signal peptide) or SEQ ID NO: 571 (with no signal peptide). In some embodiments, the anti-LIV1 CAR may be encoded by a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 527, 529, 582, or 586. In some examples, the anti-LIV1 CAR may be encoded by a nucleic acid comprising the nucleotide sequence of SEQ ID NOs: 527, 529, 582, or 586.

In some embodiments, the nucleic acid encoding the anti-LIV1 CAR is inserted into a genomic site of interest, *e.g.*, the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, or the disrupted *β2M* gene. In some embodiments, the nucleic acid encoding the CAR is inserted into the disrupted *TRAC* gene. For example, the nucleic acid encoding the CAR may replace the fragment of SEQ ID NO: 69 in the *TRAC* gene. In some instances, the disrupted TRAC gene may comprise the nucleotide sequence of any one of SEQ ID NOs: 541, 542, 585, and 589.

In some embodiments, the disrupted *Reg1* gene comprises a nucleotide sequence listed in Sequence Table 10, 12, 13, or 17. Alternatively or in addition, the disrupted *β2M* comprises a nucleotide sequence listed in Sequence Table 4. When the nucleic acid encoding the anti-LIV1 CAR is not inserted in the disrupted *TRAC* gene, the disrupted TRAC gene may comprise a nucleotide sequence listed in Sequence Table 3.

In some embodiments, the disrupted *Reg1* gene is genetically edited in exon 2 and/or exon 4. In some embodiments, 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 or exon 5.

In some embodiments, the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, and/or the disrupted *β2M* gene are genetically edited by a CRISPR/Cas-mediated gene editing system. In some embodiments, the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *TRAC* gene that comprises SEQ ID NO: 69. In some embodiments, the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *Reg1* gene that comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 320, 322, 323, and 327. In some embodiments, the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *TGFBRII* gene that comprises a nucleotide sequence of SEQ ID NOs: 269, 275, 281, 287, 293, 299, 305, 311, or 317. In some embodiments, the gRNA targeting the *Reg1* gene comprises a nucleotide sequence of SEQ ID NOs: 22, 30, 34, or 50. Alternatively or in addition, the gRNA targeting the *TGFBRII* gene comprises a nucleotide sequence of SEQ ID NOs: 270, 300, 306, or 312. Alternatively or in addition, the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 61, for example, comprising the nucleotide sequence of SEQ ID NO: 59 (unmodified or modified such as that set forth in SEQ ID NO: 58). In some embodiments, the gRNA targeting the β2M gene comprises the nucleotide sequence of SEQ ID NO: 65, for example, comprising the nucleotide sequence of SEQ ID NO: 63 (unmodified or modified such as that set forth in SEQ ID NO: 62).

In some embodiments, the engineered T cell or the population of engineered T cells may comprise: (i) a disrupted *TRAC* gene comprising a nucleic acid encoding a chimeric antigen receptor (CAR) that binds LIV1 (anti-LIV1 CAR), (ii) a disrupted *Regnase-1* (*Reg1*) gene; and (iii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene. The LIV1 CAR comprises an anti-LIV1 scFv that comprises the VH of SEQ ID NO: 568 and the VL of SEQ ID NO: 566. In some instances, the anti-LIV1 scFv comprises the amino acid sequence of SEQ ID NO: 561. In one example, the anti-LIV1 CAR comprises the amino acid sequence of SEQ ID NO: 587 (with signal peptide) or 571 (with no signal peptide) (CTX-975 shown in **Table 32** below). In specific examples, the disrupted *TRAC* gene in the engineered T cell pr the population of engineered T cells may comprise the nucleotide sequence of SEQ ID NO: 585.

The engineered T cells described herein can be, for example, a mammalian cell (*e.g.*, a human T cell). In some embodiments, the engineered T cells can be derived from one or more healthy human donors.

In some embodiments, the population of engineered T cells as described herein may contain at least 15%, 30%, 50% or 70% of the engineered T cells that express the anti-LIV1 CAR. In some examples, at least 50% (e.g., 60%, 70%, 80%, 90% or above) of the engineered T cells in the population as described herein do not express a detectable level of T cell receptor (TCR) protein. In some examples, at least 30% (e.g., 40%, 50%, 60%, 70%, 80%, or above) of the engineered T cells in the population as described herein do not express a detectable level of the Reg1 protein. In some examples, at least 30% (e.g., 40%, 50%, 60%, 70%, 80%, or above) of the engineered T cells in the population as described herein do not express a detectable level of the TGFBRII protein. In some examples, at least 30% (e.g., 40%, 50%, 60%, or above) of the engineered T cells in the population as described herein do not express a detectable level of the B2M protein.

In some embodiments, the engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 10%, 25%, 50%, 70%, 80% or 90% of the cancer cells of the population. In some embodiments, the engineered T cells of the population of cells, when co-cultured *in vitro* with a population of cancer cells that express LIV1, reduces at least 10%, 25%, 50%, 70%, 80% or 90% of the population of cancer cells (e.g., breast cancer cells). In some embodiments, at least 50% of the engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein.

In some aspects, the present disclosure provides a method of producing a population of engineered T cells as disclosed herein. The method can, in some embodiments, comprises: (a) providing a plurality of cells, wherein the plurality of cells are T cells or precursor cells thereof; (b) delivering to the plurality of cells a nucleic acid encoding a chimeric antigen receptor (CAR) that comprise (1) an ectodomain that binds specifically to LIV1, e.g., an anti-LIV1 antigen-binding fragment; (c) genetically editing the *Reg1* gene, the *TGFBRII* gene, or both; and (d) producing a population of engineered T cells expressing the CAR and having a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene. The anti-LIV1 CAR can be any of those disclosed herein, e.g., those described in **Tables 28, 29**, **31**, and **32** below. In some instances, the anti-LIV1 CAR can be CTX-975 or a functional variant thereof, which may have the same VH/VL sequences or the same anti-LIV1 scFv sequence. In other instances, the anti-LIV1 CAR can be CTX-971 or a functional variant thereof, which may have the same VH/VL sequences or the same anti-LIV1 scFv sequence.

In some embodiments, step (c) comprises genetically editing both the *Reg1* gene and the *TGFBRII* gene. In some embodiments, step (b) and/or step (c) is performed by one or more CRISPR/Cas-mediated gene editing systems. In some embodiments, step (c) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *Reg1* gene. In some embodiments, the gRNA targeting the *Reg1* gene is specific to an exon of the *Reg1* gene, which may be exon 2 or exon 4. In some embodiments, step (c) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *TGFBRII* gene. In some embodiments, the gRNA targeting the *TGFBRII* gene is specific to an exon of the *TGFBRII* gene, which may be exon 1, exon 2, exon 3, exon 4, or 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 embodiments, the nucleic acid encoding the anti-LIV1 CAR is in an AAV vector. In some embodiments, 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 of interest in the T cells, allowing for insertion of the nucleic acid into the genomic locus. The genomic locus of interest can be, for example, in the *Reg1* gene, the *TGFBRII* gene, the *TRAC* gene, or the *β2M* gene. In some embodiments, the genomic locus is in the *TRAC* gene, *e.g.*, the site targeted by the gRNA for editing the *TRAC* gene.

In some embodiments, step (b) comprising disrupting the *TRAC* gene by a CRISPR/Cas-mediated gene editing system comprising an RNA-guided nuclease and a gRNA targeting a *TRAC* gene, and the nucleic acid encoding the CAR is inserted at the site targeted by the gRNA. In some embodiments, the gRNA targeting a *TRAC* gene comprises a nucleotide sequence of SEQ ID NO: 61, for example, comprising the nucleotide sequence of SEQ ID NO: 59 (unmodified or a modified version thereof, such as that set forth in SEQ ID NO: 58). In some embodiments, the nucleic acid encoding the CAR is flanked by left and right homology arms to the *TRAC* gene. In some embodiments, the nucleic acid comprises the nucleotide sequence of any one of SEQ ID NOs: 541, 542, 585, and 589.

In some embodiments, the method comprises genetically editing the *β2M* gene. In some embodiments, genetically editing the *β2M* gene comprises delivering to plurality of cells a gRNA targeting the *β2M* gene. In some embodiments, the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 65, for example, comprising the nucleotide sequence of SEQ ID NO: 63 (unmodified or a modified version thereof such as

that set forth in SEQ ID NO: 62). The RNA-guided nuclease can, for example, be a Cas9 nuclease (e.g., a *S. pyogenes* Cas9 nuclease).

In yet other aspects, the present disclosure provides a method, comprising administering to a subject any of the engineered T cells disclosed herein or any population of engineered T cells disclosed herein. The subject can be, for example, a mammal (e.g., a human subject). Also disclosed herein includes an engineered T cell or a population of engineered T cells for use in the treatment of cancer (e.g., a LIV1+ cancer). The engineered T cell or the population of engineered T cells can be any of those as disclosed herein.

In some embodiments, the subject (e.g., a human patient) has a cancer. The cancer can be, but is not limited to, pancreatic cancer, gastric cancer, ovarian cancer, uterine cancer, breast cancer, prostate cancer, testicular cancer, thyroid cancer, nasopharyngeal cancer, non-small cell lung (NSCLC), glioblastoma, neuronal, soft tissue sarcomas, leukemia, lymphoma, melanoma, colon cancer, colon adenocarcinoma, brain glioblastoma, hepatocellular carcinoma, liver hepatocholangiocarcinoma, osteosarcoma, gastric cancer, esophagus squamous cell carcinoma, advanced stage pancreas cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell cancer, renal carcinoma, intrahepatic biliary cancer, and a combination thereof. In some embodiments, the cancer is a solid tumor cancer, for example breast cancer, prostate cancer, squamous tumor cancer, neuronal tumor cancer, or a combination thereof. The cancer comprises cancer cells expressing LIV1.

Also provided herein are uses of any of the engineered T cells or populations of engineered T cells for manufacturing a medicament for use in treating cancer, e.g., any of the LIV1+ cancers provided herein.

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

**FIG. 1A** shows an amino acid sequence alignment of scFV constructs (VL and VH) – 971 (SEQ ID NO: 532), 973 (SEQ ID NO: 560), 975 (SEQ ID NO: 561), and 977 (SEQ ID NO: 562). **FIG. 1B** shows an amino acid sequence alignment of scFV constructs (VH and VL) - 979 (SEQ ID NO: 548), 974 (SEQ ID NO: 563), 976 (SEQ ID NO: 564), 978 (SEQ ID NO: 565), and 972 (SEQ ID NO: 605).

**FIG. 2** includes a diagram showing anti-LIV1 CAR expression levels measured by flow cytometry and ddPCR.

**FIG. 3** includes a diagram showing T cell expansion of exemplary engineered anti-LIV1 CAR T cell variants over time.

**FIG. 4A-FIG. 4B** show the degree of cytotoxicity against MCF7 (**FIG. 4A**) and ZR-75-1 (**FIG. 4B**) cells that were exhibited by exemplary engineered anti-LIV1 CAR T cells and exemplary engineered anti-LIV1 CAR TGFBR2/Reg knockout (KO) T cells.

**FIG. 5A-FIG. 5D** show cytokine secretion of exemplary engineered anti-LIV1 CAR T cells and exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells when co-cultured with target MCF7 cells (FIG. 5A, FIG. 5C) and target ZR-75-1 cells (FIG. 5B, FIG. 5D).

**FIG. 6** includes a diagram showing tumor volume control by exemplary engineered anti-LIV1 CAR T cells and exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells when co-cultured with MCF7 cell lines.

**FIG. 7A-FIG. 7B** include diagrams showing tumor volume control by exemplary engineered anti-LIV1 CAR T cells and exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells when co-cultured with MCF7 cell lines.

**FIG. 8A-FIG. 8D** include diagrams showing tumor volume control by exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells when co-cultured with MCF7 cell lines.

**FIG. 9** includes an exemplary diagram showing the LIV1 editing efficiency in the generation of two LIV1 knockout breast cancer cell lines MCF7 and ZR751.

**FIG. 10A-FIG. 10B** include exemplary diagrams showing the degree of cytotoxicity against native MCF7, Liv1-2 knockout MCF7 and Liv1-4 knockout MCF7 cells that were exhibited by exemplary engineered anti-LIV1 CAR T cells (FIG. 10A) and exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells (FIG. 10B).

**FIG. 11A-FIG. 11B** include exemplary diagrams showing the degree of cytotoxicity against native ZR751, Liv1-2 knockout ZR751 and Liv1-4 knockout ZR751 cells that were exhibited by exemplary engineered anti-LIV1 CAR T cells (**FIG. 11A**) and exemplary engineered anti-LIV1 CAR TGFBR2/Reg KO T cells (**FIG. 11B**).

**FIG. 12** is a diagram showing anti-tumor activity of various anti-LIV1 CAR-T cells as indicated in a mouse model.

**FIGs. 13A-13B** include diagrams showing anti-tumor activity of various anti-LIV1 CAR-T cells as indicated in a mouse model at different doses. FIG. 13A: at the dose of $1x10^7$ CAR-T cells. FIG. 13B: at the dose of $2x10^7$ CAR-T cells.

## DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein and made part of the disclosure herein.

All patents, published patent applications, other publications, and sequences from GenBank, and other databases referred to herein are incorporated by reference in their entirety with respect to the related technology.

LIV1, a member of the ZIP family of highly conserved transmembrane zinc transporter proteins, is expressed at elevated levels in estrogen receptor-positive breast cancer and tumors of the lymph nodes. Further aberrant expression of zinc transporters such as LIV1 is known to lead to deregulated Zn intake or deficiency, leading to uncontrolled growth such that occur in cancer. Thus, LIV1 is a desirable transmembrane protein for targeting cancer. LIV-1 protein has been implicated in breast cancer, prostate cancer, squamous tumors, and neuronal tumors.

Provided herein includes an engineered T cell (*e.g.*, anti-LIV1 CAR-T cell) and a population thereof, a method of producing the engineered T cell and a method of treating a subject (e.g., a subject having a cancer) using the engineered T cell or a population of the engineered T cells.

In some aspects, disclosed herein includes an engineered T cell. The engineered T cell can comprise a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (1) an ectodomain that binds specifically to LIV1, or (2) an anti-LIV1 antigen-binding fragment. The engineered T cell can also comprise at least one of (i) a disrupted

*Regnase-1* (*Reg1*) gene, and (ii) a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene. In some embodiments, the engineered T cells comprise both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene. Disclosed herein also includes a population of cells comprising the engineered T cell disclosed herein.

Disclosed herein also includes a method of producing an engineered T cell. The method can comprise providing a plurality of cells, wherein the plurality of cells are T cells or precursor cells thereof, delivering to the plurality of cells a nucleic acid encoding a chimeric antigen receptor (CAR) that comprise (1) an ectodomain that binds specifically to LIV1, or (2) an anti-LIV1 antigen-binding fragment, genetically editing the *Reg1* gene, the *TGFBRII* gene, or both; and producing one or more engineered T cells expressing the CAR and having a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene.

Disclosed herein also includes a method for the therapeutic uses of the engineered T cell or a population of the engineered T cells herein described. The method can comprise administering to a subject the engineered T cell described herein or the population of engineered T cells described herein.

In some embodiments, engineered T cells expressing an anti-LIV1 CAR and having one or both of disrupted *Reg1* gene and disrupted *TGFBRII* gene can provide synergistically and/or advantageous enhanced anti-tumor effects as compared with non-engineered T cells or engineered T cells expressing anti-LIV1 CAR without having one or both of disrupted *Reg1* gene and disrupted *TGFBRII* gene.

**Definitions**

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. *See, e.g.* Singleton et al., Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press (Cold Spring Harbor, NY 1989). For purposes of the present disclosure, the following terms are defined below.

As used herein, the terms “nucleic acid” and “polynucleotide” are interchangeable and refer to any nucleic acid, whether composed of phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged phosphoramidate, bridged methylene

phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sultone linkages, and combinations of such linkages. The terms "nucleic acid" and "polynucleotide" also specifically include nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil).

The terms "naturally occurring" and "biologically occurring" as used herein refer to materials which are found in nature or a form of the materials that is found in nature.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the nucleotide bases or residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, Adv. Appl. Math. 2:482, 1981; Needleman & Wunsch, J. Mol. Biol. 48:443, 1970; Pearson & Lipman, Proc. Natl. Acad. Sci. USA 85:2444, 1988; Higgins & Sharp, Gene, 73:237-44, 1988; Higgins & Sharp, Cabios 5:151-3, 1989; Corpet et al., Nuc. Acids Res. 16:10881-90, 1988; Huang et al. Computer Appls. in the Biosciences 8, 155-65, 1992; Pearson et al., Meth. Mol. Bio. 24:307-31, 1994; and Altschul et al., J. Mol. Biol. 215:403-10, 1990, the content of each of which is incorporated herein in its entirety.

As used herein, the term "vector" can refer to a vehicle for carrying or transferring a nucleic acid. Non-limiting examples of vectors include viral vectors (for example, adenovirus vectors, adeno-associated virus (AAV) vectors, retrovirus vectors, lentiviral vectors, herpes virus vectors, phages, and poxvirus vectors); non-viral vectors such as liposomes, naked DNA, plasmids, cosmids; and the like.

As used herein, a "donor" refers 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.

As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) A → G, S; (b) R → K, H; (c) N → Q, H; (d) D → E, N; (e) C → S, A; (f) Q → N; (g) E → D, Q; (h) G → A; (i) H → N, Q; (j) I → L, V; (k) L → I, V; (l) K → R, H; (m) M→ L, I, Y; (n) F → Y, M, L; (o) P → A; (p) S → T; (q) T→ S; (r) W → Y, F; (s) Y → W, F; and (t) V→ I, L. Variants can be prepared according to methods for altering polypeptide sequence known to one of skill in the

art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York.

As used herein, a CDR can refer to the CDR defined by any method known in the art. Two antibodies having the same CDR means that the two antibodies have the same amino acid sequence of that CDR as determined by the same method. See, e.g., Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs.

As used herein, the term "antibody" encompasses intact (i.e., full-length) monoclonal antibodies, as well as antigen-binding fragments (such as Fab, Fab', F(ab')2, Fv), single chain variable fragment (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, single domain antibodies (*e.g.*, camel or llama VHH antibodies), multispecific antibodies (*e.g.*, bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies.

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.

As used herein, a "transmembrane domain" refers to a protein structure that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane.

Standard techniques can be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques can be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and

procedures can be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those commonly known and used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

**Anti-LIV1 Chimeric Antigen Receptors (Anti-LIV1 CARs) and T Cells Expressing Such**

The T cells disclosed herein can be engineered to express a chimeric antigen receptor (CAR) designed to target LIV1. LIV1, also known as Solute Carrier Family 39 Member 6, SLC39A6, ZIP6, and LIV-1, is a member of the ZIP family of highly conserved transmembrane zinc transporter proteins. LIV1 is expressed at elevated levels in breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and neuronal tumors. LIV1 has a restricted expression in normal tissues, *e.g.*, non-cancerous breast, prostate, and testis, which makes it a desirable transmembrane protein for targeting cancer. LIV-1 protein has been implicated in breast cancer, prostate cancer, squamous tumors, and neuronal tumors.

Anti-LIV1 CARs

An engineered T cell disclosed herein can have nucleic acid encoding a chimeric antigen receptor (CAR). In some embodiments, the CAR comprises an ectodomain that binds specifically to LIV1 or an anti-LIV1 antigen-binding fragment. In some embodiments, the anti-LIV1 antigen-binding fragment comprises an anti-LIV1 antibody.

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. In some embodiments, a CAR designed for a T cell is a chimera of a signaling domain of the T-cell receptor (TCR) complex and an antigen-recognizing domain (*e.g.*, a single chain fragment (scFv) of an antibody or other antibody fragment) (Enblad et al.,

Human Gene Therapy. 2015; 26(8):498-505). 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.

CARs typically differ in their functional properties. The CD3ζ signaling domain of the T-cell receptor, when engaged, will activate and induce proliferation of T-cells but can lead to anergy (a lack of reaction by the body's defense mechanisms, resulting in direct induction of peripheral lymphocyte tolerance). Lymphocytes are considered anergic when they fail to respond to a specific antigen. The addition of a costimulatory domain in second-generation CARs improved replicative capacity and persistence of modified T-cells. Similar antitumor effects are observed *in vitro* with CD28 or 4-1BB CARs, but preclinical *in vivo* studies suggest that 4-1BB CARs may produce superior proliferation and/or persistence. Clinical trials suggest that both of these second-generation CARs are capable of inducing substantial T-cell proliferation *in vivo*, but CARs containing the 4-1BB costimulatory domain appear to persist longer. Third generation CARs combine multiple signaling domains (costimulatory) to augment potency.

The CAR can be a first generation CAR. In some embodiments, a CAR is a second generation CAR. In yet some other embodiments, the CAR is a third generation CAR.

In some embodiments, a CAR is a fusion polypeptide comprising an extracellular domain (ectodomain) that recognizes a target antigen (*e.g.*, a single chain fragment (scFv) of an antibody or other antibody fragment) and an intracellular domain (endodomain) 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 **Sequence Table 6** below. Other signal peptides may be used.

*Ectodomain*

The ectodomain (antigen-binding extracellular domain) is the region of the CAR that is exposed to the extracellular fluid and, in some embodiments, includes an antigen binding domain, and optionally a signal peptide, a spacer domain, and/or a hinge domain.

An antibody is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule.

A typical antibody molecule comprises a heavy chain variable region (VH) and a light chain variable region (VL), which are usually involved in antigen binding. These regions/residues that are responsible for antigen-binding can be identified from amino acid sequences of the VH/VL sequences of a reference antibody (e.g., an anti-LIV1 antibody as described herein) by methods known in the art. The VH and VL regions can be further subdivided into regions of hypervariability, also known as "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, which are known as "framework regions" ("FR"). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition, all of which are well known in the art.

An antibody can, for example, specifically binds a target antigen, such as human LIV1. An antibody that "specifically binds" (used interchangeably herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody "specifically binds" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with

greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to a LIV1 epitope is an antibody that binds this LIV1 epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other LIV1 epitopes or non-LIV1 epitopes. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

In some embodiments, the equilibrium dissociation constant ($K_D$) between the antibody and LIV1 is 100 pM to 1 μM, for example, 1 nM to 100 nM.

In some embodiments, the antigen binding domain specific to LIV1 is a single-chain variable fragment (scFv) that include the light and heavy chains of immunoglobulins connected with a short linker peptide. 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. A single-chain variable fragment (scFv) is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker.

In some embodiments, the anti-LIV1 antibody for use in constructing the anti-LIV1 CAR disclosed herein may comprise VH CDRs that collectively are at least 80% (e.g., about, at least, or at least about 80%, 85%, 90%, 95%, or 98%) identical to the VH CDRs of a reference antibody having a VH of SEQ ID NO: 533 and a VL of SEQ ID NO: 534, or a reference antibody having a VH of SEQ ID NO: 568 and a VL of SEQ ID NO: 566. Alternatively or in addition, the antibody can comprise VL CDRs that collectively are at least 80% (e.g., about, at least, or at least about 80%, 85%, 90%, 95%, or 98%) identical to the VL CDRs of the reference antibody.

In some embodiments, the antibody for use in constructing the anti-LIV1 CAR comprises a VH that is at least 80% (e.g., about, at least, or at least about 80%, 85%, 90%, 95%, or 98%) identical to the VH of a reference antibody such as in VH: SEQ ID NO: 533 or 568 or 576; VL: SEQ ID NO: 534 or 566 or 606 and/or a VL variable region that is at least

80% (e.g., about, at least, or at least about 80%, 85%, 90%, 95%, or 98%) identical to the VL variable region of the reference antibody.

In some embodiments, the antibody for use in constructing the anti-LIV1 CAR comprises a VH CDR1, a VH CDR2, and a VH CDR3, which collectively contains no more than 10 amino acid variations (e.g., no more than 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH CDR1, VH CDR2, and VH CDR3 of a reference antibody such as in VH: SEQ ID NO: 533 or 568 or 576; VL: SEQ ID NO: 534 or 566 or 606. "Collectively" means that the total number of amino acid variations in all of the three VH CDRs is within the defined range. Alternatively or in addition, antibody may comprise a VL CDR1, a VL CDR2, and a VL CDR3, which collectively contains no more than 10 amino acid variations (e.g., no more than 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid variation) as compared with the VL CDR1, VL CDR2, and VL CDR3 of the reference antibody.

In some embodiments, the antibody for use in constructing the anti-LIV1 CAR comprises a VH CDR1, a VH CDR2, and a VH CDR3, at least one of which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the counterpart VH CDR of a reference antibody such as in VH: SEQ ID NO: 533 or 568 or 576; VL: SEQ ID NO: 534 or 566 or 606. In some embodiments, the antibody comprises a VH CDR3, which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the VH CDR3 of a reference antibody such as in VH: SEQ ID NO: 533 or 568 or 576; VL: SEQ ID NO: 534 or 566 or 606. Alternatively or in addition, an antibody can comprise a VL CDR1, a VL CDR2, and a VL CDR3, at least one of which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the counterpart VL CDR of the reference antibody. In some embodiments, the antibody comprises a VL CDR3, which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the LC CDR3 of the reference antibody.

The amino acid residue variations can be or comprise conservative amino acid residue substitutions. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York.

Non-limiting examples of VH and VL protein sequences that can be used to create an anti-LIV1 scFv include those provided in sequence **Tables 29** and **32**, all of which are within the scope of the present disclosure. In one example, the VH may comprise the amino acid sequence of SEQ ID NO: 533 and the VL may comprise the amino acid sequence of SEQ ID NO: 534 or 606. An anti-LIV1 scFv having such VH/VL may comprise the amino acid sequence of SEQ ID NO: 532, 548, or 605. In another example, the VH may comprise the amino acid sequence of SEQ ID NO: 576 and the VL may comprise the amino acid sequence of SEQ ID NO: 534. An anti-LIV1 scFv having such VH/VL may comprise the amino acid sequence of SEQ ID NO: 560 or 563. In yet another example, the VH may comprise the amino acid sequence of SEQ ID NO: 568 and the VL may comprise the amino acid sequence of SEQ ID NO: 566. An anti-LIV1 scFv having such VH/VL may comprise the amino acid sequence of SEQ ID NO: 561 or 564. In still another example, the VH may comprise the amino acid sequence of SEQ ID NO: 576 and the VL may comprise the amino acid sequence of SEQ ID NO: 566. An anti-LIV1 scFv having such VH/VL may comprise the amino acid sequence of SEQ ID NO: 562 or 565.

In some embodiments, the scFv of the present disclosure is humanized. In some embodiments, the scFv is fully human. In some embodiments, the scFv is a chimera (*e.g.*, of mouse and human sequence). In some embodiments, the scFv is an anti-LIV1 scFv (binds specifically to LIV1). Non-limiting examples of anti-LIV1 scFv proteins include the amino acid sequence of any one of SEQ ID NOs: 532, 548, 560, 561, 562, 563, 564, and 565. Other scFv proteins can also be used.

The signal peptide can enhance the antigen specificity of CAR binding. Signal peptides can be derived from antibodies, such as, but not limited to, CD8, as well as epitope tags such as, but not limited to, GST or FLAG. Examples of signal peptides include SEQ ID NO: 95 and SEQ ID NO: 96. Other signal peptides may be used.

A spacer domain or hinge domain can 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 spacer domain is 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 is any oligopeptide or polypeptide that functions to provide flexibility to the CAR, or domains thereof, or to prevent steric hindrance of the CAR, or domains thereof. In some embodiments, a spacer domain or 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 spacer domain(s) may be included in other regions of a CAR. In some embodiments, the hinge domain is a CD8 hinge domain. Other hinge domains can also be used.

*Transmembrane Domain*

The CAR polypeptide disclosed herein can contain a transmembrane domain, which can be a hydrophobic alpha helix that spans the membrane. A "transmembrane domain" can be 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 can be, or comprise, a CD8 transmembrane domain. In some embodiments, the transmembrane domain can be, or comprise, a CD28 transmembrane domain. In yet some other embodiments, the transmembrane domain is, or comprise, 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 **Sequence Table 6** or the sequence of SEQ ID NO: 553 or SEQ ID NO: 555 of **Sequence Table 29**. Other transmembrane domains can also be used.

*Endodomain*

The endodomain (intracellular signaling domain, e.g., CD3ζ, and optionally one or more co-stimulatory domains) is the functional end of the receptor. Following antigen recognition, receptors cluster and a signal is transmitted to the cell.

In some embodiments, an endodomain comprises CD3ζ, a 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. For example, CD28 and/or 4-1BB may be used with CD3-zeta (CD3ζ) to transmit a proliferative/survival signal.

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 some embodiments, 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 some 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. **Sequence Table 6** provides examples of signaling domains derived from 4-1BB, CD28 and CD3-zeta that can be used herein.

Exemplary Anti-LIV1 CAR-T Cells

Disclosed herein includes engineered cells (e.g., engineered cells in a population of cells) expressing CAR. For example, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, a number or a range between any two of these values, or more of the engineered T cells of the population can express the CAR. In some embodiments, at least 15%, 30%, 50% or 70% of the engineered T cells of the population express the CAR.

Immune cells (*e.g.*, T cells) disclosed herein are engineered to express a CAR comprising an antigen-binding extracellular domain that binds specifically to LIV1. In some embodiments, the antigen-binding extracellular domain comprises an anti-LIV1 antigen-binding fragment which can, for example, comprise an anti-LIV1 antibody (*e.g.*, anti-LIV1 scFv). Exemplary anti-LIV1 CAR constructs, and functional elements thereof (*e.g.*, anti-LIV1 scFvs) are provided in **Tables 29** and **32** below. Any of such constructs, as well as functional elements thereof, is within the scope of the present disclosure.

In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv encoded by a sequence comprising or consisting of any one of SEQ ID NOs: 531, 547, 575, 580, 584, 588, 592, and 596, or an anti-LIV1 scFv encoded by a nucleic acid sequence comprising or consisting of a sequence about, at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to one of SEQ ID NOs: 531, 547, 575, 580, 584, 588, 592, and 596. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv having a sequence comprising or consisting of any one of SEQ ID NOs: 532, 548, 560, 561, 562, 563, 564, and 565, or an anti-LIV1 scFv comprising or consisting of a sequence having 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to one of SEQ ID NOs: 532, 548, 560, 561, 562, 563, 564, and 565.

In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv comprising a VH comprising or consisting of an amino acid sequence of any one of SEQ ID NO: 533, 568 and 576, or an anti-LIV1 scFv comprising a VH having an amino acid sequence comprising or consisting of a sequence having 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to one of SEQ ID NOs: 533, 568 and 576. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv comprising a VL comprising or consisting the amino acid sequence of any one of SEQ ID NO: 534, 566 and 606, or an anti-LIV1 scFv comprising a VL having an amino acid sequence comprising or consisting of a sequence having 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOs: 534, 566 and 606. In some embodiments, a CAR comprising an anti-LIV1 antibody is encoded by the sequence of any one of SEQ ID NOs: 527, 529, 543, 545, 573, 578, 582, 586, 590, and 594. In some embodiments, a CAR comprising an anti-LIV7 antibody is encoded by a sequence comprising a nucleic acid that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NOs: 527, 529, 543, 545, 573, 578, 582, 586, 590, or 594. In some embodiments, a CAR comprising an anti-LIV1 antibody comprises or consists of the sequence of any one of SEQ ID NOs: 527, 529, 543, 545, 573, 578, 582, 586, 590, or 594.

In some embodiments, an anti-LIV1 CAR comprise a CAR construct selected from CTX-971 CAR, CTX-971b CAR, CTX-972 CAR, and CTX-972b CAR listed in **Sequence Table 28** with the sequences provided in **Sequence Table 29**. In some embodiments, an anti-LIV1 CAR comprise a CAR construct selected from CTX-973 CAR, CTX-974 CAR, CTX-975 CAR, CTX-976 CAR, CTX-977 CAR, CTX-978 CAR, CTX-979 CAR, or CTX-979b CAR listed in **Sequence Table 31** with the sequences provided in **Sequence Table 32.** In some embodiments the nucleic acid encoding the anti-LIV1 CARs are inserted in the *TRAC* gene.

In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a nucleotide sequence of SEQ ID NO: 527, 529, 543, 545, 573, 578, 582, 586, 590, or 594. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a nucleotide sequence that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 527, 529, 543, 545, 573, 578, 582, 586, 590, or 594. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a nucleotide sequence of SEQ ID NO: 527, 573, or 582. In some embodiments, the disrupted *TRAC* gene in the anti-

LIV1 CAR cells comprises, or consists of, a sequence that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 527, 573, or 582. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, the nucleotide sequence of SEQ ID NO: 582. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a sequence that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the nucleotide sequence of SEQ ID NO: 582.

In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a nucleotide sequence of SEQ ID NO: 541, 542, 549, 550, 577, 581, 585, 589, 593 or 597. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a sequence that is about, at least, or at least about, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 541, 542, 549, 550, 577, 581, 585, 589, 593 or 597. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells can comprise a nucleotide sequence of SEQ ID NO: 541, 542, 577, 585 or 589. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a sequence that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 541, 542, 577, 585 or 589. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, the nucleotide sequence of SEQ ID NO: 585. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells comprises, or consists of, a sequence that is about, at least or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 585.

In some embodiments, a CAR comprising an anti-LIV1 antibody comprises an anti-LIV1 antibody as described in US Patent No. 9,228,026, and WO2020/095249, the relevant disclosures of each of which are incorporated by reference for the subject matter and purpose referenced herein.

**Multi-Gene Editing**

The engineered T cells (*e.g.*, the anti-LIV1 CAR-T cells) disclosed herein can comprise one or more gene edit(s), for example, in one or more gene(s). For example, an engineered T cell can comprise a disrupted T cell receptor alpha chain constant region (*TRAC*) gene, a disrupted *TGFBRII* gene, a disrupted *Reg1* gene, or a combination thereof. In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene and at least one

of a disrupted *Reg1* gene and a disrupted *TGFBRII* gene. In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene, a disrupted *Reg1* gene, and a disrupted *TGFBRII* gene. In some embodiments, an engineered T cell can further comprise one or more of a disrupted *β2M* gene, a disrupted *CD70* gene, and a disrupted *PD-1* gene. An engineered T cell can also comprise a disrupted beta-2-microglobulin (*β2M*) gene, a disrupted programmed cell death-1 (*PD-1* or *PDCD1*) gene, a disrupted *CD70* gene, or any combination of two or more of the foregoing disrupted genes.

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 can be referred to as a knockout cell.

Provided herein, in some embodiments, are populations of cells in which a certain percentage of the cells has been edited (*e.g.*, *TRAC*, *Reg1* and/or *TGFBRII* gene), resulting in a certain percentage of cells not expressing a particular gene and/or protein. In some embodiments, at least 50% (*e.g.*, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or a number or a range between any two of these values) of the cells of a gene-edited population of cells are *TRAC*, Reg1 and/or TGFBRII knockout cells. In some embodiments, at least 50% of the cells (*e.g.*, T cells) of the population do not express detectable levels of T cell receptor (TCR) surface protein. In some embodiments, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the cells of a gene-edited population of cells can be TRAC knockout cells. In some embodiments, at least 50% of the cells (*e.g.* T cells) of the population do not express detectable levels of Reg1 and/or TGFBRII protein. In some embodiments, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the cells of a gene-edited population of cells can be Reg1 and/or TGFBRII knockout cells.

*TRAC Gene Edit*

In some embodiments, an engineered T cell herein disclosed comprises 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. In some embodiments, a disruption in the

*TRAC* gene expression is created by knocking a chimeric antigen receptor (CAR) into the *TRAC* gene (*e.g.*, using an adeno-associated viral (AAV) vector and donor template). In some embodiments, a disruption in the *TRAC* gene expression is created by gRNAs targeting the *TRAC* genomic region. In some embodiments, a genomic deletion in the *TRAC* gene is created by knocking a chimeric antigen receptor (CAR) into the *TRAC* gene (*e.g.*, using an AAV vector and donor template). In some embodiments, a disruption in the TRAC gene expression is created by gRNAs targeting the *TRAC* genomic region and knocking a chimeric antigen receptor (CAR) into the *TRAC* gene.

In some embodiments, an edited *TRAC* gene can comprise a nucleotide sequence selected from the following sequences in **Sequence Table 3**. 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 **Sequence Table 3**) may be generated by a single gRNA such as the one listed in **Sequence Table 2** (TA-1). Non-limiting examples of modified and unmodified TRAC gRNA sequences that can be used to create a genomic disruption in the *TRAC* gene are listed in **Sequence Table 2** (e.g., SEQ ID NOs: 58 and 59). *See* also International Application published as WO2019215500, which is incorporated herein by reference. Other gRNA sequences can 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 create Indels in the *TRAC* gene disrupting expression of the mRNA or protein.

In some embodiments, at least 50% of a population of engineered T cells do not express a detectable level of T cell receptor (TCR) 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 a population may not express a detectable level of TCR 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 population of engineered T cells do not express a detectable level of TCR surface protein.

In some embodiments, an engineered T cell comprises a deletion in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 15-30 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs in the *TRAC* gene relative to unmodified T cells. In

some embodiments, an engineered T cell comprises a deletion of more than 30 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 20 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of SEQ ID NO: 69 (AGAGCAACAGTGCTGTGGCC) in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion comprising SEQ ID NO: 69 (AGAGCAACAGTGCTGTGGCC) in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of SEQ ID NO: 68 in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion comprising SEQ ID NO: 68 in the *TRAC* gene relative to unmodified T cells. The *TRAC* gene editing is also described in International Patent Application published as WO2020095249, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

In some instances, the nucleic acid encoding any of the anti-LIV1 CAR may be inserted into the disrupted *TRAC* gene. Such a disrupted TRAC gene may comprise the coding sequence of the anti-LIV1 CAR.

*Reg1 Gene Edit*

In some embodiments, the engineered T cells can comprise a disrupted gene involved in mRNA decay such as 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 embodiments, the engineered T cells can 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 can 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 can 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 can be induced by the CRISPR/Cas technology using a suitable guide RNA, for example, those listed in **Sequence Table 1**. The resultant edited *Reg1* gene using a gRNA listed in **Sequence Table 1** can comprise one or more edited sequences provided in **Sequence Tables 8-17** below. The *Reg1* gene editing is also described in WO2022/064428, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

*TGFBRII Gene Editing*

The engineered T cells described herein can 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 embodiments, the engineered T cells can 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 can 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 can be identified based on the gene editing approach for use in making the genetically engineered T cells. Exemplary target sites for the genetic edits include exon 1, exon 2, exon 3, exon 4, exon 5, or a combination thereof. In some embodiments, one or more genetic editing can occur in exon 4 and/or exon 5. Such genetic editing can be induced by a gene editing technology, (*e.g.*, the CRISPR/Cas technology) using a suitable guide RNA, for example, those listed in **Sequence Table 18**. The resultant edited *TGFBRII* gene using a gRNA listed in **Sequence Table 18** can comprise one or more edited sequences provided in **Sequence Tables 19-27** below. The *TGFBRII* gene editing is also described in WO2022/064428, the relevant disclosures of which are incorporated by reference for the subject matter and purpose referenced herein.

*β2M Gene Edit*

In some embodiments, the genetically engineered T cells disclosed herein can comprise a disrupted *β2M* gene. β2M is a common (invariant) component of MHC I complexes. Disrupting its expression by gene editing can 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 can comprise a nucleotide sequence selected from the following sequences in **Sequence Table 4**. 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 **Sequence Table 4**) can be generated by a single gRNA such as the one listed in **Sequence Table 2** (β2M-1). Non-limiting examples of modified and unmodified β2M gRNA sequences that can be used herein to create a genomic disruption in the *β2M* gene are listed include, for example, SEQ ID NOs: 62 and 63). See also International Application published as WO2019215500, the relevant disclosures of which are incorporated by reference for the subject matter and purposes referenced herein. Other gRNA sequences can 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 create Indels in the *β2M* gene disrupting expression of the mRNA or protein.

In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does 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 engineered T cells of a population 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.

*PD-1 Gene Edit*

The genetically engineered T cells disclosed herein can 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.

In some embodiments, the engineered T cell herein describe can further comprise a disrupted *PD-1* gene. PD-1 is an immune checkpoint molecule that is upregulated in activated T cells and serves to dampen or stop T cell responses. Disrupting PD-1 by gene editing can lead to more persistent and/or potent therapeutic T cell responses and/or reduce immune suppression in a subject. In some embodiments, expression of the endogenous *PD-1* gene is eliminated to enhance anti-tumor efficacy of the CAR T cells of the present disclosure.

Non-limiting examples of modified and unmodified PD-1 gRNA sequences that may be used as provided herein to create a genomic deletion in the PD-1 gene are listed in **Sequence Table 2** (e.g., SEQ ID NOs: 500 and 501). See also International Application published as WO2019215500, the relevant disclosures of which are incorporated by reference for the subject matter and purposes referenced herein. Other gRNA sequences may be designed using the PD-1 gene sequence located on Chromosome 2 (GRCh38 coordinates: Chromosome 2: 241,849,881-241,858,908; Ensembl: ENSG00000188389).

In some embodiments, gRNAs targeting the PD-1 genomic region create Indels in the PD-1 gene disrupting expression of the PD-1 mRNA or protein.

In some embodiments, an engineered T cell comprises a disrupted PD-1 gene. In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does not express a detectable level of PD-1 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 engineered T cells of a population may not express a detectable level of PD-1 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 PD-1 surface protein.

*CD70 Gene Edit*

An engineered T cell disclosed herein can, in some embodiments, comprise a disrupted gene involved in cell exhaustion (e.g., T cell exhaustion). Genes involved in cell exhaustion refer to those that either positively regulate or negatively regulate this biological

process. In some embodiments, an engineered T cell comprises a disrupted Cluster of Differentiation 70 (*CD70)* gene.

Cluster of Differentiation 70 (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 has also been detected on hematological tumors and on carcinomas. CD70 is implicated in tumor cell and regulatory T cell survival through interaction with its ligand, CD27. Disrupting CD70 by gene editing increases cell expansion and reduces cell exhaustion. In some embodiments, an engineered T cell comprises a disrupted *CD70* gene. In some embodiments, expression of the endogenous *CD70* gene is eliminated to enhance anti-tumor efficacy of the CAR T cells of the present disclosure. In some embodiments, gRNAs targeting the *CD70* genomic region create Indels in, or around, the *CD70* gene disrupting expression of the CD70 mRNA and/or protein.

In some embodiments, the gRNA targeting CD70 listed in **Sequence Table 2** (CD70-7) can be used for disrupting the CD70 gene via CRISPR/Cas9 gene editing. In some examples, an edited *CD70* gene can comprise a nucleotide sequence selected from the following sequences in **Sequence Table 5**. Other gRNA sequences can be designed using the CD70 gene sequence located on Chromosome 19 (GRCh38 coordinates: Chromosome 19: 6,583,183-6,604,103; Ensembl: ENSG00000125726).

In some embodiments, the engineered T cells can 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 can 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 can be identified based on the gene editing approach for use in making the genetically engineered T cells.

In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does 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%, or at least 95% 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%, or 90%-100% of the engineered T cells of a population does not express a detectable level of CD70 surface protein.

**Engineered T cells**

Provided herein also includes a population of 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-LIV1 CAR. The anti-LIV1 CAR can comprise an ectodomain that binds specifically to LIV1 or an anti-LIV1 antigen-binding fragment (e.g., an anti-LIV1 antibody or a fragment thereof). In some instances, the population of engineered immune cells (*e.g.*, T cells such as human T cells) comprise both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, and express an anti-LIV1 CAR, *e.g.*, those disclosed herein. In some embodiments, the anti-LIV1 CAR-T cells disclosed herein, which express any of the anti-LIV1 CAR disclosed herein (*e.g.*, the anti-LIV1 CAR comprising the amino acid sequence provided in Tables 29 and 32), can also comprise a disrupted *TRAC* gene and/or a disrupted *β2M* gene as also disclosed herein. In some embodiments, the engineered T cell is a human T cell.

In some embodiments, the anti-LIV1 CAR T cell comprise a construct of CTX-971 CAR, CTX-971b CAR, CTX-972 CAR, or CTX-972b CAR of **Sequence Table 28** with the sequences provided in **Sequence Table 29**. In some embodiments, the anti-LIV1 CAR T cell comprise a construct of CTX-973 CAR, CTX-974 CAR, CTX-975 CAR, CTX-976 CAR, CTX-977 CAR, CTX-978 CAR, CTX-979 CAR, or CTX-979b CAR of **Sequence Table 31** with the sequences provided in **Sequence Table 32.** In some embodiments the nucleic acid encoding the anti-LIV1 CAR is inserted in the *TRAC* gene.

In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells can comprise a nucleotide sequence of SEQ ID NO: 527, 529, 543, 545, 573, 578, 582, 586, 590, or 594. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells can comprise a nucleotide sequence of SEQ ID NO: 527, 573, or 582. In some embodiments, the disrupted *TRAC* gene in the anti-LIV1 CAR cells can comprise the nucleotide sequence of SEQ ID NO: 582.

In some embodiments, the population of engineered T cells are anti-LIV1 CAR cells that further comprise a disrupted *Reg-1* gene. In some examples, anti-LIV1 CAR cells are LIV1 directed T cells having disrupted *TRAC* gene and *β2M* gene. The nucleic acid encoding the anti-LIV1 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-LIV1 CAR, thereby disrupting expression of the *TRAC* gene. The disrupted *TRAC* gene in the anti-LIV1 CAR cells can comprise the nucleotide sequence of SEQ ID NO: 582.

Anti-LIV1 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-LIV1 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-LIV1 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-LIV1 CAR construct. CRISPR-Cas9-mediated gene editing involves at least a sgRNA targeting *TGFBRII* (*e.g.*, those listed in **Sequence Table 18,** e.g., 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-LIV1 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-LIV1 CAR construct. CRISPR-Cas9-mediated gene editing involves at least a sgRNA targeting *TGFBRII* (*e.g.*, those listed in **Sequence Table 18**) and a sgRNA targeting *Reg1* (*e.g.*, those listed in **Sequence Table 1**), 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-LIV1 CAR T cells are composed of an anti-LIV1 single-chain antibody fragment (scFv, which can comprise the amino acid sequence of SEQ ID NO: 532, 548, 561, or 564), followed by a CD8 hinge and transmembrane domain (*e.g.*, comprising the amino acid sequence of SEQ ID NO: 575) that is fused to an intracellular co-signaling domain of

CD28 (*e.g.*, SEQ ID NO: 579) and a CD3ζ signaling domain (*e.g.*, SEQ ID NO: 581). In some embodiments, the anti-LIV1 CAR disclosed herein may comprise the amino acid sequence of SEQ ID NO: 528, 574, or 583, or the counterpart thereof without the N-terminus signal peptide (see **Table 29** and **Table 32** below).

In some embodiments, at least 30% of a population of anti-LIV1 CAR T cells express a detectable level of the anti-LIV1 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-LIV1 CAR T cells express a detectable level of the anti-LIV1 CAR.

In some embodiments, at least 50% of a population of anti-LIV1 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-LIV1 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, in some embodiment at least 50% of a population of anti-LIV1 CAR T cells do 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-LIV1 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-LIV1 CAR T cells do not express a detectable TRAC surface protein.

A substantial percentage of the population of anti-LIV1 CAR T cells described herein can, in some embodiments, 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, in some embodiments, at least 50% of a population of anti-LIV1 CAR T cells do 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-LIV1 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-LIV1 CAR T cells do not express a detectable level of TRAC and β2M surface proteins.

The population of anti-LIV1 CAR T cells described herein can, in some embodiments, 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-LIV1 CAR T cells may comprise a disrupted *TRAC* gene *via* the CRISPR/Cas technology using the TA-1 *TRAC* gRNA. In some embodiments, the anti-LIV1 CAR T cells can comprise a deletion in the *TRAC* gene relative to unmodified T cells. For example, the anti-LIV1 CAR T cells can 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-LIV1 CAR (*e.g.*, SEQ ID NO: 527, 573, or 582.). Alternatively or in addition, in some embodiments the population of anti-LIV1 CAR T cells can comprise a disrupted *β2M* gene *via* CRISPR/Cas9 technology using the gRNA of β2M-1. Such anti-LIV1 CAR T cells can 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-LIV1 CAR T cells comprise ≥ 30% $CAR^+$ T cells, ≤ 50% $\beta2M^+$ cells, and ≤ 30% $TCR\alpha\beta^+$ cells. In additional specific examples, anti-LIV1 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 some embodiments, the engineered T cell population can be the anti-LIV1 CAR T cells disclosed herein that further comprise a disrupted *Reg1* gene. The disrupted *Reg 1* gene can comprise any of the sequences provided in **Sequence Tables 29-38** below. In some examples, the anti-LIV1 CAR T cells can 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 some embodiments, the engineered T cell population can be the anti-LIV1 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene. For example, the disrupted *TGFBRII* gene can comprise a nucleotide sequence selected from those listed in **Sequence Tables 40-48** below. In some examples, the anti-LIV1 CAR T cells can 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 embodiments, the genetically engineered T cell population can be the anti-LIV1 CAR T cells disclosed herein that further comprise a disrupted *TGFBRII* gene and a disrupted *Reg1* gene. The disrupted *Reg 1* gene can comprise any of the sequences provided in **Sequence Tables 29-38** below. Alternatively or in addition, the disrupted *TGFBRII* gene may comprise a nucleotide sequence selected from those listed in **Sequence Tables 40-48** below. In some examples, the anti-LIV1 CAR T cells can 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-LIV1 CAR T cells can 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-LIV1 CAR T cells can 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 embodiments, such a population of genetically engineered T cells can 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 can also contain at least 50% (*e.g.*, at least 60%) cells expressing the anti-LIV1 CAR.

In some embodiments, one or more gene edits within a population of cells results in a phenotype associated with changes in cellular proliferative capacity, cellular exhaustion, cellular viability, cellular lysis capability (*e.g.*, increase cytokine production and/or release), anti-tumor effects, or any combination thereof.

In some embodiments, engineered T cells expressing a CAR (e.g., anti-LIV1 CAR) and having one or both of *Reg1* gene and *TGFBRII* gene can provide synergistic effects as compared with non-engineered T cells or engineered T cells expressing a CAR without having one or both of disrupted *Reg1* gene and disrupted *TGFBRII* gene.

In some embodiments, the engineered T cells disclosed herein exhibit an at least 20% increase in cellular lysis capability (i.e., kill at least 20% more target cells), relative to engineered T cells not having one or both of *Reg1* gene and *TGFBRII* gene. For example, the engineered T cells can exhibit about, at least, or at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95% or a number between any two of the values, increase in cellular lysis capability, relative to non-engineered T cells or engineered T cells without having one or both of *Reg1* gene and *TGFBRII* gene. In some embodiments, the engineered T cells exhibit about, at least or at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold,

90-fold, 100-fold, or a number or a range between any of these values, increase in cellular lysis capability, relative to non-engineered T cells or engineered T cells without having one or both of *Reg1* gene and *TGFBRII* gene. For example, the level of cytokines (*e.g.*, IL-2 and/or IFN-gamma) secreted by the engineered T cells can be at least 2-fold (*e.g.*, at least 3-fold, at least 4-fold, or at least 5-fold) greater than the level of cytokines secreted by engineered T cells not having one or both of *Reg1* gene and *TGFBRII* gene.

The engineered T cells can exhibit enhanced anti-tumor effects such as reduction of tumor size and/or elongated survival rates. In some embodiments, a combination of an anti-LIV1 gene and one or both of a disrupted *Reg1* gene and a disrupted *TGFBRII* gene (e.g., a disrupted *Reg1* gene and a disrupted *TGFBRII* gene) in an engineered T cell can result in significantly enhanced efficacy against cancer (e.g., breast cancer), causing tumor regression and cancer survival. Surprisingly, the resulted tumor regression and cancer survival rate/duration by the combination is more than additive, i.e., superior to the cumulated anti-tumor efficacy caused by T cells expressing an anti-LIV1 CAR and T cells having one or both of a disrupted *Reg1* gene and a disrupted *TGFBRII* gene, separately. In some embodiments, the inhibition of tumor progression is enhanced or synergistic, that is, the inhibition is greater than the combined inhibition of progression caused by T cells expressing anti-LIV1 alone plus T cells having one or both disrupted *Reg1* gene and disrupted *TGFBRII* gene alone. In some embodiments, anti-LIV1 CAR T cells having a disrupted *Reg1* gene and a disrupted *TGFBRII* gene show significantly enhanced anti-tumor activities relative to control T cells (e.g., anti-LIV1 CAR T Cells without a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene). In some embodiments, the engineered T cells exhibit an about, at least, at least about, at most, or at most about, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 210%, 220%, 230%, 240%, 250%, 260%, 270%, 280%, 290%, 300%, or a combination thereof, increase in anti-tumor activities, relative to control T cells (e.g., engineered T cells without one or both of *Reg1* gene and *TGFBRII* gene). In some embodiments, engineered T cells of the present disclosure exhibit an at least about 2-fold (e.g., 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, or a number or a range between any of these values) increase in anti-tumor activities, relative to control T cells (*e.g.*, engineered T cells without one or both of *Reg1* gene and *TGFBRII* gene).

In some embodiments, the engineered T cells disclosed herein exhibit at least 20% greater cellular proliferative capacity, relative to control T cells. For example, engineered T cells can exhibit about, at least, or at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% greater cellular proliferative capacity, relative to control T cells. In some embodiments, engineered T cells of the present disclosure exhibit 20%-100%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-100%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, 30%-50%, 40%-100%, 40%-90%, 40%-80%, 40%-70%, 40%-60%, 40%-50%, 50%-100%, 50%-90%, 50%-80%, 50%-70%, or 50%-60% greater cellular proliferative capacity, relative to control T cells.

In some embodiments, the engineered T cells disclosed herein exhibit an at least 20% increase in cellular viability, relative to control cells. For example, engineered T cells can exhibit about, at least, or at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90% or a number between any two of the values, increase in cellular viability, relative to control cells. In some embodiments, engineered T cells exhibit a 20%-100%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-100%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, 30%-50%, 40%-100%, 40%-90%, 40%-80%, 40%-70%, 40%-60%, 40%-50%, 50%-100%, 50%-90%, 50%-80%, 50%-70%, or 50%-60% increase in cellular viability, relative to control cells.

Control T cells can be engineered T cells (*e.g.*, gene edited T cells). In some embodiments, control T cells are engineered T cells that comprise a disrupted *TRAC* gene, a nucleic acid encoding a CAR (*e.g.*, an anti-LIV1 CAR) inserted into the *TRAC* gene, and/or a disrupted *β2M* gene. In some embodiments, control T cells are unedited T cells. In some embodiments, control T cells do not comprise both a disrupted *Reg1* gene and a disrupted *TGFBRII* gene.

**Methods of Producing Engineered T cells**

Provided herein includes a method of producing an engineered T cell or a population thereof. The method can comprise providing a plurality of cells, wherein the plurality of cells are T cells or precursor cells thereof. The method can also comprise delivering to the plurality of cells a nucleic acid encoding a chimeric antigen receptor (CAR) that comprise (1) an ectodomain that binds specifically to LIV1, or (2) an anti-LIV1 antigen-binding fragment, genetically editing the *Reg1* gene, the *TGFBRII* gene, or both, and producing one or more

engineered T cells expressing the CAR and having a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene.

The plurality of cells (e.g., T cells) can 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 can 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*. 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. For example, T cells are 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 embodiments, 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, the engineered T cell populations do not express or do not substantially express one or more of the following markers: CD70, CD57, CD244, CD160, PD-1, CTLA4, HM3, and LAG3. In some embodiments, subpopulations of T cells can be isolated by positive or negative selection prior to genetic engineering and/or post genetic engineering.

In some embodiments, an isolated population of T cells can 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 embodiments, 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 can 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. Patent Nos. 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, at least, at least about, at most, or at most about 4 hours, 6 hours, 12 hours, 24 hours, 1 day to 4 days, 1 day to 3 days, 1 day to 2 days, 2 days to 3 days, 2 days to 4 days, 3 days to 4 days, or 2 days, 3 days, or 4 days prior to introduction of the genome editing compositions into the T cells. For example, 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 embodiments, the T cell population can be expanded and/or activated after the genetic editing. 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.

The method herein described can comprise delivering to the plurality of cells (e.g., T cells or precursor cells thereof described above) a nucleic acid encoding a CAR. The nucleic acid encoding a CAR can comprise an ectodomain that binds specifically to LIV1 or an anti-LIV1 antigen-binding fragment. The ectodomain that binds specifically to LIV1 comprises an anti-LIV1 antigen-binding fragment, and optionally the anti-LIV1 antigen-binding fragment comprises an anti-LIV1 antibody. The nucleic acid encoding a CAR can be delivered to the cells via conventional viral and non-viral based gene transfer methods known to a skilled person. In some embodiments, a nucleic acid encoding a CAR construct can be delivered to a cell using an AAV such as AAV6. In some embodiments, a nucleic acid encoding a CAR can be designed to insert into a genomic site of interest in the host T cells via a donor template. 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 AAV vector) can be designed such that it can insert into a

location within a *TRAC* gene to disrupt the *TRAC* gene in the engineered T cells and express the CAR polypeptide. 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 AAV vector) can be designed such that it can insert into a location within a *Reg1* gene, a *TGFBRII* gene, or a *β2M* gene.

The method can comprise genetically editing one or more genes herein described using gene editing methods known in the art. For example, the method can comprise genetically editing one or both of the *Reg1* gene and/or the *TGFBRII* gene. In some embodiments, the method comprises genetically editing both *Reg1* gene and/or *TGFBRII* gene. In some embodiments, genetically editing one or more genes herein described is performed by one or more CRISPR/Cas-mediated gene editing systems described below in details, which involves the use of an RNA-guided nuclease and one or more guide RNA targeting the one or more genes to be edited.

The engineered T cells having a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene and further expressing a chimeric antigen receptor (CAR), optionally having additional disrupted genes, *e.g.*, *β2M*, *CD70*, or *PD-1* can be produced by sequential targeting of the genes of interest. For example, in some embodiments, the *Reg1* gene can be disrupted first, followed by disruption of *TRAC* and *β2M* genes and CAR insertion. In other embodiments, *TGFBRII* and/or *β2M* genes can be disrupted first, followed by CAR insertion and disruption of the *Reg1* or other target gene. In some embodiments, the genetically engineered T cells can be produced by multiple, sequential electroporation events with multiple ribonucleoproteins (RNPs, formed by guide RNs and the Cas protein, such as a CRISPR/Cas complex) targeting the genes of interest, including but not limited to, *Reg1*, *β2M*, *TRAC*, and *CD70*. In some embodiments, the engineered CAR T cells can be produced by a single electroporation event with an RNP complex comprising an RNA-guided nuclease and multiple gRNAs targeting the genes of interest, including but not limited to, *Reg1*, *TGFBRII*, *β2M*, *TRAC*, *CD70*, and *PD-1*.

Gene Editing

Gene editing (including genomic editing) is 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 can be knocked-out or knocked-down due to the sequence alteration. Therefore, targeted editing can 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.

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 can 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.

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.

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. Patent 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. Patent Nos. 7,888,121 and 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 Publication US2011/0145940. 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.

Other non-limiting examples of targeted nucleases include naturally-occurring and recombinant nucleases, *e.g.*, CRISPR/Cas9, restriction endonucleases, meganucleases homing endonucleases, and the like.

*CRISPR-Cas9 Gene Editing System*

In some embodiments, one or more CRISPR-Cas9 gene editing systems are used to genetically edit one or more target genes herein disclosed. The CRISPR-Cas9 system is a naturally-occurring defense mechanism in prokaryotes that has been repurposed as a 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, single-guide RNA (sgRNA), 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.

In some embodiments, CRISPR-Cas9 gene editing system comprises an RNA-guided nuclease and one or more guide RNAs targeting one or more target genes.

***RNA-guided Nuclease***

The Cas9 (CRISPR associated protein 9) endonuclease can be used in a CRISPR method for genetically editing the one or more genes disclosed herein. The Cas9 enzyme can be one from *Streptococcus pyogenes*, although other Cas9 homologs may be used. It should be understood, that wild-type Cas9 can be used or modified versions of Cas9 can be used (*e.g.*, evolved versions of Cas9, or Cas9 orthologues or variants), as provided herein. In some embodiments, Cas9 can 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.

The Cas nuclease can be 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.

The Cas nuclease can comprise more than one nuclease domain. For example, a Cas9 nuclease can comprise at least one RuvC-like nuclease domain (*e.g.*, Cpf1) and at least one HNH-like nuclease domain (*e.g.*, Cas9). The Cas9 nuclease can, for example, introduce 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 some 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). In some embodiments, a Cas9 nuclease has an amino acid sequence of SEQ ID NO: 1 or a sequence having about, at least or at least about 85%, 90%, or 95% sequence identity to SEQ ID NO: 1.

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)***

In some embodiments, the CRISPR/Cas-mediated gene editing system comprises a genome-targeting nucleic acid that can direct the activities of an associated polypeptide (*e.g.*, a site-directed polypeptide) to a specific target sequence within a target nucleic acid. The genome-targeting nucleic acid can be an 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 of interest, and a CRISPR repeat sequence.

In Type II systems, the gRNA also comprises a second RNA called the tracrRNA sequence. In the Type II guide RNA (gRNA), the CRISPR repeat sequence and tracrRNA sequence hybridize to each other to form a duplex. In the Type V guide RNA (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 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). The

genome-targeting nucleic acid can be a double-molecule guide RNA, or a single-molecule guide RNA.

A double-molecule guide RNA comprises two strands of RNA. 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 "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 can 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 used herein, the spacer sequence can be 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 can perfectly match the target sequence or can 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.

The target nucleic acid sequence can vary in length, for example, 20 nucleotides in length, less than 20 nucleotides in length, or 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 can 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, at least, at least about, at most or at most about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, 100% or a number between any two of the values. In some embodiments, the spacer sequence of the guide RNA and the target sequence in the target gene is 100% complementary. In some 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.

The length of the spacer sequence in any of the gRNAs disclosed herein can 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 can 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 can have 18-24 nucleotides in length. In some embodiments, the targeting sequence can have 19-21 nucleotides in length. In some embodiments, the spacer sequence can comprise 20 nucleotides in length.

The gRNA can be an sgRNA, which can comprise a 20-nucleotide spacer sequence at the 5' end of the sgRNA sequence. In some embodiments, the sgRNA can comprise a less than 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. In some embodiments, the sgRNA can 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 **Sequence Table 2** below. In these exemplary sequences, the fragment of "n" refers to the spacer sequence at the 5' end.

In some embodiments, the sgRNA comprises no uracil at the 3' end of the sgRNA sequence. In some embodiments, the sgRNA can 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.

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. Any of the gRNAs disclosed herein, including any of the sgRNAs, can be unmodified. Alternatively, it can 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 can be located at either the 5' end, the 3' end, or both.

More than one guide RNAs can be used with a CRISPR/Cas nuclease system. Each guide RNA can 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 can 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 *TRAC* gene. *See* also WO2019097305 and WO 2020095249, the relevant disclosures of which are incorporated by reference herein for the subject matter and purpose referenced herein. Other gRNA sequences can 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 *TRAC* gene are provided in **Sequence Table 2** below.

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 **Sequence Table 1** 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 **Sequence Table 18** 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 **Sequence Table 2** 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 and WO2020/095249, the relevant disclosures of which are incorporated by reference herein for the purpose and subject matter referenced herein. Other gRNA sequences can 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. Exemplary spacer sequences and gRNAs targeting a *β2M* gene are provided in **Sequence Table 2** below.

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 can be 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 some embodiments, 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. In some embodiments, non-natural modified nucleobases can be introduced into any of the gRNAs disclosed herein during synthesis or post-synthesis. The modifications can be on internucleoside linkages, purine or pyrimidine bases, or sugar. The modification can be 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, including but

not limited to targeting or delivery agents, fluorescent labels, liquids, and nanoparticles. 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 can include at least one guide RNA. In some examples, the CRISPR/Cas nuclease system can contain multiple gRNAs, for example, 2, 3, or 4 gRNAs. Such multiple gRNAs can target different sites in a same target gene. Alternatively, the multiple gRNAs can target different genes. In some embodiments, the guide RNA(s) and the Cas protein can form a ribonucleoprotein (RNP), *e.g.*, a CRISPR/Cas complex. The guide RNA(s) can 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, can 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, which comprise 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 any conventional method known to a skilled person. In some embodiments, components of a CRISPR/Cas nuclease system can be delivered to a target cell separately, either simultaneously or sequentially. In some embodiments, the components of the CRISPR/Cas nuclease system can be delivered into a target together (*e.g.*, as a complex). In some embodiments, gRNA and a RNA-guided nuclease can be pre-complexed together to form a ribonucleoprotein (RNP), which can then be delivered into a target cell.

RNPs can be used for the delivery of guide RNAs and nuclease to T cells. 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 some embodiments, 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 can be through direct injection or cell transfection using known methods, for example, electroporation or chemical transfection. Other cell transfection methods may be used.

***Delivery Methods and Constructs for Delivery***

Nucleases and/or a nucleic acid (e.g., a nucleic acid encoding a CAR) can 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.

A nucleic acid encoding a CAR can be introduced into any of the engineered T cells disclosed herein by methods known to those of skill in the art. For example, a coding

sequence of the CAR can be cloned into a vector, which can 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.

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. AAVs do not provoke an immune response upon administration to mammals, including humans. AAVs are effectively delivered to target cells, particularly when consideration is given to selecting the appropriate AAV serotype. AAVs have the ability to infect both dividing and non-dividing cells because the genome can persist in the host cell without integration.

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.

A nucleic acid encoding a CAR can be designed to insert into a genomic site of interest in the host T cells via a donor template. A donor template as disclosed herein can

contain a coding sequence for a CAR. In some embodiments, the CAR-coding sequence is 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 and described herein. In some embodiments, 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.

In some embodiments, a donor template has no regions of homology to the targeted location in the DNA and can 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 (*e.g.*, 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 **Sequence Table 7** and SEQ ID NO: 557 provided in **Sequence Table 30** below.

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

In some embodiments, a nucleic acid encoding a CAR (*e.g.*, via a donor template) can be designed such that it can insert into the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, or the disrupted *β2M*.

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 can 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 *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.

In some embodiments, one or more nucleic acid encoding one or more CARs can be designed such that it can insert into one or more target locations, such as CD70, to disrupt one or more target genes in the engineered T cells.

In some embodiments, a donor template for delivering an anti-LIV1 CAR can be an AAV vector inserted with a nucleic acid fragment comprising the coding sequence of the anti-LIV1 CAR, and optionally regulatory sequences for expression of the anti-LIV1 CAR (*e.g.*, a promoter such as the EF1a promoter provided in the sequence Table provided herein), 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 some embodiments, the nucleic acid can 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-LIV1 CAR can comprise a nucleotide sequence of SEQ ID NO: 528, 574, or 583, which can be inserted into a disrupted *TRAC* gene, for example, replacing the fragment of SEQ ID NO: 69. In some embodiments, the donor template for delivering the anti-LIV1 CAR can comprise a nucleotide sequence of SEQ ID NO: 583, which can be inserted into a disrupted *TRAC* gene, for example, replacing the fragment of SEQ ID NO: 69.

**Treatment Methods**

Provided herein also include a method for treating cancer. The method can comprise administering to a subject an engineered T cell herein described or a population of the engineered T cells.

Non-limiting examples of cancers that can be treated as provided herein include: breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous

tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and neuronal tumors. In some embodiments, the methods comprise delivering the CAR T cells (e.g., anti-LIV1 CAR T cells) of the present disclosure to a subject having cancer, including, breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and/or neuronal tumors.

The engineered T cells, methods and kits disclosed herein can be used to various types of cancer, including but are not limited to, melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g., clear cell carcinoma), prostate cancer (e.g., hormone refractory prostate adenocarcinoma), pancreatic adenocarcinoma, breast cancer, colon cancer, lung cancer (e.g., non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC)), esophageal cancer, squamous cell carcinoma of the head and neck, liver cancer, ovarian cancer, cervical cancer, thyroid cancer, glioblastoma, glioma, leukemia, lymphoma, and other neoplastic malignancies. Additionally, the disease or condition provided herein includes refractory or recurrent malignancies whose growth may be inhibited using the methods and compositions disclosed herein. In some embodiments, the cancer is carcinoma, squamous carcinoma, adenocarcinoma, sarcomata, endometrial cancer, breast cancer, ovarian cancer, cervical cancer, fallopian tube cancer, primary peritoneal cancer, colon cancer, colorectal cancer, squamous cell carcinoma of the anogenital region, melanoma, renal cell carcinoma, lung cancer, non-small cell lung cancer, squamous cell carcinoma of the lung, stomach cancer, bladder cancer, gall bladder cancer, liver cancer, thyroid cancer, laryngeal cancer, salivary gland cancer, esophageal cancer, head and neck cancer, glioblastoma, glioma, squamous cell carcinoma of the head and neck, prostate cancer, pancreatic cancer, mesothelioma, sarcoma, hematological cancer, leukemia, lymphoma, neuroma, or a combination thereof. In some embodiments, the cancer is carcinoma, squamous carcinoma (e.g., cervical canal, eyelid, tunica conjunctiva, vagina, lung, oral cavity, skin, urinary bladder, tongue, larynx, and gullet), and adenocarcinoma (for example, prostate, small intestine, endometrium, cervical canal, large intestine, lung, pancreas, gullet, rectum, uterus, stomach, mammary gland, and ovary). In some embodiments, the cancer is sarcomata (e.g., myogenic sarcoma), leukosis, neuroma, melanoma, and lymphoma.

In some embodiments, the cancer can include pancreatic cancer, gastric cancer, ovarian cancer, uterine cancer, breast cancer, prostate cancer, testicular cancer, thyroid cancer, nasopharyngeal cancer, non-small cell lung (NSCLC), glioblastoma, neuronal, soft

tissue sarcomas, leukemia, lymphoma, melanoma, colon cancer, colon adenocarcinoma, brain glioblastoma, hepatocellular carcinoma, liver hepatocholangiocarcinoma, osteosarcoma, gastric cancer, esophagus squamous cell carcinoma, advanced stage pancreas cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell cancer, renal carcinoma, intrahepatic biliary cancer, and a combination thereof.

The cancer can be a solid tumor, a liquid tumor, or a combination thereof. In some embodiments, the cancer is a solid tumor, including but are not limited to, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, Merkel cell carcinoma, brain and central nervous system cancers, and any combination thereof. In some embodiments, the cancer is a liquid tumor. In some embodiments, the cancer is a hematological cancer, including but not limited to, Diffuse large B cell lymphoma ("DLBCL"), Hodgkin's lymphoma ("HL"), Non-Hodgkin's lymphoma ("NHL"), Follicular lymphoma ("FL"), acute myeloid leukemia ("AML"), and Multiple myeloma ("MM").

In some embodiments, the cancer is breast cancer, prostate cancer, squamous tumor cancer, neuronal tumor cancer, or a combination thereof. In some embodiments, the cancer comprises cancer cells expressing LIV1.

The step of administering can include introducing (*e.g.*, transplantation) the cells, *e.g.*, an engineered T cell or a population thereof described herein, into a subject, by a method or route that results in at least partial localization of the introduced cells at a desired site, such as tumor, such that a desired effect(s) is produced. Engineered 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. In some aspects as described herein, an effective amount of engineered T cells is administered via a systemic route of administration, such as an intraperitoneal or intravenous route.

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

The engineered T cell population being administered according to the methods described herein can 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. 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 can used, such as those obtained from genetically identical donors (*e.g.*, identical twins). The cells can be 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. A donor can be 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 does not induce toxicity in the subject, e.g., the engineered T cells do not induce toxicity in non-cancer cells. In some embodiments, an engineered T cell population being administered does not trigger complement mediated lysis or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC).

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.

The effective amount of cells (*e.g.*, engineered T cells) can comprise about, at least, or at least about, $10^2$ cells, $5 \times 10^2$ cells, $10^3$ cells, $5 \times 10^3$ cells, $10^4$ cells, $5 \times 10^4$ cells, $10^5$ cells, $2 \times 10^5$ cells, $3 \times 10^5$ cells, $4 \times 10^5$ cells, $5 \times 10^5$ cells, $6 \times 10^5$ cells, $7 \times 10^5$ cells, $8 \times 10^5$ cells, $9 \times 10^5$ cells, $1 \times 10^6$ cells, $2 \times 10^6$ cells, $3 \times 10^6$ cells, $4 \times 10^6$ cells, $5 \times 10^6$ cells, $6 \times 10^6$ cells, $7 \times 10^6$ cells, $8 \times 10^6$ cells, $9 \times 10^6$ cells, $10^7$ cells, $1.2 \times 10^7$ cells, $1.4 \times 10^7$ cells, $1.6 \times 10^7$ cells, $1.8 \times 10^7$ cells, $2 \times 10^7$ cells, $2.5 \times 10^7$ cells, $3 \times 10^7$ cells, or a number between any two of the values. The cells are derived from one or more donors, or are obtained from an autologous source. In some embodiments described herein, the cells are expanded in culture prior to administration to a subject in need thereof.

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. In some embodiments, engineered T cells are administered systemically. Systemic administration refers to the administration of a population of cells other than directly into a target site, tissue, or organ, such that it enters the subject's circulatory system and, thus, is subject to metabolism and other like processes.

The efficacy of a treatment comprising a composition for the treatment of a medical condition can be determined by the skilled clinician. A treatment is considered "effective treatment," 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.

Combinational Cancer Therapy

The engineered T cells, methods, and kits disclosed herein can be used with additional cancer therapeutics or therapy to treat cancer. In some embodiments, the treatment can comprise administration of at least one additional cancer therapeutics or cancer therapy. The treatment can comprise administration a therapeutically effective amount of at least one additional cancer therapeutics or cancer therapy. The engineered T cells herein described and the cancer therapeutics or cancer therapy can, for example, co-administered simultaneously or sequentially. Examples of the cancer therapies include, but are not limited to, surgery, chemotherapy, radiation therapy, hormonal therapy, immunotherapy, complementary or alternative therapy, and any combination thereof.

**Kits**

Provided herein also includes kits for use in producing the engineered T cells and for therapeutic uses. The kit provided herein can, for example, comprise components for performing genetic edit of one or more of *TRAC* gene, *Reg1* gene, *TGFBRII* gene, *β2M* gene, *CD90* gene and/or *PD-1* gene. A kit also comprises a nucleic acid encoding a CAR, wherein the CAR comprises an ectodomain that binds specifically to LIV1 or an anti-LIV1 antigen-binding fragment, and components for delivery of the nucleic acid. In some embodiments, the nucleic acid encoding an anti-LIV1 CAR is part of a donor template as disclosed herein, which can contain homologous arms flanking the anti-LIV1 CAR coding sequence. In some embodiments, the donor template is carried by a viral vector such as an AAV vector. The components for genetically editing one or more of the target genes can 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 can comprise a Cas enzyme such as Cas 9 and one or more gRNAs targeting a *TRAC* gene, a *Reg1* gene, and/or a *TGFBRII* gene. In some embodiments, the kit comprises gRNAs specific to *TRAC* gene for inserting the anti-LIV1 CAR sequence into the *TRAC* gene. Any of the gRNAs specific to these target genes can be included in the kit. Such a kit can further comprise components for additional gene editing, for example, gRNAs and optionally additional endonucleases for editing other target genes such as *β2M*, *CD90* and/or *PD-1*.

A kit can, for example, comprise a population of immune cells to which the genetic editing will be performed (*e.g.*, a leukopak). A leukopak sample can be an enriched leukapheresis product collected from peripheral blood, which typically contains a variety of blood cells including monocytes, lymphocytes, platelets, plasma, and red cells. In some embodiments, a kit disclosed herein can comprise a population of therapeutic T cells as disclosed for the intended therapeutic purposes.

Kit disclosed herein can further comprise instructions for making the engineered T cells, or therapeutic applications of the therapeutic T cells. In some examples, the included instructions can comprise a description of using the gene editing components to genetically engineer one or more of the target genes (*e.g.*, *TRAC*, *Reg1*, *TGFBRII*, *β2M*, *PD-1*, *CD70*, or a combination thereof). In other examples, the included instructions can comprise a description of how to introduce a nucleic acid encoding a CAR construction into the T cells for making therapeutic T cells.

Alternatively and in addition, the kit can comprise instructions for administration of the engineered T cells as disclosed herein to achieve the intended activity, *e.g.*, eliminating disease cells targeted by the anti-LIV1 CAR expressed on the therapeutic T cells. The kit can 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 can 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 can have a sterile access port (for example, the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container can also have a sterile access port.

## EXAMPLES

Some aspects of the embodiments discussed above are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the present disclosure.

### Example 1: CAR T Cell Generation and CAR Expression

Allogeneic human T cells that lack expression of the TRAC gene, β2M gene, TGFBRII gene and Regnase-1 gene, and express a chimeric antigen receptor (CAR) targeting Liv1 were produced.

Activated primary human T cells were electroporated with Cas9:sgRNA RNP complexes and adeno-associated adenoviral vectors (AAVs) to generate TRAC-/β2M-/TGFBRII-/REG-1- anti-Liv1 CAR+ T cells. The sgRNAs, which form RNPs with the Cas9 enzyme, can be introduced into the T cells in a single electroporation event. After the electroporation, the cells were transduced with the recombinant AAVs to introduce the donor template encoding for the anti- Liv1 CAR. Recombinant AAV serotype 6 (AAV6) comprising one of the nucleotide sequences encoding an anti-Liv1 CAR (971 (SEQ ID NO:

527), 975 (SEQ ID NO: 582), and 976 (SEQ ID NO: 586), were delivered with Cas9:sgRNA RNPs (1 μM Cas9, 5 μM gRNA) to activated allogeneic human T cells. The following sgRNAs were used: TRAC (SEQ ID NO: 61), β2M (SEQ ID NO: 64), TGFBRII (SEQ ID NO: 10) and REG-1 (SEQ ID NO: 327). The unmodified versions (or other modified versions) of the sgRNAs may also be used (e.g., SEQ ID NOS: 14-53 and 264-315). Table 33 presents in different edited T cells that were produced. '+' indicates an intact gene; '-' indicates gene disruption. CTX971, CTX975 and CTX976 are previously described in WO2020/095249, which is incorporated herein by reference.

**Table 33**: **Edited T cells**

| Cell name | TRAC | β2M | TGFBRII | Regnase | Anti-Liv1a CAR |
|---|---|---|---|---|---|
| CTX971 | - | - | + | + | SEQ ID NO: 527, 528 |
| TGFBRII/Reg 971 | - | - | - | - | SEQ ID NO: 527, 528 |
| CTX975 | - | - | + | + | SEQ ID NO: 582, 583 |
| TGFBRII/Reg 975 | - | - | - | - | SEQ ID NO: 582, 583 |
| CTX976 | - | - | + | + | SEQ ID NO: 586, 589 |
| TGFBRII/Reg 976 | - | - | - | - | SEQ ID NO: 586, 589 |

About one (1) week post electroporation, cells were processed ddPCR to assess anti-Liv1 CAR expression levels at the cell surface of the edited cell population. The results shown in Table 34 demonstrate comparable expressions of the CAR in all edited cells via ddPCR analysis. **FIG. 2** also includes diagrams showing the expressions of CAR in edited cells via ddPCR and flow cytometry.

**Table 34**: **Anti-Liv1 CAR Expression Levels (%)**

| Cell name | ddPCR |
|---|---|
| CTX971 | 71 |
| TGFBRII/Reg 971 | 63 |
| CTX975 | 71 |
| TGFBRII/Reg 975 | 67 |
| CTX976 | 74 |
| TGFBRII/Reg 976 | 63 |

The cells were also counted at regular intervals to assess T-cell expansion. All the CAR T cells demonstrated similar T-cell expansion, as presented in **Table 35**. **FIG. 3** also includes diagrams showing the T cell numbers of the engineered CAR T cells.

**Table 35: T-cell Numbers (x $10^8$)**

| Cell name | Day 2 | Day 3 | Day 7 | Day 9 | Day 10 |
|---|---|---|---|---|---|
| CTX971 | 120 | 207 | 672 | 1154 | 1500 |
| TGFBRII/Reg 971 | 104 | 146 | 624 | 1019 | 1190 |
| CTX975 | 111 | 178 | 828 | 1272 | 1280 |
| TGFBRII/Reg 975 | 91 | 140 | 687 | 999 | 1260 |
| CTX976 | 107 | 156 | 830 | 1231 | 1400 |
| TGFBRII/Reg 976 | 82 | 127 | 598 | 995 | 1030 |

**Example 2: Cytotoxicity of Anti-Liv1 CAR T Cells**

A cell killing (cytotoxicity) assay was used to assess the ability of the anti-Liv1 $CAR^+$ T cells to cause cellular lysis in breast cancer cell lines (MCF7 and ZR-75-1).

MCF7 cells were cultured in Eagle's Minimum Essential Media (EMEM) with 10% fetal bovine serum (FBS). ZR-75-1 cells were cultured in RPMI-1640 Medium (ATCC Modification) with 10% FBS. All cells were cultured at 37°C with 5% $CO_2$. During the following day, T cells were added to the wells containing target cancer cells at ratios of 0.5: 1, 1: 1, 2: 1, and 4: 1 T cell:target cell. CAR- T cells that were electroporated with Cas9:sgRNA RNP complexes but were not transduced with AAV, and thus do not express an anti-Liv1 CAR, served as negative controls. After approximately 24 hours, 100 µL of supernatant was removed for cytokine quantification (see below) and T cells were removed from the culture by aspiration. PBS washes ensured the removal of all T cells. 100 µL CellTiter-Glo® (Promega) was added to each well of the plate to assess the number of remaining viable cells and incubated for 10 minutes at 37°C. The luminescence from each well was then quantified using a plate reader.

As presented in **Tables 36A** and **36B** (see also **FIGs. 4A** and **4B**), CTX975 and TGFBRII/Reg 975 cells exhibited the highest degree of cytotoxicity against the MCF7 and ZR-75-1 cell lines. The EP only control does not have any CAR transduction but has TRAC/β2M edits. The TGFBRII/Reg EP only control does not have any CAR transduction but has TRAC/ β2M/TGFBRII/Regnase edits.

**Table 36A: Cytotoxicity Against MCF7 (% lysis normalized to 0: 1)**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | -9 | -5 | -2 | -12 |
| TGFBRII/Reg 971 | -12 | -5 | -6 | -15 |
| CTX975 | 10 | 32 | 38 | 41 |
| TGFBRII/Reg 975 | 17 | 42 | 51 | 39 |
| CTX976 | -5 | 1 | 3 | 3 |

| | | | | |
|---|---|---|---|---|
| TGFBRII/Reg 976 | -5 | 5 | 16 | 23 |
| EP only | -4 | -16 | -16 | -24 |
| TGFBRII/Reg EP only | -12 | -11 | -15 | -22 |

**Table 36B: Cytotoxicity against ZR-75-1 (% lysis normalized to 0: 1)**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | -8 | -6 | -7 | 0 |
| TGFBRII/Reg 971 | -6 | -9 | -6 | -3 |
| CTX975 | 25 | 50 | 77 | 90 |
| TGFBRII/Reg 975 | 50 | 72 | 90 | 95 |
| CTX976 | 0 | 1 | 7 | 25 |
| TGFBRII/Reg 976 | 9 | 13 | 29 | 60 |
| EP only | -6 | -21 | -20 | -21 |
| TGFBRII/Reg EP only | -16 | -17 | -11 | -12 |

**Example 3: Effector Cytokine Secretion**

The MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay kit (Millipore, catalog # HCYTOMAG-60K) using magnetic microspheres, anti-human IFNγ bead (Millipore, catalog # HCYIFNG-MAG) and anti-human IL-2 bead (Millipore, catalog # HIL2-MAG), respectively, was used to quantify IFN-γ and IL-2 secretion in samples from the cytotoxicity assay. The assay was conducted following manufacturer's protocol. MILLIPLEX® standard and quality control (QC) samples were reconstituted, and serial dilutions of the working standards from 10,000 pg/mL to 3.2 pg/mL were prepared. MILLIPLEX® standards, QCs and cell supernatants were added to each plate, and assay media was used to dilute the supernatants. All samples were incubated with anti-human IFNγ and anti-human IL-2 beads for 2 hours. After incubation, the plate was washed using an automated magnetic plate washer. Human cytokine/chemokine detection antibody solution was added to each well and incubated for 1 hour followed by incubation with Streptavidin-Phycoerythrin for 30 minutes. The plate was subsequently washed, samples were resuspended with 150 µL Sheath Fluid, and agitated on a plate shaker for 5 minutes. The samples were read using the Luminex® 100/200™ instrument with xPONENT® software and data acquisition and analysis was completed using MILLIPLEX® Analyst software. The Median Fluorescent Intensity (MFI) data was automatically analyzed using a 5-parameter logistic curve-fitting method for calculating the cytokine concentration measured in the unknown samples.

As shown in **Tables 37-40**, TGFBRII/Reg 975 T cells secreted the effector cytokines interferon-γ and interleukin-2 when co-cultured with the target cells lines MCF7 and ZR-75-1 at levels significantly higher than the other CAR T cells. See also **FIGs. 5A-5D**.

**Table 37. IFN-γ (pg/mL) after co-culture with different ratios of T cell: MCF7**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | 67 | 147 | 273 | 481 |
| TGFBRII/Reg 971 | 118 | 315 | 738 | 1478 |
| CTX975 | 3152 | 5404 | 8258 | 9154 |
| TGFBRII/Reg 975 | 11241 | 23181 | 38615 | 50975 |
| CTX976 | 287 | 582 | 1058 | 1819 |
| TGFBRII/Reg 976 | 1142 | 3655 | 12013 | 24308 |
| EP only | 1 | 2 | 3 | 5 |
| TGFBRII/Reg EP only | 4 | 7 | 13 | 33 |

**Table 38. IFN-γ (pg/mL) after co-culture with different ratios of T cell: ZR-75-1**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | 37 | 85 | 113 | 243 |
| TGFBRII/Reg 971 | 76 | 163 | 314 | 677 |
| CTX975 | 2897 | 4558 | 4562 | 6679 |
| TGFBRII/Reg 975 | 17108 | 28908 | 31542 | 39990 |
| CTX976 | 104 | 240 | 405 | 663 |
| TGFBRII/Reg 976 | 868 | 3817 | 8333 | 18887 |
| EP only | 3 | 3 | 4 | 6 |
| TGFBRII/Reg EP only | 5 | 6 | 14 | 24 |

**Table 39. IL2 (pg/mL) after co-culture with different ratios of T cell: MCF7**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | 1 | 5 | 2 | 2 |
| TGFBRII/Reg 971 | 6 | 9 | 8 | 7 |
| CTX975 | 987 | 1320 | 1575 | 1282 |
| TGFBRII/Reg 975 | 3030 | 4123 | 2840 | 1797 |
| CTX976 | 19 | 21 | 18 | 12 |
| TGFBRII/Reg 976 | 13 | 200 | 248 | 111 |
| EP only | 0 | 0 | 0 | 0 |
| TGFBRII/Reg EP only | 0 | 0 | 0 | 0 |

**Table 40. IL2 (pg/mL) after co-culture with different ratios of T cell: ZR-75-1**

| Cell name | 0.5: 1 | 1: 1 | 2: 1 | 4: 1 |
|---|---|---|---|---|
| CTX971 | 1 | 2 | 3 | 2 |
| TGFBRII/Reg 971 | 9 | 10 | 5 | 5 |
| CTX975 | 957 | 1195 | 777 | 424 |
| TGFBRII/Reg 975 | 4010 | 3164 | 2140 | 732 |
| CTX976 | 7 | 11 | 9 | 5 |
| TGFBRII/Reg 976 | 102 | 247 | 229 | 125 |
| EP only | 0 | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| TGFBRII/Reg EP only | 0 | 0 | 0 | 0 |

**Example 4: Generation of Liv1 KO Breast Cancer Cell Lines**

To test the specificity of the anti-Liv1 CAR T cells, MCF-7 cells or ZR751 cells were electroporated with Cas9:sgRNA RNP complexes using sgRNA targeting the Liv1 gene. After the electroporation, the cells were expanded and Liv1 editing was confirmed to be 75% by sequencing and subsequent TIDE. The cells were seeded at 10 cells per well in a 96-well plate and allowed to expand. Upon expansion of the individual wells, genomic DNA was isolated and subsequent sequencing and TIDE confirmed 99% editing in a population of cells. These cells were further expanded and utilized in a cytotoxicity assay. The MCF7 cells were seeded in a 96 well plate in Eagle's Minimum Essential Media (EMEM) with 10% fetal bovine serum (FBS) at 37°C with 5% $CO_2$. The following day, CTX975 TGFBRII/Reg KO T cells were added to the wells containing target cancer cells at ratios of 1: 1 and 2: 1 T cell:target cell. After approximately 24 hours, the T cells were removed from the culture by aspiration. PBS washes ensured the removal of all T cells. 100 µL CellTiter-Glo® (Promega) was added to each well of the plate to assess the number of remaining viable cells and incubated for 10 minutes at 37°C. The luminescence from each well was then quantified using a plate reader.

The Liv1 editing efficiencies in the breast cell lines MCF7 and ZR751 are shown in **FIG. 9**.

The data presented in **Table 41** below shows the decrease in cytotoxicity of the TGFBRII/Reg 975 CAR T cells in the Liv1 KO MCF7 culture, demonstrating the specificity of the CAR T cells against Liv1-positive cells. See also **FIGs. 10A-10B** and **11A-11B**. Similar results were observed in Liv1 KO ZR751 cells.

**Table 41**: **% lysis of MCF7 cells versus Liv1-edited MCF7 cells by TGFBRII/Reg 975 CAR T cells**

| | MCF7 | Liv1 KO MCF7 |
|---|---|---|
| 1: 1 | 37 | -3 |
| 2: 1 | 43 | 5 |

**Example 5: In Vivo Cytotoxicity of Anti-Liv1 CAR T cells**

The CAR T cells generated in **Example 1** were evaluated in the MCF-7 human breast tumor xenograft model in NSG mice. Female NSG mice aged 5-8 weeks were implanted with one 90-day 0.72mg β-estradiol pellet three days prior to MCF-7 cell inoculation. After three

days, 1e7 MCF-7 cells per mouse were inoculated into the mammary fat pad of each mouse. Tumors grew until they reached ~50mm$^3$. At that point, mice were randomized and injected intravenously with CAR T cells at 1e7 or 2e7 CAR+ cells per mouse in a total volume of 200 uL. Tumor volumes and body weight were evaluated every few days. Tumor volumes are presented in **Table 42**. See also **FIGs. 6, 7A-7B,** and **8A-8D**.

**Table 42. Tumor volume (mm$^3$)**

| Cell name and number | Day 1 | Day 8 | Day 15 | Day 26 | Day 33 | Day 40 | Day 47 | Day 50 | Day 55 |
|---|---|---|---|---|---|---|---|---|---|
| Untreated | 53 | 68 | 91 | 126 | 174 | 251 | 375 | 497 | 604 |
| CTX971 (1 x 10$^6$) | 53 | 55 | 69 | 112 | 139 | 182 | 262 | 361 | 300 |
| CTX971 (2 x 10$^6$) | 53 | 65 | 116 | 158 | 211 | 308 | 477 | 900 | 265 |
| TGFBRII/Reg 971 (1 x 10$^6$) | 52 | 69 | 93 | 121 | 106 | 142 | 174 | 417 | 107 |
| TGFBRII/Reg 971 (2 x 10$^6$) | 52 | 62 | 90 | 120 | 143 | 178 | 221 | 469 | 245 |
| CTX975 (1 x 10$^6$) | 52 | 74 | 136 | 201 | 265 | 376 | 470 | 815 | 471 |
| CTX975 (2 x 10$^6$) | 54 | 76 | 109 | 137 | 151 | 219 | 303 | 652 | 153 |
| TGFBRII/Reg 975 (1 x 10$^6$) | 53 | 74 | 76 | 79 | 89 | 128 | 152 | 259 | 302 |
| TGFBRII/Reg 975 (2 x 10$^6$) | 52 | 75 | 84 | 93 | 102 | 111 | 193 | 315 | 361 |
| CTX976 (1 x 10$^6$) | 53 | 101 | 159 | 190 | 196 | 233 | 275 | 456 | 257 |
| CTX976 (2 x 10$^6$) | 54 | 75 | 95 | 117 | 136 | 182 | 238 | 408 | 181 |
| TGFBRII/Reg 976 (1 x 10$^6$) | 53 | 75 | 94 | 135 | 165 | 198 | 243 | 395 | 382 |
| TGFBRII/Reg 976 (2 x 10$^6$) | 54 | 80 | 112 | 128 | 153 | 189 | 265 | 362 | 391 |

## Example 6: *In Vivo* Cytotoxicity of Anti-Liv1 CAR-T Cells with *TGFBRII* and/or *Regnase-1* Edits

Female NSG mice were subcutaneously inoculated with 1e7 MDA-MB-231 cells per mouse into the right flank. Tumors were allowed to grow until they reached a mean tumor size between 25-75 mm3 with a target of 50 mm3. Upon reaching the target tumor volume, mice were randomized into 9 groups. Group 1 received no treatment. Groups 2-9 were given a single dose of CAR T cells on Day 1 by intravenous administration. CAR T cell dose was based off the percentage of CAR+ cells as measured by flow cytometry. Each CAR product was dosed at both 1e7 and 2e7 CAR+ cells per mouse in a total volume of 200 µL. Tumor

volumes and body weights were measured twice a week and gross observations were made daily. Treatment details are provided in **Tables 42-43** below.

**Table 42. CAR-T Cells and Doses**

| CAR T cell product | Percentage CAR+ Cells | Cells administered for 1e7 CAR+ dose | Cells administered for 2e7 CAR+ dose |
|---|---|---|---|
| 28_CTX975 | 93.9 | 1.08e7 | 2.15e7 |
| 28_CTX975 TGFBRII KO | 93.7 | 1.08e7 | 2.15e7 |
| 28_CTX975 Regnase-1 KO | 93.8 | 1.08e7 | 2.15e7 |
| 28_CTX975 TGFBRII & Regnase-1 KO | 92.6 | 1.08e7 | 2.15e7 |

5

**Table 43. Mouse Groups**

| | | CAR T cell product | | | |
|---|---|---|---|---|---|
| **GROUP** | **N** | **28_CTX975** | **28_CTX975 TGFBRII KO** | **28_CTX975 Regnase-1 KO** | **28_CTX975 TGFBRII & Regnase-1 KO** |
| No Treatment | 5 | | | | |
| 28_CTX975 1e7 CAR+ cells/mouse | 5 | X | | | |
| 28_CTX975 2e7 CAR+ cells/mouse | 5 | X | | | |
| 28_CTX975 TGFBRII KO 1e7 CAR+ cells/mouse | 5 | | X | | |
| 28_CTX975 TGFBRII KO 2e7 CAR+ cells/mouse | 5 | | X | | |
| 28_CTX975 Regnase-1 KO 1e7 CAR+ cells/mouse | 5 | | | X | |
| 28_CTX975 Regnase-1 KO 2e7 CAR+ cells/mouse | 5 | | | X | |
| 28_CTX975 TGFBRII & Regnase-1 KO 1e7 CAR+ cells/mouse | 5 | | | | X |
| 28_CTX975 TGFBRII & Regnase-1 KO 2e7 CAR+ cells/mouse | 5 | | | | X |

Tumor volumes in mice treated by anti-Liv1 CAR-T cells, with or without TGFBRII and/or Regnase-1 edits are shown in **FIG. 12** and **FIG. 13A-13B**. The results indicate that

the additional gene editing of the *TGFBRII* gene and the *Regnase-1* gene, either alone or in combination, improves the potency of the anti-Liv1 CAR-T cells.

## Sequence Tables

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

**Sequence Table 1.** Exemplary sgRNA Sequences and Target Gene Sequences for Reg1.

| Name | Unmodified Sequence | Modified Sequence | Target Sequences (PAM) |
|---|---|---|---|
| REG1-Z01 sgRNA (EX2_T1) | GGUCAUCGAUGGGAGCAAC Gguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 14) | G*G*U*CAUCGAUGGGAGCAACG guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 15) | GGTCATCGATGGGAGCA ACG (TGG) (SEQ ID NO: 171)<br><br>GGTCATCGATGGGAGCA ACG (SEQ ID NO: 318) |
| REG1-Z01 sgRNA (EX2_T1) spacer | GGUCAUCGAUGGGAGCAAC G (SEQ ID NO: 16) | G*G*U*CAUCGAUGGGAGCAACG (SEQ ID NO: 17) | |
| REG1-Z02 sgRNA (EX2_T2) | CACCACCCCGCGGGACUAG Aguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 18) | C*A*C*CACCCCGCGGGACUAGA guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 19) | CACCACCCCGCGGGACT AGA (GGG) (SEQ ID NO: 172)<br><br>CACCACCCCGCGGGACT AGA (SEQ ID NO: 319) |
| REG1-Z02 sgRNA (EX2_T2) spacer | CACCACCCCGCGGGACUAG A (SEQ ID NO: 20) | C*A*C*CACCCCGCGGGACUAGA (SEQ ID NO: 21) | |
| REG1-Z03 sgRNA (EX2_T3) | GGUCUGGCGCUCCCGCUCG Gguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 22) | G*G*U*CUGGCGCUCCCGCUCGG guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 23) | GGTCTGGCGCTCCCGCT CGG (TGG) (SEQ ID NO: 173)<br><br>GGTCTGGCGCTCCCGCT CGG (SEQ ID NO: 320) |
| REG1-Z03 sgRNA (EX2_T3) spacer | GGUCUGGCGCUCCCGCUCG G (SEQ ID NO: 24) | G*G*U*CUGGCGCUCCCGCUCGG (SEQ ID NO: 25) | |
| REG1-Z04 sgRNA (EX4_T1) | UUCACACCAUCACGACGCG Uguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 26) | U*U*C*ACACCAUCACGACGCGU guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 27) | TTCACACCATCACGACG CGT (GGG) (SEQ ID NO: 174)<br><br>TTCACACCATCACGACG CGT (SEQ ID NO: 321) |

| | | | |
|---|---|---|---|
| REG1-Z04 sgRNA (EX4_T1) spacer | UUCACACCAUCACGACGCG U (SEQ ID NO: 28) | U*U*C*ACACCAUCACGACGCGU (SEQ ID NO: 29) | |
| REG1-Z05 sgRNA (EX4_T2) | ACACCAUCACGACGCGUGG Gguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 30) | A*C*A*CCAUCACGACGCGUGGG guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 31) | ACACCATCACGACGCGT GGG (TGG) (SEQ ID NO: 175)<br><br>ACACCATCACGACGCGT GGG (SEQ ID NO: 322) |
| REG1-Z05 sgRNA (EX4_T2) spacer | ACACCAUCACGACGCGUGG G (SEQ ID NO: 32) | A*C*A*CCAUCACGACGCGUGGG (SEQ ID NO: 33) | |
| REG1-Z06 sgRNA (EX4_T3) | CUACGAGUCUGACGGGAUC Gguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 34) | C*U*A*CGAGUCUGACGGGAUCG guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 35) | CTACGAGTCTGACGGGA TCG (TGG) (SEQ ID NO: 176)<br><br>CTACGAGTCTGACGGGA TCG (SEQ ID NO: 323) |
| REG1-Z06 sgRNA (EX4_T3) spacer | CUACGAGUCUGACGGGAUC G (SEQ ID NO: 36) | C*U*A*CGAGUCUGACGGGAUCG (SEQ ID NO: 37) | |
| REG1-Z07 sgRNA (EX4_T4) | UUGCCACCCACGCGUCGUG Aguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 38) | U*U*G*CCACCCACGCGUCGUGA guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U(SEQ ID NO: 39) | TTGCCACCCACGCGTCG TGA (TGG) (SEQ ID NO: 177)<br><br>TTGCCACCCACGCGTCG TGA (SEQ ID NO: 324) |
| REG1-Z07 sgRNA (EX4_T4) spacer | UUGCCACCCACGCGUCGUG A (SEQ ID NO: 40) | U*U*G*CCACCCACGCGUCGUGA (SEQ ID NO: 41) | |
| REG1-Z08 sgRNA (EX4_T5) | GUUCACACCAUCACGACGC Gguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 42) | G*U*U*CACACCAUCACGACGCG guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 43) | GTTCACACCATCACGAC GCG (TGG) (SEQ ID NO: 178)<br><br>GTTCACACCATCACGAC GCG (SEQ ID NO: 325) |
| REG1-Z08 sgRNA (EX4_T5) spacer | GUUCACACCAUCACGACGC G (SEQ ID NO: 44) | G*U*U*CACACCAUCACGACGCG (SEQ ID NO: 45) | |
| REG1-Z09 sgRNA (EX4_T6) | CACGAUCCCGUCAGACUCG Uguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 46) | C*A*C*GAUCCCGUCAGACUCGU guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 47) | CACGATCCCGTCAGACT CGT (AGG) (SEQ ID NO: 179)<br><br>CACGATCCCGTCAGACT CGT (SEQ ID NO: 326) |
| REG1-Z09 sgRNA | CACGAUCCCGUCAGACUCG U (SEQ ID NO: 48) | C*A*C*GAUCCCGUCAGACUCGU (SEQ ID NO: 49) | |

| | | | |
|---|---|---|---|
| (EX4_T6) spacer | | | |
| REG1-Z10 sgRNA (EX4_T7) | ACGACGCGUGGGUGGCAAG Cguuuuagagcuagaaaua gcaaguuaaaauaaggcua guccguuaucaacuugaaa aaguggcaccgagucggug cUUUU (SEQ ID NO: 50) | A*C*G*ACGCGUGGGUGGCAAGC guuuuagagcuagaaauagcaag uuaaaauaaggcuaguccguuau caacuugaaaaaguggcaccgag ucggugcU*U*U*U (SEQ ID NO: 51) | ACGACGCGTGGGTGGCA AGC(GGG) (SEQ ID NO: 180)<br><br>ACGACGCGTGGGTGGCA AGC (SEQ ID NO: 327) |
| REG1-Z10 sgRNA (EX4_T7) spacer | ACGACGCGUGGGTGGCAAG C (SEQ ID NO: 52) | A*C*G*ACGCGUGGGUGGCAAGC (SEQ ID NO: 53) | |

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

**Sequence Table 2**. Exemplary sgRNA Sequences and Target Gene Sequences for *TRAC*, *β2M*, and *CD70*.

| sgRNA Sequences | | | SEQ ID NO: |
|---|---|---|---|
| ***CD70* sgRNA (CD70-7)** | Modified | G*C*U*UUGGUCCCAUUGGUCGCguuuuagagcuagaaauag caaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcU*U*U*U | 54 |
| | Unmodified | GCUUUGGUCCCAUUGGUCGCguuuuagagcuagaaauagcaa guuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcUUUU | 55 |
| ***CD70* sgRNA spacer** | Modified | G*C*U*UUGGUCCCAUUGGUCGC | 56 |
| | Unmodified | GCUUUGGUCCCAUUGGUCGC | 57 |
| ***TRAC* sgRNA (TA-1)** | Modified | A*G*A*GCAACAGUGCUGUGGCCguuuuagagcuagaaauag caaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcU*U*U*U | 58 |
| | Unmodified | AGAGCAACAGUGCUGUGGCCguuuuagagcuagaaauagcaa guuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcUUUU | 59 |
| ***TRAC* sgRNA spacer** | Modified | A*G*A*GCAACAGUGCUGUGGCC | 60 |
| | Unmodified | AGAGCAACAGUGCUGUGGCC | 61 |
| ***β2M* sgRNA (β2M-1)** | Modified | G*C*U*ACUCUCUCUUUCUGGCCguuuuagagcuagaaauag caaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcU*U*U*U | 62 |
| | Unmodified | GCUACUCUCUCUUUCUGGCCguuuuagagcuagaaauagcaa guuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcUUUU | 63 |
| ***β2M* sgRNA spacer** | Modified | G*C*U*ACUCUCUCUUUCUGGCC | 64 |
| | Unmodified | GCUACUCUCUCUUUCUGGCC | 65 |
| ***PD-1* sgRNA** | Modified | CUGCAGCUUCUCCAACACAUguuuuagagcuagaaauagcaa guuaaaauaaggcuaguccguuaucaacuugaaaaaguggca ccgagucggugcUUUU | 500 |
| | Unmodified | C*U*G*CAGCUUCUCCAACACAUguuuuagagcuagaaauag caaguuaaaauaaggcuaguccguuaucaacuugaaaaagug gcaccgagucggugcU*U*U*U | 510 |
| ***PD-1* sgRNA spacer** | Modified | CUGCAGCUUCUCCAACACAU | 501 |
| | Unmodified | C*U*G*CAGCUUCUCCAACACAU | 511 |
| **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 |
| ***PD*-1 target sequence** | CTGCAGCTTCTCCAACACAT (CGG) | 520 |
| **Exemplary sgRNA Formulas** | | |
| **sgRNA sequence** | nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuu | 72 |
| **sgRNA sequence** | nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugc | 73 |
| **sgRNA sequence** | n(17-30)guuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu(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 3**. Exemplary 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 4**. Exemplary 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<br>TATCCAGCGTGAGTCTCTCCTACCCTCCCGCT | 86 |
| *β2M* gene-edit | CGTGGCCTTAGCTGTGCTCGC----------<br>GCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT | 87 |
| *β2M* gene-edit | CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTC**TG**TGGCCTGGAGGCTA<br>TCCAGCGTGAGTCTCTCCTACCCTCCCGCT | 88 |

**Sequence Table 5.** Exemplary Edited *CD70* Gene Sequence.

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

**Sequence Table 6**. Exemplary Chimeric Antigen Receptor components

| **SEQ ID NO** | **Description** | **Sequence** |
|---|---|---|
| 95 | signal peptide | MLLLVTSLLLCELPHPAFLLIP |
| 96 | signal peptide | MALPVTALLLPLALLLHAARP |
| 97 | CD8a transmembrane domain | IYIWAPLAGTCGVLLLSLVITLY |
| 98 | 4-1BB nucleotide sequence | AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTA<br>TGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCG<br>ATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG |
| 99 | 4-1BB amino acid sequence | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL |
| 100 | CD28 nucleotide sequence | TCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTC<br>CTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCCC<br>CCCACGAGACTTCGCTGCGTACAGGTCC |
| 101 | CD28 amino acid sequence | SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS |
| 102 | CD3-zeta nucleotide sequence | CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAG<br>GACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGA<br>GTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATGGGG<br>GGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAAC<br>TCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAA<br>GGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGG<br>TTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAGG<br>CCCTGCCTCCCAGA |

| | | |
|---|---|---|
| 103 | CD3-zeta amino acid sequence | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR |

**Sequence Table 7**. Exemplary AAV Donor Template Sequences.

| SEQ ID NO | Description | Sequence |
|---|---|---|
| 161 | Left ITR (5' ITR) | TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAA AGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGC GCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT |
| 162 | Left ITR (5' ITR) (alternate) | CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCGTCGGG CGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT GGCCAACTCCATCACTAGGGGTTCCT |
| 163 | Right ITR (3' ITR) | AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT CACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCG GCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA |
| 164 | Right ITR (3' ITR) (alternate) | AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCT CACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCG GCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG |
| 165 | *TRAC*-LHA (800bp) | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGG TAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTAT CAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACC ACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCC TGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATT AAATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTT GAGTGGCAGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTG GCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAG CTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCA GCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGG GTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCC CACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAA TCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATG TGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGA CATGAGGTCTATGGACTTCA |
| 166 | *TRAC*-RHA (800bp) | TGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTA TTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTT CGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGG TCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAG AGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACG TGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCA GACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCA AGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCAC TAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCG GCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGG GGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTG GGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGA AAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCT ACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT GGGACAGGAGCTCAATGAGAAAGG |
| 167 | EF1a | GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGA AGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGG GGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGT GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCA ACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCC TGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACTGGC |

| | | |
|---|---|---|
| | | TGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAG<br>TTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCC<br>TGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCT<br>GTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGC<br>TGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTG<br>CACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGT<br>CCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAAT<br>CGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCG<br>CCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAG<br>TTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAA<br>ATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGG<br>AAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACC<br>GGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTC<br>TTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGG<br>GTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT<br>TTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGT<br>TCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA |

**Sequence Table 8.** 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

**Sequence Table 9**. 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**GAGGGAGCT | 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

**Sequence Table 10.** 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

**Sequence Table 11.** 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

**Sequence Table 12.** 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

**Sequence Table 13.** 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

**Sequence Table 14.** 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

**Sequence Table 15.** 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

**Sequence Table 16.** 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

**Sequence Table 17.** 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

**Sequence Table 18**. Exemplary TGFBRII gRNA Sequences/Target Sequences

| **Name** | **Unmodified Sequence** | **Modified Sequence** | **Target Sequence (PAM)** |
|---|---|---|---|
| TGFBRII sgRNA (EX1_T1) | CCGACUUCUGAACGU GCGGUguuuuagagcuagaa auagcaaguuaaaauaaggcuag uccguuaucaacuugaaaaagu ggcaccgagucggugcUUUU (SEQ ID NO: 264) | C*C*G*ACUUCUGAACG UGCGGUGGGguuuuagagc uagaaauagcaaguuaaaauaagg cuaguccguuaucaacuugaaaaa guggcaccgagucggugcU*U* U*U<br><br>(SEQ ID NO: 265) | CCGACTTCTGAACGTGCG GT<br>(GGG) (SEQ ID NO: 2)<br><br>CCGACTTCTGAACGTGCG GT (SEQ ID NO: 269) |

| | | | |
|---|---|---|---|
| TGFBRII sgRNA (EX1_T1) spacer | CCGACUUCUGAACGUGCGGU (SEQ ID NO: 266) | C*C*C*GACUUCUGAACGUGCGGU (SEQ ID NO: 267) | |
| TGFBRII sgRNA (EX1_T2) | UGCUGGCGAUACGCGUCCACguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 270) | U*G*C*UGGCGAUACGCGUCCACguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcmU*U*U*U (SEQ ID NO: 271) | TGCTGGCGATACGCGTCCAC (AGG) (SEQ ID NO: 3)<br><br>TGCTGGCGATACGCGTCCAC (SEQ ID NO: 275) |
| TGFBRII sgRNA (EX1_T2) spacer | UGCUGGCGAUACGCGUCCAC<br><br>(SEQ ID NO: 272) | U*G*C*UGGCGAUACGCGUCCAC<br><br>(SEQ ID NO: 273) | |
| TGFBRII sgRNA (EX1_T3) | UCGGUCUAUGACGAGCAGCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU<br><br>(SEQ ID NO: 276) | U*C*G*GUCUAUGACGAGCAGCGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 277) | TCGGTCTATGACGAGCAGCG(GGG) (SEQ ID NO: 4)<br><br>TCGGTCTATGACGAGCAGCG (SEQ ID NO: 281) |
| TGFBRII sgRNA (EX1_T3) spacer | UCGGUCUAUGACGAGCAGCG (SEQ ID NO: 278) | U*C*G*GUCUAUGACGAGCAGCG (SEQ ID NO: 279) | |
| TGFBRII sgRNA (EX2_T1) | AUGGGCAGUCCUAUUACAGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 282) | A*U*G*GGCAGUCCUAUUACAGCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 283) | ATGGGCAGTCCTATTACAGC (TGG) (SEQ ID NO: 5)<br><br>ATGGGCAGTCCTATTACAGC (SEQ ID NO: 287) |
| TGFBRII sgRNA (EX2_T1) spacer | AUGGGCAGUCCUAUUACAGC (SEQ ID NO: 284) | A*U*G*GGCAGUCCUAUUACAGC (SEQ ID NO: 285) | |
| TGFBRII sgRNA (EX3_T1) | AUUGUUCACUUGUUAGCCCCguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 288) | A*U*U*GUUCACUUGUUAGCCCCAGGguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 289) | ATTGTTCACTTGTTAGCCCC (AGG) (SEQ ID NO: 6)<br><br>ATTGTTCACTTGTTAGCCCC (SEQ ID NO: 293) |
| TGFBRII sgRNA (EX3_T1) spacer | AUUGUUCACUUGUUAGCCCC (SEQ ID NO: 290) | A*U*U*GUUCACUUGUUAGCCCC (SEQ ID NO: 291) | |
| TGFBRII sgRNA (EX3_T2) | GCUGAAGAACUGCCUCUAUAguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 294) | G*C*U*GAAGAACUGCCUCUAUAguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 295) | GCTGAAGAACTGCCTCTATA (TGG) (SEQ ID NO: 7)<br><br>GCTGAAGAACTGCCTCTATA (SEQ ID NO: 299) |
| TGFBRII sgRNA | GCUGAAGAACUGCCUCUAUA (SEQ ID NO: 296) | G*C*U*GAAGAACUGCCUCUAUA (SEQ ID NO: 297) | |

| | | | |
|---|---|---|---|
| (EX3_T2) spacer | | | |
| TGFBRII sgRNA (EX4_T1) | GCAGGAUUUCUGGUUGUCACguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 300) | G*C*A*GGAUUUCUGGUUGUCACguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 301) | GCAGGATTTCTGGTTGTCAC (AGG) (SEQ ID NO: 8)<br><br>GCAGGATTTCTGGTTGTCAC (SEQ ID NO: 305) |
| TGFBRII sgRNA (EX4_T1) spacer | GCAGGAUUUCUGGUUGUCAC (SEQ ID NO: 302) | G*C*A*GGAUUUCUGGUUGUCAC (SEQ ID NO: 303) | |
| TGFBRII sgRNA (EX4_T2) | CUCCAUCUGUGAGAAGCCACguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 306) | C*U*C*CAUCUGUGAGAAGCCACguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 307) | CTCCATCTGTGAGAAGCCAC (AGG) (SEQ ID NO: 9)<br><br>CTCCATCTGTGAGAAGCCAC (SEQ ID NO: 311) |
| TGFBRII sgRNA (EX4_T2) spacer | CUCCAUCUGUGAGAAGCCAC (SEQ ID NO: 308) | C*U*C*CAUCUGUGAGAAGCCAC (SEQ ID NO: 309) | |
| TGFBRII sgRNA (EX5_T1) | CCCCUACCAUGACUUUAUUCguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcUUUU (SEQ ID NO: 312) | C*C*C*CUACCAUGACUUUAUUCguuuuagagcuagaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcU*U*U*U (SEQ ID NO: 313) | CCCCTACCATGACTTTATTC (TGG) (SEQ ID NO: 10)<br><br>CCCCTACCATGACTTTATTC (SEQ ID NO: 317) |
| TGFBRII sgRNA (EX5_T1) spacer | CCCCUACCAUGACUUUAUUC (SEQ ID NO: 314) | C*C*C*CUACCAUGACUUUAUUC (SEQ ID NO: 315) | |

*: 2'-O-methyl phosphorothioate residue

**Sequence Table 19.** 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 | CTGAACGTGCCGGTGGGATCG | 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

**Sequence Table 20**. 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

**Sequence Table 21**. 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

**Sequence Table 22.** 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

**Sequence Table 23.** 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

**Sequence Table 24**. 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

**Sequence Table 25.** 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

**Sequence Table 26**. 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**T**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

**Sequence Table 27.** 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

**Sequence Table 28**. CAR structures of CTX-971 CAR, CTX-971b CAR, CTX-972 CAR and CTX-972b CAR

| CAR | CAR structure | SEQ ID NO: |
|---|---|---|
| CTX-971 CAR | CD8[signal peptide]-VL-linker-VH-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ | 527 (nt),<br>528 (aa with signal peptide),<br>600 (aa with no signal peptide) |
| CTX-971b CAR | CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 529 (nt)<br>530 (aa with signal peptide)<br>601 (aa with no signal peptide) |
| CTX-972 CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ | 543 (nt)<br>603 (aa with signal peptide)<br>602 (aa with no signal peptide) |
| CTX-972b CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]- 41BB[co-stimulatory domain]-CD3ζ | 545 (nt)<br>604 (aa with signal peptide)<br>607 (aa with no signal peptide) |

**Sequence Table 29.** CAR Components of CTX-971 CAR, CTX-971b CAR, CTX-972 CAR and CTX-972b CAR with a CAR Structure: CD8[signal peptide]-anti-LIV1[scFV]-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ; or CD8[signal peptide]-anti-LIV1[scFV]-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ

| Name | Sequence | SEQ ID NO: |
|---|---|---|
| CTX-971 CAR CD28 co-stim | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCA<br>CGCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACT<br>CTCGGACAACCGGCTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATAGCA<br>GTGGTAACACTTATCTTGAGTGGTACCAACAGCGGCCCGGCCAATCTCCTAGGCC<br>CCTGATATATAAGATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGG<br>TCTGGGAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACG<br>TGGGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGG<br>CACAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAGGT<br>GGTGGGTCACAAGTACAGCTCGTGCAATCAGGGGCGGAGGTGAAGAAACCAGGGG<br>CGTCTGTGAAGGTAAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACAT<br>GCATTGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGAT<br>CCTGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGA<br>CTCGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCGA<br>TGACACGGCCGTTTATTATTGCGCCGTACACAATGCGCACTACGGGACATGGTTC<br>GCGTATTGGGGTCAAGGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTG<br>TCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGAC<br>ACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGA<br>CCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT<br>ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTAT<br>TACTTTGTATTGTAATCACAGGAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCC<br>GATTACATGAATATGACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAAC<br>CCTATGCCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCG<br>AAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTG<br>AATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGGAGAGACC<br>CGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGA<br>ACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAA<br>CGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCA<br>AAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT | 527 |
| CTX-971 CAR CD28 co-stim | *MALPVTALLLPLALLLHAARP*DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSG<br>NTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVG<br>VYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGAS<br>VKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTR<br>DTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVP<br>VFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI<br>WAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY<br>APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE<br>MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD<br>TYDALHMQALPPR | 528<br><br>600 (no signal peptide) |
| CTX-971b CAR 41BB co-stim | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCA<br>CGCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACT<br>CTCGGACAACCGGCTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATAGCA<br>GTGGTAACACTTATCTTGAGTGGTACCAACAGCGGCCCGGCCAATCTCCTAGGCC<br>CCTGATATATAAGATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGG<br>TCTGGGAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACG<br>TGGGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGG<br>CACAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAGGT<br>GGTGGGTCACAAGTACAGCTCGTGCAATCAGGGGCGGAGGTGAAGAAACCAGGGG<br>CGTCTGTGAAGGTAAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACAT<br>GCATTGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGAT<br>CCTGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGA | 529 |

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| | CTCGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCGA<br>TGACACGGCCGTTTATTATTGCGCCGTACACAATGCGCACTACGGGACATGGTTC<br>GCGTATTGGGGTCAAGGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTG<br>TCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGAC<br>ACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGA<br>CCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT<br>ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTAT<br>TACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTAT<br>ATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCT<br>GTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTT<br>TTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAAC<br>GAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGA<br>GAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTA<br>CAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAG<br>GGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGG<br>CAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT | |
| CTX-971b<br>CAR<br>41BB co-stim | *MALPVTALLLPLALLLHAARP*DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSG<br>NTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVG<br>VYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGAS<br>VKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTR<br>DTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVP<br>VFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI<br>WAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCS<br>CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD<br>PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT<br>KDTYDALHMQALPPR | 530<br><br>601 (no signal peptide) |
| CTX-971 and<br>CTX-971b<br>scFv | GACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACTCTCGGACAACCGG<br>CTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATAGCAGTGGTAACACTTA<br>TCTTGAGTGGTACCAACAGCGGCCCGGCCAATCTCCTAGGCCCCTGATATATAAG<br>ATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTCTGGGAGTGGTA<br>CAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACGTGGGTGTTTATTA<br>CTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGGCACAAAAGTCGAG<br>ATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAGGTGGTGGGTCACAAG<br>TACAGCTCGTGCAATCAGGGGCGGAGGTGAAGAAACCAGGGGCGTCTGTGAAGGT<br>AAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACATGCATTGGGTCCGC<br>CAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGATCCTGAAAATGGGG<br>ATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGACTCGAGATACTAG<br>CATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCGATGACACGGCCGTT<br>TATTATTGCGCCGTACACAATGCGCACTACGGGACATGGTTCGCGTATTGGGGTC<br>AAGGAACGCTCGTTACTGTCTCAAGT | 531 |
| CTX-971 and<br>CTX-971b<br>scFv<br>(linker<br>underlined) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYK<br>ISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVE<br>IK**GGGGSGGGGSGGGGS**QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVR<br>QAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAV<br>YYCAVHNAHYGTWFAYWGQGTLVTVSS | 532 |
| CTX-971 and<br>CTX-971b<br>scFv VH<br>CDRs- in bold | QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPGQGLEWMG**WIDPEN<br>GDTEYGPKFQG**RVTMTRDTSINTAYMELSRLRSDDTAVYYCAV**HNAHYGTWFAY**W<br>GQGTLVTVSS | 533 |
| CTX-971 and<br>CTX-971b<br>scFv VL<br>CDRs – in<br>bold | DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQRPGQSPRPLIY**K<br>ISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHVPYT**FGGGTKVE<br>IK | 534 |
| CTX-971 and<br>CTX-971b<br>VH CDR1 | DYYMH | 535 |

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| CTX-971 and CTX-971b VH CDR2 | WIDPENGDTEYGPKFQG | 536 |
| CTX-971 and CTX-971b VH CDR3 | HNAHYGTWFAY | 537 |
| CTX-971 and CTX-971b VL CDR1 | RSSQSLLHSSGNTYLE | 538 |
| CTX-971 and CTX-971b VL CDR2 | KISTRFS | 539 |
| CTX-971 and CTX-971b VL CDR3 | FQGSHVPYT | 540 |
| CTX-971 Donor LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAG<br>TGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCAATGA<br>GAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACATACCAT<br>AAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCC<br>AAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCC<br>AGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAG<br>TATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGC<br>CGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTG<br>TCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTA<br>TAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCAC<br>TGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCT<br>AACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCA<br>GCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGAT<br>TCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAA<br>CTGTGCTAGACATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcag<br>agcgcacatcgcccacagtccccgagaagttgggggggagggtcggcaattgaac<br>cggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactgg<br>ctccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccg<br>tgaacgttctttttcgcaacgggtttgccgccagaacacaggtaagtgccgtgtg<br>tggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaatt<br>acttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgg<br>gtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt<br>gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcg<br>cctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacctgc<br>tgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcac<br>actggtatttcggttttttggggccgcgggcggcgacggggcccgtgcgtcccagc<br>gcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggg<br>gtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc<br>gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaa<br>gatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcggcgctc<br>gggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctca<br>gccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgatt<br>agttctcgagcttttggagtacgtcgtctttaggttgggggggaggggttttatgc<br>gatggagtttccccacactgagtgggtggagactgaagttaggccagcttggcac<br>ttgatgtaattctccttggaatttgccctttttgagtttggatcttggttcattc<br>tcaagcctcagacagtggttcaaagtttttttcttccatttcaggtgtcgtgaCC<br>ACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCACG<br>CAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACTCT<br>CGGACAACCGGCTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATAGCAGT<br>GGTAACACTTATCTTGAGTGGTACCAACAGCGGCCCGGCCAATCTCCTAGGCCCC<br>TGATATATAAGATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTC<br>TGGGAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACGTG<br>GGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGGCA | 541 |

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| | CAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAGGTGG<br>TGGGTCACAAGTACAGCTCGTGCAATCAGGGGCGGAGGTGAAGAAACCAGGGGCG<br>TCTGTGAAGGTAAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACATGC<br>ATTGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGATCC<br>TGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGACT<br>CGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCGATG<br>ACACGGCCGTTTATTATTGCGCCGTACACAATGCGCACTACGGGACATGGTTCGC<br>GTATTGGGGTCAAGGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTGTC<br>CCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACAC<br>CCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC<br>CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTAC<br>ATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTA<br>CTTTGTATTGTAATCACAGGAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGA<br>TTACATGAATATGACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCC<br>TATGCCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAA<br>GCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAA<br>TTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCG<br>GAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAAC<br>TCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACG<br>ACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAA<br>GATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATC<br>GCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCT<br>GACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCT<br>TCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTC<br>AGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTG<br>ATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAAC<br>AGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGA<br>GAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCT<br>GCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATT<br>CTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATC<br>TTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACT<br>GATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCA<br>GATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCA<br>GCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAG<br>AAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTAC<br>TTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGAC<br>AGGAGCTCAATGAGAAAGG | |
| CTX-971b<br>Donor<br>LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAG<br>TGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCAATGA<br>GAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACATACCAT<br>AAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCC<br>AAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCC<br>AGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAG<br>TATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGC<br>CGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTG<br>TCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTA<br>TAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCAC<br>TGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCT<br>AACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCA<br>GCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGAT<br>TCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAA<br>CTGTGCTAGACATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcag<br>agcgcacatcgcccacagtccccgagaagttgggggggagggtcggcaattgaac<br>cggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactgg<br>ctccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccg<br>tgaacgttcttttcgcaacgggtttgccgccagaacacaggtaagtgccgtgtg<br>tggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaatt<br>acttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgg<br>gtgggagagttcgaggccttgcgcttaaggagcccttcgcctcgtgcttgagtt<br>gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcg | 542 |

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| | cctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacctgc<br>tgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcac<br>actggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagc<br>gcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggg<br>gtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc<br>gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaa<br>gatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcggcgctc<br>gggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctca<br>gccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgatt<br>agttctcgagcttttggagtacgtcgtctttaggttgggggggagggggttttatgc<br>gatggagtttccccacactgagtgggtggagactgaagttaggccagcttggcac<br>ttgatgtaattctccttggaatttgccctttttgagtttggatcttggttcattc<br>tcaagcctcagacagtggttcaaagtttttttcttccatttcaggtgtcgtgaCC<br>ACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCACG<br>CAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACTCT<br>CGGACAACCGGCTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATAGCAGT<br>GGTAACACTTATCTTGAGTGGTACCAACAGCGGCCCGGCCAATCTCCTAGGCCCC<br>TGATATATAAGATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTC<br>TGGGAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACGTG<br>GGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGGCA<br>CAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAGGTGG<br>TGGGTCACAAGTACAGCTCGTGCAATCAGGGGCGGAGGTGAAGAAACCAGGGGCG<br>TCTGTGAAGGTAAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACATGC<br>ATTGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGATCC<br>TGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGACT<br>CGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCGATG<br>ACACGGCCGTTTATTATTGCGCCGTACACAATGCGCACTACGGGACATGGTTCGC<br>GTATTGGGGTCAAGGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTGTC<br>CCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACAC<br>CCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC<br>CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTAC<br>ATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTA<br>CTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATAT<br>ATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGT<br>AGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTT<br>CCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGA<br>ACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGA<br>GACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACA<br>ATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGG<br>CGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCA<br>ACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAAT<br>AAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAAC<br>AAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACA<br>CCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCT<br>TGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACT<br>CCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTA<br>AGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGA<br>TGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGA<br>GTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGC<br>CTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAA<br>AAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCA<br>ATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAA<br>AAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCAT<br>CTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGG<br>GTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAA<br>TGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCC<br>TGGGACAGGAGCTCAATGAGAAAGG | |
| CTX-972 CAR CD28 co-stim | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCA<br>CGCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCC<br>GGCGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGATTATT | 543 |

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| | ACATGCATTGGGTCAGACAGGCACCCGGACAGGGATTGGAGTGGATGGGTTGGAT<br>CGACCCGGAAAACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACA<br>ATGACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCT<br>CTGATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTG<br>GTTCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTGGA<br>AGCGGGGGAGGGGGTAGCGGAGGTGGCGGAAGTGATGTTGTTATGACACAGAGTC<br>CCCTGTCATTGCCCGTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAG<br>TCAAAGCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGG<br>CCAGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGTG<br>TTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAG<br>CAGGGTTGAAGCTGAGGACGTAGGGGTATATTATTGTTTTCAGGGCAGTCACGTG<br>CCGTACACTGGGGGCGGAACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCC<br>CGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACC<br>CGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCC<br>GCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACA<br>TTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTAC<br>TTTGTATTGTAATCACAGGAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGAT<br>TACATGAATATGACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCT<br>ATGCCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAAT<br>TTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGG<br>AAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACT<br>CCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGA<br>CGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAG<br>ATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT | |
| CTX-972 CAR<br>CD28 co-stim | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYM<br>HWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSD<br>DTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPL<br>SLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVP<br>DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTGGGTKVEIKSAAAFVPV<br>FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW<br>APLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA<br>PPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM<br>GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT<br>YDALHMQALPPR | 603<br><br>602 (no signal peptide) |
| CTX-972b<br>CAR<br>41BB co-stim | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCA<br>CGCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCC<br>GGCGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGATTATT<br>ACATGCATTGGGTCAGACAGGCACCCGGACAGGGATTGGAGTGGATGGGTTGGAT<br>CGACCCGGAAAACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACA<br>ATGACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCT<br>CTGATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTG<br>GTTCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTGGA<br>AGCGGGGGAGGGGGTAGCGGAGGTGGCGGAAGTGATGTTGTTATGACACAGAGTC<br>CCCTGTCATTGCCCGTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAG<br>TCAAAGCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGG<br>CCAGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGTG<br>TTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAG<br>CAGGGTTGAAGCTGAGGACGTAGGGGTATATTATTGTTTTCAGGGCAGTCACGTG<br>CCGTACACTGGGGGCGGAACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCC<br>CGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACC<br>CGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCC<br>GCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACA<br>TTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTAC<br>TTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATA<br>TTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTA<br>GCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTC<br>CCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAA<br>CTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAG<br>ACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA | 545 |

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| | TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGC<br>GAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAA<br>CCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT | |
| CTX-972b CAR 41BB co-stim | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYM<br>HWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSD<br>DTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPL<br>SLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVP<br>DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTGGGTKVEIKSAAAFVPV<br>FLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIW<br>APLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC<br>RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP<br>EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK<br>DTYDALHMQALPPR | 604<br>607 (no signal peptide) |
| CTX-972 and CTX-972b scFv | CAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCCGGCGCGAGCGTAA<br>AAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGATTATTACATGCATTGGGT<br>CAGACAGGCACCCGGACAGGGATTGGAGTGGATGGGTTGGATCGACCCGGAAAAC<br>GGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAATGACACGAGATA<br>CGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCTCTGATGATACAGC<br>AGTTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTGGTTCGCGTACTGG<br>GGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTGGAAGCGGGGGAGGGG<br>GTAGCGGAGGTGGCGGAAGTGATGTTGTTATGACACAGAGTCCCCTGTCATTGCC<br>CGTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAGTCAAAGCCTCCTT<br>CACAGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGGCCAGGGCAATCTC<br>CTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGTGTTCCCGACCGCTT<br>CAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAGCAGGGTTGAAGCT<br>GAGGACGTAGGGGTATATTATTGTTTTCAGGGCAGTCACGTGCCGTACACTGGGG<br>GCGGAACCAAAGTCGAGATAAAG | 547 |
| CTX-972 and CTX-972b scFv (linker underlined) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPEN<br>GDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYW<br>GQGTLVTVSS**GGGGSGGGGSGGGGS**DVVMTQSPLSLPVTLGQPASISCRSSQSLL<br>HSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEA<br>EDVGVYYCFQGSHVPYTGGGTKVEIK | 605 |
| CTX-972 and CTX-972b scFv VH CDRs- in bold | QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPGQGLEWMG**WIDPEN<br>GDTEYGPKFQG**RVTMTRDTSINTAYMELSRLRSDDTAVYYCAV**HNAHYGTWFAY**W<br>GQGTLVTVSS | 533 |
| CTX-972 and CTX-972b scFv VL CDRs – in bold | DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQRPGQSPRPLIY**K<br>ISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHVPYT**GGGTKVEI<br>K | 606 |
| CTX-972 and CTX-972b VH CDR1 | DYYMH | 535 |
| CTX-972 and CTX-972b VH CDR2 | WIDPENGDTEYGPKFQG | 536 |
| CTX-972 and CTX-972b VH CDR3 | HNAHYGTWFAY | 537 |
| CTX-972 and CTX-972b VL CDR1 | RSSQSLLHSSGNTYLE | 538 |
| CTX-972 and CTX-972b VL CDR2 | KISTRFS | 539 |

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| CTX-972 and CTX-972b VL CDR3 | FQGSHVPYT | 540 |
| CTX-972 Donor LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAG<br>TGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCAATGA<br>GAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACATACCAT<br>AAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCC<br>AAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCC<br>AGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAG<br>TATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGC<br>CGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTG<br>TCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTA<br>TAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCAC<br>TGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCT<br>AACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCA<br>GCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGAT<br>TCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAA<br>CTGTGCTAGACATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcag<br>agcgcacatcgcccacagtccccgagaagttgggggggagggtcggcaattgaac<br>cggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactgg<br>ctccgccttttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccg<br>tgaacgttctttttcgcaacgggtttgccgccagaacacaggtaagtgccgtgtg<br>tggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaatt<br>acttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgg<br>gtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt<br>gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcg<br>cctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacctgc<br>tgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcac<br>actggtatttcggtttttgggccgcgggcggcgacggggcccgtgcgtcccagc<br>gcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggg<br>gtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc<br>gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaa<br>gatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcggcgctc<br>gggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctca<br>gccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgatt<br>agttctcgagcttttggagtacgtcgtctttaggttgggggggagggtttttatgc<br>gatggagtttccccacactgagtgggtggagactgaagttaggccagcttggcac<br>ttgatgtaattctccttggaatttgccctttttgagtttggatcttggttcattc<br>tcaagcctcagacagtggttcaaagtttttttcttccatttcaggtgtcgtgaCC<br>ACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCACG<br>CAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCCGG<br>CGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGATTATTAC<br>ATGCATTGGGTCAGACAGGCACCCGGACAGGGATTGGAGTGGATGGGTTGGATCG<br>ACCCGGAAAACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAAT<br>GACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCTCT<br>GATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTGGT<br>TCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTGGAAG<br>CGGGGGAGGGGGTAGCGGAGGTGGCGGAAGTGATGTTGTTATGACACAGAGTCCC<br>CTGTCATTGCCCGTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAGTC<br>AAAGCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGGCC<br>AGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGTGTT<br>CCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAGCA<br>GGGTTGAAGCTGAGGACGTAGGGGTATATTATTGTTTTCAGGGCAGTCACGTGCC<br>GTACACTGGGGGCGGAACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCG<br>GTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCG<br>CTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGC<br>CGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATT<br>TGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTT<br>TGTATTGTAATCACAGGAATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGATTA | 549 |

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| | CATGAATATGACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTAT<br>GCCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAAGCG<br>CAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAATTT<br>GGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAA<br>ATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACTCC<br>AGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACG<br>ACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGAT<br>ACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATCGCT<br>ATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAATCTGAC<br>TTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCC<br>CCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGG<br>AATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATT<br>GGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGT<br>GAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAA<br>GGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCC<br>TGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTA<br>AGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTT<br>CCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGAT<br>TGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCT<br>GGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAA<br>ACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTG<br>AAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGG<br>AGCTCAATGAGAAAGG | |
| CTX-972b<br>Donor<br>LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAG<br>TGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCAATGA<br>GAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACATACCAT<br>AAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCC<br>AAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCC<br>AGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAAGCAG<br>TATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGC<br>CGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTG<br>TCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCTAAGATGCTATTTCCCGTA<br>TAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCAC<br>TGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCT<br>AACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCA<br>GCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGAT<br>TCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAA<br>CTGTGCTAGACATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcag<br>agcgcacatcgcccacagtccccgagaagttgggggggagggtcggcaattgaac<br>cggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactgg<br>ctccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccg<br>tgaacgttctttttcgcaacgggtttgccgccagaacacaggtaagtgccgtgtg<br>tggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaatt<br>acttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgg<br>gtgggagagttcgaggccttgcgcttaaggagcccttcgcctcgtgcttgagtt<br>gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcg<br>cctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacctgc<br>tgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcac<br>actggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagc<br>gcacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacgggg<br>gtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc<br>gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaa<br>gatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcggcgctc<br>gggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctca<br>gccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgatt<br>agttctcgagcttttggagtacgtcgtctttaggttgggggggagggttttatgc<br>gatggagtttccccacactgagtgggtggagactgaagttaggccagcttggcac<br>ttgatgtaattctccttggaatttgccctttttgagtttggatcttggttcattc<br>tcaagcctcagacagtggttcaaagtttttttcttccatttcaggtgtcgtgaCC | 550 |

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|---|---|---|
| | ACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCACG<br>CAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCCGG<br>CGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGATTATTAC<br>ATGCATTGGGTCAGACAGGCACCCGGACAGGGATTGGAGTGGATGGGTTGGATCG<br>ACCCGGAAAACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAAT<br>GACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCTCT<br>GATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTGGT<br>TCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTGGAAG<br>CGGGGGAGGGGGTAGCGGAGGTGGCGGAAGTGATGTTGTTATGACACAGAGTCCC<br>CTGTCATTGCCCGTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAGTC<br>AAAGCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGGCC<br>AGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGTGTT<br>CCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAGCA<br>GGGTTGAAGCTGAGGACGTAGGGGTATATTATTGTTTTCAGGGCAGTCACGTGCC<br>GTACACTGGGGGCGGAACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCG<br>GTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCG<br>CTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGC<br>CGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATT<br>TGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTT<br>TGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATT<br>CAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGC<br>TGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCC<br>GAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACT<br>GAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATG<br>AACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGA<br>ACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACC<br>AAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAA<br>ATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCAACAAA<br>TCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCT<br>TCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGC<br>TTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCT<br>CTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGA<br>AACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGA<br>AGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTT<br>CCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTC<br>ATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAA<br>ATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATC<br>ACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAG<br>TCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTG<br>TCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTT<br>GAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGG<br>GACAGGAGCTCAATGAGAAAGG | |
| CD8 signal peptide | MALPVTALLLPLALLLHAARP | 551 |
| CD8a transmembrane + 5' Linker (underlined) | GCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCC<br>CGCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCC<br>CGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTC<br>GCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGT<br>TGTCACTCGTTATTACTTTGTATTGTAATCACAGGAATCGC | 552 |
| CD8a transmembrane + 5' Linker (underlined) | SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD<br>FACDIYIWAPLAGTCGVLLLSLVITLYCNHRNR | 553 |
| CD8a transmembrane | TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTC<br>CGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATG<br>CCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGAT | 554 |

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|---|---|---|
| (without linker) | ATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCG TTATTACTTTGTATTGTAATCACAGGAATCGC | |
| CD8a transmembrane (without linker) | FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD IYIWAPLAGTCGVLLLSLVITLYCNHRNR | 555 |
| CD28 co-stimulatory | TCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTCCTCGCCGGC CTGGGCCGACAAGAAAACATTACCAACCCTATGCCCCCCCACGAGACTTCGCTGC GTACAGGTCC | 523 |
| CD28 co-stimulatory | SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS | 524 |
| 41BB co-stimulatory | AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAG TACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGA AGGAGGATGTGAACTG | 521 |
| 41BB co-stimulatory | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL | 522 |
| CD3ζ | CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATC AGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAA ACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAA GAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAA TAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGG GTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCT CCCAGA | 525 |
| CD3ζ | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR | 526 |

**Sequence Table 30.** Donor Components with a donor structure: TRAC[LHA]-EF1a[promoter]-CAR-polyA–TRAC[RHA]

| Name | Sequence | SEQ ID NO: |
|---|---|---|
| TRAC-LHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGG TAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTAT CAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACC ACTCCAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCC TGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATT AAATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTT GAGTGGCAGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTG GCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAG CTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCA GCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGG GTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCC CACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAA TCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATG TGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGA CATGAGGTCTATGGACTTCA | 556 |
| EF1α promoter | GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGA AGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGG GGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGT GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCA ACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCC TGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACTGGC TGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAG TTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCC TGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCT | 557 |

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|---|---|---|
| | GTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGC<br>TGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTG<br>CACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGT<br>CCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAAT<br>CGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCG<br>CCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAG<br>TTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAA<br>ATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGG<br>AAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACC<br>GGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTC<br>TTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGG<br>GTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAAT<br>TTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGT<br>TCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA | |
| Synthetic poly(A) signal | AATAAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTG | 558 |
| TRAC–RHA | TGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTA<br>TTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTT<br>CGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGG<br>TCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCC<br>ACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAG<br>AGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACG<br>TGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCA<br>GACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCA<br>AGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCAC<br>TAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCG<br>GCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGG<br>GGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTG<br>GGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGA<br>AAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCT<br>ACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | 559 |

**Sequence Table 31**. Exemplary CAR structures of CTX-973 CAR, CTX-974 CAR, CTX-975 CAR, CTX-976 CAR, CTX-977 CAR, CTX-978 CAR, CTX-979 CAR and CTX-979b CAR.

| CAR | CAR structure | SEQ ID NO: |
|---|---|---|
| CTX-973 CAR | CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 573 (nt)<br>574 (aa with signal peptide)<br>567 (aa with no signal peptide) |
| CTX-974 CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 578 (nt)<br>579 (aa with signal peptide)<br>569 (aa with no signal peptide) |
| CTX-975 CAR | CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 582 (nt)<br>583 (aa with signal peptide)<br>570 (aa with no signal peptide) |
| CTX-976 CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 586 (nt)<br>587 (aa with signal peptide)<br>571 (aa with no signal peptide) |
| CTX-977 CAR | CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 590 (nt)<br>591 (aa with signal peptide)<br>572 (aa with no signal peptide) |

| | | |
|---|---|---|
| CTX-978 CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 594 (nt)<br>595 (aa with signal peptide)<br>598 (aa with no signal peptide) |
| CTX-979 CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ | 546 (aa with signal peptide)<br>599 (aa with no signal peptide) |
| CTX-979b CAR | CD8[signal peptide]-VH-linker-VL-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ | 544 (aa with signal peptide)<br>608 (aa with no signal peptide) |

**Sequence Table 32**. Exemplary CAR Components of CTX-973 CAR, CTX-974 CAR, CTX-975 CAR, CTX-976 CAR, CTX-977 CAR, CTX-978 CAR, CTX-979 CAR and CTX-979b CAR

| Name | Sequence | SEQ ID NO: |
|---|---|---|
| CTX-973 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGGATGTCGTTATGACACAATCTCCCTTGAGTTTGCCGGT<br>TACCTTGGGACAACCTGCTAGTATTTCATGTAGGAGTTCTCAAAGTCTCTTGC<br>ACTCCTCAGGGAACACCTACCTCGAATGGTACCAACAACGCCCTGGCCAAAGC<br>CCGCGGCCCTTGATATACAAAATATCAACAAGATTTAGCGGGGTACCCGATAG<br>ATTCAGCGGCTCTGGCAGCGGGACGGATTTTACCCTGAAAATTAGTCGCGTAG<br>AAGCTGAAGACGTTGGTGTGTATTACTGCTTTCAAGGGAGCCATGTGCCTTAC<br>ACATTTGGAGGAGGCACCAAGGTCGAGATTAAGGGAGGGGGTGGATCAGGTGG<br>GGGTGGGTCCGGAGGCGGCGGCAGTCAAGTGCAGTTGGTTCAATCAGGAGCTG<br>AAGTTAAAAAGCCAGGAGCTTCAGTCAAGGTTTCATGCAAGGCGTCCGGTCTC<br>ACTATAGAGGATTACTACATGCACTGGGTGCGGCAAGCTCCAGGCCAGGGGCT<br>GGAGTGGATGGGATGGATTGATCCGGAAAACGGGGACACAGAGTATGGGCCCA<br>AATTCCAAGGCCGGGTGACAATGACCAGAGATACTAGTATTTCAACAGCATAC<br>ATGGAGCTGTCACGGCTGAGGTCAGACGATACGGCAGTCTACTATTGTGCAGT<br>ACATAACGCACATTATGGTACGTGGTTCGCTTATTGGGGTCAAGGTACCCTGG<br>TCACGGTAAGTTCAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG<br>GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | 573 |
| CTX-973 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*DVVMTQSPLSLPVTLGQPASISCRSSQSLLHS<br>SGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEA<br>EDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEV<br>KKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKF<br>QGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVT<br>VSSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 574<br><br>567 (no signal peptide) |
| CTX-973 scFv (nt) | GATGTCGTTATGACACAATCTCCCTTGAGTTTGCCGGTTACCTTGGGACAACC<br>TGCTAGTATTTCATGTAGGAGTTCTCAAAGTCTCTTGCACTCCTCAGGGAACA<br>CCTACCTCGAATGGTACCAACAACGCCCTGGCCAAAGCCCGCGGCCCTTGATA | 575 |

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|---|---|---|
| | TACAAAATATCAACAAGATTTAGCGGGGTACCCGATAGATTCAGCGGCTCTGG<br>CAGCGGGACGGATTTTACCCTGAAAATTAGTCGCGTAGAAGCTGAAGACGTTG<br>GTGTGTATTACTGCTTTCAAGGGAGCCATGTGCCTTACACATTTGGAGGAGGC<br>ACCAAGGTCGAGATTAAGGGAGGGGGTGGATCAGGTGGGGGTGGGTCCGGAGG<br>CGGCGGCAGTCAAGTGCAGTTGGTTCAATCAGGAGCTGAAGTTAAAAAGCCAG<br>GAGCTTCAGTCAAGGTTTCATGCAAGGCGTCCGGTCTCACTATAGAGGATTAC<br>TACATGCACTGGGTGCGGCAAGCTCCAGGCCAGGGGCTGGAGTGGATGGGATG<br>GATTGATCCGGAAAACGGGGACACAGAGTATGGGCCCAAATTCCAAGGCCGGG<br>TGACAATGACCAGAGATACTAGTATTTCAACAGCATACATGGAGCTGTCACGG<br>CTGAGGTCAGACGATACGGCAGTCTACTATTGTGCAGTACATAACGCACATTA<br>TGGTACGTGGTTCGCTTATTGGGGTCAAGGTACCCTGGTCACGGTAAGTTCA | |
| CTX-973<br>scFv (aa) (linker underlined) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS | 560 |
| CTX-973<br>scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 576 |
| CTX-973<br>scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 534 |
| CTX-973<br>Donor (nt)<br>LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA<br>GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA<br>GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA<br>GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT<br>CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG<br>GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA<br>CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc<br>gagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggcg<br>cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg<br>gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgc<br>aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc<br>tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct<br>gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt<br>cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg<br>cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct<br>cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga<br>cgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact<br>ggtatttcggtttttgggggccgcgggcggcgacggggcccgtgcgtcccagcg<br>cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg<br>ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt<br>atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc<br>ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc<br>ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt<br>ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag<br>gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg<br>aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt<br>aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt<br>tggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttc<br>catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC | 577 |

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|---|---|---|
| | CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGATGTCGTTATGACACAATCT<br>CCCTTGAGTTTGCCGGTTACCTTGGGACAACCTGCTAGTATTTCATGTAGGAG<br>TTCTCAAAGTCTCTTGCACTCCTCAGGGAACACCTACCTCGAATGGTACCAAC<br>AACGCCCTGGCCAAAGCCCGCGGCCCTTGATATACAAAATATCAACAAGATTT<br>AGCGGGGTACCCGATAGATTCAGCGGCTCTGGCAGCGGGACGGATTTTACCCT<br>GAAAATTAGTCGCGTAGAAGCTGAAGACGTTGGTGTGTATTACTGCTTTCAAG<br>GGAGCCATGTGCCTTACACATTTGGAGGAGGCACCAAGGTCGAGATTAAGGGA<br>GGGGGTGGATCAGGTGGGGGTGGGTCCGGAGGCGGCGGCAGTCAAGTGCAGTT<br>GGTTCAATCAGGAGCTGAAGTTAAAAAGCCAGGAGCTTCAGTCAAGGTTTCAT<br>GCAAGGCGTCCGGTCTCACTATAGAGGATTACTACATGCACTGGGTGCGGCAA<br>GCTCCAGGCCAGGGGCTGGAGTGGATGGGATGGATTGATCCGGAAAACGGGGA<br>CACAGAGTATGGGCCCAAATTCCAAGGCCGGGTGACAATGACCAGAGATACTA<br>GTATTTCAACAGCATACATGGAGCTGTCACGGCTGAGGTCAGACGATACGGCA<br>GTCTACTATTGTGCAGTACATAACGCACATTATGGTACGTGGTTCGCTTATTG<br>GGGTCAAGGTACCCTGGTCACGGTAAGTTCAAGTGCTGCTGCCTTTGTCCCGG<br>TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC<br>GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC<br>CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT<br>ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT<br>ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT<br>GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG<br>ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA<br>GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA<br>GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA<br>AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC<br>CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC<br>AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT<br>ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG<br>GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG<br>TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT<br>CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA<br>GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC<br>CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT<br>TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT<br>GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA<br>GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC<br>TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC<br>TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA<br>GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG<br>TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-974 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGCAGGTGCAGCTGGTCCAAAGCGGCGCCGAGGTTAAGAA<br>ACCAGGCGCATCCGTCAAGGTTTCATGTAAAGCAAGTGGCTTGACTATAGAAG<br>ACTACTACATGCATTGGGTACGGCAAGCCCCTGGGCAGGGGCTGGAATGGATG<br>GGGTGGATCGACCCGGAGAATGGTGATACAGAGTACGGACCTAAGTTCCAGGG<br>ACGAGTTACCATGACGCGAGATACATCCATCTCCACGGCATACATGGAGCTGA<br>GTCGACTGCGGAGCGATGATACAGCTGTCTATTATTGTGCTGTCCACAATGCG<br>CACTACGGCACCTGGTTCGCTTATTGGGGACAAGGTACCCTGGTCACAGTCAG<br>CTCTGGGGGTGGCGGCAGTGGAGGGGGTGGTTCTGGTGGCGGGGGTTCCGATG<br>TTGTAATGACTCAAAGCCCTCTTTCTTTGCCAGTCACTCTCGGACAACCCGCG<br>AGCATATCTTGCAGGTCTTCACAATCACTCCTTCACAGTAGCGGGAATACTTA<br>CTTGGAGTGGTATCAGCAGCGGCCTGGTCAGTCCCCTAGACCGCTTATATATA<br>AGATCTCCACTAGGTTCAGTGGAGTGCCGGACCGCTTTTCAGGCTCAGGTTCC<br>GGGACGGACTTTACATTGAAAATATCCAGGGTGGAGGCGGAGGACGTCGGAGT<br>CTACTATTGCTTCCAAGGCTCCCACGTCCCATACACTTTCGGTGGCGGTACAA | 578 |

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| | AAGTGGAAATAAAAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG<br>GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | |
| CTX-974 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVV<br>MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKI<br>STRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKV<br>EIKSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 579<br><br>569 (no signal peptide) |
| CTX-974 scFv (nt) | CAGGTGCAGCTGGTCCAAAGCGGCGCCGAGGTTAAGAAACCAGGCGCATCCGT<br>CAAGGTTTCATGTAAAGCAAGTGGCTTGACTATAGAAGACTACTACATGCATT<br>GGGTACGGCAAGCCCCTGGGCAGGGGCTGGAATGGATGGGGTGGATCGACCCG<br>GAGAATGGTGATACAGAGTACGGACCTAAGTTCCAGGGACGAGTTACCATGAC<br>GCGAGATACATCCATCTCCACGGCATACATGGAGCTGAGTCGACTGCGGAGCG<br>ATGATACAGCTGTCTATTATTGTGCTGTCCACAATGCGCACTACGGCACCTGG<br>TTCGCTTATTGGGGACAAGGTACCCTGGTCACAGTCAGCTCTGGGGGTGGCGG<br>CAGTGGAGGGGGTGGTTCTGGTGGCGGGGGTTCCGATGTTGTAATGACTCAAA<br>GCCCTCTTTCTTTGCCAGTCACTCTCGGACAACCCGCGAGCATATCTTGCAGG<br>TCTTCACAATCACTCCTTCACAGTAGCGGGAATACTTACTTGGAGTGGTATCA<br>GCAGCGGCCTGGTCAGTCCCCTAGACCGCTTATATATAAGATCTCCACTAGGT<br>TCAGTGGAGTGCCGGACCGCTTTTCAGGCTCAGGTTCCGGGACGGACTTTACA<br>TTGAAAATATCCAGGGTGGAGGCGGAGGACGTCGGAGTCTACTATTGCTTCCA<br>AGGCTCCCACGTCCCATACACTTTCGGTGGCGGTACAAAAGTGGAAATAAAA | 580 |
| CTX-974 scFv (aa) (linker underlined) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCR<br>SSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFT<br>LKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK | 563 |
| CTX-974 scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 576 |
| CTX-974 scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 534 |
| CTX-974 Donor (nt) LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA | 581 |

GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA
GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA
GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT
CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG
GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA
CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc
gagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggcg
cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagg
gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgc
aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc
tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct
gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt
cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg
cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct
cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga
cgcttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact
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cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg
ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt
atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc
ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc
ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt
ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag
gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg
aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt
aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt
tggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttc
catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC
CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTGCAGCTGGTCCAAAGC
GGCGCCGAGGTTAAGAAACCAGGCGCATCCGTCAAGGTTTCATGTAAAGCAAG
TGGCTTGACTATAGAAGACTACTACATGCATTGGGTACGGCAAGCCCCTGGGC
AGGGGCTGGAATGGATGGGGTGGATCGACCCGGAGAATGGTGATACAGAGTAC
GGACCTAAGTTCCAGGGACGAGTTACCATGACGCGAGATACATCCATCTCCAC
GGCATACATGGAGCTGAGTCGACTGCGGAGCGATGATACAGCTGTCTATTATT
GTGCTGTCCACAATGCGCACTACGGCACCTGGTTCGCTTATTGGGGACAAGGT
ACCCTGGTCACAGTCAGCTCTGGGGGTGGCGGCAGTGGAGGGGGTGGTTCTGG
TGGCGGGGGTTCCGATGTTGTAATGACTCAAAGCCCTCTTTCTTTGCCAGTCA
CTCTCGGACAACCCGCGAGCATATCTTGCAGGTCTTCACAATCACTCCTTCAC
AGTAGCGGGAATACTTACTTGGAGTGGTATCAGCAGCGGCCTGGTCAGTCCCC
TAGACCGCTTATATATAAGATCTCCACTAGGTTCAGTGGAGTGCCGGACCGCT
TTTCAGGCTCAGGTTCCGGGACGGACTTTACATTGAAAATATCCAGGGTGGAG
GCGGAGGACGTCGGAGTCTACTATTGCTTCCAAGGCTCCCACGTCCCATACAC
TTTCGGTGGCGGTACAAAAGTGGAAATAAAAAGTGCTGCTGCCTTTGTCCCGG
TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC
GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC
CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT
ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT
ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT
GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG
ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA
GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA
GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA
AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC
CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC
AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT
ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG
GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG
TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT
CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA
GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC

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|---|---|---|
| | CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT<br>TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT<br>GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA<br>GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC<br>TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC<br>TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA<br>GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG<br>TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-975 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGGATGTAGTTATGACCCAGAGTCCGCTCTCTTTGCCGGT<br>GACGCTCGGCCAACCGGCGTCTATTTCTTGCAGAAGTAGTCAATCACTTCTGC<br>ACTCTAGCGGTAACACTTATTTGGAGTGGTATCTCCAACGACCAGGGCAAAGC<br>CCCAAGCCGTTGATTTATAAGATCTCTACAAGATTCAGCGGAGTGCCCGACAG<br>ATTTTCCGGGAGTGGGTCCGGTACTGATTTCACTTTGAAAATTTCCCGCGTCG<br>AGGCTGAAGATGTTGGTGTCTACTACTGCTTTCAGGGGAGCCATGTTCCATAT<br>ACCTTTGGAGGTGGGACTAAGGTAGAAATTAAAGGTGGGGGTGGATCAGGGGG<br>TGGCGGCAGCGGGGGAGGGGGCTCACAAGTGCAACTTGTGCAAAGTGGGGCCG<br>AGGTGAAAAAACCCGGTGCAAGTGTAAAGGTCTCATGCAAAGCGTCTGGTTTG<br>ACAATTGAAGACTATTATATGCATTGGGTGAGACAGGCCCCGGGCCAAGGCTT<br>GGAATGGATGGGATGGATAGACCCCGAAAACGGTGACACGGAGTACGGACCTA<br>AATTTCAAGGAAGAGTGACAATGACACGCGATACATCTATTAACACGGCTTAT<br>ATGGAACTGAGCCGACTTCGGAGTGATGACACTGCTGTATATTATTGCGCCGT<br>CCACAACGCACATTATGGCACCTGGTTTGCGTACTGGGGACAGGGAACTTTGG<br>TTACAGTATCAAGCAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG<br>GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | 582 |
| CTX-975 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*DVVMTQSPLSLPVTLGQPASISCRSSQSLLHS<br>SGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEA<br>EDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEV<br>KKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKF<br>QGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVT<br>VSSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 583<br><br>570 (no signal peptide) |
| CTX-975 scFv (nt) | GATGTAGTTATGACCCAGAGTCCGCTCTCTTTGCCGGTGACGCTCGGCCAACC<br>GGCGTCTATTTCTTGCAGAAGTAGTCAATCACTTCTGCACTCTAGCGGTAACA<br>CTTATTTGGAGTGGTATCTCCAACGACCAGGGCAAAGCCCCAAGCCGTTGATT<br>TATAAGATCTCTACAAGATTCAGCGGAGTGCCCGACAGATTTTCCGGGAGTGG<br>GTCCGGTACTGATTTCACTTTGAAAATTTCCCGCGTCGAGGCTGAAGATGTTG<br>GTGTCTACTACTGCTTTCAGGGGAGCCATGTTCCATATACCTTTGGAGGTGGG<br>ACTAAGGTAGAAATTAAAGGTGGGGGTGGATCAGGGGGTGGCGGCAGCGGGGG | 584 |

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| | AGGGGGCTCACAAGTGCAACTTGTGCAAAGTGGGGCCGAGGTGAAAAAACCCG<br>GTGCAAGTGTAAAGGTCTCATGCAAAGCGTCTGGTTTGACAATTGAAGACTAT<br>TATATGCATTGGGTGAGACAGGCCCCGGGCCAAGGCTTGGAATGGATGGGATG<br>GATAGACCCCGAAAACGGTGACACGGAGTACGGACCTAAATTTCAAGGAAGAG<br>TGACAATGACACGCGATACATCTATTAACACGGCTTATATGGAACTGAGCCGA<br>CTTCGGAGTGATGACACTGCTGTATATTATTGCGCCGTCCACAACGCACATTA<br>TGGCACCTGGTTTGCGTACTGGGGACAGGGAACTTTGGTTACAGTATCAAGC | |
| CTX-975<br>scFv (aa) (linker<br>underlined) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS | 561 |
| CTX-975<br>scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 568 |
| CTX-975<br>scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 566 |
| CTX-975<br>Donor (nt)<br>LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA<br>GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA<br>GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA<br>GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT<br>CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG<br>GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA<br>CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc<br>gagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggcg<br>cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg<br>gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgc<br>aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc<br>tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct<br>gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt<br>cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg<br>cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct<br>cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga<br>cgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact<br>ggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagcg<br>cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg<br>ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt<br>atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc<br>ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc<br>ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt<br>ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag<br>gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg<br>aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt<br>aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt<br>tggatcttggttcattctcaagcctcagacagtggttcaaagttttttttcttc<br>catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC<br>CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGATGTAGTTATGACCCAGAGT<br>CCGCTCTCTTTGCCGGTGACGCTCGGCCAACCGGCGTCTATTTCTTGCAGAAG<br>TAGTCAATCACTTCTGCACTCTAGCGGTAACACTTATTTGGAGTGGTATCTCC<br>AACGACCAGGGCAAAGCCCCAAGCCGTTGATTTATAAGATCTCTACAAGATTC | 585 |

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|---|---|---|
| | AGCGGAGTGCCCGACAGATTTTCCGGGAGTGGGTCCGGTACTGATTTCACTTT<br>GAAAATTTCCCGCGTCGAGGCTGAAGATGTTGGTGTCTACTACTGCTTTCAGG<br>GGAGCCATGTTCCATATACCTTTGGAGGTGGGACTAAGGTAGAAATTAAAGGT<br>GGGGGTGGATCAGGGGGTGGCGGCAGCGGGGGAGGGGGCTCACAAGTGCAACT<br>TGTGCAAAGTGGGGCCGAGGTGAAAAAACCCGGTGCAAGTGTAAAGGTCTCAT<br>GCAAAGCGTCTGGTTTGACAATTGAAGACTATTATATGCATTGGGTGAGACAG<br>GCCCCGGGCCAAGGCTTGGAATGGATGGGATGGATAGACCCCGAAAACGGTGA<br>CACGGAGTACGGACCTAAATTTCAAGGAAGAGTGACAATGACACGCGATACAT<br>CTATTAACACGGCTTATATGGAACTGAGCCGACTTCGGAGTGATGACACTGCT<br>GTATATTATTGCGCCGTCCACAACGCACATTATGGCACCTGGTTTGCGTACTG<br>GGGACAGGGAACTTTGGTTACAGTATCAAGCAGTGCTGCTGCCTTTGTCCCGG<br>TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC<br>GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC<br>CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT<br>ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT<br>ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT<br>GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG<br>ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA<br>GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA<br>GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA<br>AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC<br>CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC<br>AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT<br>ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG<br>GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG<br>TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT<br>CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA<br>GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC<br>CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT<br>TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT<br>GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA<br>GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC<br>TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC<br>TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA<br>GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG<br>TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-976 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGCAGGTTCAACTGGTTCAGAGTGGAGCAGAGGTAAAAAA<br>GCCCGGAGCGTCCGTCAAAGTGTCATGTAAAGCCTCTGGACTTACTATCGAAG<br>ACTACTACATGCACTGGGTGAGGCAGGCGCCTGGCCAAGGTCTCGAGTGGATG<br>GGTTGGATTGACCCTGAAAATGGAGATACAGAATACGGCCCTAAGTTTCAAGG<br>GCGAGTAACTATGACTCGAGATACGTCAATTAATACGGCATACATGGAGTTGT<br>CTCGGCTCCGATCTGATGACACTGCAGTTTACTATTGTGCCGTCCACAATGCT<br>CATTACGGGACATGGTTCGCTTACTGGGGGCAAGGGACACTCGTAACGGTTAG<br>CTCTGGGGGAGGAGGGTCTGGTGGAGGGGGCTCAGGAGGGGGTGGTAGCGACG<br>TAGTAATGACCCAGTCACCTCTGTCTTTGCCGGTCACGTTGGGCCAGCCTGCA<br>TCCATATCCTGCAGATCCAGCCAGAGCCTCCTGCACAGTAGTGGCAACACGTA<br>TTTGGAATGGTACCTGCAGAGGCCGGGTCAAAGTCCAAAACCGCTGATCTATA<br>AGATATCTACGCGATTTTCAGGGGTGCCGGACCGATTTAGCGGATCAGGAAGT<br>GGAACCGACTTTACGCTCAAGATCAGCCGGGTTGAAGCCGAAGATGTCGGCGT<br>TTACTACTGTTTCCAAGGAAGCCACGTACCCTATACGTTTGGTGGCGGCACGA<br>AGGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG | 586 |

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|---|---|---|
| | GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | |
| CTX-976 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVV<br>MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKI<br>STRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKV<br>EIKSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 587<br><br>571 (no signal peptide) |
| CTX-976 scFv (nt) | CAGGTTCAACTGGTTCAGAGTGGAGCAGAGGTAAAAAAGCCCGGAGCGTCCGT<br>CAAAGTGTCATGTAAAGCCTCTGGACTTACTATCGAAGACTACTACATGCACT<br>GGGTGAGGCAGGCGCCTGGCCAAGGTCTCGAGTGGATGGGTTGGATTGACCCT<br>GAAAATGGAGATACAGAATACGGCCCTAAGTTTCAAGGGCGAGTAACTATGAC<br>TCGAGATACGTCAATTAATACGGCATACATGGAGTTGTCTCGGCTCCGATCTG<br>ATGACACTGCAGTTTACTATTGTGCCGTCCACAATGCTCATTACGGGACATGG<br>TTCGCTTACTGGGGGCAAGGGACACTCGTAACGGTTAGCTCTGGGGGAGGAGG<br>GTCTGGTGGAGGGGGCTCAGGAGGGGGTGGTAGCGACGTAGTAATGACCCAGT<br>CACCTCTGTCTTTGCCGGTCACGTTGGGCCAGCCTGCATCCATATCCTGCAGA<br>TCCAGCCAGAGCCTCCTGCACAGTAGTGGCAACACGTATTTGGAATGGTACCT<br>GCAGAGGCCGGGTCAAAGTCCAAAACCGCTGATCTATAAGATATCTACGCGAT<br>TTTCAGGGGTGCCGGACCGATTTAGCGGATCAGGAAGTGGAACCGACTTTACG<br>CTCAAGATCAGCCGGGTTGAAGCCGAAGATGTCGGCGTTTACTACTGTTTCCA<br>AGGAAGCCACGTACCCTATACGTTTGGTGGCGGCACGAAGGTCGAGATAAAG | 588 |
| CTX-976 scFv (aa) (linker underlined) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCR<br>SSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFT<br>LKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK | 564 |
| CTX-976 scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 568 |
| CTX-976 scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 566 |
| CTX-976 Donor (nt) LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA<br>GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA<br>GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA<br>GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT<br>CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG | 589 |

GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA
CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc
gagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggcg
cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg
gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttttcgc
aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc
tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct
gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt
cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg
cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct
cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga
cgcttttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact
ggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagcg
cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg
ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt
atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc
ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc
ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt
ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag
gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg
aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt
aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt
tggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttc
catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC
CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTTCAACTGGTTCAGAGT
GGAGCAGAGGTAAAAAAGCCCGGAGCGTCCGTCAAAGTGTCATGTAAAGCCTC
TGGACTTACTATCGAAGACTACTACATGCACTGGGTGAGGCAGGCGCCTGGCC
AAGGTCTCGAGTGGATGGGTTGGATTGACCCTGAAAATGGAGATACAGAATAC
GGCCCTAAGTTTCAAGGGCGAGTAACTATGACTCGAGATACGTCAATTAATAC
GGCATACATGGAGTTGTCTCGGCTCCGATCTGATGACACTGCAGTTTACTATT
GTGCCGTCCACAATGCTCATTACGGGACATGGTTCGCTTACTGGGGGCAAGGG
ACACTCGTAACGGTTAGCTCTGGGGGAGGAGGGTCTGGTGGAGGGGGCTCAGG
AGGGGGTGGTAGCGACGTAGTAATGACCCAGTCACCTCTGTCTTTGCCGGTCA
CGTTGGGCCAGCCTGCATCCATATCCTGCAGATCCAGCCAGAGCCTCCTGCAC
AGTAGTGGCAACACGTATTTGGAATGGTACCTGCAGAGGCCGGGTCAAAGTCC
AAAACCGCTGATCTATAAGATATCTACGCGATTTTCAGGGGTGCCGGACCGAT
TTAGCGGATCAGGAAGTGGAACCGACTTTACGCTCAAGATCAGCCGGGTTGAA
GCCGAAGATGTCGGCGTTTACTACTGTTTCCAAGGAAGCCACGTACCCTATAC
GTTTGGTGGCGGCACGAAGGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCGG
TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC
GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC
CGCCGCCGGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT
ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT
ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT
GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG
ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA
GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA
GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA
AACGCCGGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC
CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC
AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT
ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG
GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG
TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT
CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA
GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC
CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT
TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT
GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA
GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC

| | | |
|---|---|---|
| | TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC<br>TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA<br>GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG<br>TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-977 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGGACGTTGTGATGACGCAGTCTCCTCTGAGCCTGCCAGT<br>TACGTTGGGGCAACCCGCATCAATATCTTGTAGGTCCAGTCAGAGCCTGCTTC<br>ACAGCTCTGGCAACACTTACTTGGAATGGTACCTCCAGAGACCTGGACAGAGT<br>CCCAAGCCATTGATTTACAAGATTTCAACGCGATTTAGTGGAGTGCCCGATCG<br>ATTCTCTGGGAGTGGCTCTGGGACTGATTTCACACTTAAAATAAGTAGGGTGG<br>AGGCTGAAGATGTGGGTGTATATTATTGTTTTCAAGGGTCCCATGTCCCTTAC<br>ACTTTCGGCGGCGGCACCAAAGTTGAGATCAAAGGTGGTGGTGGGTCCGGCGG<br>TGGAGGCAGTGGGGGTGGCGGGTCACAAGTTCAACTTGTCCAGTCAGGGGCTG<br>AAGTAAAAAAGCCTGGTGCATCAGTTAAAGTTTCATGTAAGGCTTCCGGCCTT<br>ACCATTGAAGATTACTATATGCACTGGGTTAGACAAGCTCCTGGACAAGGTCT<br>GGAGTGGATGGGCTGGATAGACCCCGAGAATGGTGACACAGAATACGGGCCTA<br>AGTTCCAGGGTAGGGTAACAATGACGCGGGATACATCCATTTCCACAGCTTAC<br>ATGGAACTGAGTAGACTCAGATCTGACGACACTGCTGTCTACTATTGTGCCGT<br>CCATAACGCGCATTATGGCACTTGGTTCGCATATTGGGGGCAAGGCACTCTTG<br>TTACAGTGTCCTCAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG<br>GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG<br>CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | 590 |
| CTX-977 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*DVVMTQSPLSLPVTLGQPASISCRSSQSLLHS<br>SGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEA<br>EDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEV<br>KKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKF<br>QGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVT<br>VSSSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 591<br><br>572 (no signal peptide) |
| CTX-977 scFv (nt) | GACGTTGTGATGACGCAGTCTCCTCTGAGCCTGCCAGTTACGTTGGGGCAACC<br>CGCATCAATATCTTGTAGGTCCAGTCAGAGCCTGCTTCACAGCTCTGGCAACA<br>CTTACTTGGAATGGTACCTCCAGAGACCTGGACAGAGTCCCAAGCCATTGATT<br>TACAAGATTTCAACGCGATTTAGTGGAGTGCCCGATCGATTCTCTGGGAGTGG<br>CTCTGGGACTGATTTCACACTTAAAATAAGTAGGGTGGAGGCTGAAGATGTGG<br>GTGTATATTATTGTTTTCAAGGGTCCCATGTCCCTTACACTTTCGGCGGCGGC<br>ACCAAAGTTGAGATCAAAGGTGGTGGTGGGTCCGGCGGTGGAGGCAGTGGGGG<br>TGGCGGGTCACAAGTTCAACTTGTCCAGTCAGGGGCTGAAGTAAAAAAGCCTG<br>GTGCATCAGTTAAAGTTTCATGTAAGGCTTCCGGCCTTACCATTGAAGATTAC<br>TATATGCACTGGGTTAGACAAGCTCCTGGACAAGGTCTGGAGTGGATGGGCTG<br>GATAGACCCCGAGAATGGTGACACAGAATACGGGCCTAAGTTCCAGGGTAGGG | 592 |

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|---|---|---|
| | TAACAATGACGCGGGATACATCCATTTCCACAGCTTACATGGAACTGAGTAGA<br>CTCAGATCTGACGACACTGCTGTCTACTATTGTGCCGTCCATAACGCGCATTA<br>TGGCACTTGGTTCGCATATTGGGGGCAAGGCACTCTTGTTACAGTGTCCTCA | |
| CTX-977<br>scFv (aa) (linker<br>underlined) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS | 562 |
| CTX-977<br>scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 576 |
| CTX-977<br>scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 566 |
| CTX-977<br>Donor (nt)<br>LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA<br>GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA<br>GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA<br>GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT<br>CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG<br>GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA<br>CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc<br>gagaagttgggggggaggggtcggcaattgaaccggtgcctagagaaggtggcg<br>cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg<br>gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgc<br>aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc<br>tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct<br>gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt<br>cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg<br>cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct<br>cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga<br>cgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact<br>ggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagcg<br>cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg<br>ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt<br>atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc<br>ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc<br>ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt<br>ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag<br>gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg<br>aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt<br>aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt<br>tggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttc<br>catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC<br>CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGACGTTGTGATGACGCAGTCT<br>CCTCTGAGCCTGCCAGTTACGTTGGGGCAACCCGCATCAATATCTTGTAGGTC<br>CAGTCAGAGCCTGCTTCACAGCTCTGGCAACACTTACTTGGAATGGTACCTCC<br>AGAGACCTGGACAGAGTCCCAAGCCATTGATTTACAAGATTTCAACGCGATTT<br>AGTGGAGTGCCCGATCGATTCTCTGGGAGTGGCTCTGGGACTGATTTCACACT<br>TAAAATAAGTAGGGTGGAGGCTGAAGATGTGGGTGTATATTATTGTTTTCAAG<br>GGTCCCATGTCCCTTACACTTTCGGCGGCGGCACCAAAGTTGAGATCAAAGGT<br>GGTGGTGGGTCCGGCGGTGGAGGCAGTGGGGGTGGCGGGTCACAAGTTCAACT | 593 |

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|---|---|---|
| | TGTCCAGTCAGGGGCTGAAGTAAAAAAGCCTGGTGCATCAGTTAAAGTTTCAT<br>GTAAGGCTTCCGGCCTTACCATTGAAGATTACTATATGCACTGGGTTAGACAA<br>GCTCCTGGACAAGGTCTGGAGTGGATGGGCTGGATAGACCCCGAGAATGGTGA<br>CACAGAATACGGGCCTAAGTTCCAGGGTAGGGTAACAATGACGCGGGATACAT<br>CCATTTCCACAGCTTACATGGAACTGAGTAGACTCAGATCTGACGACACTGCT<br>GTCTACTATTGTGCCGTCCATAACGCGCATTATGGCACTTGGTTCGCATATTG<br>GGGGCAAGGCACTCTTGTTACAGTGTCCTCAAGTGCTGCTGCCTTTGTCCCGG<br>TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC<br>GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC<br>CGCCGCCGGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT<br>ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT<br>ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT<br>GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG<br>ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA<br>GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA<br>GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA<br>AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC<br>CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC<br>AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT<br>ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG<br>GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG<br>TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT<br>CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA<br>GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC<br>CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT<br>TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT<br>GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA<br>GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC<br>TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC<br>TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA<br>GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG<br>TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT<br>GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-978 CAR 41BB co-stim (nt) | CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTC<br>CACGCAGCAAGGCCGCAGGTACAACTCGTTCAGAGCGGTGCAGAGGTTAAGAA<br>ACCGGGCGCCAGTGTCAAAGTATCATGCAAGGCGAGTGGTCTGACCATCGAAG<br>ATTATTATATGCATTGGGTGAGACAAGCACCGGGGCAGGGGCTCGAATGGATG<br>GGTTGGATCGACCCCGAAAATGGTGATACGGAGTATGGCCCGAAATTTCAGGG<br>TCGGGTCACGATGACCCGCGATACAAGCATCAGTACTGCATACATGGAGCTCT<br>CTCGCTTGCGGAGTGATGATACCGCCGTTTATTATTGCGCGGTTCACAACGCT<br>CATTATGGCACTTGGTTCGCGTATTGGGGCCAAGGAACACTGGTTACAGTGAG<br>CAGTGGAGGGGGTGGCTCTGGTGGCGGCGGGAGCGGCGGAGGGGGCAGTGATG<br>TTGTGATGACACAGTCACCCCTGAGTCTCCCGGTCACTCTTGGGCAACCAGCC<br>AGCATAAGCTGTCGCAGTTCTCAGAGCTTGCTCCATAGCTCCGGGAATACCTA<br>CCTCGAATGGTATCTCCAAAGACCCGGTCAATCTCCAAAGCCTTTGATTTACA<br>AGATTAGTACACGATTTAGTGGGGTCCCAGATAGATTTTCAGGTAGTGGATCT<br>GGTACAGATTTCACATTGAAAATATCACGCGTCGAGGCGGAGGATGTCGGGGT<br>CTACTATTGCTTTCAAGGTAGTCACGTGCCCTACACGTTTGGTGGCGGTACGA<br>AGGTCGAAATCAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA<br>CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTC<br>TCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTG<br>TTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG<br>GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAA<br>TCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC<br>CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGA<br>TTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAG | 594 |

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|---|---|---|
| | CGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGA<br>ATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGAGAC<br>CCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA<br>TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGG<br>GCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACG<br>GCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATA<br>AT | |
| CTX-978 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVV<br>MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKI<br>STRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKV<br>EIKSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 595<br><br>598 (no signal peptide) |
| CTX-978 scFv (nt) | CAGGTACAACTCGTTCAGAGCGGTGCAGAGGTTAAGAAACCGGGCGCCAGTGT<br>CAAAGTATCATGCAAGGCGAGTGGTCTGACCATCGAAGATTATTATATGCATT<br>GGGTGAGACAAGCACCGGGGCAGGGGCTCGAATGGATGGGTTGGATCGACCCC<br>GAAAATGGTGATACGGAGTATGGCCCGAAATTTCAGGGTCGGGTCACGATGAC<br>CCGCGATACAAGCATCAGTACTGCATACATGGAGCTCTCTCGCTTGCGGAGTG<br>ATGATACCGCCGTTTATTATTGCGCGGTTCACAACGCTCATTATGGCACTTGG<br>TTCGCGTATTGGGGCCAAGGAACACTGGTTACAGTGAGCAGTGGAGGGGGTGG<br>CTCTGGTGGCGGCGGGAGCGGCGGAGGGGGCAGTGATGTTGTGATGACACAGT<br>CACCCCTGAGTCTCCCGGTCACTCTTGGGCAACCAGCCAGCATAAGCTGTCGC<br>AGTTCTCAGAGCTTGCTCCATAGCTCCGGGAATACCTACCTCGAATGGTATCT<br>CCAAAGACCCGGTCAATCTCCAAAGCCTTTGATTTACAAGATTAGTACACGAT<br>TTAGTGGGGTCCCAGATAGATTTTCAGGTAGTGGATCTGGTACAGATTTCACA<br>TTGAAAATATCACGCGTCGAGGCGGAGGATGTCGGGGTCTACTATTGCTTTCA<br>AGGTAGTCACGTGCCCTACACGTTTGGTGGCGGTACGAAGGTCGAAATCAAG | 596 |
| CTX-978 scFv (aa) (linker underlined) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCR<br>SSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFT<br>LKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK | 565 |
| CTX-978 scFv VH (aa) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSS | 576 |
| CTX-978 scFv VL (aa) | DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLI<br>YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG<br>TKVEIK | 566 |
| CTX-978 Donor (nt) LHA to RHA | GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGT<br>AGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTCAAAACCTCTATCA<br>ATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA<br>TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTC<br>CAGATTCCAAGATGTACAGTTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCT<br>TTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAA<br>AAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGC<br>AGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCAAGAT<br>TGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTTTCT<br>AAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGA<br>GCCCCGCCCTTGTCCATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAA<br>GAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCA<br>GAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGT<br>CTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG<br>GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGGA<br>CTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtcccc<br>gagaagttgggggggagggtcggcaattgaaccggtgcctagagaaggtggcg<br>cggggtaaactgggaaagtgatgtcgtgtactggctccgccttttttcccgagg | 597 |

gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgc
aacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcc
tggcctctttacgggttatggcccttgcgtgccttgaattacttccactggct
gcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagtt
cgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctgg
cctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtct
cgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcga
cgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacact
ggtatttcggtttttgggggccgcgggcggcgacggggcccgtgcgtcccagcg
cacatgttcggcgaggcggggcctgcgagcgcggccaccgagaatcggacggg
ggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgt
atcgccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagc
ggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgc
ggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttt
ccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccag
gcacctcgattagttctcgagcttttggagtacgtcgtctttaggttgggggg
aggggttttatgcgatggagtttccccacactgagtgggtggagactgaagtt
aggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtt
tggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttc
catttcaggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCC
CCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTACAACTCGTTCAGAGC
GGTGCAGAGGTTAAGAAACCGGGCGCCAGTGTCAAAGTATCATGCAAGGCGAG
TGGTCTGACCATCGAAGATTATTATATGCATTGGGTGAGACAAGCACCGGGGC
AGGGGCTCGAATGGATGGGTTGGATCGACCCCGAAAATGGTGATACGGAGTAT
GGCCCGAAATTTCAGGGTCGGGTCACGATGACCCGCGATACAAGCATCAGTAC
TGCATACATGGAGCTCTCTCGCTTGCGGAGTGATGATACCGCCGTTTATTATT
GCGCGGTTCACAACGCTCATTATGGCACTTGGTTCGCGTATTGGGGCCAAGGA
ACACTGGTTACAGTGAGCAGTGGAGGGGGTGGCTCTGGTGGCGGCGGGAGCGG
CGGAGGGGGCAGTGATGTTGTGATGACACAGTCACCCCTGAGTCTCCCGGTCA
CTCTTGGGCAACCAGCCAGCATAAGCTGTCGCAGTTCTCAGAGCTTGCTCCAT
AGCTCCGGGAATACCTACCTCGAATGGTATCTCCAAAGACCCGGTCAATCTCC
AAAGCCTTTGATTTACAAGATTAGTACACGATTTAGTGGGGTCCCAGATAGAT
TTTCAGGTAGTGGATCTGGTACAGATTTCACATTGAAAATATCACGCGTCGAG
GCGGAGGATGTCGGGGTCTACTATTGCTTTCAAGGTAGTCACGTGCCCTACAC
GTTTGGTGGCGGTACGAAGGTCGAAATCAAGAGTGCTGCTGCCTTTGTCCCGG
TATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC
GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC
CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTT
ACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT
ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAAACTCCT
GTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAG
ATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGA
GTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCA
GCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA
AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCC
CAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTC
AGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT
ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG
GCCCTGCCTCCCAGATAATAATAAAATCGCTATCCATCGAAGATGGATGTGTG
TTGGTTTTTTGTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTT
CAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCA
GCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGC
CCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCT
TATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCT
GGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGA
GAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCC
TTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCC
TTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCA
GCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG
TGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGAT

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|---|---|---|
| | GAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG<br>CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGA<br>GAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGC<br>TACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCT<br>GGGACAGGAGCTCAATGAGAAAGG | |
| CTX-979 CAR 41BB co-stim (aa) | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVV<br>MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKI<br>STRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKV<br>EIKSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPF<br>MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE<br>RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | 546<br><br>599 (no signal peptide) |
| CTX-979b CAR CD28 co-stim (aa) | *MALPVTALLLPLALLLHAARP*QVQLVQSGAEVKKPGASVKVSCKASGLTIEDY<br>YMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSR<br>LRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVV<br>MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKI<br>STRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKV<br>EIKSAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH<br>TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMT<br>PRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR<br>REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR<br>RGKGHDGLYQGLSTATKDTYDALHMQALPPR | 544<br><br>608 (no signal peptide) |
| CTX-979 and CTX-979b scFv (aa) (linker underlined) | QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP<br>ENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTW<br>FAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCR<br>SSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFT<br>LKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK | 548 |
| CTX-979 and CTX-979b scFv VH (aa) CDRs - in bold | QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPGQGLEWMG**WIDP<br>ENGDTEYGPKFQG**RVTMTRDTSINTAYMELSRLRSDDTAVYYCAV**HNAHYGTW<br>FAY**WGQGTLVTVSS | 533 |
| CTX-979 and CTX-979b scFv VL (aa) CDRs - in bold | DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQRPGQSPRPLI<br>Y**KISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGSHVPYT**FGGG<br>TKVEIK | 534 |

## OTHER EMBODIMENTS

In at least some of the previously described embodiments, one or more elements used in an embodiment can interchangeably be used in another embodiment unless such a replacement is not technically feasible. It will be appreciated by those skilled in the art that various other omissions, additions and modifications may be made to the methods and structures described above without departing from the scope of the claimed subject matter. All such modifications and changes are intended to fall within the scope of the subject matter, as defined by the appended claims.

With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to

the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Any reference to "or" herein is intended to encompass "and/or" unless otherwise stated.

It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to "at least one of A, B, and C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., " a system having at least one of A, B, and C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to "at least one of A, B, or C, etc." is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., " a system having at least one of A, B, or C" would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C

together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms.

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

**WHAT IS CLAIMED IS:**

1. An engineered T cell, comprising:

a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an ectodomain that binds specifically to LIV1; and

at least one of

(i) a disrupted *Regnase-1* (*Reg1*) gene, and

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

2. The engineered T cell of claim 1, comprising both (i) and (ii).

3. The engineered T cell of claim 1 or 2, further comprising a disrupted T cell receptor alpha chain constant region (*TRAC*) gene.

4. The engineered T cell of any one of claims 1-3, further comprising a disrupted beta-2-microglobulin (*β2M*) gene.

5. The engineered T cell of any one of claims 1-4, wherein the ectodomain of the CAR comprises an anti-LIV1 antigen-binding fragment.

6. The engineered T cell of claim 5, wherein the anti-LIV1 antigen-binding fragment is an anti-LIV1 single-chain variable fragment (scFv).

7. The engineered T cell of claim 6, wherein the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody, wherein the reference antibody comprises:

(i) a VH comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 533 and a VL comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 534;

(ii) a VH comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 568 and a VL comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 566.

8. The engineered T cell of claim 7, wherein the reference antibody comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 533 and a VL comprising the amino acid sequence of SEQ ID NO: 534 or

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 568 and a VL comprising the amino acid sequence of SEQ ID NO: 566.

9. The engineered T cell of claim 6, wherein the anti-LIV1 scFv comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 532, 548, 561, and 564.

10. The engineered T cell of claim 9, wherein the anti-LIV1 scFv comprises an amino acid sequence having the amino acid sequence of any one of SEQ ID NOs: 532, 548, 561, and 564.

11. The engineered T cell of any one of claims 1-10, wherein the CAR further comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

12. The engineered T cell of claim 10 or claim 11, wherein the CAR further comprises a CD3ζ cytoplasmic signaling domain, a CD8 transmembrane domain, or both.

13. The engineered T cell of claim 1, wherein the CAR comprises an amino acid sequence selected from any one of SEQ ID NOs: 600, 601, 570, and 571; optionally wherein the CAR comprises an amino acid sequence selected from any one of SEQ ID NOs: 528, 530, 583, and 587.

14. The engineered T cell of claim 13, wherein the CAR is encoded by a nucleotide sequence comprising a nucleic acid sequence that is at least 90% identical to any

one of SEQ ID NOs: 527, 529, 582, or 586; optionally wherein the CAR is encoded by the nucleotide sequence of any one of SEQ ID NOs: 527, 529, 582, and 586.

15. The engineered T cell of any one of claims 4-13, wherein the nucleic acid encoding the CAR is inserted into the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, or the disrupted *β2M* gene.

16. The engineered T cell of claim 14, wherein the nucleic acid encoding the CAR is inserted into the disrupted *TRAC* gene, optionally wherein the nucleic acid encoding the CAR replaces a fragment comprising SEQ ID NO: 69 in the *TRAC* gene.

17. The engineered T cell of any one of claims 1-15, wherein the nucleic acid encoding the CAR comprises the nucleotide sequence of any one of SEQ ID NOs: 527, 529, 582, and 586; optionally wherein the nucleic acid comprises the nucleotide sequence of any one of SEQ ID NOs: 541, 542, 585, and 589.

18. The engineered T cell of any one of claims 1-16, wherein the disrupted *Reg1* gene comprises a nucleotide sequence listed in Sequence Table 10, 12, 13, or 17; and/or the disrupted *β2M* comprises a nucleotide sequence listed in Sequence Table 4.

19. The engineered T cell of any one of claims 1-16, wherein the disrupted *Reg1* gene is genetically edited in exon 2 and/or exon 4.

20. The engineered T cell of any one of claims 1-19, 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.

21. The engineered T cell of any one of claims 1-20, wherein the disrupted *Reg1* gene, the disrupted *TGFBRII* gene, the disrupted *TRAC* gene, and/or the disrupted *β2M* gene are genetically edited by a CRISPR/Cas-mediated gene editing system.

22. The engineered T cell of claim 21, wherein the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *TRAC* gene that comprises SEQ ID NO: 69.

23. The engineered T cell of claim 21 or 22, wherein the CRISPR/Cas-mediated gene editing system comprises a guide RNA (gRNA) targeting a site in the *Reg1* gene that comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 320, 322, 323, and 327.

24. The engineered T cell of any one of 21-23, 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.

25. The engineered T cell of any one of claims 21-24, wherein:

the gRNA targeting the *Reg1* gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 22, 30, 34, and 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, and 312; and/or

the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 59.

26. The engineered T cell of any one of 1-25, wherein the T cell is a human T cell.

27. An engineered T cell, comprising:

(i) a disrupted *TRAC* gene comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the disrupted *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 585;

(ii) a disrupted *Regnase-1* (*Reg1*) gene; and

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

28. A population of engineered T cells, wherein the engineered T cells collectively comprise:

a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an ectodomain that binds specifically to LIV1; and

at least one of

(i) a disrupted *Regnase-1* (*Reg1*) gene, and

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

29. The population of engineered T cells of claim 28, wherein the engineered T cells further comprise a disrupted *TRAC* gene.

29. The population of engineered T cells of claim 29, wherein the engineered T cells further comprise a disrupted *β2M* gene.

30. The population of engineered T cells of any one of claims 28-29, wherein the CAR is set forth in any one of claims 5-14.

31. The population of engineered T cells of claim 29-30, wherein the nucleic acid encoding the CAR is inserted in the disrupted *TRAC* gene, the disrupted TGFBRII gene, the disrupted Reg1 gene, or the disrupted *β2M* gene; optionally wherein the nucleic acid encoding the CAR is inserted in the disrupted TRAC gene.

32. The population of engineered T cells of claim 29, which comprises engineered T cells set forth in any one of claims 1-27.

33. A population of engineered T cells, wherein the engineered T cells collectively comprise:

a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises the amino acid sequence of SEQ ID NO: 570 or 583;

a disrupted *TRAC* gene, which comprises the nucleic acid encoding the CAR;

a disrupted *Regnase-1* (*Reg1*) gene,

a disrupted Transforming Growth Factor Beta Receptor II *(TGFBRII)* gene; and optionally

a disrupted *B2M* gene.

34. The population of engineered T cells, wherein the nucleic acid encoding the CAR is inserted into the disrupted *TRAC* gene; optionally wherein the disrupted *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 585.

35. The population of any one of claims 28-34, wherein at least 15%, 30%, 50% or 70% of the engineered T cells of the population express the CAR.

36. The population of cells of any one of claims 28-35, wherein at least 50% of the engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein; optionally wherein at least 30% of the engineered T cells of the population do not express a detectable B2M surface protein.

37. A method of producing engineered T cells, comprising:

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

(b) delivering to the plurality of cells a nucleic acid encoding a chimeric antigen receptor (CAR) that comprise (1) an ectodomain that binds specifically to LIV1;

(c) genetically editing the *Reg1* gene, the *TGFBRII* gene, or both; and

(d) producing engineered T cells expressing the CAR and having a disrupted *Reg1* gene and/or a disrupted *TGFBRII* gene.

38. The method of claim 37, wherein step (c) comprises genetically editing both the *Reg1* gene and the *TGFBRII* gene.

39. The method of claim 37 or38, wherein step (b) and/or step (c) is performed by one or more CRISPR/Cas-mediated gene editing systems.

40. The method of any one of claims 37-39, wherein step (c) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *Reg1* gene.

41. The method of claim 40, 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.

42. The method of any one of claims 37-41, wherein step (c) is performed by delivering to the plurality of cells an RNA-guided nuclease and a gRNA targeting the *TGFBRII* gene.

43. The method of claim 42, 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; and optionally wherein the gRNA targeting the *TGFBRII* gene is specific to exon 4 or exon 5.

44. The method of any one of claims 37-43, wherein the CAR is set forth in any one of claims 5-14.

45. The method of any one of claims 37-44, wherein the nucleic acid encoding the CAR is in an AAV vector.

46. The method of any one of claims 37-45, 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.

47. The method of claim 46, wherein the genomic locus is in the *Reg1* gene, in the *TGFBRII* gene, in the *TRAC* gene, or in the *β2M* gene; optionally wherein the genomic locus is in the *TRAC* gene.

48. The method of claim 47, wherein step (b) comprising disrupting the *TRAC* gene by a CRISPR/Cas-mediated gene editing system comprising an RNA-guided nuclease and a gRNA targeting a *TRAC* gene, and the nucleic acid encoding the CAR is inserted at the site targeted by the gRNA.

49. The method of claim48, wherein the gRNA targeting a *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 61; optionally wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 59.

50. The method of claim 48 or claim 49, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the site of the *TRAC* gene that is targeted by the gRNA.

51. The method of any one of claims 37-50, wherein the nucleic acid comprises the nucleotide sequence of any one of SEQ ID NOs: 541, 542, 585, and 589.

52. The method of any one of claims 37-51, further comprising genetically editing the *β2M* gene.

53. The method of claim 52, wherein genetically editing the *β2M* gene comprises delivering to the plurality of cells a gRNA targeting the *β2M* gene.

54. The method of claim 53, wherein the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 65; optionally wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 63.

55. The method of any one of claims 37-54, wherein the RNA-guided nuclease is a Cas9 nuclease, optionally wherein the Cas9 nuclease is a *S. pyogenes* Cas9 nuclease.

56. A method for inhibiting LIV1$^+$ cells in a subject, comprising administering to a subject in need there of the engineered T cell of any one of claims 1-27 or the population of engineered T cells of any one of claims 28-36.

57. The method of claim 56, wherein the subject is a human subject.

58. The method of claim 56 or claim 57, wherein the subject has a $LIV1^+$ cancer.

59. The method of claim 58, wherein the cancer is a solid tumor cancer.

60. The method of claim 59, wherein the solid tumor cancer is breast cancer, prostate cancer, squamous tumor cancer, or neuronal tumor cancer.

61. An engineered T cell or a population of engineered T cells for use in the treatment of cancer, wherein the engineered T cell is set forth in any one of claims 1-27, and wherein the population of engineered T cells is set forth in any one of claims 28-36.

FIG. 1A


971 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPR
973 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPR
975 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPK
977 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPK
PLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP
YTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCK
ASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD
TSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS
TSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS

FIG. 1B


979 QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
974 QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
976 QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
978 QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
972 QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
979 IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
974 IDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHN
976 IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
978 IDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHN
972 IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
979 AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
974 AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
976 AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
978 AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
972 AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
979 GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPKPLIYKISTRFSGVPD
974 GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPKPLIYKISTRFSGVPD
976 GQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPD
978 GQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPD
972 GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPKPLIYKISTRFSGVPD
979 RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
974 RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
976 RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
978 RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
972 RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT-GGGTKVEIK

FIG. 2

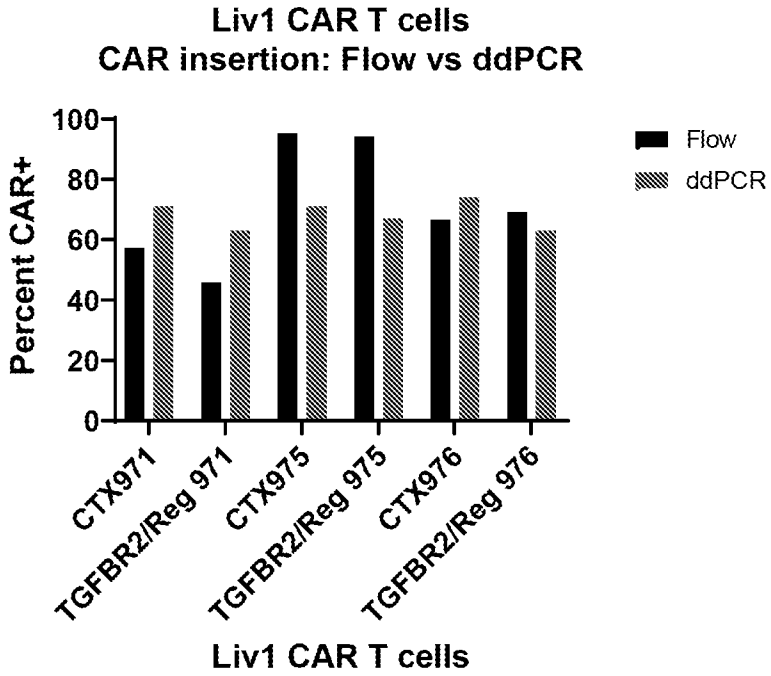

Liv1 CAR T cells
CAR insertion: Flow vs ddPCR
Percent CAR+
100
80
60
40
20
0
Flow
ddPCR
CTX971
TGFBR2/Reg 971
CTX975
TGFBR2/Reg 975
CTX976
TGFBR2/Reg 976
Liv1 CAR T cells

FIG. 3

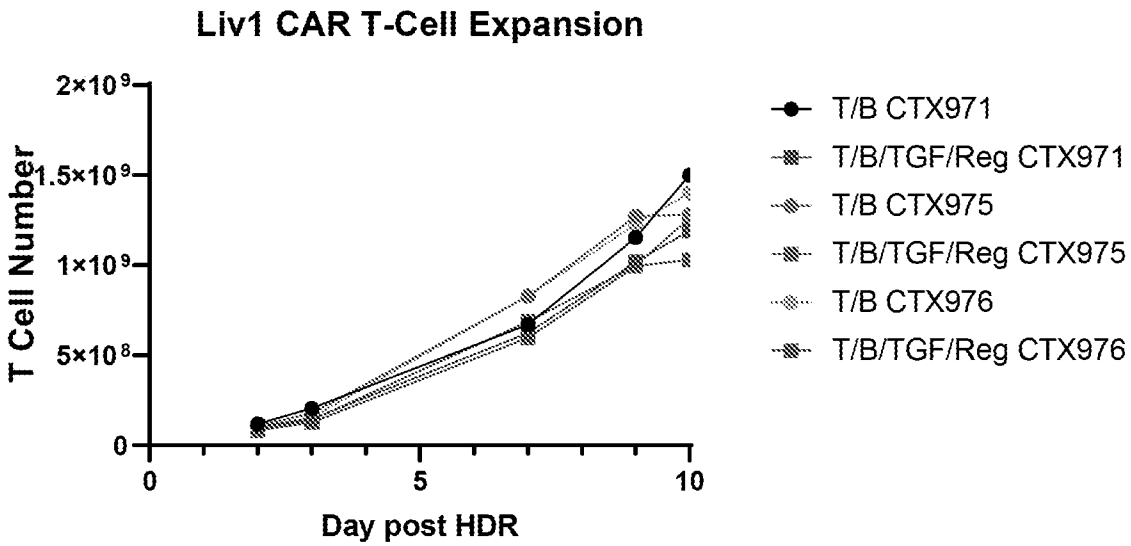

Liv1 CAR T-Cell Expansion
T Cell Number
2×10⁹
1.5×10⁹
1×10⁹
5×10⁸
0
0
5
10
Day post HDR
T/B CTX971
T/B/TGF/Reg CTX971
T/B CTX975
T/B/TGF/Reg CTX975
T/B CTX976
T/B/TGF/Reg CTX976

FIG. 4A

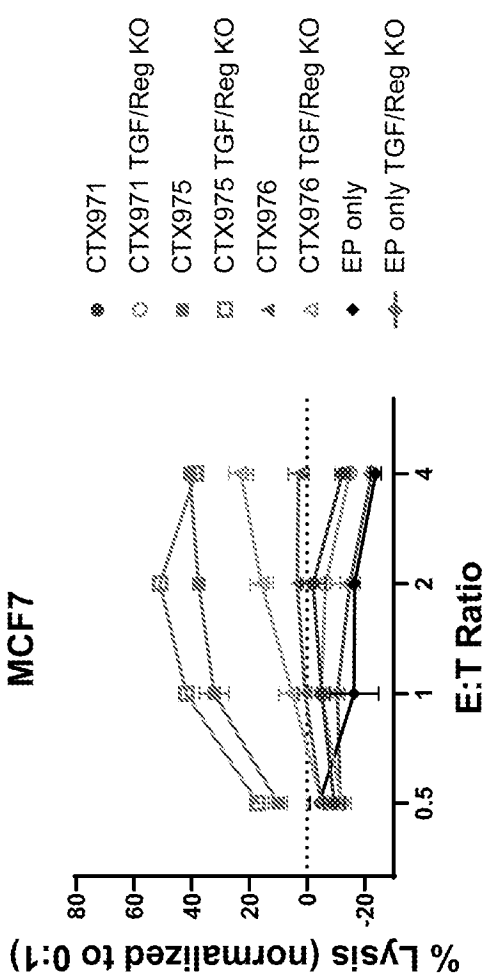

MCF7
CTX971
CTX971 TGF/Reg KO
CTX975
CTX975 TGF/Reg KO
CTX976
CTX976 TGF/Reg KO
EP only
EP only TGF/Reg KO
% Lysis (normalized to 0:1)
80
60
40
20
0
-20
0.5
1
2
4
E:T Ratio

FIG. 4B

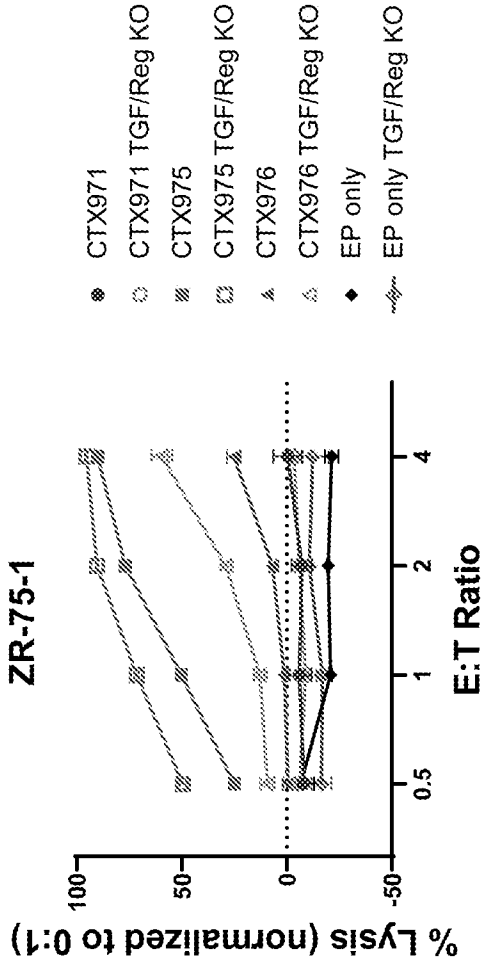

ZR-75-1
CTX971
CTX971 TGF/Reg KO
CTX975
CTX975 TGF/Reg KO
CTX976
CTX976 TGF/Reg KO
EP only
EP only TGF/Reg KO
% Lysis (normalized to 0:1)
100
50
0
-50
0.5
1
2
4
E:T Ratio

FIG. 5A

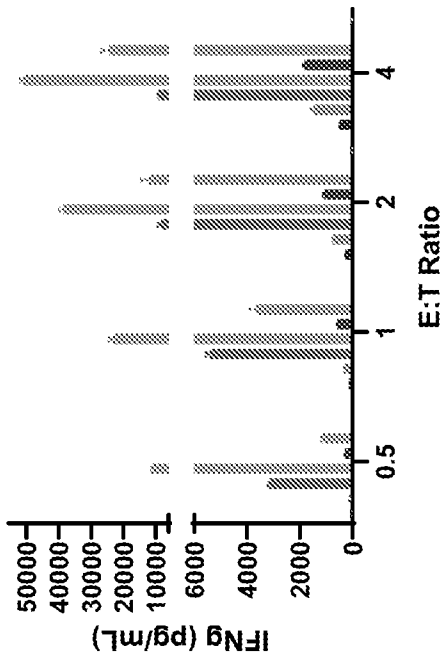

MCF7 Co-culture
IFNg Secretion
IFNg (pg/mL)
50000
40000
30000
20000
10000
6000
4000
2000
0
0.5
1
2
4
E:T Ratio

FIG. 5B

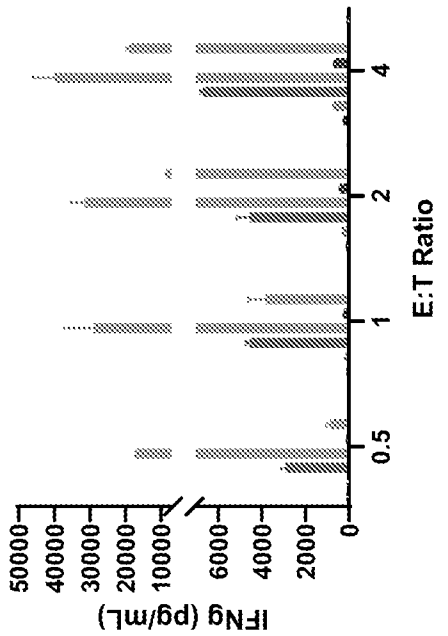

ZR-75-1 Co-culture
IFNg Secretion
IFNg (pg/mL)
50000
40000
30000
20000
10000
6000
4000
2000
0
0.5
1
2
4
E:T Ratio

FIG. 5C

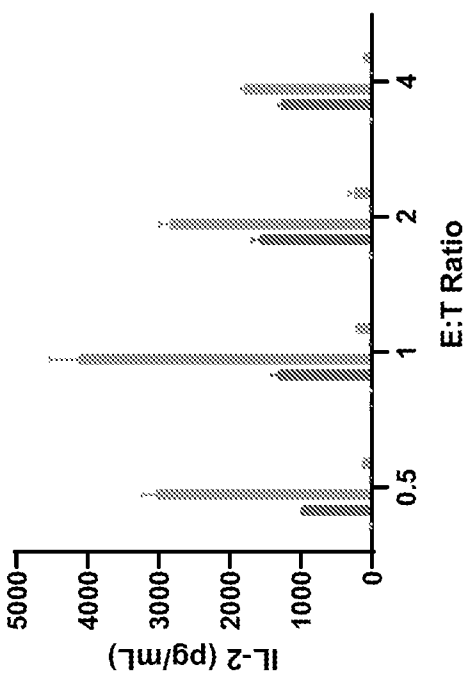

MCF7 Co-culture
IL2 Secretion
IL-2 (pg/mL)
5000
4000
3000
2000
1000
0
0.5
1
2
4
E:T Ratio

FIG. 5D

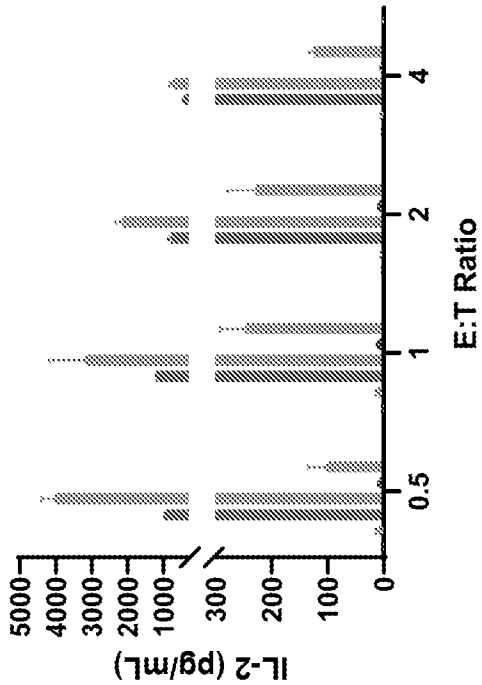

ZR-75-1 Co-culture
IL2 Secretion
IL-2 (pg/mL)
5000
4000
3000
2000
1000
300
200
100
0
0.5
1
2
4
E:T Ratio

FIG. 6

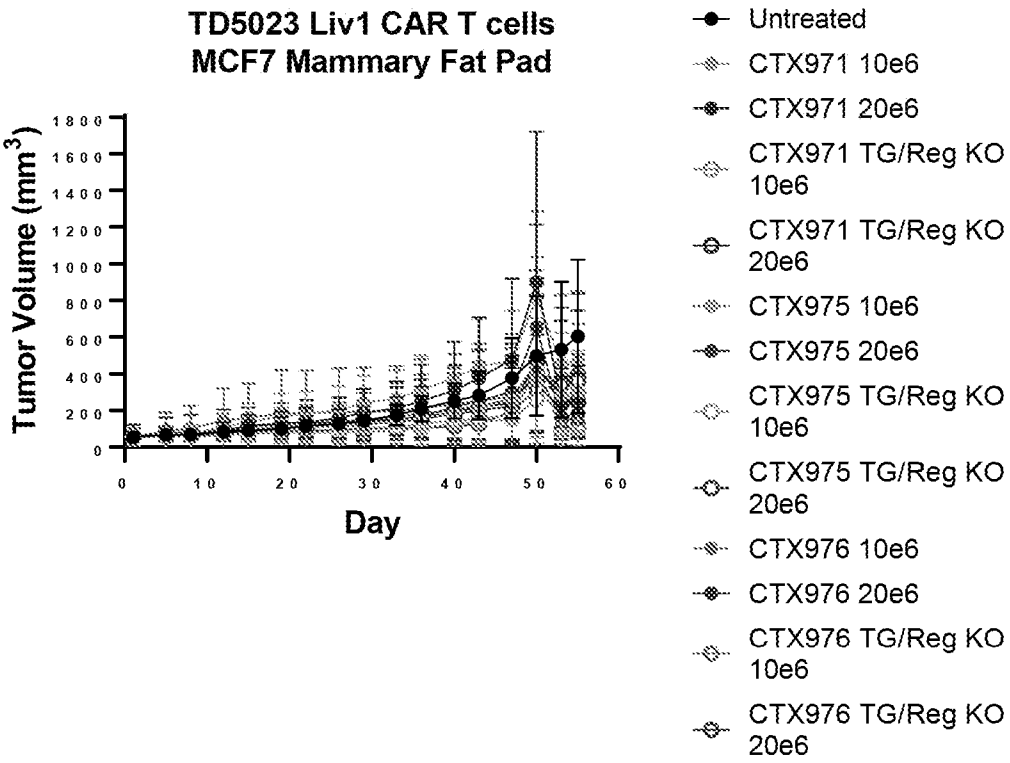

TD5023 Liv1 CAR T cells
MCF7 Mammary Fat Pad
Tumor Volume (mm3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
Day
Untreated
CTX971 10e6
CTX971 20e6
CTX971 TG/Reg KO 10e6
CTX971 TG/Reg KO 20e6
CTX975 10e6
CTX975 20e6
CTX975 TG/Reg KO 10e6
CTX975 TG/Reg KO 20e6
CTX976 10e6
CTX976 20e6
CTX976 TG/Reg KO 10e6
CTX976 TG/Reg KO 20e6

FIG. 7A

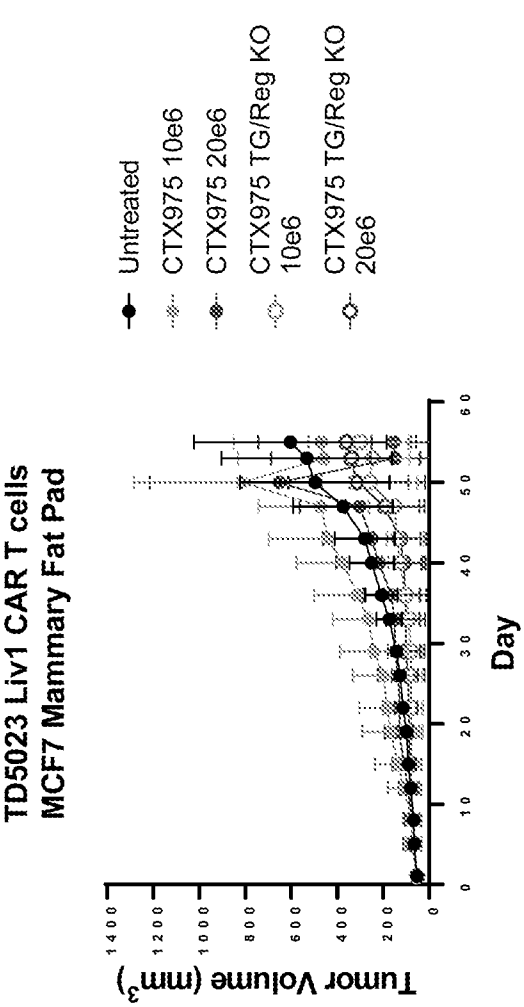

TD5023 Liv1 CAR T cells
MCF7 Mammary Fat Pad
Untreated
CTX975 10e6
CTX975 20e6
CTX975 TG/Reg KO 10e6
CTX975 TG/Reg KO 20e6
Tumor Volume (mm3)
Day
0
200
400
600
800
1000
1200
1400
0
10
20
30
40
50
60

FIG. 7B

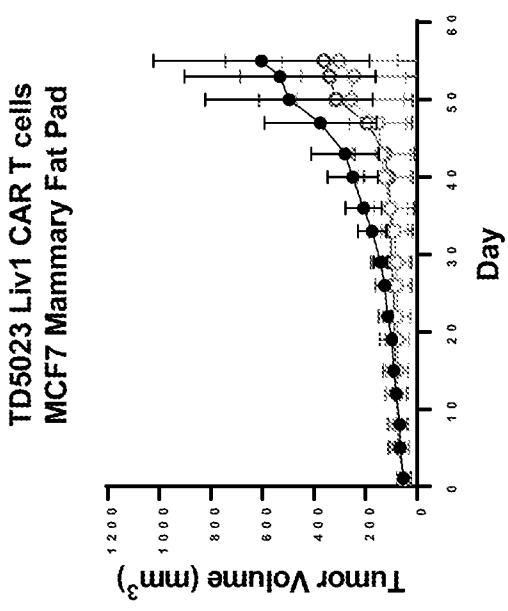

TD5023 Liv1 CAR T cells
MCF7 Mammary Fat Pad
Untreated
CTX975 TG/Reg KO 10e6
CTX975 TG/Reg KO 20e6
Tumor Volume (mm3)
Day
0
200
400
600
800
1000
1200
0
10
20
30
40
50
60

FIG. 8A

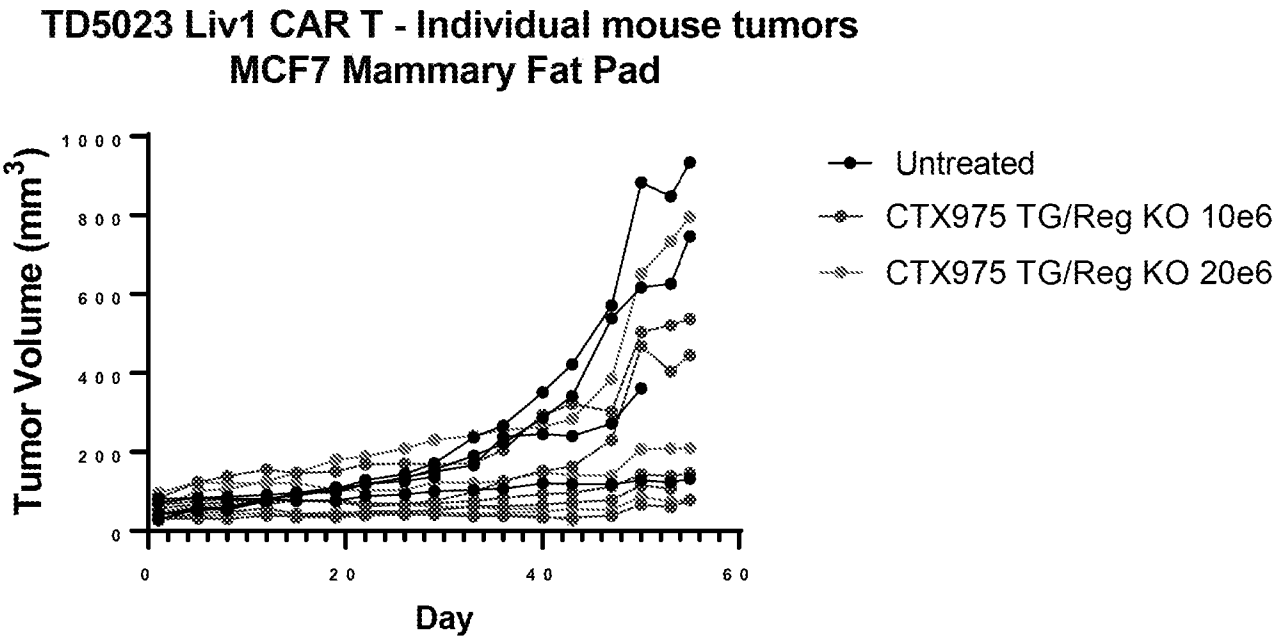

TD5023 Liv1 CAR T - Individual mouse tumors
MCF7 Mammary Fat Pad
Tumor Volume (mm3)
1000
800
600
400
200
0
0
20
40
60
Day
Untreated
CTX975 TG/Reg KO 10e6
CTX975 TG/Reg KO 20e6

FIG. 8B

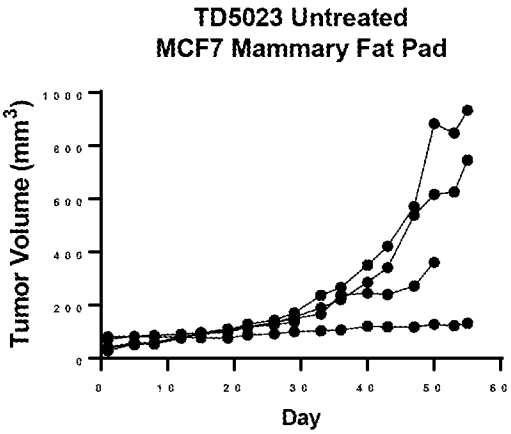



FIG. 8C

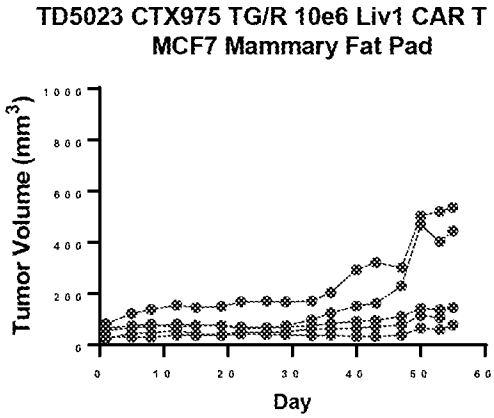



FIG. 8D

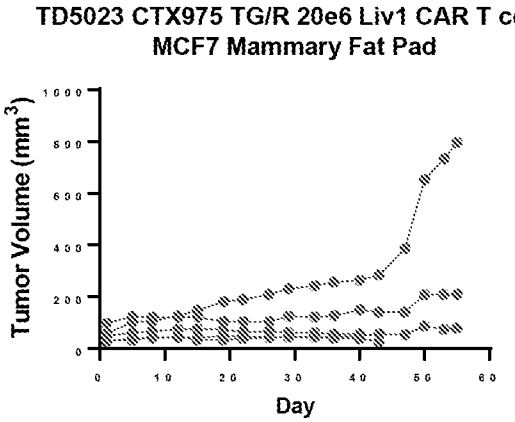


FIG. 9

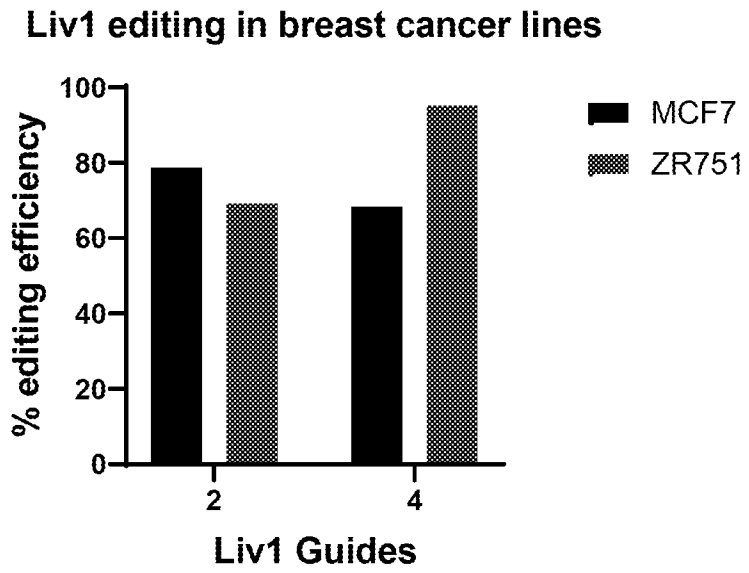

Liv1 editing in breast cancer lines
% editing efficiency
100
80
60
40
20
0
2
4
Liv1 Guides
MCF7
ZR751

FIG. 10A

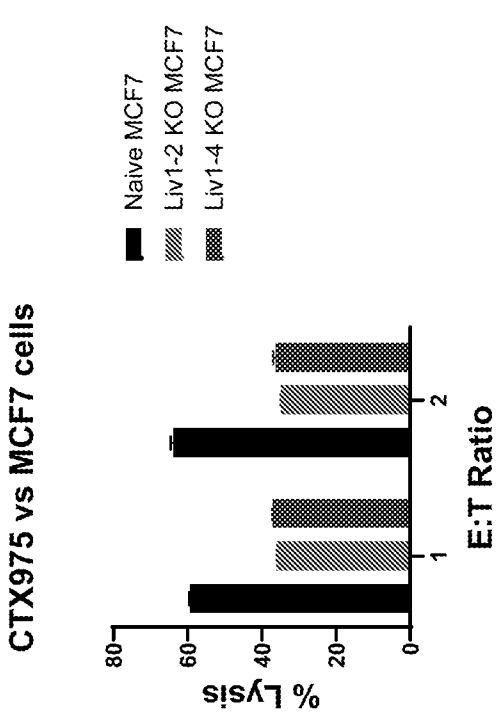



FIG. 10B

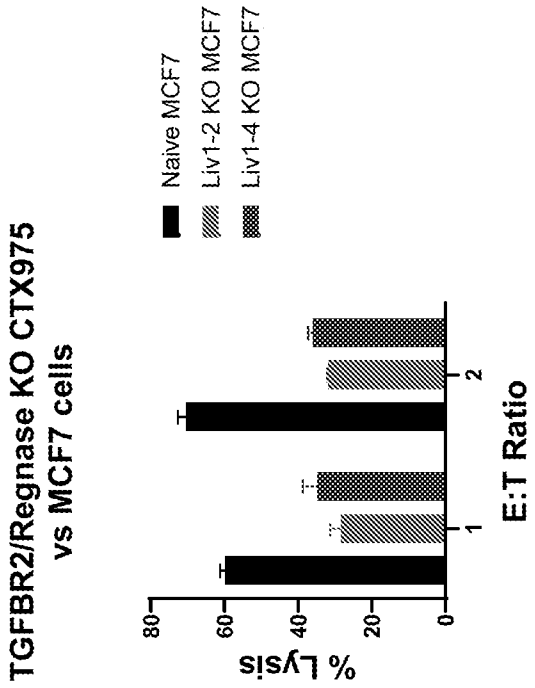


FIG. 11A

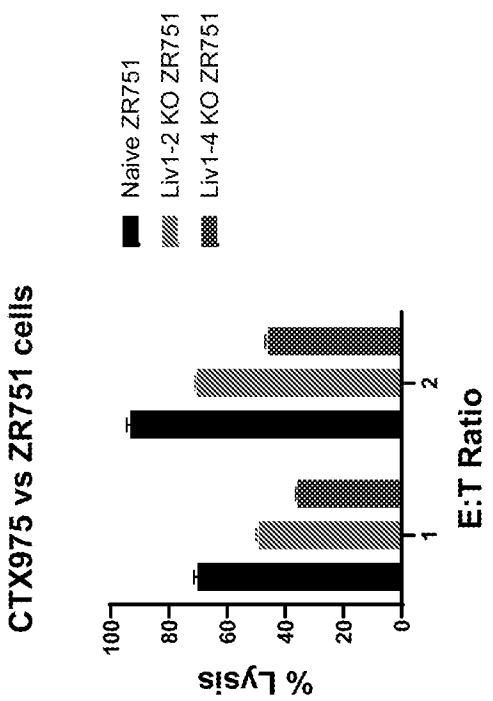



FIG. 11B

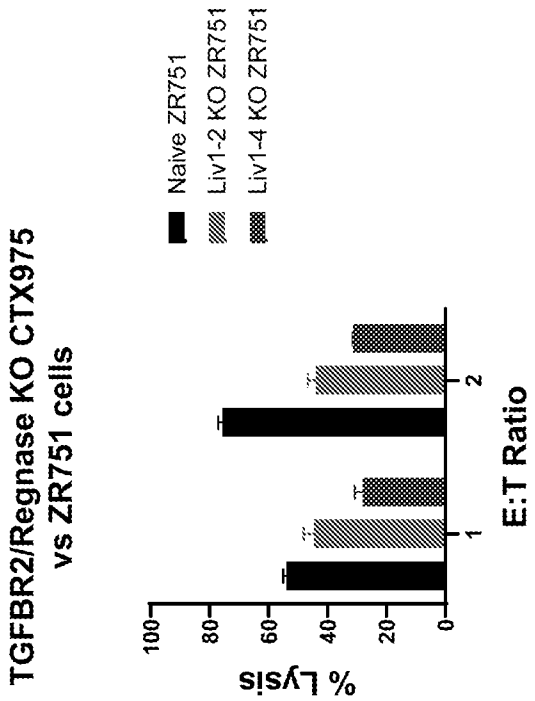


FIG. 12

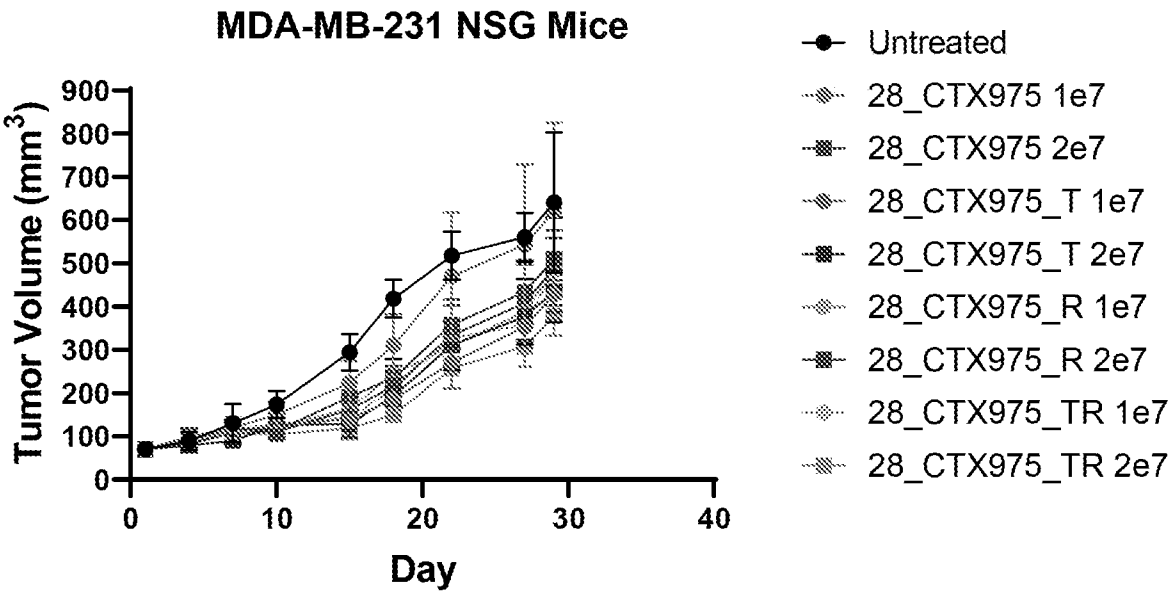

MDA-MB-231 NSG Mice
Tumor Volume (mm3)
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
Day
Untreated
28_CTX975 1e7
28_CTX975 2e7
28_CTX975_T 1e7
28_CTX975_T 2e7
28_CTX975_R 1e7
28_CTX975_R 2e7
28_CTX975_TR 1e7
28_CTX975_TR 2e7

FIG. 13A

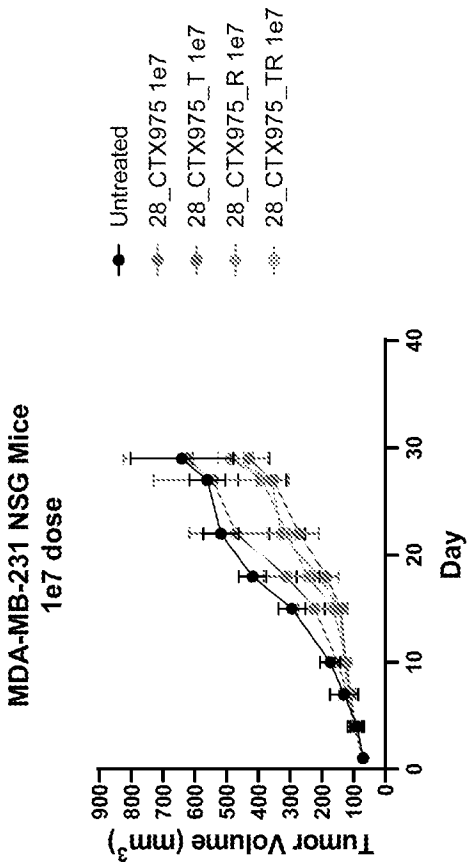
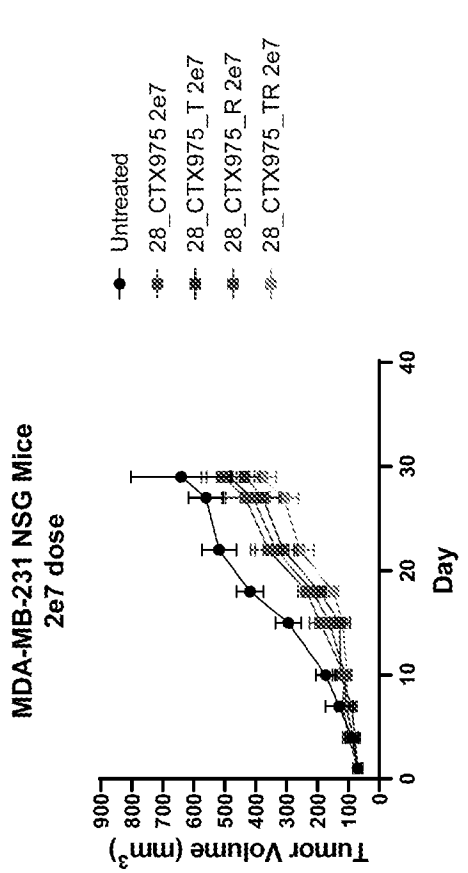



FIG. 13B



# INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/062244

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K39/00 C07K14/725 C12N15/113
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K C12N A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2020/095249 A1 (CRISPR THERAPEUTICS AG [CH]) 14 May 2020 (2020-05-14) cited in the application the whole document | 1-61 |
| Y | WO 2020/206248 A1 (PREC BIOSCIENCES INC [US]) 8 October 2020 (2020-10-08) the whole document | 1-61 |
| Y | WO 2020/223535 A1 (JUNO THERAPEUTICS INC [US]; EDITAS MEDICINE INC [US]) 5 November 2020 (2020-11-05) the whole document | 1-61 |

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 April 2023 | 21/04/2023 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Manu, Dominique |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2022/062244

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2022/062244

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WEI JUN ET AL: "Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy", NATURE, NATURE PUBLISHING GROUP UK, LONDON, vol. 576, no. 7787, 1 December 2019 (2019-12-01), pages 471-476, XP036974188, ISSN: 0028-0836, DOI: 10.1038/S41586-019-1821-Z [retrieved on 2019-12-11] the whole document | 1-61 |
| Y | WO 2020/219682 A2 (ST JUDE CHILDRENS RES HOSPITAL INC [US]) 29 October 2020 (2020-10-29) the whole document | 1-61 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2022/062244

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | US 2022041987 A1 | 10-02-2022 |
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| WO 2020219682 A2 | 29-10-2020 | US 2022226380 A1 | 21-07-2022 |
| | | WO 2020219682 A2 | 29-10-2020 |