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(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 5th Floor, Oakland, CA 94607 (US).

(72) Inventors: **PRUSSAK, Charles, E.**; C/o The Regents Of The University Of California, 1111 Franklin Street, 5th Floor, Oakland, CA 94607 (US). **KIPPS, Thomas, J.**; C/o The Regents Of The University Of California, 1111 Franklin Street, 5th Floor, Oakland, CA 94607 (US). **WIDHOPF, George, F.**; C/o The Regents Of The University Of California, 1111 Franklin Street, 5th Floor, Oakland, CA 94607 (US). **OH, Christopher, S.**; C/o The Regents Of The University Of California, 1111 Franklin Street, 5th Floor, Oakland, CA 94607 (US).

(74) Agent: **HETZER-EGGER, Claudia** et al.; Mintz Levin Cohn Ferris Glovsky And Popeo, P.C., 3580 Carmel Mountain Road, Suite 300, San Diego, CA 92130 (US).

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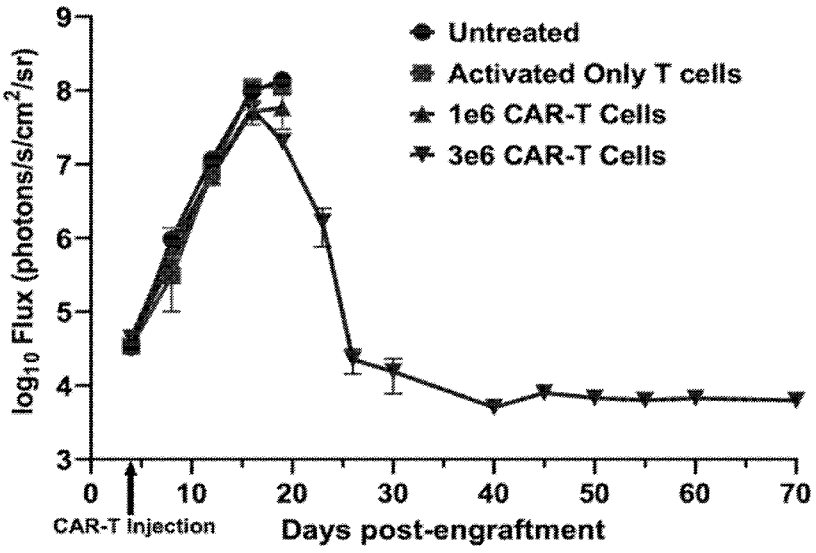

FIG. 13

(57) Abstract: Provided herein is a chimeric antigen receptor (CAR) and CAR-expressing immune cells that target human ROR1 expressed aberrantly on a tumor cancers.

[Continued on next page]

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Chimeric Antigen Receptor Modified T-Cells (CAR-T) for the Treatment of Hematological and Solid Tumor Cancers

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/824,080, filed March 26, 2019, U.S. Provisional Application No. 62/931,103, filed November 5, 2019 and U.S. Provisional Application No. 62/969,569, filed February 3, 2020, which are hereby incorporated by reference in their entirety and for all purposes.

SEQUENCE LISTING

[0002] The Sequence Listing written in file 048537-623001WO_SEQUENCE_LISTING_ST25.TXT, created on March 23, 2020, 69,632 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference.

BACKGROUND

[0003] The utility of chimeric antigen receptor modified T-cells (CAR-T) to treat cancer has been demonstrated in a number of clinical studies including the approval by the FDA of two of these products for the treatment of pediatric acute lymphocytic leukemia and transformed non-Hodgkins Lymphoma. These successful CAR-Ts, tisagenlecleucel and axicabtagene ciloleucel developed by Novartis and Kite Pharmaceuticals, respectively, are autologous products that target the B-cell antigen CD-19. Although they target the same cancer antigen, the CAR-T products they created have distinct structures and production methods. Along with these approved products, many additional CAR-T clinical studies, sponsored by a variety of domestic and foreign pharmaceutical and biotech companies, are ongoing that target the same antigen and like the approved products, involve unique structures and manufacturing methods. Along with the CD-19 targeting CARs, a number of additional cancer-associated antigens are targeted in ongoing clinical studies by CARs variably employing unique structures, T-cell targets, activation and manufacturing methods and patient pretreatment protocols. The large number of clinical studies employing these differing agents and approaches highlight the complex pharmacology of CAR-Ts. The compositions and methods provided herein address current needs in the art for ROR-1 specific CARs and their use in therapeutics and diagnostics.

SUMMARY

[0004] Described herein are chimeric antigen receptors that target human ROR-1, cell compositions expressing the chimeric antigen receptors, and methods and uses of the chimeric antigen receptors and/or the cell compositions. The chimeric antigen receptors described herein can be expressed by the T lymphocytes isolated from an individual afflicted with cancer and re-administered to the individual. Administration of T cells expressing the CARs described herein serves as an effective therapeutic treatment for cancers that express ROR-1. Further, described herein are optimized intracellular signaling domains and membrane spacers that result in improved function of anti- ROR-1 CAR-T cells.

[0005] Aspects disclosed herein provide a chimeric antigen receptor targeting ROR-1. The chimeric receptor comprises i) an antigen binding region, wherein the antigen binding region specifically binds ROR-1 and wherein the antigen binding region comprises a light chain variable domain and a heavy chain variable domain, ii) a spacer domain, wherein the spacer domain comprises a spacer of between 10 and 240 amino acids in length, iii) a transmembrane domain, and iv) an intracellular domain. The light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45, and the heavy chain variable domain comprises a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48. Alternatively, the light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51, and the heavy chain variable domain comprises a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54.

[0006] In some instances, the light chain variable domain is coupled to N-terminus or C-terminus of the heavy chain variable domain. In some embodiments, the light chain variable domain is covalently coupled to the heavy chain variable domain through a polypeptide linker. In some embodiments, the polypeptide linker comprises an amino acid sequence of SEQ ID NO:24. In some embodiments, the light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:21. In some embodiments, the light chain variable domain consists of an sequence of SEQ ID NO:21. In some embodiments, the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:27. In some embodiments, the heavy chain variable domain consists of an amino acid sequence of SEQ ID NO:27. In some embodiments, the light chain variable domain comprises an amino acid sequence at least about

90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:19. In some embodiments, the light chain variable domain consists of an amino acid sequence of SEQ ID NO:19. In some embodiments, the light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:20. In some embodiments, the light chain variable domain consists of an amino acid sequence of SEQ ID NO:20. In some embodiments, the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:25. In some embodiments, the heavy chain variable domain consists of the sequence of SEQ ID NO:25. In some embodiments, the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:26. In some embodiments, the heavy chain variable domain consists of the sequence of SEQ ID NO:26.

[0007] In some instances, the spacer domain comprises an antibody domain. In some embodiments, the antibody domain comprises an immunoglobulin hinge domain, an immunoglobulin constant heavy chain 3 (CH3) domain, an immunoglobulin constant heavy chain 2 (CH2) domain, or a combination thereof. In some embodiments, the antibody domain consists of the immunoglobulin hinge domain. In some embodiments, the antibody domain consists of the immunoglobulin hinge domain and the immunoglobulin constant heavy chain 3 (CH3) domain. In some embodiments, the antibody domain consists of the immunoglobulin hinge domain, the immunoglobulin constant heavy chain 3 (CH3) domain and the immunoglobulin constant heavy chain 2 (CH2) domain.

[0008] In some instances, the spacer domain is between 14 and 120 amino acids in length. In some embodiments, the spacer domain comprises an amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42. In some embodiments, the spacer domain consists of an amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[0009] In some instances, the transmembrane domain comprises a CD8α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain, a CD3ζ transmembrane domain, or any combination thereof. In some embodiments, transmembrane domain is a CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises an amino acid sequence of SEQ ID NO:32. In some embodiments, the CD28 transmembrane domain consists of an amino acid sequence of SEQ ID NO:32.

[0010] In some instances, the intracellular domain comprises an intracellular co-stimulatory signaling domains, an intracellular T-cell signaling domains, or a combination thereof. In some embodiments, the intracellular co-stimulatory signaling domains is a 4-1BB intracellular co-

stimulatory signaling domain, a CD28 intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain, or any combination thereof. In some embodiments, the 4-1BB intracellular co-stimulatory signaling domain comprises an amino acid sequence of SEQ ID NO:33. In some embodiments, the 4-1BB intracellular co-stimulatory signaling domain consists of an amino acid sequence of SEQ ID NO:33. In some embodiments, the intracellular costimulatory signaling domain comprises a CD28 intracellular co-stimulatory signaling domain and a 4-1BB intracellular co-stimulatory signaling domain. In some embodiments, the intracellular costimulatory signaling domain comprises an intracellular T-cell signaling domain. In some embodiments, the intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain. In some embodiments, the CD3ζ intracellular T-cell signaling domain comprises an amino acid sequence of SEQ ID NO:34. In some embodiments, the CD3ζ intracellular T-cell signaling domain consists of an amino acid sequence of SEQ ID NO:34. In some embodiments, the chimeric antigen receptor binds to a cell expressing ROR-1.

[0011] Aspects disclosed herein provide a nucleic acid encoding the chimeric antigen receptor described herein. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is a lentiviral vector.

[0012] Aspects disclosed herein provide a cell comprising the nucleic acid described herein. Aspects disclosed herein also provide a cell expressing the chimeric antigen receptor described herein. In some embodiments, the cell is a T lymphocyte. In some embodiments, the T lymphocyte is a CD4+ T lymphocyte or a CD8 + T lymphocyte. In some embodiments, the cell is a natural killer cell, a genetically engineered natural killer cell or a CD56+ cell.

[0013] Aspects disclosed herein provide a pharmaceutical composition comprising a therapeutically effective amount of the cells described herein and a pharmaceutically acceptable diluent, carrier, or excipient. In some embodiments, the composition is formulated for intravenous injection.

[0014] Aspects disclosed herein provide a method of treating cancer in an individual in need thereof by administering to the individual the pharmaceutical composition as described herein. In some embodiments, the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia, adult acute myeloid leukemia, acute lymphoblastic leukemia, a mantle cell lymphoma, ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer. In some embodiments, the method further comprises administering cirmtuzumab to the individual. In some embodiments,

the cirmtuzumab and the pharmaceutical composition are administered separately.

[0015] Aspects disclosed herein provide a method of treating cancer in an individual in need thereof or the use of the chimeric antigen receptor or chimeric antigen receptor T cells for a metod of treating cancer in an indiviudal, wherein the cancer is a CD19 negative cancer or has reduced CD19 expression as a result of a prior treatment that targets CD19. In some embodiments, the individual has previously been treated with a therapeutic that targets CD19. In some embodiments, the therapeutic that targets CD19 is an antibody that binds to CD19. In some embodiments, the therapeutic that targets CD19 is a chimeric antigen receptor T cell that targets CD19. In some embodiments, the therapeutic that targets CD19 is a chimeric antigen receptor NK cell that targets CD19. In some embodiments, the cancer expresses ROR1.

[0016] Aspects disclosed herein provide a pharmaceutical composition as described herein for use in a method of treating cancer in an individual. In some embodiments, the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia, adult acute myeloid leukemia, acute lymphoblastic leukemia, a mantle cell lymphoma, ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1A** illustrates an anti-ROR-1 CAR-T cell interacting with an ROR-1 expressing chronic lymphocytic leukemia (CLL) cell.

[0018] **FIG. 1B** illustrates alternative embodiments of CARs according to this disclosure.

[0019] **FIG. 1C** illustrates a 2nd generation CAR introduced into healthy human donor T lymphocytes by a 2nd generation lentiviral vector. The activity of this highly active and specific CAR product is shown in **FIG. 8A** and FIG. **9A**.

[0020] **FIG. 2A** and FIG. 2**B** illustrate vector schematics for a coding region of a CAR of this disclosure.

[0021] **FIG. 3** illustrates schematics for lentiviral and transposon-based gene delivery vectors that can be used to transduce immune cells with the CARs of this disclosure.

[0022] **FIG. 4A** and **FIG. 4B** illustrate two different methods of transducing immune cells with the CARs.

[0023] **FIG. 5A** and **FIG. 5B** show flow cytometry of total PBMC before (**A**) and after (**B**) T cell isolation.

[0024] FIG. 6A shows activation profile of different T cell subsets after 3 days. **FIG. 6B** shows transduction efficiency with lentivirus at an MOI of 1 or 3.

[0025] FIG. 7A and **FIG. 7B** show a time course of CAR cell-surface expression after transduction.

[0026] FIG. 8A and **FIG. 8B** show chromium release assays of 2nd generation CARs utilizing different spacers between the scFV and the transmembrane domain of the CAR against MEC-1 cells that express ROR-1 (**A**), or lack ROR-1 expression (**B**).

[0027] FIG. 8C and **FIG. 8D** shows a comparison of 2nd and 3rd generation CARs in a chromium release assay using MEC-1 cells that express ROR-1 (**C**), or lack expression of ROR-1 (**D**); for both 8C and 8D Left to Right: activated only; CH3 only 2nd gen CAR; CH3 only 3rd gen CAR; hinge only 2nd gen CAR; hinge only 3rd gen CAR.

[0028] FIG. 8E shows *in vitro* cell killing activity of ROR1 CAR-T cells in a 4 hr chromium release assay from T cells of two healthy donors transduced with a second-generation CAR. Left-bar, Activated cells only; middle-bar, donor one; right-bar, donor two. Asterisk indicates bars not visible due to low level of killing.

[0029] FIG. 9A and **FIG. 9B** show graphs of cell viability or death rate of MCF7 target cells that express ROR-1 **(FIG. 9A),** or lack ROR-1 expression **(FIG. 9B**) upon treatment with ROR1 CAR-T. Data shown is an impedance-based cytotoxicity assay.

[0030] FIG. 9C shows a graph of cell death rate of MCF7 target cells that express ROR-1 by CAR-T cells generated from two different donors.

[0031] FIG. 10 shows a graph of cell viability of MEC-1 or Jeko cells upon treatment with anti-ROR-1 CAR-T cells at different effector to target ratios.

[0032] FIG. 11A shows that MEC-1/ROR-1 cells persist in bone marrow, kidney, and spleen of mice injected with $1x10^6$ MEC-1/ROR-1-luciferase cells.

[0033] FIG. 11B illustrates a schematic of the *in vivo* experiments and bioluminescence in mice that were treated with $3x10^6$ activated cells only (bottom left), or anti-ROR-1 CAR-T cells (bottom right).

[0034] FIG. 12 shows bioluminescence imaging of mice inoculated with MEC1-ROR1 cells and with ROR-1 CAR T-cells. Animals treated with ROR-1 CAR-T cells had reduced tumor burden compared to controls. The highest dose ($3x10^6$ CAR-Ts) cohort had only minimal amounts of disease at the end of the study.

[0035] FIG. 13 shows a time course of tumor volume (photon flux) in mice untreated, treated with activated cells only, $1x10^6$, or $3x10^6$ anti-ROR-1 CAR T cells. Mice treated with $3x10^6$

ROR-1 CAR-T reduced the leukemic burden to background levels by day 30 and that this minimal amount of disease was maintained for the duration of the study. Animals in the control groups (untreated, activated cells, or $1x10^6$ dose) had to be sacrificed on day 20.

[0036] FIG. 14 shows ROR-1 CAR-T expression in mouse tissues at various times in mice. Following ROR-1 CAR-T administration, animals were sacrificed on days 11 (top), and 25 (bottom), blood and organs were collected and subjected to flow analysis for CAR expression and confirmatory ROR1 binding activity. The ROR-1 CAR-T cell number was substantially greater in mice bearing MEC-1 ROR1 cells (CAR + MEC1-ROR1) vs control (CAR only) demonstrating elevated expansion of ROR-1 CAR-T cells in animals with tumor burden. Bars represent the mean values from the five mice in each group and error bars represent the S.E of the mean.

DETAILED DESCRIPTION

DEFINITIONS

[0037] While various embodiments and aspects of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[0038] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0039] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0040] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. 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 Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or

equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0041] As used herein, the term "about" means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, the term "about" means within a standard deviation using measurements generally acceptable in the art. In embodiments, about means a range extending to +/- 10% of the specified value. In embodiments, about means the specified value.

[0042] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term "polynucleotide" refers to a linear sequence of nucleotides. The term "nucleotide" typically refers to a single unit of a polynucleotide, *i.e.*, a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof. Examples of polynucleotides contemplated herein include single and double stranded DNA, single and double stranded RNA (including siRNA), and hybrid molecules having mixtures of single and double stranded DNA and RNA. Nucleic acid as used herein also refers to nucleic acids that have the same basic chemical structure as a naturally occurring nucleic acid. Such analogues have modified sugars and/or modified ring substituents, but retain the same basic chemical structure as the naturally occurring nucleic acid. A nucleic acid mimetic refers to chemical compounds that have a structure that is different from the general chemical structure of a nucleic acid, but that functions in a manner similar to a naturally occurring nucleic acid. Examples of such analogues include, without limitation, phosphorothiolates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

[0043] The term "gene" means the segment of DNA involved in producing a protein; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer, as well as the introns, include regulatory elements that are necessary during the transcription and the translation of a gene. Further, a "protein gene product" is a protein expressed from a particular gene.

[0044] The word "expression" or "expressed" as used herein in reference to a gene means the transcriptional and/or translational product of that gene. The level of expression of a DNA molecule in a cell may be determined on the basis of either the amount of corresponding mRNA that is present within the cell or the amount of protein encoded by that DNA produced by the

cell. The level of expression of non-coding nucleic acid molecules may be detected by standard PCR or Northern blot methods well known in the art. *See*, Sambrook *et al.*, 1989 *Molecular Cloning: A Laboratory Manual*, 18.1-18.88.

[0045] Expression of a transfected gene can occur transiently or stably in a cell. During "transient expression" the transfected gene is not transferred to the daughter cell during cell division. Since its expression is restricted to the transfected cell, expression of the gene is lost over time. In contrast, stable expression of a transfected gene can occur when the gene is co-transfected with another gene that confers a selection advantage to the transfected cell. Such a selection advantage may be a resistance towards a certain toxin that is presented to the cell.

[0046] The terms "transfection", "transduction", "transfecting" or "transducing" are used interchangeably throughout and are defined as a process of introducing a nucleic acid molecule or a protein to a cell. Nucleic acids are introduced to a cell using non-viral or viral-based methods. The nucleic acid molecules may be gene sequences encoding complete proteins or functional portions thereof. Non-viral methods of transfection include any appropriate transfection method that does not use viral DNA or viral particles as a delivery system to introduce the nucleic acid molecule into the cell. Exemplary non-viral transfection methods include calcium phosphate transfection, liposomal transfection, nucleofection, sonoporation, transfection through heat shock, magnetifection, and electroporation. In some embodiments, the nucleic acid molecules are introduced into a cell using electroporation following standard procedures well known in the art. For viral-based methods of transfection any useful viral vector may be used in the methods described herein. Examples for viral vectors include, but are not limited to retroviral, adenoviral, lentiviral and adeno-associated viral vectors. In some embodiments, the nucleic acid molecules are introduced into a cell using a retroviral vector following standard procedures well known in the art. The terms "transfection" or "transduction" also refer to introducing proteins into a cell from the external environment. Typically, transduction or transfection of a protein relies on attachment of a peptide or protein capable of crossing the cell membrane to the protein of interest. *See, e.g.*, Ford *et al.* (2001) *Gene Therapy* 8:1-4 and Prochiantz (2007) *Nat. Methods* 4:119-20.

[0047] The term "plasmid" or "expression vector" refers to a nucleic acid molecule that encodes for genes and/or regulatory elements necessary for the expression of genes. Expression of a gene from a plasmid can occur in cis or in trans. If a gene is expressed in cis, gene and regulatory elements are encoded by the same plasmid. Expression in trans refers to the instance where the gene and the regulatory elements are encoded by separate plasmids.

[0048] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.,* hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.,* homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.,* norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0049] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0050] The terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given amino acid or polynucleotide sequence, refer to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. An amino acid residue in a protein "corresponds" to a given residue when it occupies the same essential structural position within the protein as the given residue. One skilled in the art will immediately recognize the identity and location of residues corresponding to a specific position in a protein (*e.g.,* ROR-1) in other proteins with different numbering systems. For example, by performing a simple sequence alignment with a protein (*e.g.,* ROR-1) the identity and location of residues corresponding to specific positions of the protein are identified in other protein sequences aligning to the protein. For example, a selected residue in a selected protein corresponds to glutamic acid at position 138 when the selected residue occupies the same essential spatial or other structural relationship as a glutamic acid at position 138. In some embodiments, where a selected protein is aligned for maximum homology with a protein, the position in the aligned selected protein aligning with glutamic acid 138 is the to correspond to glutamic acid 138. Instead of a primary sequence alignment, a three dimensional structural alignment can also be used, *e.g.,* where the structure of the selected protein is aligned for maximum correspondence with the glutamic acid at position

138, and the overall structures compared. In this case, an amino acid that occupies the same essential position as glutamic acid 138 in the structural model is the to correspond to the glutamic acid 138 residue.

[0051] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may optionally be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a chimeric protein encoding two or more separate protein sequences that are recombinantly expressed as a single moiety.

[0052] The term "recombinant" when used with reference, for example, to a cell, a nucleic acid, a protein, or a vector, indicates that the cell, nucleic acid, protein or vector has been modified by or is the result of laboratory methods. Thus, for example, recombinant proteins include proteins produced by laboratory methods. Recombinant proteins can include amino acid residues not found within the native (non-recombinant) form of the protein or can be include amino acid residues that have been modified (*e.g.,* labeled).

[0053] The term "isolated", when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified.

[0054] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity over a specified region, *e.g.,* of the entire polypeptide sequences of the invention or individual domains of the polypeptides of the invention), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then the to be "substantially identical." This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 50 nucleotides in

length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length.

[0055] "Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0056] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0057] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of, *e.g.,* a full length sequence or from 20 to 600, about 50 to about 200, or about 100 to about 150 amino acids or nucleotides in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Any methods of alignment of sequences for comparison well known in the art are contemplated. Optimal alignment of sequences for comparison can be conducted, *e.g.,e.g.,* by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g.,* Ausubel *et al.*, *Current Protocols in Molecular Biology* (1995 supplement)).

[0058] An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul

et al. (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0059] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.,* Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0060] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross-reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described

below. Thus, a polypeptide is typically or substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0061] Antibodies are large, complex molecules (molecular weight of ~150,000 or about 1320 amino acids) with intricate internal structure. A natural antibody molecule contains two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. Each light chain and heavy chain in turn consists of two regions: a variable ("V") region involved in binding the target antigen, and a constant ("C") region that interacts with other components of the immune system. The light and heavy chain variable regions come together in 3-dimensional space to form a variable region that binds the antigen (for example, a receptor on the surface of a cell). Within each light or heavy chain variable region, there are three short segments (averaging 10 amino acids in length) called the complementarity determining regions ("CDRs"). The six CDRs in an antibody variable domain (three from the light chain and three from the heavy chain) fold up together in 3-dimensional space to form the actual antibody binding site which docks onto the target antigen. The position and length of the CDRs have been precisely defined by Kabat, E. et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1983, 1987. The part of a variable region not contained in the CDRs is called the framework ("FR"), which forms the environment for the CDRs.

[0062] An "antibody variant" as provided herein refers to a polypeptide capable of binding to an antigen and including one or more structural domains (*e.g.,* light chain variable domain, heavy chain variable domain) of an antibody or fragment thereof. Non-limiting examples of antibody variants include single-domain antibodies or nanobodies, monospecific Fab_2, bispecific Fab_2, trispecific Fab_3, monovalent IgGs, scFv, bispecific antibodies, bispecific diabodies, trispecific triabodies, scFv-Fc, minibodies, IgNAR, V-NAR, hcIgG, VhH, or peptibodies. A "peptibody" as provided herein refers to a peptide moiety attached (through a covalent or non-covalent linker) to the Fc domain of an antibody. Further non-limiting examples of antibody variants known in the art include antibodies produced by cartilaginous fish or camelids. A general description of antibodies from camelids and the variable regions thereof and methods for their production, isolation, and use may be found in references WO97/49805 and WO 97/49805 which are incorporated by reference herein in their entirety and for all purposes. Likewise, antibodies from

cartilaginous fish and the variable regions thereof and methods for their production, isolation, and use may be found in WO2005/118629, which is incorporated by reference herein in its entirety and for all purposes.

[0063] The terms "CDR L1", "CDR L2" and "CDR L3" as provided herein refer to the complementarity determining regions (CDR) 1, 2, and 3 of the variable light (L) chain of an antibody. In embodiments, the variable light chain provided herein includes in N-terminal to C-terminal direction a CDR L1, a CDR L2 and a CDR L3. Likewise, the terms "CDR H1", "CDR H2" and "CDR H3" as provided herein refer to the complementarity determining regions (CDR) 1, 2, and 3 of the variable heavy (H) chain of an antibody. In embodiments, the variable light chain provided herein includes in N-terminal to C-terminal direction a CDR L1, a CDR L2 and a CDR L3.

[0064] The term "antibody" is used according to its commonly known meaning in the art. Antibodies exist, *e.g.,* as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_{H1} by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (*e.g.,* single chain Fv) or those identified using phage display libraries (see, *e.g.,* McCafferty *et al.*, *Nature* 348:552-554 (1990)).

[0065] The term "antigen" as provided herein refers to molecules capable of binding to the antibody binding domain provided herein. An "antigen binding domain" as provided herein is a region of an antibody that binds to an antigen (epitope). As described above, the antigen binding domain is generally composed of one constant and one variable domain of each of the heavy and the light chain (V_L, V_H, C_L and C_H1, respectively). The paratope or antigen-binding site is formed on the N-terminus of the antigen binding domain. The two variable domains of an antigen binding domain typically bind the epitope on an antigen.

[0066] Antibodies exist, for example, as intact immunoglobulins or as a number of well-

characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'2 dimer into an Fab' monomer. The Fab' monomer is essentially the antigen binding portion with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g.,*e.g.,* single chain Fv) or those identified using phage display libraries (see, e.g.,*e.g.,* McCafferty et al., Nature 348:552-554 (1990)).

[0067] A single-chain variable fragment (scFv) is typically a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of 10 to about 25 amino acids. The linker may usually be rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa.

[0068] The epitope of an antibody is the region of its antigen to which the antibody binds. Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1x, 5x, 10x, 20x or 100x excess of one antibody inhibits binding of the other by at least 30% but preferably 50%, 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.,* Junghans *et al.*, Cancer Res. 50:1495, 1990). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0069] The term "ROR-1" or "ROR1" as used herein refers to any of the recombinant or naturally-occurring forms of tyrosine kinase-like orphan receptor 1 (ROR-1) or variants or homologs thereof that maintain ROR-1 activity (*e.g.,* within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to ROR-1). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (*e.g.,* a 50, 100, 150 or 200 continuous

amino acid portion) compared to a naturally occurring ROR-1 protein. In embodiments, the ROR-1 protein is substantially identical to the protein identified by Accession No. NP_005003.1 or a variant or homolog having substantial identity thereto. In embodiments, the ROR-1 protein includes the amino acid sequence of SEQ ID NO:55. In embodiments, the ROR-1 protein is the amino acid sequence of SEQ ID NO: 55. In embodiments, the ROR-1 protein includes the amino acid sequence of SEQ ID NO:56. In embodiments, the ROR-1 protein includes the amino acid sequence of SEQ ID NO:57.

[0070] The terms "cirmtuzumab", "UC-961", and "99961.1" refer to a humanized monoclonal antibody capable of binding the extracellular domain of the human receptor tyrosine kinase-like orphan receptor 1 (ROR-1). In embodiments, cirmtuzumab is any one of the antibodies or fragments thereof disclosed in US Patent Application No. 14/422,519, which is incorporated by reference herein in its entirety and for all purposes.

[0071] The term "CD28 transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD28, or variants or homologs thereof that maintain CD28 transmembrane activity (*e.g.* within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD28 transmembrane domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (*e.g.* a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD28 transmembrane domain polypeptide. In embodiments, the CD28 transmembrane domain is a human CD28 transmembrane domain protein. In embodiments, a variant or mutant of the CD28 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD28 transmembrane domain protein. In embodiments, a variant or mutant of the CD28 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD28 transmembrane domain protein. In embodiments, a variant or mutant of the CD28 transmembrane domain protein does not include deletions compared to the naturally occurring CD28 transmembrane domain protein. In embodiments, a variant or mutant of the CD28 transmembrane domain protein does not include insertions compared to the naturally occurring CD28 transmembrane domain protein. In embodiments, a variant or mutant of the CD28 transmembrane domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD28 transmembrane domain protein. In embodiments, the CD28 transmembrane domain includes all or a portion of the protein as identified by NCBI sequence reference NP_001230006.1, or an

isoform or naturally occurring mutant or variant thereof. In embodiments, the CD28 transmembrane domain includes all or a portions of the protein as identified by the NCBI sequence reference NP_ 001230007.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD28 transmembrane domain includes all or a portion of the protein as identified by the NCBI sequence reference NP_006130.1, or an isoform or naturally occurring mutant or variant thereof.

[0072] The term "CD4 transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD4, or variants or homologs thereof that maintain CD4 transmembrane domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD4 transmembrane domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD4 transmembrane domain polypeptide. In embodiments, the CD4 transmembrane domain is a human CD4 transmembrane domain protein. In embodiments, a variant or mutant of the CD4 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD4 transmembrane domain protein. In embodiments, a variant or mutant of the CD4 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD4 transmembrane domain protein. In embodiments, a variant or mutant of the CD4 transmembrane domain protein does not include deletions compared to the naturally occurring CD4 transmembrane domain protein. In embodiments, a variant or mutant of the CD4 transmembrane domain protein does not include insertions compared to the naturally occurring CD4 transmembrane domain protein. In embodiments, a variant or mutant of the CD4 transmembrane domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD4 transmembrane domain protein. In embodiments, the CD4 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_000607.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD4 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001181943.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD4 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001181944.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD4 transmembrane domain includes all or a portion of

the protein identified by the NCBI sequence reference NP_001181945.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD4 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001181946.1, or an isoform or naturally occurring mutant or variant thereof.

[0073] The term "CD8 transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD8, or variants or homologs thereof that maintain CD8 transmembrane domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD8 transmembrane domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD8 transmembrane domain polypeptide. In embodiments, the CD8 transmembrane domain is CD8A transmembrane domain. In embodiments, the CD8 transmembrane domain is a CD8B transmembrane domain. In embodiments, the CD8 transmembrane domain is a human CD8 transmembrane domain protein. In embodiments, a variant or mutant of the CD8 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD8 transmembrane domain protein. In embodiments, a variant or mutant of the CD8 transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD8 transmembrane domain protein. In embodiments, a variant or mutant of the CD8 transmembrane domain protein does not include deletions compared to the naturally occurring CD8 transmembrane domain protein. In embodiments, a variant or mutant of the CD8 transmembrane domain protein does not include insertions compared to the naturally occurring CD8 transmembrane domain protein. In embodiments, a variant or mutant of the CD8 transmembrane domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD8 transmembrane domain protein. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001139345.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001181943.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001181944.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified

by the NCBI sequence reference NP_001181945.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_741969.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001759.3, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference XP_011531466.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001171571.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_757362.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_742100.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_742099.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD8 transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_004922.1, or an isoform or naturally occurring mutant or variant thereof.

[0074] The term "CD3-zeta transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD3-zeta, or variants or homologs thereof that maintain CD3-zeta transmembrane domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD3-zeta transmembrane domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD3-zeta transmembrane domain polypeptide. In embodiments, the CD3-zeta transmembrane domain is a human CD3- zeta transmembrane domain protein. In embodiments, a variant or mutant of the CD3-zeta transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD3-zeta transmembrane domain protein. In embodiments, a variant or mutant of the CD3-zeta transmembrane domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD3-

zeta transmembrane domain protein. In embodiments, a variant or mutant of the CD3-zeta transmembrane domain protein does not include deletions compared to the naturally occurring CD3-zeta transmembrane domain protein. In embodiments, a variant or mutant of the CD3-zeta transmembrane domain protein does not include insertions compared to the naturally occurring CD3-zeta transmembrane domain protein. In embodiments, a variant or mutant of the CD3-zeta transmembrane domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD3-zeta transmembrane domain protein. In embodiments, the CD3-zeta transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_000725.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD3-zeta transmembrane domain includes all or a portion of the protein identified by the NCBI sequence reference NP_932170.1, or an isoform or naturally occurring mutant or variant thereof.

[0075] The term "CD28 co-stimulatory domain" as provided herein includes any of the recombinant or naturally-occurring forms of the co-stimulatory domain of CD28, or variants or homologs thereof that maintain CD28 co-stimulatory domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD28 co-stimulatory domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD28 co-stimulatory domain polypeptide. In embodiments, the CD28 co-stimulatory domain is a human CD28 co-stimulatory domain protein. In embodiments, a variant or mutant of the CD28 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD28 co-stimulatory domain protein. In embodiments, a variant or mutant of the CD28 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD28 co-stimulatory domain protein. In embodiments, a variant or mutant of the CD28 co-stimulatory domain protein does not include deletions compared to the naturally occurring CD28 co-stimulatory domain protein. In embodiments, a variant or mutant of the CD28 co-stimulatory domain protein does not include insertions compared to the naturally occurring CD28 co-stimulatory domain protein. In embodiments, a variant or mutant of the CD28 co-stimulatory domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD28 co-stimulatory domain protein. In embodiments, the CD28 co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001230006.1, or an

isoform or naturally occurring mutant or variant thereof. In embodiments, the CD28 co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001230007.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD28 co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_006130.1, or an isoform or naturally occurring mutant or variant thereof.

[0076] The term "4-1BB co-stimulatory domain" as provided herein includes any of the recombinant or naturally-occurring forms of the co-stimulatory domain of 4-1BB, or variants or homologs thereof that maintain 4-1BB co-stimulatory domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the 4-1BB co-stimulatory domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring 4-1BB co-stimulatory domain polypeptide. In embodiments, the 4-1BB co-stimulatory domain is a human 4-1BB co-stimulatory domain protein. In embodiments, a variant or mutant of the 4-1BB co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring 4-1BB co-stimulatory domain protein. In embodiments, a variant or mutant of the 4-1BB co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring 4-1BB co-stimulatory domain protein. In embodiments, a variant or mutant of the 4-1BB co-stimulatory domain protein does not include deletions compared to the naturally occurring 4-1BB co-stimulatory domain protein. In embodiments, a variant or mutant of the 4-1BB co-stimulatory domain protein does not include insertions compared to the naturally occurring 4-1BB co-stimulatory domain protein. In embodiments, a variant or mutant of the 4-1BB co-stimulatory domain protein includes substitutions that are conservative substitutions compared to the naturally occurring 4-1BB co-stimulatory domain protein. In embodiments, the 4-1BB co-stimulatory domain protein amino acid sequence has at least or about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identify to SEQ ID NO:14. In embodiments, the 4-1BB co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_001552.2 or an isoform or naturally occurring mutant or variant thereof.

[0077] The term "ICOS co-stimulatory domain" as provided herein includes any of the recombinant or naturally-occurring forms of the co-stimulatory domain of ICOS, or variants or homologs thereof that maintain ICOS co-stimulatory domain activity (e.g. within at least 50%,

80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the ICOS co-stimulatory domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring ICOS co-stimulatory domain polypeptide. In embodiments, the ICOS co-stimulatory domain is a human ICOS co-stimulatory domain protein. In embodiments, a variant or mutant of the ICOS co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring ICOS co-stimulatory domain protein. In embodiments, a variant or mutant of the ICOS co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring ICOS co-stimulatory domain protein. In embodiments, a variant or mutant of the ICOS co-stimulatory domain protein does not include deletions compared to the naturally occurring ICOS co-stimulatory domain protein. In embodiments, a variant or mutant of the ICOS co-stimulatory domain protein does not include insertions compared to the naturally occurring ICOS co-stimulatory domain protein. In embodiments, a variant or mutant of the ICOS co-stimulatory domain protein includes substitutions that are conservative substitutions compared to the naturally occurring ICOS co-stimulatory domain protein. In embodiments, the ICOS co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_036224.1, or an isoform or naturally occurring mutant or variant thereof.

[0078] The term "OX-40 co-stimulatory domain" as provided herein includes any of the recombinant or naturally-occurring forms of the co-stimulatory domain of OX-40, or variants or homologs thereof that maintain OX-40 co-stimulatory domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the OX-40 co-stimulatory domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring OX-40 co-stimulatory domain polypeptide. In embodiments, the OX-40 co-stimulatory domain is a human OX-40 co-stimulatory domain protein. In embodiments, a variant or mutant of the OX-40 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring OX-40 co-stimulatory domain protein. In embodiments, a variant or mutant of the OX-40 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring OX-40 co-stimulatory domain protein. In embodiments, a variant or mutant of the OX-40 co-stimulatory domain protein does

not include deletions compared to the naturally occurring OX-40 co-stimulatory domain protein. In embodiments, a variant or mutant of the OX-40 co-stimulatory domain protein does not include insertions compared to the naturally occurring OX-40 co-stimulatory domain protein. In embodiments, a variant or mutant of the OX-40 co-stimulatory domain protein includes substitutions that are conservative substitutions compared to the naturally occurring OX-40 co-stimulatory domain protein. In embodiments, the OX-40 co-stimulatory domain includes all or a portion of the protein identified by the NCBI sequence reference NP_003318.1, or an isoform or naturally occurring mutant or variant thereof.

[0079] The term "CTLA-4 co-stimulatory domain" as provided herein includes any of the recombinant or naturally-occurring forms of the co-stimulatory domain of CTLA-4, or variants or homologs thereof that maintain CTLA-4 co-stimulatory domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CTLA-4 co-stimulatory domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CTLA-4 co-stimulatory domain polypeptide. In embodiments, CTLA-4 co-stimulatory domain protein is a human CTLA-4 co-stimulatory domain protein. In embodiments, the CTLA-4 co-stimulatory domain includes no more than 5, 4, 3, 2, or 1 deletions. In embodiments, the CTLA-4 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions. In embodiments, the CTLA-4 co-stimulatory domain protein does not include deletions. In embodiments, CTLA-4 co-stimulatory domain protein does not include insertions. In embodiments, the CTLA-4 co-stimulatory domain protein includes substitutions that are conservative substitutions. In embodiments, the CTLA-4 co-stimulatory domain is a human CTLA-4 co-stimulatory domain protein. In embodiments, a variant or mutant of the CTLA-4 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CTLA-4 co-stimulatory domain protein. In embodiments, a variant or mutant of the CTLA-4 co-stimulatory domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CTLA-4 co-stimulatory domain protein. In embodiments, a variant or mutant of the CTLA-4 co-stimulatory domain protein does not include deletions compared to the naturally occurring CTLA-4 co-stimulatory domain protein. In embodiments, a variant or mutant of the CTLA-4 co-stimulatory domain protein does not include insertions compared to the naturally occurring CTLA-4 co-stimulatory domain protein. In embodiments, a variant or mutant of the CTLA-4 co-stimulatory domain protein includes

substitutions that are conservative substitutions compared to the naturally occurring CTLA-4 co-stimulatory domain protein.

[0080] The term " CD3ζ intracellular T-cell signaling domain " as provided herein includes any of the recombinant or naturally-occurring forms of the CD3ζ intracellular T-cell signaling domain, or variants or homologs thereof that maintain CD3ζ intracellular T-cell signaling domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD3ζ intracellular T-cell signaling domain). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring CD3ζ intracellular T-cell signaling domain polypeptide. In embodiments, the CD3ζ intracellular T-cell signaling domain is a human CD3ζ intracellular T-cell signaling domain protein. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain protein includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain protein. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain protein includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain protein. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain protein does not include deletions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain protein. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain protein does not include insertions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain protein. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain protein includes substitutions that are conservative substitutions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain protein. In embodiments, the CD3ζ intracellular T-cell signaling domain includes all or a portion of the protein identified by the NCBI sequence reference NP_000725.1, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD3ζ intracellular T-cell signaling domain includes all or a portion of the protein identified by the NCBI sequence reference NP_932170.1, or an isoform or naturally occurring mutant or variant thereof. Non-limiting examples of human CD3-zeta amino acid sequences available under NCBI sequence references are identified *supra*.

[0081] In embodiments, the CD3ζ intracellular T-cell signaling domain is encoded by all or a portion of the nucleic acid sequence identified by the NCBI sequence reference NM_000734.3, or an isoform or naturally occurring mutant or variant thereof. In embodiments, the CD3ζ

intracellular T-cell signaling domain is encoded by all or a portion of the nucleic acid sequence identified by the NCBI sequence reference NM_198053.2, or an isoform or naturally occurring mutant or variant thereof. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain nucleic acid sequence includes no more than 5, 4, 3, 2, or 1 deletions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain nucleic acid sequence. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain nucleic acid sequence includes no more than 5, 4, 3, 2, or 1 insertions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain nucleic acid sequence. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain nucleic acid sequence does not include deletions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain nucleic acid sequence. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain nucleic acid sequence does not include insertions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain nucleic acid sequence. In embodiments, a variant or mutant of the CD3ζ intracellular T-cell signaling domain nucleic acid sequence includes substitutions that are conservative substitutions compared to the naturally occurring CD3ζ intracellular T-cell signaling domain nucleic acid sequence.

[0082] A "control" sample or value refers to a sample that serves as a reference, usually a known reference, for comparison to a test sample. For example, a test sample can be taken from a test condition, *e.g.,* in the presence of a test compound, and compared to samples from known conditions, *e.g.,* in the absence of the test compound (negative control), or in the presence of a known compound (positive control). A control can also represent an average value gathered from a number of tests or results. One of skill in the art will recognize that controls can be designed for assessment of any number of parameters. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.,* half-life) or therapeutic measures (*e.g.,* comparison of side effects). Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant.

[0083] As used herein, the term "cancer" or “tumor” refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans), including leukemias, lymphomas, carcinomas and sarcomas. Exemplary cancers that may be treated with a compound or method provided herein include brain cancer, glioma, glioblastoma, neuroblastoma, prostate cancer, colorectal cancer, pancreatic cancer, Medulloblastoma, melanoma, cervical cancer, gastric cancer, ovarian cancer, lung cancer, cancer of the head, Hodgkin's Disease, and Non-Hodgkin's

Lymphomas. Exemplary cancers that may be treated with a compound or method provided herein include cancer of the thyroid, endocrine system, brain, breast, cervix, colon, head & neck, liver, kidney, lung, ovary, pancreas, rectum, stomach, and uterus. Additional examples include, thyroid carcinoma, cholangiocarcinoma, pancreatic adenocarcinoma, skin cutaneous melanoma, colon adenocarcinoma, rectum adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, breast invasive carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, non-small cell lung carcinoma, mesothelioma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, or prostate cancer.

[0084] "Treating" or "treatment" as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject's condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease's transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, "treatment" as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease's spread; relieve the disease's symptoms.,, fully or partially remove the disease's underlying cause, shorten a disease's duration, or do a combination of these things.

[0085] "Treating" and "treatment" as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease

over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient. In embodiments, the treating or treatment is no prophylactic treatment.

[0086] "Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0087] A "effective amount" is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (*e.g.,* achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A "prophylactically effective amount" of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "activity decreasing amount," as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A "function disrupting amount," as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th

Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0088] For any compound described herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art.

[0089] As is well known in the art, therapeutically effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring compounds effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[0090] The term "therapeutically effective amount," as used herein, refers to that amount of the therapeutic agent sufficient to ameliorate the disorder, as described above. For example, for the given parameter, a therapeutically effective amount will show an increase or decrease of at least 5%, 10%, 15%, 20%, 25%, 40%, 50%, 60%, 75%, 80%, 90%, or at least 100%. Therapeutic efficacy can also be expressed as "-fold" increase or decrease. For example, a therapeutically effective amount can have at least a 1.2-fold, 1.5-fold, 2-fold, 5-fold, or more effect over a control.

[0091] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present disclosure, should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[0092] As used herein, the term "administering" means oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release

device, *e.g.,* a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (*e.g.,* buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, *e.g.,* intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, *etc.* In embodiments, the administering does not include administration of any active agent other than the recited active agent.

[0093] "Co-administer" it is meant that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds provided herein can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present disclosure can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0094] An "anticancer agent" as used herein refers to a molecule (e.g. compound, peptide, protein, nucleic acid, 0103) used to treat cancer through destruction or inhibition of cancer cells or tissues. Anticancer agents may be selective for certain cancers or certain tissues. In embodiments, anticancer agents herein may include epigenetic inhibitors and multi-kinase inhibit "Anti-cancer agent" and "anticancer agent" are used in accordance with their plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (*e.g.,* cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g., mechloroethamine, cyclophosphamide, chlorambucil, meiphalan), ethylenimine and

methylmelamines (e.g., hexamethlymelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomusitne, semustine, streptozocin), triazenes (decarbazine)), anti-metabolites (*e.g.,* 5- azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (*e.g.,* vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (*e.g.,* irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (*e.g.,* doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (*e.g.* cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossyphol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C;

camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-

based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth

inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin I1 (including recombinant interleukin II, or rlL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone

hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of

Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, lnanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tuiarik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desaetyleleutherobin, lsoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (*e.g.,* dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethlystilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (*e.g.,* Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (*e.g.,* anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (*e.g.,* anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (*e.g.,* anti-CD20 monoclonal antibody conjugated to ^{111}In, ^{90}Y, or ^{131}I, *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa ™), erlotinib (Tarceva ™), cetuximab (Erbitux™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0095] "Selective" or "selectivity" or the like of a compound refers to the compound's ability to discriminate between molecular targets (e.g. a compound having selectivity toward ROR1).

[0096] "Specific", "specifically", "specificity", or the like of a compound refers to the compound's ability to cause a particular action, such as inhibition, to a particular molecular target with minimal or no action to other proteins in the cell.

[0097] An "ROR1 inhibitor" refers to a compound (e.g. compounds described herein) that reduces the activity of ROR1 when compared to a control, such as absence of the compound or a compound with known inactivity.

[0098] "Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0099] As defined herein, the term "inhibition", "inhibit", "inhibiting" and the like in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In embodiments, inhibition refers to a reduction in the activity of a particular protein target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation). A "ROR1 inhibitor" is a compound that negatively affects (e.g. decreases) the activity or function of ROR1 relative to the activity or function of ROR1 in the absence of the inhibitor.

[00100] The term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting protein (*e.g.,* ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, *etc.*).

[00101] The term "associated" or "associated with" in the context of a substance or substance activity or function associated with a disease (e.g. a protein associated disease, a cancer associated with ROR1 activity, ROR1 associated cancer, ROR1 associated disease (*e.g.,* cancer, inflammatory disease, autoimmune disease, or infectious disease)) means that the disease (e.g. cancer, inflammatory disease, autoimmune disease, or infectious disease) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease. For example, a cancer associated with ROR1 activity or function or a ROR1 associated disease (*e.g.,* cancer, inflammatory disease, autoimmune disease, or infectious disease), may be treated with a ROR1 modulator or ROR1 inhibitor, in the instance where increased ROR1 activity or function (e.g. signaling pathway activity) causes the disease (*e.g.,* cancer, inflammatory disease, autoimmune disease, or infectious disease). For example, an inflammatory disease associated with ROR1 activity or function or an ROR1 associated inflammatory disease, may be treated with an ROR1 modulator or ROR1 inhibitor, in the instance where increased ROR1 activity or function (e.g. signaling pathway activity) causes the disease.

[00102] The term "signaling pathway" as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propagated to other signaling pathway components.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

COMPOSITIONS

[00103] Provided herein are recombinant protein/chimeric antigen receptor (these terms are used interchangeably throughout) compositions and methods of using the same that are, *inter alia*, useful for treating hematological malignancies. Applicants have discovered that chimeric antigen receptors (CARs) directed to ROR-1 provide for highly active and efficient immunotherapeutic compositions. Without being bound to any particular theory, antibodies known to inhibit the receptor they bind and downregulate its surface expression are generally not

considered good clinical candidates for CARs. Therefore, it was very surprising that CARs including CDRs of ROR-1 antibodies described herein including embodiments thereof exhibit effective cancer-specific cytotoxicity when expressed by T cells.

[00104] In one aspect, there is provided a recombinant protein or a composition comprising a recombinant protein, which includes an antigen binding region, preferably a ROR-1 binding domain, and a transmembrane domain. In embodiments, the ROR-1 binding domain is an antibody. In embodiments, the ROR-1 binding domain is an antibody fragment.

[00105] In one aspect, there is provided a recombinant protein including (i) an antibody region including: (a) a light chain variable domain comprising a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and (b) a heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; and (ii) a transmembrane domain.

[00106] In one aspect a chimeric antigen receptor is provided. The chimeric antigen receptor includes: i. an antigen binding region, wherein the antigen binding region specifically binds ROR-1, a transmembrane domain, a spacer domain that couples the antigen binding region and the transmembrane domain, and an intracellular domain. The terms "antibody region", "antigen binding region", or "antigen binding domain" as provided herein are used interchangeably throughout and refer to a monovalent or multivalent protein moiety that forms part of the protein (i.e. recombinant protein, chimeric antigen receptor) provided herein including embodiments thereof. A person of ordinary skill in the art would therefore immediately recognize that the antibody region or antigen binding region is a protein moiety capable of binding an antigen (epitope). In embodiments, the ROR-1 binding domain includes an antibody region including a light chain variable region (V_L) and a heavy chain variable region (V_H). In embodiments, the light chain variable domain includes a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45, and the heavy chain variable domain including a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48. It is noted that those CDRs are of or are derived from cirmtuzumab (also known as UC-961 or 99961.1). The development and structure of cirmtuzumab is disclosed in US Patent Application No.14/422,519, which is incorporated by reference herein in its entirety and for all purposes. In embodiments, the ROR-1 binding domain includes an antibody region including CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51, and the

heavy chain variable domain includes a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54.

[00107] In one aspect a chimeric antigen receptor is provided. The chimeric antigen receptor includes: i. an antigen binding region, wherein the antigen binding region specifically binds ROR-1, and wherein the antigen binding region includes a light chain variable domain and a heavy chain variable domain; (a) wherein the light chain variable domain includes a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and the heavy chain variable domain includes a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; or (b) wherein the light chain variable domain includes a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and the heavy chain variable domain includes a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54; ii. a spacer domain, wherein the spacer domain includes a spacer of between 10 and 240 amino acids in length; iii. a transmembrane domain; and iv. an intracellular domain.

[00108] In one aspect a chimeric antigen receptor is provided. The chimeric antigen receptor includes: i. an antigen binding region, wherein the antigen binding region specifically binds ROR-1 and wherein the antigen binding region includes a light chain variable domain and a heavy chain variable domain; (a) wherein the light chain variable domain includes a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and the heavy chain variable domain includes a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; or (b) wherein said light chain variable domain includes a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and the heavy chain variable domain including a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54; ii. a transmembrane domain; and iii. an intracellular domain, wherein the intracellular domain includes an intracellular T cell signaling domain and an intracellular co-stimulatory domain selected from, 4-1BB, ICOS, OX-40, and combinations thereof.

[00109] The terms "antibody region" and “antigen binding region” as provided herein are used interchangeably throughout and refer to a monovalent or multivalent protein moiety that forms part of the protein (i.e. recombinant protein, chimeric antigen receptor) provided herein including embodiments thereof. A person of ordinary skill in the art would therefore immediately

recognize that the antibody region or antigen binding region is a protein moiety capable of binding an antigen (epitope). The antibody region provided herein may include a domain of an antibody or fragment (e.g., Fab) thereof. Thus, the antibody region may include a light chain variable domain (VL) and/or a heavy chain variable domain (VH).

[00110] A light chain variable (VL) domain as provided includes CDR sequences and framework region (FR) sequences of the light chain of an antibody, an antibody variant or fragment thereof. In embodiments, the antibody region or antigen binding region includes a variable light chain domain and a variable heavy chain domain. A "variable light chain domain" as provided herein refers to a polypeptide included in (forming part of) a light chain variable (VL) region. In embodiments, the variable light chain region is a light chain variable (VL) domain. A "variable heavy chain domain" as provided herein refers to a polypeptide included in (forming part of) a heavy chain variable (VH) region. In embodiments, the variable heavy chain region is a heavy chain variable (VH) domain. In embodiments, the light chain variable (VL) domain includes CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45). In embodiments, the heavy chain variable (VH) domain includes CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48). It is noted that those CDRs are of or are derived from cirmtuzumab (also known as UC-961 or 99961.1). The development and structure of cirmtuzumab is disclosed in US Patent Application No.14/422,519 which is incorporated by reference herein in its entirety and for all purposes.

[00111] In embodiments, the light chain variable domain includes the amino acid sequence of SEQ ID NO:21, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21. In embodiments, the light chain variable domain has the amino acid sequence of SEQ ID NO:21, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21. In embodiments, the heavy chain variable domain includes the amino acid sequence of SEQ ID NO:27, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27. In embodiments, the heavy chain variable domain has the amino acid sequence of SEQ ID NO:27, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27. In embodiments

[00112] , the light chain variable domain includes the amino acid sequence of SEQ ID NO:19, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19. In embodiments, the light chain variable domain has the amino acid sequence of SEQ ID NO:19, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19. In embodiments, the light chain variable domain includes the amino acid sequence of SEQ ID

NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20. In embodiments, the light chain variable domain has the amino acid sequence of SEQ ID NO:20, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20. In embodiments, the heavy chain variable domain includes the amino acid sequence of SEQ ID NO:25, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:25. In embodiments, the heavy chain variable domain has the amino acid sequence of SEQ ID NO:25, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:25. In embodiments, the heavy chain variable domain includes the amino acid sequence of SEQ ID NO:26, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:26. In embodiments, the heavy chain variable domain has the amino acid sequence of SEQ ID NO:26, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:26.

[00113] In embodiments, the C-terminus of the light chain variable domain is bound to the N-terminus of the heavy chain variable domain. In embodiments, the N-terminus of the light chain variable domain is bound to the C-terminus of the heavy chain variable domain. In embodiments, the light chain variable domain is covalently bound to the heavy chain variable domain through a chemical linker. A "chemical linker," as provided herein, is a covalent linker, a non-covalent linker, a peptide linker (a linker including a peptide moiety), a cleavable peptide linker, a substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene or substituted or unsubstituted heteroarylene or any combination thereof. Thus, a chemical linker as provided herein may include a plurality of chemical moieties, wherein each of the plurality of chemical moieties is chemically different. Alternatively, the chemical linker may be a non-covalent linker. Examples of non-covalent linkers include without limitation, ionic bonds, hydrogen bonds, halogen bonds, van der Waals interactions (e.g. dipole-dipole, dipole-induced dipole, London dispersion), ring stacking (pi effects), and hydrophobic interactions. In embodiments, a chemical linker is formed using conjugate chemistry including, but not limited to nucleophilic substitutions (*e.g.,* reactions of amines and alcohols with acyl halides, active esters), electrophilic substitutions (*e.g.,* enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (*e.g.,* Michael reaction, Diels-Alder addition). In embodiments, the chemical linker is a peptide linker, which comprises 5-100, 5-80, 5-70, 5-60, 5-50, 10-50, 10-40, or 10-30 amino acids in length. Any sequence that provides sufficient flexibility between the light chain variable domain and the heavy chain variable domain is contemplated for the recombinant proteins provided herein including embodiments

thereof. In embodiments, the peptide linker includes or has the amino acid sequence of SEQ ID NO:24. In embodiments, the peptide linker has an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:24.

[00114] Any transmembrane domain capable of anchoring the proteins provided herein including embodiments thereof are contemplated. While any suitable transmembrane domains are contemplated herein, exemplary, but non-limiting examples of transmembrane domains include the transmembrane domains of CD28, CD8, CD4, CD3ζ, or CD8α. In preferred embodiments, the transmembrane domain is a CD28 transmembrane domain. The term "CD28 transmembrane domain" as provided herein includes any of the recombinant or naturally-occurring forms of the transmembrane domain of CD28, or variants or homologs thereof that maintain CD28 transmembrane domain activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the CD28 transmembrane domain). In some aspects, the variants or homologs have at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity across the whole sequence or a portion of the a naturally occurring CD28 transmembrane domain polypeptide. In embodiments, the CD28 transmembrane domain includes an amino acid sequence of SEQ ID NO:32. In embodiments, the CD28 transmembrane domain is an amino acid sequence of SEQ ID NO:32. In embodiments, the CD28 transmembrane domain in the ROR1 CAR described herein has a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:32.

[00115] In embodiments, the C-terminus of the heavy chain variable domain is coupled to the N-terminus of the transmembrane domain. In embodiments, the C-terminus of the light chain variable domain is coupled to the N-terminus of the transmembrane domain. In embodiments, the heavy chain variable domain or light chain variable domain is covalently bound to the transmembrane domain through a spacer domain. In embodiments, the binding affinity of the antigen binding region to the antigen is increased in the CAR construct having the spacer domain. In embodiments, the activity of the CAR having the spacer domain is higher than the CAR without the spacer domain. In embodiments, the flexibility of the antigen binding region of the CAR is increased by the presence of the spacer domain.

[00116] Any suitable chemical or biological moieties that can provide a space or flexibility to the antigen binding region or antigen binding domain, without providing excessive steric hindrance to the antigen binding domain, are contemplated. Preferably, the spacer domain comprises a polypeptides that are in length of between 10-250 amino acids, between 12-250 amino acids, between 12-200 amino acids, between 14-250 amino acids, between 14-200 amino

acids, between 14-150 amino acids. Thus, exemplary spacer domains herein include, but not limited to, immunoglobulin molecules or fragments thereof (*e.g.,* IgG1, IgG2, IgG3, IgG4, or constant heavy chain 1 (CH1), constant heavy chain 2 (CH2), or constant heavy chain 3(CH3) domain(s) of the IgG1, IgG2, IgG3 or IgG4) or immunoglobulin molecules or fragments thereof (*e.g.,* IgG1, IgG2, IgG3, IgG4) including mutations affecting Fc receptor binding. Alternatively and/or additionally, some contemplated spacer domains include all or parts of an immunoglobulin (*e.g.,* IgG1, IgG2, IgG3, IgG4) hinge region, *i.e.,* the sequence that falls between the CH1 and CH2 domains of an immunoglobulin, *e.g.,* an IgG4 Fc hinge or a CD8 hinge. Some spacer domains include an immunoglobulin CH3 domain or both a CH3 domain and a CH2 domain. The immunoglobulin derived sequences can include one or more amino acid modifications, for example, 1, 2, 3, 4 or 5 substitutions (*e.g.,* substitutions that reduce off-target binding) or addition of less than 10, less than 5, less than 3 amino acid sequences at its N terminal or C terminal ends.

[00117] In embodiments, the spacer domain includes a hinge domain, and optionally one of or both of CH3 domain and CH2 domain of an immunoglobulin molecule. Thus, in embodiments, a spacer domain has or consists of a hinge region or a modified hinge region (*e.g.,* with one or more amino acid substitution or addition). In embodiments, a spacer domain has or consists of a hinge region coupled to the CH3 domain of hIgG4 at the C-terminus of the hinge region. In embodiments, a spacer domain has or consists of a hinge region coupled to the CH2 domain of hIgG4 at the C-terminus of the hinge region. Alternatively, a spacer domain has or consists of a hinge region coupled to the CH2-CH3 domain of the hIgG4 at the C-terminus of the hinge region. In embodiments, the hinge region used herein comprises or consist of an amino acid sequence of SEQ ID NO:29, or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:29. In embodiments, the spacer domain includes the amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42. In embodiments, the spacer domain has or consists of the amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00118] In embodiments, the recombinant protein (*e.g.*, the intracellular domain) further includes one or more intracellular co-stimulatory signaling domains. An "intracellular co-stimulatory signaling domain" as provided herein includes amino acid sequences capable of providing co-stimulatory signaling in response to binding of an antigen to the antibody region

provided herein including embodiments thereof. In embodiments, the signaling of the co-stimulatory signaling domain results in production of cytokines and proliferation of the T cell expressing the same. In embodiments, the intracellular co-stimulatory signaling domain comprises at least one or more of a CD28 intracellular co-stimulatory signaling domain, a 4-1BB (CD137) intracellular co-stimulatory signaling domain, an ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain, or any combinations thereof. For example, a preferred intracellular co-stimulatory signaling domain comprises or consists of at least a portion of CD28 intracellular co-stimulatory signaling domain, or at least a portion of a 4-1BB (CD137) intracellular co-stimulatory signaling domain. Another preferred intracellular co-stimulatory signaling domain comprises or consists of at least a portion of CD28 intracellular co-stimulatory signaling domain coupled with at least a portion of a 4-1BB (CD137) intracellular co-stimulatory signaling domain. In embodiments, at least a portion of the CD28 intracellular domain comprises or consists of the amino acid sequence of SEQ ID NO:32 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:32. In embodiments, at least a portion of the CD28 intracellular domain comprises or consists of the amino acid sequence of SEQ ID NO:32 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:32. In embodiments, at least a portion of the 4-1BB intracellular co-stimulatory signaling domain comprises or consists of the amino acid sequence of SEQ ID NO:33 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:33. In embodiments, at least a portion of the 4-1BB intracellular co-stimulatory signaling domain comprises or consists of the amino acid sequence of SEQ ID NO:33 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:29. In embodiments, the chimeric antigen receptor disclosed herein or the recombinant protein includes an amino acid sequence of a combination of SEQ ID NO: 32 and SEQ ID NO:33 (either SEQ ID NO: 32 present at the N'-terminus of SEQ ID NO: 33, or SEQ ID NO: 33 present at the N'-terminus of SEQ ID NO: 32), or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to a combination of SEQ ID NO: 32 and SEQ ID NO:33 (either SEQ ID NO: 32 present at the N'-terminus of SEQ ID NO: 33, or SEQ ID NO: 33 present at the N'-terminus of SEQ ID NO: 32).

[00119] In embodiments, the recombinant protein further includes an intracellular T-cell signaling domain. An "intracellular T-cell signaling domain" as provided herein includes amino acid sequences capable of providing primary signaling in response to binding of an antigen to the antibody region provided herein including embodiments thereof. In embodiments, the signaling of the intracellular T-cell signaling domain results in activation of the T cell expressing the same.

In embodiments, the signaling of the intracellular T-cell signaling domain results in proliferation (cell division) of the T cell expressing the same. In embodiments, the signaling of the intracellular T-cell signaling domain results expression by the T cell of proteins known in the art to characteristic of activated T cell (*e.g.,* CTLA-4, PD-1, CD28, CD69). In embodiments, the intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain. In embodiments, the CD3ζ intracellular T-cell signaling domain includes the amino acid sequence of SEQ ID NO:34 or an amino acid sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:34 that generates a CD3ζ intracellular T-cell signaling domain having at least 80%, 85%, 90%, or 95% activity of the CD3ζ intracellular T-cell signaling domain having SEQ ID NO:34. In embodiments, the CD3ζ intracellular T-cell signaling domain has the amino acid sequence of SEQ ID NO:34 or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:34 that generates a peptide having at least 80%, 85%, 90%, or 95% activity of the CD3ζ intracellular T-cell signaling domain having SEQ ID NO:34.

[00120] In embodiments, the ROR-1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH2 and CH3 domain, iii) CD28 transmembrane domain, iv) 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00121] Alternatively, in embodiments, the ROR1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3

(SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH2 domain, iii) CD28 transmembrane domain, iv) 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00122] Alternatively, in embodiments, the ROR1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH3 domain, iii) CD28 transmembrane domain, iv) 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00123] Alternatively, in embodiments, the ROR1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and a portion of CH3 domain (*e.g.,* a half of CH3 domain, N-terminal 43 amino acids of CH3 domain, etc.), iii) CD28 transmembrane domain, iv) 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00124] Alternatively, in embodiments, the ROR1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge domain, iii) CD28 transmembrane domain, iv) 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00125] Consequently, in embodiments, the ROR1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH2 and CH3 domain, iii) CD28 transmembrane domain, iv) CD28 costimulatory domain coupled to 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00126] Alternatively, in embodiments, the ROR-1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or

alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH2 domain, iii) CD28 transmembrane domain, iv) CD28 costimulatory domain coupled to 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00127] Alternatively, in embodiments, the ROR-1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge and CH3 domain, iii) CD28 transmembrane domain, iv) CD28 costimulatory domain coupled to 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00128] Alternatively, in embodiments, the ROR-1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer

domain having a hinge and a portion of CH3 domain (*e.g.,* a half of CH3 domain, N-terminal 43 amino acids of CH3 domain, etc.), iii) CD28 transmembrane domain, iv) CD28 costimulatory domain coupled to 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00129] Alternatively, in embodiments, the ROR-1 CAR disclosed herein includes or consists of i) ROR1 scFv (having CDR L1 (SEQ ID NO:43), CDR L2 (SEQ ID NO:44), and CDR L3 (SEQ ID NO:45), CDR H1 (SEQ ID NO:46), CDR H2 (SEQ ID NO:47), and CDR H3 (SEQ ID NO:48), or VL domain having the sequence of SEQ ID NO:21, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:21 and the VH domain having the sequence of SEQ ID NO:27, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:27) or alternatively ROR1 scFv (CDR L1 (SEQ ID NO:49), CDR L2 (SEQ ID NO:50), and CDR L3 (SEQ ID NO:51), CDR H1 (SEQ ID NO:52), CDR H2 (SEQ ID NO:53), and CDR H3 (SEQ ID NO:54), or VL domain having the sequence of SEQ ID NO:19, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:19 and the VH domain having the sequence of SEQ ID NO:20, or a sequence at least 80%, 85%, 90%, or 95% identical to SEQ ID NO:20), ii) a spacer domain having a hinge domain, iii) CD28 transmembrane domain, iv) CD28 costimulatory domain coupled to 4-1BB (CD137) costimulatory domain, and v) CD3Z T cell activation domain.

[00130] It is contemplated that the chimeric antigen receptor described here binds to amino acids 130-160 of ROR-1 or a fragment thereof, preferably to a peptide including a glutamic acid at a position corresponding to position 138 of ROR-1 polypeptide. Alternatively and/or additionally, the chimeric antigen receptor described herein specifically binds either the 3' or middle Ig-like region of the extracellular domain of the ROR-1 protein, preferably to 3' end of the Ig-like region of the extracellular domain of ROR-1 protein from position 1-147.

[00131] Consequently, the ROR-1 CAR disclosed herein binds to a ROR-1 expressing cells and can initiate or induce immune response against the ROR-1 expressing cells, including a cancer cell, such as leukemia cell, a lymphoma cell, a chronic lymphocytic leukemia (CLL) cell, an adult acute myeloid leukemia (AML) cell, an acute lymphoblastic leukemia (ALL) cell, a mantle cell lymphoma cell, an ovarian cancer cell, a colon cancer cell, a lung cancer cell, a skin cancer cell, a pancreatic cancer cell, a testicular cancer cell, a bladder cancer cell, a uterine cancer cell, a prostate cancer cell, or an adrenal cancer cell. wherein the recombinant protein forms part of a cell.

[00132] In embodiments, the recombinant protein provided herein, including embodiments

thereof, further includes a detectable domain. A "detectable domain" as provided herein is a peptide moiety detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, a detectable domain as provided herein may be a protein or other entity which can be made detectable, *e.g.,* by incorporating a radiolabel or being reactive to an antibody specifically. Any appropriate method known in the art for conjugating an antibody to the label may be employed, *e.g.,* using methods described in Hermanson, Bioconjugate Techniques 1996, Academic Press, Inc., San Diego. In the present invention, a detectable domain is used to confirm transfection of T cells.

[00133] In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 500 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 550 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 600 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 650 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 700 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 750 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 800 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 850 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 900 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 950 pM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1.5 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 2 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 2.5 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 3 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 3.5 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 4 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding

affinity of about 4.5 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 5 nM to about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 5.5 nM to about 6 nM.

[00134] In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 500 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 500 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 550 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 550 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 600 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 600 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 650 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 650 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 700 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 700 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 750 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 750 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 800 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 800 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 850 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 850 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 900 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 900 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 950 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 950 pM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 1 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 1.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a

binding affinity of 1.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 2 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 2 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 2.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 2.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 3 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 3 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 3.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 3.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 4 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 4 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 4.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 4.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 5.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 5.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor has a binding affinity of 6 nM.

[00135] In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 40 nM (*e.g.*, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1 nM). In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 40 nM (*e.g.*, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1 nM). In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 35 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 35 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 30 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 30 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein

with a K_D of less than about 25 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 25 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 20 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 20 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 15 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 15 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 10 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 10 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 9 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 9 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 8 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 8 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 7 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 7 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 6 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 5 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 5 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 4 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 4 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 3 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 3 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 2 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 2 nM. In embodiments, the recombinant protein or

chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 1 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 1 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 0.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 0.5 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 0.25 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 0.25 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than about 0.1 nM. In embodiments, the recombinant protein or chimeric antigen receptor binds to an ROR-1 protein with a K_D of less than 0.1 nM.

[00136] It is contemplated that the chimeric antigen receptor described herein is expressed on the surface of T lymphocytes or T cells (e.g.,*e.g.,* CD8+ T cells, CD4+ T cells) by genetically engineering the T cells to express the heterologous nucleic acid sequences encoding the chimeric nucleic acid receptor. Such generated CAR-T cells specifically bind to ROR-1-expressing cells, preferably, ROR1 expressing cancer cells, to elicit an immune response against the ROR-1 expressing cancer cells. In some embodiments, the chimeric antigen receptor described herein is expressed on the surface of natural killer cells (NK cells or genetically modified or engineered NK cells). In certain embodiments, the NK cells are CD56 positive cells. CD56 positivity can be determined by for example flow cytometry analysis of acell population and defined as cells at least 10x, 100x, or 1,000x compared to cells stained with an isotype control antibody. In certain embodiments, the NK cells are primary NK cells. In certain embodiments, the NK cells comprise an NK cell line. In certain embodiments, the NK cells are autologous to an individual being treated with a CAR expressing NK cell of this disclosure. In certain embodiments, the NK cells are heterologous to an individual being treated with a CAR expressing NK cell of this disclosure. In certain embodiments, the NK cells are allogeneic to an individual being treated with a CAR expressing NK cell of this disclosure. NK cells can be derived from any suitbale source bone marrow, induced pluripotent stem cells, peripheral blood mononuvlear cells, fetal or placental cells.

[00137] Further provided herein are recombinant nucleic acids encoding the recombinant protein or chimeric antigen receptor provided herein including embodiments thereof. In some embodiments, the chimeric antigen receptor is encoded by a single recombinant nucleic acid that forms part of an expression vector. Any suitable expression vector that is capable of being

transfected into and expressed in an immune cells (*e.g.,* CD8+ T cells, CD4+ T cells, CD56+ immune cells, NK cells, or genetically modified or engineered NK cells) are contemplated. Exemplary and/or preferred expression vectors may include a viral expression vector (*e.g.,* expression vectors for adenovirus, adeno-associated viruses, alphaviruses, herpes viruses, lentiviruses, etc.). In some embodiments, an adenovirus is a replication deficient and non-immunogenic virus, which is typically accomplished by targeted deletion of selected viral proteins (*e.g.,* E1, E2b, E3 proteins). For example, the recombinant nucleic acid can be placed in a non-viral vector (*e.g.,* mammalian expression vector) and transfected to the T cells using any generally used transfection method. For other example, the recombinant nucleic acid in a viral vector such that the viral particle including recombinant nucleic acid infect the T cells to deliver the recombinant nucleic acid into the T cells. For still other example, where the recombinant nucleic acid is a self-replicating RNA-based vector, self-replicating RNA-based vector can be formulated with a pharmaceutically acceptable carrier (*e.g.,* in a buffer or cell culture medium, preferably with RNAase inhibitors)) such that the self-replicating RNA-based vector can be delivered in a naked form to the cell by contacting directly to the cell membrane. In some embodiments, the self-replicating RNA-based vector can be coupled to a carrier molecule. Exemplary carrier molecules includes protein A, protein G, protein Z, albumin, refolded albumin, a nanoparticle (*e.g.,* quantum dots, gold nanoparticles, magnetic nanoparticles, nanotubes, polymeric nanoparticles, dendrimers, etc.), or a bead (*e.g.,* polystyrene bead, latex bead, dynabead, etc.). Preferably, the nanoparticle and/or beads have a dimension below 1 μm, preferably below 100 nm. In other embodiments, the self-replicating RNA-based vector can be coupled with a micro particle (*e.g.,* PLG RG503 (50:50 lactide/glycolide molar ratio), where the self-replicating RNA-based vector can be absorbed to the micro particle for further delivery to the cell. In still other embodiments, the self-replicating RNA-based vector can be encapsulated in a liposome (*e.g.,* PEG-based liposome, etc.) to protect the self-replicating RNA-based vector from RNAase digestion and deliver the RNA-based vector by fusing the liposome to the target cell membrane.

[00138] In embodiments, the recombinant nucleic acids encoding the recombinant protein or chimeric antigen receptor provided herein can be inserted into a vector having a cassette for gene editing (e.g., CRISPR-CAS expression vector). Another exemplary expression vectors may include transposon-based expression system (*e.g.,* Sleeping Beauty system, as disclosed in Deninger et al., PLOS ONE, June 1, 2015).

PHARMACEUTICAL COMPOSITIONS

[00139] The immune cells expressing chimeric antigen receptor(s) and/or the recombinant nucleic acid encoding the chimeric antigen receptor(s) may further formulated as a pharmaceutical composition, optionally with any pharmaceutically acceptable carrier (*e.g.,* as a sterile injectable composition for immune cells expressing chimeric antigen receptor(s), a pharmaceutically acceptable salt for recombinant nucleic acid encoding the chimeric antigen receptor(s), etc.). While the dose or cell titer of the pharmaceutical composition may vary depending on the treatment protocols, treatment regimens, treatment conditions, etc., one example of the dose or cell titer of the pharmaceutical composition may include a cell titer of at least 1×10^3 cells/ml, preferably at least 1×10^5 cells/ml, more preferably at least 1×10^6 cells/ml, and at least 1 ml, preferably at least 5ml, more preferably and at least 20 ml per dosage unit.

[00140] In some embodiments, the pharmaceutical composition may include a homogenous cell or plurality thereof (*e.g.,* CD8+ T cells expressing the chimeric antigen receptor, CD4+ T cells expressing the chimeric antigen receptor NK cells expressing the chimeric antigen receptor, etc.). In other embodiments, the composition can comprise a mixture of heterogeneous cells (*e.g.,* mixture of CD8+ T cells expressing the chimeric antigen receptor and NK cells expressing the chimeric antigen receptor, etc., in a ratio of 1:1, 1:2, 1:3, 1:4, 4:1, 3:1, 2:1, etc.). In this pharmaceutical composition, the ratio of different types of cells may vary based on the type of cancer, age, gender, or health status of the patient, size of tumor, and the cell counts of the patient. The actual amount effective for a particular application will depend, *inter alia,* on the condition being treated. When administered in methods to treat a disease, the recombinant proteins described herein will contain an amount of active ingredient effective to achieve the desired result, *e.g.,* modulating the activity of a target molecule, and/or reducing, eliminating, or slowing the progression of disease symptoms. Determination of a therapeutically effective amount of a compound of the invention is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[00141] The dosage and frequency (single or multiple doses) administered to a mammal can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated (e.g. symptoms of cancer and severity of such symptoms), kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can

be used in conjunction with the methods and compounds of the invention. Adjustment and manipulation of established dosages (*e.g.,* frequency and duration) are well within the ability of those skilled in the art.

[00142] For any composition (*e.g.,* recombinant protein, nucleic acid) provided herein, the therapeutically effective amount can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of achieving the methods described herein, as measured using the methods described herein or known in the art. As is well known in the art, effective amounts for use in humans can also be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be adjusted by monitoring effectiveness and adjusting the dosage upwards or downwards, as described above. Adjusting the dose to achieve maximal efficacy in humans based on the methods described above and other methods is well within the capabilities of the ordinarily skilled artisan.

[00143] Dosages may be varied depending upon the requirements of the patient and the compound being employed. The dose administered to a patient, in the context of the present invention should be sufficient to affect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached.

[00144] Dosage amounts and intervals can be adjusted individually to provide levels of the administered compound effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

[00145] Utilizing the teachings provided herein, an effective prophylactic or therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred

[00146] "Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject

and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates such as lactose, amylose or starch, fatty acid esters, hydroxymethycellulose, polyvinyl pyrrolidine, and colors, and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[00147] The term "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like.

[00148] The term "preparation" is intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly, cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

[00149] The pharmaceutical preparation is optionally in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The unit dosage form can be of a frozen dispersion.

METHODS OF TREATMENT

[00150] The CAR-T cell compositions described herein are useful for treating a cancer or a tumor in an individual. Thus, described herein is a method of treating cancer in a subject in need thereof. The method includes administering to a subject a therapeutically effective amount of a CAR-T cell provided herein including embodiments thereof, thereby treating the cancer or

tumor in the subject. CAR T-cell compositions are generally administered with one or more pharmaceutically acceptable excipients, carriers, or diluents.

[00151] In certain embodiments, disclosed herein, are anti-ROR-1 CAR T-cells and anti-ROR-1 CAR-T cell compositions useful for the treatment of a cancer or tumor. Treatment refers to a method that seeks to improve or ameliorate the condition being treated. With respect to cancer, treatment includes, but is not limited to, reduction of tumor volume, reduction in growth of tumor volume, increase in progression-free survival, or overall life expectancy. In certain embodiments, treatment will effect remission of a cancer being treated. In certain embodiments, treatment encompasses use as a prophylactic or maintenance dose intended to prevent reoccurrence or progression of a previously treated cancer or tumor. It is understood by those of skill in the art that not all individuals will respond equally or at all to a treatment that is administered, nevertheless these individuals are considered to be treated.

[00152] In certain embodiments, the cancer or tumor treated using the anti ROR-1 CAR-T cells described herein is a solid cancer or tumor. In certain embodiments, the cancer or tumor is a blood cancer or tumor. In certain embodiments, the blood cancer is a leukemia or lymphoma. In certain embodiments, the leukemia or lymphoma comprises a B cell leukemia, lymphoma, chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), multiple myeloma, B cell lymphoblastic acute lymphoblastic leukemia (B-ALL), mantle cell lymphoma (MCL), T cell lymphoblastic acute lymphoblastic leukemia T-ALL, and combinations thereof. In certain embodiments, the solid cancer/tumor comprises ovarian cancer, colon cancer, lung cancer, breast cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, adrenal cancer, and combinations thereof. In certain embodiments, the cancer is relapsed or is refractory to at least one other treatment.

[00153] In certain embodiments, the anti-ROR-1 CAR T-cells and anti-ROR-1 CAR-T cell compositions can be administered to a subject in need thereof by any route suitable for the administration of cell-containing pharmaceutical compositions, such as, for example, subcutaneous, intraperitoneal, intravenous, intramuscular, intratumoral, or intracerebral, etc. In certain embodiments, the antibodies are administered intravenously. In certain embodiments, the antibodies are administered subcutaneously. In certain embodiments, the antibodies are administered intratumoral.

[00154] The anti-ROR-1 CAR T-cells and anti-ROR-1 CAR-T cell compositions can be administered according to a suitable dosage schedule. In certain embodiments, the CAR T-cells are administered once, with subsequent doses depending on clinical criteria. If an individual does

not respond or only partially responds said patient can have an anti-ROR-1 CAR-T cell composition administered a second, third, or fourth time until the desired clinical response is observed. A dosage of CAR-T cells will generally comprise at least $1x10^6$ cells, but no more than $5x10^8$ cells. Cells can be administered based upon a total amount of an individual's viable PBMC that were transduced with a CAR construct. In certain embodiments, a single dosage comprises 1 million transduced PBMCs to 100 million transduced PBMCs. In certain embodiments, a single dosage comprises 1 million transduced PBMCs to 2 million transduced PBMCs, 1 million transduced PBMCs to 3 million transduced PBMCs, 1 million transduced PBMCs to 4 million transduced PBMCs, 1 million transduced PBMCs to 5 million transduced PBMCs, 1 million transduced PBMCs to 6 million transduced PBMCs, 1 million transduced PBMCs to 7 million transduced PBMCs, 1 million transduced PBMCs to 8 million transduced PBMCs, 1 million transduced PBMCs to 9 million transduced PBMCs, 1 million transduced PBMCs to 10 million transduced PBMCs, 1 million transduced PBMCs to 50 million transduced PBMCs, 1 million transduced PBMCs to 100 million transduced PBMCs, 2 million transduced PBMCs to 3 million transduced PBMCs, 2 million transduced PBMCs to 4 million transduced PBMCs, 2 million transduced PBMCs to 5 million transduced PBMCs, 2 million transduced PBMCs to 6 million transduced PBMCs, 2 million transduced PBMCs to 7 million transduced PBMCs, 2 million transduced PBMCs to 8 million transduced PBMCs, 2 million transduced PBMCs to 9 million transduced PBMCs, 2 million transduced PBMCs to 10 million transduced PBMCs, 2 million transduced PBMCs to 50 million transduced PBMCs, 2 million transduced PBMCs to 100 million transduced PBMCs, 3 million transduced PBMCs to 4 million transduced PBMCs, 3 million transduced PBMCs to 5 million transduced PBMCs, 3 million transduced PBMCs to 6 million transduced PBMCs, 3 million transduced PBMCs to 7 million transduced PBMCs, 3 million transduced PBMCs to 8 million transduced PBMCs, 3 million transduced PBMCs to 9 million transduced PBMCs, 3 million transduced PBMCs to 10 million transduced PBMCs, 3 million transduced PBMCs to 50 million transduced PBMCs, 3 million transduced PBMCs to 100 million transduced PBMCs, 4 million transduced PBMCs to 5 million transduced PBMCs, 4 million transduced PBMCs to 6 million transduced PBMCs, 4 million transduced PBMCs to 7 million transduced PBMCs, 4 million transduced PBMCs to 8 million transduced PBMCs, 4 million transduced PBMCs to 9 million transduced PBMCs, 4 million transduced PBMCs to 10 million transduced PBMCs, 4 million transduced PBMCs to 50 million transduced PBMCs, 4 million transduced PBMCs to 100 million transduced PBMCs, 5 million transduced PBMCs to 6 million transduced PBMCs, 5 million transduced PBMCs to 7 million transduced

PBMCs, 5 million transduced PBMCs to 8 million transduced PBMCs, 5 million transduced PBMCs to 9 million transduced PBMCs, 5 million transduced PBMCs to 10 million transduced PBMCs, 5 million transduced PBMCs to 50 million transduced PBMCs, 5 million transduced PBMCs to 100 million transduced PBMCs, 6 million transduced PBMCs to 7 million transduced PBMCs, 6 million transduced PBMCs to 8 million transduced PBMCs, 6 million transduced PBMCs to 9 million transduced PBMCs, 6 million transduced PBMCs to 10 million transduced PBMCs, 6 million transduced PBMCs to 50 million transduced PBMCs, 6 million transduced PBMCs to 100 million transduced PBMCs, 7 million transduced PBMCs to 8 million transduced PBMCs, 7 million transduced PBMCs to 9 million transduced PBMCs, 7 million transduced PBMCs to 10 million transduced PBMCs, 7 million transduced PBMCs to 50 million transduced PBMCs, 7 million transduced PBMCs to 100 million transduced PBMCs, 8 million transduced PBMCs to 9 million transduced PBMCs, 8 million transduced PBMCs to 10 million transduced PBMCs, 8 million transduced PBMCs to 50 million transduced PBMCs, 8 million transduced PBMCs to 100 million transduced PBMCs, 9 million transduced PBMCs to 10 million transduced PBMCs, 9 million transduced PBMCs to 50 million transduced PBMCs, 9 million transduced PBMCs to 100 million transduced PBMCs, 10 million transduced PBMCs to 50 million transduced PBMCs, 10 million transduced PBMCs to 100 million transduced PBMCs, or 50 million transduced PBMCs to 100 million transduced PBMCs. In certain embodiments, a single dosage comprises 1 million transduced PBMCs, 2 million transduced PBMCs, 3 million transduced PBMCs, 4 million transduced PBMCs, 5 million transduced PBMCs, 6 million transduced PBMCs, 7 million transduced PBMCs, 8 million transduced PBMCs, 9 million transduced PBMCs, 10 million transduced PBMCs, 50 million transduced PBMCs, or 100 million transduced PBMCs. In certain embodiments, a single dosage comprises at least 1 million transduced PBMCs, 2 million transduced PBMCs, 3 million transduced PBMCs, 4 million transduced PBMCs, 5 million transduced PBMCs, 6 million transduced PBMCs, 7 million transduced PBMCs, 8 million transduced PBMCs, 9 million transduced PBMCs, 10 million transduced PBMCs, or 50 million transduced PBMCs. In certain embodiments, a single dosage comprises at most 2 million transduced PBMCs, 3 million transduced PBMCs, 4 million transduced PBMCs, 5 million transduced PBMCs, 6 million transduced PBMCs, 7 million transduced PBMCs, 8 million transduced PBMCs, 9 million transduced PBMCs, 10 million transduced PBMCs, 50 million transduced PBMCs, or 100 million transduced PBMCs.

[00155] More or less cells may be used depending on the transduction efficiency of an individual's T cells on a case by case basis. In certain embodiments, a single dosage comprises 1

million CAR-T cells to 100 million CAR-T cells. In certain embodiments, a single dosage comprises 1 million CAR-T cells to 2 million CAR-T cells, 1 million CAR-T cells to 3 million CAR-T cells, 1 million CAR-T cells to 4 million CAR-T cells, 1 million CAR-T cells to 5 million CAR-T cells, 1 million CAR-T cells to 6 million CAR-T cells, 1 million CAR-T cells to 7 million CAR-T cells, 1 million CAR-T cells to 8 million CAR-T cells, 1 million CAR-T cells to 9 million CAR-T cells, 1 million CAR-T cells to 10 million CAR-T cells, 1 million CAR-T cells to 50 million CAR-T cells, 1 million CAR-T cells to 100 million CAR-T cells, 2 million CAR-T cells to 3 million CAR-T cells, 2 million CAR-T cells to 4 million CAR-T cells, 2 million CAR-T cells to 5 million CAR-T cells, 2 million CAR-T cells to 6 million CAR-T cells, 2 million CAR-T cells to 7 million CAR-T cells, 2 million CAR-T cells to 8 million CAR-T cells, 2 million CAR-T cells to 9 million CAR-T cells, 2 million CAR-T cells to 10 million CAR-T cells, 2 million CAR-T cells to 50 million CAR-T cells, 2 million CAR-T cells to 100 million CAR-T cells, 3 million CAR-T cells to 4 million CAR-T cells, 3 million CAR-T cells to 5 million CAR-T cells, 3 million CAR-T cells to 6 million CAR-T cells, 3 million CAR-T cells to 7 million CAR-T cells, 3 million CAR-T cells to 8 million CAR-T cells, 3 million CAR-T cells to 9 million CAR-T cells, 3 million CAR-T cells to 10 million CAR-T cells, 3 million CAR-T cells to 50 million CAR-T cells, 3 million CAR-T cells to 100 million CAR-T cells, 4 million CAR-T cells to 5 million CAR-T cells, 4 million CAR-T cells to 6 million CAR-T cells, 4 million CAR-T cells to 7 million CAR-T cells, 4 million CAR-T cells to 8 million CAR-T cells, 4 million CAR-T cells to 9 million CAR-T cells, 4 million CAR-T cells to 10 million CAR-T cells, 4 million CAR-T cells to 50 million CAR-T cells, 4 million CAR-T cells to 100 million CAR-T cells, 5 million CAR-T cells to 6 million CAR-T cells, 5 million CAR-T cells to 7 million CAR-T cells, 5 million CAR-T cells to 8 million CAR-T cells, 5 million CAR-T cells to 9 million CAR-T cells, 5 million CAR-T cells to 10 million CAR-T cells, 5 million CAR-T cells to 50 million CAR-T cells, 5 million CAR-T cells to 100 million CAR-T cells, 6 million CAR-T cells to 7 million CAR-T cells, 6 million CAR-T cells to 8 million CAR-T cells, 6 million CAR-T cells to 9 million CAR-T cells, 6 million CAR-T cells to 10 million CAR-T cells, 6 million CAR-T cells to 50 million CAR-T cells, 6 million CAR-T cells to 100 million CAR-T cells, 7 million CAR-T cells to 8 million CAR-T cells, 7 million CAR-T cells to 9 million CAR-T cells, 7 million CAR-T cells to 10 million CAR-T cells, 7 million CAR-T cells to 50 million CAR-T cells, 7 million CAR-T cells to 100 million CAR-T cells, 8 million CAR-T cells to 9 million CAR-T cells, 8 million CAR-T cells to 10 million CAR-T cells, 8 million CAR-T cells to 50 million CAR-T cells, 8 million CAR-T cells to 100 million CAR-T cells, 9 million CAR-T

cells to 10 million CAR-T cells, 9 million CAR-T cells to 50 million CAR-T cells, 9 million CAR-T cells to 100 million CAR-T cells, 10 million CAR-T cells to 50 million CAR-T cells, 10 million CAR-T cells to 100 million CAR-T cells, or 50 million CAR-T cells to 100 million CAR-T cells . In certain embodiments, a single dosage comprises 1 million CAR-T cells, 2 million CAR-T cells, 3 million CAR-T cells, 4 million CAR-T cells, 5 million CAR-T cells, 6 million CAR-T cells, 7 million CAR-T cells, 8 million CAR-T cells, 9 million CAR-T cells, 10 million CAR-T cells, 50 million CAR-T cells, or 100 million CAR-T cells . In certain embodiments, a single dosage comprises at least 1 million CAR-T cells, 2 million CAR-T cells, 3 million CAR-T cells, 4 million CAR-T cells, 5 million CAR-T cells, 6 million CAR-T cells, 7 million CAR-T cells, 8 million CAR-T cells, 9 million CAR-T cells, 10 million CAR-T cells, or 50 million CAR-T cells . In certain embodiments, a single dosage comprises at most 2 million CAR-T cells, 3 million CAR-T cells, 4 million CAR-T cells, 5 million CAR-T cells, 6 million CAR-T cells, 7 million CAR-T cells, 8 million CAR-T cells, 9 million CAR-T cells, 10 million CAR-T cells, 50 million CAR-T cells, or 100 million CAR-T cells .

[00156] In certain embodiments, a population of the anti-ROR-1 CAR-T cells of the current disclosure are included in a pharmaceutical composition comprising one or more pharmaceutically acceptable excipients, carriers, and diluents. In certain embodiments, the CAR-T cells of the current disclosure are administered suspended in a sterile isotonic solution. In certain embodiments, the solution comprises about 0.9% NaCl. In certain embodiments, the solution comprises about 5.0% dextrose. In certain embodiments, the solution further comprises one or more of: buffers, for example, acetate, citrate, histidine, succinate, phosphate, bicarbonate and hydroxymethylaminomethane (Tris); surfactants, for example, polysorbate 80 (Tween 80), polysorbate 20 (Tween 20), and poloxamer 188; polyol/disaccharide/polysaccharides, for example, glucose, dextrose, mannose, mannitol, sorbitol, sucrose, trehalose, and dextran 40; amino acids, for example, glycine or arginine; antioxidants, for example, ascorbic acid, methionine; or chelating agents, for example, EDTA or EGTA. The CAR-T cells when formulated, can be buffered at a certain pH, generally between about 7.0 and about 8.0. In certain embodiments, the cells are buffered at a physiological pH of about 7.4 (averaging between about 7.35 and 7.45.

[00157] Also described herein are methods of treating an individual that has developed one or more serious adverse events associated with CAR T-cell treatment comprising administering a bolus injection of cirmtuzumab. Such an administration blocks CAR T-cells from interacting with their targets and can attenuate their activity.

[00158] Also described herein are kits comprising one or more of the nucleic acids encoding the CAR-t cells described herein in a suitable container and one or more additional components selected from: instructions for use; a diluent, an excipient, a carrier, and a device for administration.

[00159] In certain embodiments, described herein is a method of preparing a cancer treatment comprising admixing one or more pharmaceutically acceptable excipients, carriers, or diluents and an antibody of the current disclosure. In certain embodiments, described herein is a method of preparing a cancer treatment for storage or shipping comprising lyophilizing one or more antibodies of the current disclosure.

EXAMPLES

[00160] The following illustrative examples are representative of embodiments of compositions and methods described herein and are not meant to be limiting in any way.

EXAMPLE 1: CHIMERIC ANTIGEN RECEPTOR MODIFIED T-CELLS (CAR-T) THAT TARGET ROR-1

[00161] A series of monoclonal antibodies have previously been described that target the ROR-1 antigen, this target is the subject of ongoing clinical trials in patients with CLL, MCL and breast cancer employing the anti-human ROR-1 mAb UC-961 (cirmtuzumab) combined with standard chemotherapies. The CARs constructed and tested in these examples utilize the complementarity determining regions (CDRs) and variable regions (V_H/V_L) of the cirmtuzumab antibody. The cirmtuzumab antibody and sequences encoding it's CDRS and VH/VL are disclosed in WO 2014/031174.

[00162] There are several unmet needs in the CAR-T space for treatment of cancer. The CAR T cells described herein solve these unmet needs.

Treatment failures

[00163] There is an increasing number of patient relapses following CAR-T therapy, frequently due to mutations or loss of target tumor antigen (e.g., CD19), evading CAR-T cells. Using ROR-1 as a target potentially provides for fewer antigen negative relapses, as ROR-1 expression is associated with aggressive tumor phenotype, and ROR-1 mutation or antigen loss

might render cancer cells less aggressive and susceptible to chemotherapy.

Safety concerns

[00164] Persistent CAR-T safety issues include deaths potentially related to activation by normal cells expressing the target antigen ("on-target/off-tumor"). Previous studies have shown Cirmtuzumab did not bind to normal human tissues in GLP tissue cross-reactivity studies, and there are no serious adverse events related to cirmtuzumab-only reported in clinical studies.

[00165] An additional avenue of therapeutic intervention is to employ the antigen binding domains of these anti-human ROR-1 antibodies, including UC-961, as the targeting moieties of a CAR. **FIG. 1A** shows an artist's rendering of an anti-ROR-1 CAR-T cell interacting with an ROR-1 expressing cancer cell (CLL). **FIG. 1B** shows a schematic of different non-limiting embodiments of CARs of this disclosure. The CARs of this disclosure describe light chain CDRs of the anti-human ROR-1 mAbs 4A5 or UC-961 attached to the heavy chain CDRs of the same mAbs by specific linkers that generate high-affinity single-chain (scFv) molecules that specifically bind human ROR-1 with a sufficient affinity to activate intracellular signaling and cytotoxicity in the transduced T-lymphocytes. To attach the scFv to a transmembrane domain, a series of protein spacers generated from IgG4 have been created. These spacers allow the anti-ROR-1 scFv binding domain sufficient flexibility to optimally bind the target ROR-1 antigen.

[00166] Still referring to **FIG. 1B,** the extracellular antigen binding domain from the CARs is bound to a CD28 transmembrane domain. This transmembrane domain is then attached to an intracellular activating domain derived from CD28 and/or CD137, which are employed singularly (2^{nd}-gen CAR) or in combination (3^{rd}-gen CAR). These activating domains are then attached to the T-cell receptor activating domain contained within the CD3 zeta chain (CD3 ζ-chain). Therefore, the anti-ROR-1 CARs (shown in **FIG. 1A**, **1B**, **1C**) are expressed as a single polypeptide that from the N-terminal end contains in order: a leader that directs the construct to the cell surface, the light chain CDR of either the 4A5 or UC-961 mAbs, a linker that attaches the light and heavy chains, the heavy chain CDR of 4A5 or UC-961 mAbs that are complimented by the corresponding light chain molecules, a spacer of defined length generated from IgG4 including the IgG4 hinge region, the CD28 transmembrane region, the intracellular activation domains of CD28 and 41-BB (CD137)-individually or in tandem attached to the T-cell ζ-chain which extends to the carboxy terminus of the molecule. **FIG. 2A** and **2B** show vector schematics illustrating CARS with different spacer domains.

[00167] The CARs described herein can be included in a transposon based or lentiviral vector, as shown in **FIG. 3** and introduced into target lymphocytes employing standard transfection or transduction techniques. To maximize stable transduction and long-term expression of the CAR product, retroviral delivery systems are used including murine gamma and human lentivirus. Currently, a third generation lentiviral, four plasmid system (Addgene, Inc.) is used to render T lymphocytes transgenic for an ROR-1 expressing CAR. This third-generation system is based on the lentiviral vector system described in Naldini et al, "Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector" *Proc Natl Acad Sci* USA. 1996 Oct 15; 93(21): 11382–11388. This four-plasmid system comprises: plasmid 1-gag/pol; plasmid 2-rev; plasmid 3 VSV-G protein; and plasmid 4, the transfer plasmid, which comprises a nucleic acid sequence encoding an anti-ROR1 CAR, which has been inserted using the restriction sites in the poly-linker.

[00168] **FIG. 4A** and **4B** illustrate steps of making an anti-ROR-1 CAR expressing T cell. Referring to **FIG. 4A**, T cells are isolated from peripheral blood mononuclear cells (PBMC), followed by activation with CD3/Cd28 nanoparticles, transduction with lentivirus, and optionally, analysis steps to verify CAR expression and specificity.

[00169] **FIG. 5A** shows flow cytometry analysis of whole-blood PBMC. **FIG. 5B** shows CD8+ and CD4+ populations after purification. **FIG. 6A** shows that activation by CD3/CD28 results in activation of both CD4+ (80%) and CD8+ (63%) cells, and **FIG. 6B** shows that lentiviral transduction results in dose dependent transduction efficiency of CD8+ T cells of 10% (MOI 1) or 28% (MOI 3); or of CD4+ T cells of 15% (MOI 1) or 34% (MOI 3). **FIG. 7A** and **7B** show a time course of anti-ROR-1 CAR expression after lentiviral transduction of T lymphocytes.

EXAMPLE 2: *IN VITRO* ACTIVITY OF CHIMERIC ANTIGEN RECEPTOR MODIFIED T-CELLS (CAR-T) THAT TARGET ROR-1

[00170] Several chimeric antigen receptors (CARs) that target the cancer associated antigen ROR-1 were created according to the above scheme and used to transduce cytotoxic T lymphocytes.

[00171] To generate an anti-human ROR1 T-cell CAR, multiple candidate 2nd and 3rd generation CAR constructs were generated with different signaling and spacer domains. To test these constructs, the candidate CAR-Ts were produced and expanded in a serum-free growth

media supplemented with IL-2. Using this protocol, CAR-T cells from over 20 healthy donors have been generated and their activity has been examined against lymphoid cancers in vitro using chromium release and impedance assays. The 2nd generation CARs using the 4-1BB co-stimulation domain had activity even at low effector to target (E:T) ratios, eliminated both primary B-cell leukemias and lymphomas, as well as tumor cell lines that express the ROR1 target.

[00172] These T cell CARs demonstrated specific activity in targeting ROR-1 expressed on both hematological and solid tumor cancers. As shown in **FIG. 8A**, T-lymphocytes expressing anti-ROR-1 CAR are effective at killing leukemic MEC-1/ROR1 cells at different effector to target (E:T) ratios. These results were generated through the use of a 4-hour chromium release assay. In this study anti-ROR1 CARs expressing the hinge and CH3 spacers had the greatest activity. For **FIG. 8A**, "Activated Only" (leftmost bar; ET9) are T-cells isolated from PBMC or blood product which have undergone the identical activation and cell culture process as CAR-T-cells, but without transduction of lentiviral CAR; "Long UC961" (second bar from the left) refers to a construct utilizing the CH2CH3 spacer, comprising Spacer #3 (SEQ ID NO:29) and both the CH2 and CH3 regions of hIgG4; "CH3" (third bar from left) refers to CAR-T construct utilizing spacer CH3, comprising Spacer #3 and the CH3 region of human IgG4; and "Hinge" (fourth bar from the left) refers to a CAR-T construct utilizing Spacer #3 (Hinge) without human IgG4 CH2 and CH3 domains. **FIG. 8B** shows 4-hour chromium release assay with non-ROR-1 expressing MEC-1 cells. This data indicates that ROR-1 CARs are specific and kill only based on the presence of ROR-1 at the cell surface of a target cell. Bars in **FIG. 8B** are ordered as in **FIG. 8A**.

[00173] **FIG. 8C** shows the effect on cytotoxicity of a 2nd or 3rd generation anti-ROR-1 CAR. **FIG. 8C** shows a similar experimental setup as in **FIG. 8A** and **8B** except comparing the hinge or CH3 as a 2nd generation CAR (4-1BB intracellular signaling domain only) or a 3rd generation CAR (both 4-1BB and CD28 intracellular signaling domains). There is no difference between a 2nd and 3rd generation CAR for constructs with the full CH3-hinge, however the 3rd generation format showed improved killing compared to the 2nd generation format for constructs that have hinge only and no CH3. **FIG. 8D** shows the absence of killing of MEC-1 cells that do not express ROR-1, indicating that these CARs are specific as well. **FIG 8E**. cell killing of MEC1-ROR1 cells by CAR T from two different donors.

[00174] As shown in **FIG. 9A** anti-ROR-1 CARs kill MCF-7 ROR-1 cells. This figure shows the specific killing by anti-ROR-1 T-cell CARs with different spacer lengths, generated as

described in Example 1, against MCF-7/ROR-1 breast cancer cells. These results were generated through the use of a 96-h impedance assay (ACEA). In this study anti-ROR-1 CARs that express the hinge and CH3 domains have the greatest activity against the ROR-1 positive cells. As used in **FIG. 9A**, "CH3" refers to a CAR-T construct utilizing Spacer CH3, containing Spacer #3 (SEQ ID NO:29) and the CH3 region of human IgG4; "Hinge" refers to CAR-T construct utilizing Spacer #3 (Hinge) without human IgG4 CH2 or CH3; "ET1" refers to an effector to target ratio of 1 to 1 for anti-ROR-1 CAR-T cells against the target cells in the cytotoxicity assay. **FIG 9B** shows that the killing observed in **FIG. 9A** is specific as MCF-7 cells that do not express ROR-1 are not killed by anti-ROR-1 CAR-T cells. FIG. 9C shows killing from CAR T cells generated from two different healthy donors.

[00175] **FIG. 10** illustrates that T lymphocytes expressing anti-ROR-1 CARs kill MEC1/ROR-1 cells and Jeko cells, which naturally express ROR-1, at different effector to target ratios.

EXAMPLE 3: *IN VIVO* ACTIVITY OF CHIMERIC ANTIGEN RECEPTOR MODIFIED T-CELLS (CAR-T) THAT TARGET ROR-1

[00176] To test the ROR1 T-cell CARs *in vivo*, luciferase/RFP bi-specific lentiviral expression vectors to modify human MEC1-ROR1 leukemia cells (Yu et al., J Clin Invest, 2015) were created. When injected into immune-deficient NSG mice, MEC1-ROR1 cells infiltrate the marrow (femur), kidney, spleen, and liver, generating a progressive and fatal leukemia/lymphoma. As shown in **FIG. 11A**, when injected into immune-deficient mice, this highly active cell-line rapidly expands into the bone marrow (femur), kidney, spleen, liver and blood with the animals succumb to the leukemia within about 3-weeks. *In vivo* experiments were conducted as in **FIG. 11B** (top), briefly, mice were injected with $1x10^6$ ROR-1/MEC1-luciferase cells on day 1, followed by $1x10^6$ or $3x10^6$ anti ROR-1 CAR-T cells on day 4. Tumor growth was measured using bioluminescence of the modified MEC cells at subsequent timepoints.

[00177] Mice that were injected with the tumor cells responded in a dose dependent manner to 2nd generation CAR-T cells from the *in vitro* studies. $3x10^6$ anti-ROR-1 CAR T cells were able to clear luciferase expressing ROR-1/MEC cells as shown in **FIG. 12** and **13.** By week 3, the rapidly expanding leukemic cells are cleared from the marrow, the remaining leukemic cells are undetectable by week 4, and animals survive greater than 90 days vs ~21 days in the absence of these CAR-T cells. **FIG. 14** shows that anti-ROR1 CAR-T cells were major components of the

animals' marrow and kidneys by day 11 and by day 18. Additionally, the concentration of the CAR-T cells was substantially greater in the mice that were pretreated with MEC-1 ROR1 cells (left panels) vs mock control (right panels) demonstrating the elevated expansion of the CAR-T in animals bearing leukemic cells.

[00178] To summarize the results presented here:

[00179] A single injection of $3x10^6$ anti-ROR1 4-1BB CAR T-cells unsupported, without conditioning or cytokine supplementation regimens, expanded in the animals bearing MEC1-ROR1 xenografts and homed to the sites of MEC1-ROR1 disease activity.

[00180] By week four, the rapidly expanding leukemic cells were cleared from the major tissue reservoirs including the marrow, kidneys and spleen.

[00181] The CAR-T treated animals survived greater than 90 days vs ~21 days for those animals that received only non-transduced activated T-cells or were untreated controls.

[00182] The administered CAR T-products were highly active and detected in mouse tissues months after injection

[00183] These studies have been expanded to other lymphoid tumor cell lines including the ROR-1 expressing JeKo cells.

[00184] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[00185] All publications, patent applications, issued patents, and other documents referred to in this specification are herein incorporated by reference as if each individual publication, patent application, issued patent, or other document was specifically and individually indicated to be incorporated by reference in its entirety. Definitions that are contained in text incorporated by reference are excluded to the extent that they contradict definitions in this disclosure.

INFORMAL SEQUENCE LISTING:

[00186] **UC961 VL:**

DIVMTQTPLSLPVTPGEPASISCRASKSISKYLAWYQQKPGQAPRLLIYSGSTLQSGIPP RFSGSGYGTDFTLTINNIESEDAAYYFCQQHDESPYTFGEGTKVEIK (SEQ ID NO:21)

[00187] **Linker #3:**

GGGGSGSTSGSGKPGSGEGSTKGGGGGS (SEQ ID NO:24)

[00188] **UC961 VH:**

QVQLQESGPGLVKPSQTLSLTCTVSGYAFTAYNIHWVRQAPGQGLEWMGSFDPYDG GSSYNQKFKDRLTISKDTSKNQVVLTMTNMDPVDTATYYCARGWYYFDYWGHGT LVTVSS (SEQ ID NO:27)

[00189] **Spacer #3 (or "Hinge/Short"):**

VDESKYGPPCPPCP (SEQ ID NO:29)

[00190] **Spacer CH3:**

VDESKYGPPCPPCPLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO:41)

[00191] **Spacer CH2CH3 (or "Long/Full"):**

VDESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS LSLGK (SEQ ID NO:42)

[00192] **UC961 CDR L1:**

KSISKY (SEQ ID NO:43)

[00193] **UC961 CDR L2:**

SGS (SEQ ID NO:44)

[00194] **UC961 CDR L3:**

QQHDESPY (SEQ ID NO:45)

[00195] **UC961 CDR H1:**

GYAFTAYN (SEQ ID NO:46)

[00196] **UC961 CDR H2:**

FDPYDGGS (SEQ ID NO:47)

[00197] **UC961 CDR H3:**

GWYYFDY (SEQ ID NO:48)

[00198] **4A5 CDR L1:**

PDINSY (SEQ ID NO:49)

[00199] **4A5 CDR L2:**

RAN (SEQ ID NO:50)

[00200] **4A5 CDR L3:**

LQYDEFPYT (SEQ ID NO:51)

[00201] **4A5 CDR H1:**

GFTFSSYA (SEQ ID NO:52)

[00202] **4A5 CDR H2:**

ISRGGTT (SEQ ID NO:53)

[00203] **4A5 CDR H3:**

YDYDGYYAMDY (SEQ ID NO:54)

[00204] Full-length human ROR-1 Protein (SEQ ID NO:55):

MHRPRRRGTRPPLLALLAALLLAARGAAAQETELSVSAELVPTSSWNISSELNKDSY
LTLDEPMNNITTSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQEPRRLSFRSTIYGSR
LRIRNLDTTDTGYFQCVATNGKEVVSSTGVLFVKFGPPPTASPGYSDEYEEDGFCQP
YRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHY
AFPYCDETSSVPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPE
SPEAANCIRIGIPMADPINKNHKCYNSTGVDYRGTVSVTKSGRQCQPWNSQYPHTHT
FTALRFPELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACDSKDSKEKNKM
EILYILVPSVAIPLAIALLFFFICVCRNNQKSSSAPVQRQPKHVRGQNVEMSMLNAYK
PKSKAKELPLSAVRFMEELGECAFGKIYKGHLYLPGMDHAQLVAIKTLKDYNNPQQ
WMEFQQEASLMAELHHPNIVCLLGAVTQEQPVCMLFEYINQGDLHEFLIMRSPHSD
VGCSSDEDGTVKSSLDHGDFLHIAIQIAAGMEYLSSHFFVHKDLAARNILIGEQLHVK
ISDLGLSREIYSADYYRVQSKSLLPIRWMPPEAIMYGKFSSDSDIWSFGVVLWEIFSFG
LQPYYGFSNQEVIEMVRKRQLLPCSEDCPPRMYSLMTECWNEIPSRRPRFKDIHVRL
RSWEGLSSHTSSTTPSGGNATTQTTSLSASPVSNLSNPRYPNYMFPSQGITPQGQIAGF
IGPPIPQNQRFIPINGYPIPPGYAAFPAAHYQPTGPPRVIQHCPPPKSRSPSSASGSTSTG

HVTSLPSSGSNQEANIPLLPHMSIPNHPGGMGITVFGNKSQKPYKIDSKQASLLGDAN IHGHTESMISAEL

[00205] 21 amino acid stretch of human ROR-1 including glutamic acid at position 138 (SEQ ID NO:56): VATNGKEVVSSTGVLFVKFGP

[00206] 15 amino acid stretch of human ROR-1 including glutamic acid at position 138 (SEQ ID NO:57): EVVSSTGVLFVKFGP

P EMBODIMENTS

[00207] P Embodiment 1. A recombinant protein comprising: (i) an antibody region comprising: (a) a light chain variable domain comprising a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and (b) a heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; and (ii) a transmembrane domain.

[00208] P embodiment 2. A recombinant protein comprising: (i) an antibody region comprising: (a) a light chain variable domain comprising a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and (b) a heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54; and (ii) a transmembrane domain.

[00209] P embodiment 3. The recombinant protein of P embodiment 1, wherein the C-terminus of said light chain variable domain is bound to the N-terminus of said heavy chain variable domain.

[00210] P embodiment 4. The recombinant protein of any one of P embodiments 1-3, wherein said light chain variable domain is covalently bound to said heavy chain variable domain through a chemical linker.

[00211] P embodiment 5. The recombinant protein of any one of P embodiments 1-4, wherein said chemical linker is a peptide linker.

[00212] P embodiment 6. The recombinant protein of P embodiment 5, wherein said peptide linker has the sequence of SEQ ID NO:24.

[00213] P embodiment 7. The recombinant protein of any one of P embodiments 1-6, wherein the C-terminus of said heavy chain variable domain is bound to the N-terminus of said transmembrane domain.

[00214] P embodiment 8. The recombinant protein of any one of P embodiments 1-7, wherein said heavy chain variable domain is covalently bound to said transmembrane domain through a spacer domain.

[00215] P embodiment 9. The recombinant protein of P embodiment 8, wherein said spacer domain comprises an antibody domain.

[00216] P embodiment 10. The recombinant protein of P embodiment 9, wherein said antibody domain comprises a hinge domain, a constant heavy chain 3 (CH3) domain, a

constant heavy chain 2 (CH2) domain or any combination thereof.

[00217] P embodiment 11. The recombinant protein of P embodiment 9 or 10, wherein said antibody domain consists of a hinge domain.

[00218] P embodiment 12. The recombinant protein of P embodiment 9 or 10, wherein said antibody domain consists of a hinge domain and a constant heavy chain 3 (CH3) domain.

[00219] P embodiment 13. The recombinant protein of P embodiment 9 or 10, wherein said antibody domain consists of a hinge domain, a constant heavy chain 3 (CH3) domain and a constant heavy chain 2 (CH2) domain.

[00220] P embodiment 14. The recombinant protein of any one of P embodiments 9-13, wherein said spacer domain comprises the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00221] P embodiment 15. The recombinant protein of any one of P embodiments 9-13, wherein said spacer domain has the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00222] P embodiment 16. The recombinant protein of any one of P embodiments 1-15, wherein said light chain variable domain comprises the sequence of SEQ ID NO:21.

[00223] P embodiment 17. The recombinant protein of any one of P embodiments 1-16, wherein said light chain variable domain has the sequence of SEQ ID NO:21.

[00224] P embodiment 18. The recombinant protein of any one of P embodiments 1-17, wherein said heavy chain variable domain comprises the sequence of SEQ ID NO:27.

[00225] P embodiment 19. The recombinant protein of any one of P embodiments 1-18, wherein said heavy chain variable domain has the sequence of SEQ ID NO:27.

[00226] P embodiment 20. The recombinant protein of any one of P embodiments 2-13, wherein said light chain variable domain comprises the sequence of SEQ ID NO:19.

[00227] P embodiment 21. The recombinant protein of any one of P embodiments 2-13 or 20, wherein said light chain variable domain has the sequence of SEQ ID NO:19.

[00228] P embodiment 22. The recombinant protein of any one of P embodiments 2-13, wherein said light chain variable domain comprises the sequence of SEQ ID NO:20.

[00229] P embodiment 23. The recombinant protein of any one of P embodiments 2-13 or 22, wherein said light chain variable domain has the sequence of SEQ ID NO:20.

[00230] P embodiment 24. The recombinant protein of any one of P embodiments 2-13, wherein said heavy chain variable domain comprises the sequence of SEQ ID NO:25.

[00231] P embodiment 25. The recombinant protein of any one of P embodiments 2-13 or

24, wherein said heavy chain variable domain has the sequence of SEQ ID NO:25.

[00232] P embodiment 26. The recombinant protein of any one of P embodiments 2-13, wherein said heavy chain variable domain comprises the sequence of SEQ ID NO:26.

[00233] P embodiment 27. The recombinant protein of any one of P embodiments 2-13 or 26, wherein said heavy chain variable domain has the sequence of SEQ ID NO:26.

[00234] P embodiment 28. The recombinant protein of any one of P embodiments 1-27, wherein said transmembrane domain is a CD8α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain or a CD3ζ transmembrane domain or any combination thereof.

[00235] P embodiment 29. The recombinant protein of any one of P embodiments 1-28, wherein said transmembrane domain is a CD28 transmembrane domain.

[00236] P embodiment 30. The recombinant protein of P embodiment 29, wherein said CD28 transmembrane domain comprises the sequence of SEQ ID NO:32.

[00237] P embodiment 31. The recombinant protein of P embodiment 29, wherein said CD28 transmembrane domain has the sequence of SEQ ID NO:32.

[00238] P embodiment 32. The recombinant protein of any one of P embodiments 1-31, further comprising one or more intracellular co-stimulatory signaling domains.

[00239] P embodiment 33. The recombinant protein of P embodiment 32, wherein said one or more intracellular co-stimulatory signaling domains are a 4-1BB intracellular co-stimulatory signaling domain, a CD28 intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain or any combination thereof.

[00240] P embodiment 34. The recombinant protein of P embodiment 32 or 33, comprising a 4-1BB intracellular co-stimulatory signaling domain.

[00241] P embodiment 35. The recombinant protein of P embodiment 34, wherein said 4-1BB intracellular co-stimulatory signaling domain comprises the sequence of SEQ ID NO:33.

[00242] P embodiment 36. The recombinant protein of P embodiment 34 or 35, wherein said 4-1BB intracellular co-stimulatory signaling domain has the sequence of SEQ ID NO:33.

[00243] P embodiment 37. The recombinant protein of P embodiment 32, comprising a CD28 intracellular co-stimulatory signaling domain and a 4-1BB intracellular co-stimulatory signaling domain.

[00244] P embodiment 38. The recombinant protein of any one of P embodiments 1-37, further comprising an intracellular T-cell signaling domain.

[00245] P embodiment 39. The recombinant protein of P embodiment 38, wherein said intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain.

[00246] P embodiment 40. The recombinant protein of P embodiment 39, wherein said CD3ζ intracellular T-cell signaling domain comprises the sequence of SEQ ID NO:34.

[00247] P embodiment 41. The recombinant protein of P embodiment 39, wherein said CD3ζ intracellular T-cell signaling domain has the sequence of SEQ ID NO:34.

[00248] P embodiment 42. The recombinant protein of any one of P embodiments 1-41, wherein said recombinant protein binds to a cell.

[00249] P embodiment 43. The recombinant protein of P embodiment 42, wherein said cell is a cancer cell.

[00250] P embodiment 44. The recombinant protein of P embodiment 43, wherein said cancer cell is a leukemia cell, a lymphoma cell, a chronic lymphocytic leukemia (CLL) cell, an adult acute myeloid leukemia (AML) cell, an acute lymphoblastic leukemia (ALL) cell, a mantle cell lymphoma (MCL) cell, a multiple myeloma cell, an ovarian cancer cell, a colon cancer cell, a lung cancer cell, a skin cancer cell, a pancreatic cancer cell, a testicular cancer cell, a bladder cancer cell, a uterine cancer cell, a prostate cancer cell, a breast cancer cell, or an adrenal cancer cell.

[00251] P embodiment 45. The recombinant protein any one of P embodiments 1-44, wherein said recombinant protein forms part of a cell.

[00252] P embodiment 46. The recombinant protein any one of P embodiments 1-46, wherein said recombinant protein forms part of a T cell.

[00253] P embodiment 47. An isolated nucleic acid encoding a recombinant protein of any one of P embodiments 1-46.

[00254] P embodiment 48. A pharmaceutical composition comprising a therapeutically effective amount of a recombinant protein of one of P embodiments 1-46, and a pharmaceutically acceptable excipient.

[00255] P embodiment 49. A method of treating cancer in a subject in need thereof, said method comprising administering to a subject a therapeutically effective amount of a recombinant protein of one of P embodiments 1-46, thereby treating cancer in said subject.

EMBODIMENTS

[00256] Embodiment 1. A chimeric antigen receptor comprising:

an antigen binding region, wherein said antigen binding region specifically binds ROR-1 wherein said antigen binding region comprises a light chain variable domain and a heavy chain variable domain;

wherein said light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and said heavy chain variable domain comprises a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; or

wherein said light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and said heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54;

a spacer domain, wherein said spacer domain comprises a spacer of between 10 and 240 amino acids in length;

a transmembrane domain; and

an intracellular domain.

[00257] Embodiment 2. The chimeric antigen receptor of embodiment 1, wherein the spacer is between 14 and 120 amino acids in length.

[00258] Embodiment 3. The chimeric antigen receptor of embodiment 1 or 2, wherein said light chain variable domain is N-terminal of said heavy chain variable domain.

[00259] Embodiment 4. The chimeric antigen receptor of embodiment 3, wherein said light chain variable domain is covalently coupled to said heavy chain variable domain through a polypeptide linker.

[00260] Embodiment 5. The chimeric antigen receptor of embodiment 4, wherein said polypeptide linker consists of the sequence of SEQ ID NO:24.

[00261] Embodiment 6. The chimeric antigen receptor of any one of embodiments 1 to 5, wherein said spacer domain comprises an antibody domain.

[00262] Embodiment 7. The chimeric antigen receptor of embodiment 6, wherein said antibody domain comprises an immunoglobulin hinge domain, an immunoglobulin constant heavy chain 3 (CH3) domain, an immunoglobulin constant heavy chain 2 (CH2) domain, or

any combination thereof.

[00263] Embodiment 8. The chimeric antigen receptor of embodiment 7, wherein said antibody domain consists of said immunoglobulin hinge domain.

[00264] Embodiment 9. .The chimeric antigen receptor of embodiment 7, wherein said antibody domain consists of an immunoglobulin hinge domain and said immunoglobulin constant heavy chain 3 (CH3) domain.

[00265] Embodiment 10. The chimeric antigen receptor of embodiment 7, wherein said antibody domain consists of said immunoglobulin hinge domain, said immunoglobulin constant heavy chain 3 (CH3) domain and said immunoglobulin constant heavy chain 2 (CH2) domain.

[00266] Embodiment 11. The chimeric antigen receptor of embodiment 7, wherein said spacer domain comprises the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00267] Embodiment 12. The chimeric antigen receptor of embodiment 7, wherein said spacer domain consists of the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00268] Embodiment 13. The chimeric antigen receptor of any one of embodiments 7 to 12, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:21.

[00269] Embodiment 14. The chimeric antigen receptor of any one of embodiments 7 to 12, wherein said light chain variable domain consists of the sequence of SEQ ID NO:21.

[00270] Embodiment 15. The chimeric antigen receptor of any one of embodiments 7 to 12, wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:27.

[00271] Embodiment 16. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:27.

[00272] Embodiment 17. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:19.

[00273] Embodiment 18. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said light chain variable domain consists of the sequence of SEQ ID NO:19.

[00274] Embodiment 19. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:20.

[00275] Embodiment 20. The chimeric antigen receptor of any one of embodiments 1 to

12, wherein said light chain variable domain consists of the sequence of SEQ ID NO:20.

[00276] Embodiment 21. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:25.

[00277] Embodiment 22. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:25.

[00278] Embodiment 23. The chimeric antigen receptor of any one of embodiments 1 to 12 wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:26.

[00279] Embodiment 24. The chimeric antigen receptor of any one of embodiments 1 to 12, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:26.

[00280] Embodiment 25. The chimeric antigen receptor of any one of embodiments 1 to 24, wherein said transmembrane domain comprises a CD8α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain, a CD3ζ transmembrane domain, or any combination thereof.

[00281] Embodiment 26. The chimeric antigen receptor of embodiment 25, wherein said transmembrane domain is a CD28 transmembrane domain.

[00282] Embodiment 27. The chimeric antigen receptor of embodiment 25, wherein said CD28 transmembrane domain comprises the sequence of SEQ ID NO:32.

[00283] Embodiment 28. The chimeric antigen receptor of embodiment 25, wherein said CD28 transmembrane domain consists of the sequence of SEQ ID NO:32.

[00284] Embodiment 29. The chimeric antigen receptor of any one of embodiments 1 to 28, wherein said intracellular domain comprises, one or more intracellular co-stimulatory signaling domains, one or more intracellular T-cell signaling domains, or one or more intracellular co-stimulatory signaling domains and one or more intracellular T-cell signaling domains.

[00285] Embodiment 30. The chimeric antigen receptor of embodiment 29, wherein said one or more intracellular co-stimulatory signaling domains are a 4-1BB intracellular co-stimulatory signaling domain, a CD28 intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain or any combination thereof.

[00286] Embodiment 31. The chimeric antigen receptor of embodiment 29, comprising a 4-1BB intracellular co-stimulatory signaling domain.

[00287] Embodiment 32. The chimeric antigen receptor of embodiment 31, wherein said 4-1BB intracellular co-stimulatory signaling domain comprises the sequence of SEQ ID NO:33.

[00288] Embodiment 33. The chimeric antigen receptor of embodiment 31, wherein said 4-1BB intracellular co-stimulatory signaling domain consists of the sequence of SEQ ID NO:33.

[00289] Embodiment 34. The chimeric antigen receptor of embodiment 29 comprising a CD28 intracellular co-stimulatory signaling domain and a 4-1BB intracellular co-stimulatory signaling domain.

[00290] Embodiment 35. The chimeric antigen receptor of any one of embodiments 1 to 34, further comprising an intracellular T-cell signaling domain.

[00291] mbodiment 36. The chimeric antigen receptor of embodiment 35, wherein said intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain.

[00292] Embodiment 37. The chimeric antigen receptor of embodiment 36, wherein said CD3ζ intracellular T-cell signaling domain comprises the sequence of SEQ ID NO:34.

[00293] Embodiment 38. The chimeric antigen receptor of embodiment 36, wherein said CD3ζ intracellular T-cell signaling domain consists of the sequence of SEQ ID NO:34.

[00294] Embodiment 39. The chimeric antigen receptor of any one of embodiments 1 to 38, wherein said chimeric antigen receptor binds to a cell expressing ROR-1.

[00295] Embodiment 40. A nucleic acid encoding the chimeric antigen receptor of any one of embodiments 1 to 39.

[00296] Embodiment 41. The nucleic acid of embodiment 40, wherein the nucleic acid is a viral vector,

[00297] Embodiment 42. The nucleic acid of embodiment 41, wherein the nucleic acid vector is a lentiviral vector.

[00298] Embodiment 43. A cell comprising the nucleic acid of any one of embodiments 40 to 42.

[00299] Embodiment 44. A cell expressing the chimeric antigen receptor of any one of embodiments 1 to 39.

[00300] Embodiment 45. The cell of embodiment 43 or 44, wherein the cell is a T lymphocyte.

[00301] Embodiment 46. The cell of embodiment 45, wherein said T lymphocyte is a CD8 positive T lymphocyte.

[00302] Embodiment 47. The cell of embodiment 43 or 44, wherein the cell is a natural killer cell.

[00303] Embodiment 48. A pharmaceutical composition comprising a therapeutically effective amount of a population of cells of any one of embodiments 43 to 47 and a pharmaceutically acceptable diluent, carrier, or excipient.

[00304] Embodiment 49. The pharmaceutical composition of embodiment 48, formulated for intravenous injection.

[00305] Embodiment 50. A method of treating cancer in an individual in need thereof, said method comprising administering to said individual the pharmaceutical composition of embodiment 48 or 49.

[00306] Embodiment 51. The method of embodiment 50, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia (CLL), adult acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

[00307] Embodiment 52. The method of embodiment 50 or 51, wherein the method further comprises administering cirmtuzumab to the individual.

[00308] Embodiment 53. The method of embodiment 52, wherein said cirmtuzumab and said pharmaceutical composition of embodiment 48 or 49 are administered separately.

[00309] Embodiment 54. The pharmaceutical composition of embodiment 48 or 49 for use in a method of treating cancer in an individual.

[00310] Embodiment 55. The pharmaceutical composition for the use of embodiment 54, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia (CLL), adult acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

[00311] Embodiment 56. A chimeric antigen receptor comprising:
an antigen binding region, wherein said antigen binding region specifically binds ROR-1 wherein said antigen binding region comprises a light chain variable domain and a heavy chain variable domain;

wherein said light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and

said heavy chain variable domain comprises a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; or

wherein said light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and said heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54;

a transmembrane domain; and

an intracellular domain, wherein said intracellular domain comprises an intracellular T cell signaling domain and an intracellular co-stimulatory domain selected from, 4-1BB, ICOS, OX-40, and combinations thereof.

[00312] Embodiment 57. The chimeric antigen receptor of embodiment 56, comprising a spacer a spacer domain, wherein said spacer domain comprises a spacer of between 10 and 200 amino acids in length

[00313] Embodiment 58. The chimeric antigen receptor of embodiment 57, wherein the spacer is between 14 and 120 amino acids in length.

[00314] mbodiment 59. The chimeric antigen receptor of any one of embodiments 56 or 58, wherein said light chain variable domain is N-terminal of said heavy chain variable domain.

[00315] Embodiment 60. The chimeric antigen receptor of embodiment 59, wherein said light chain variable domain is covalently coupled to said heavy chain variable domain through a polypeptide linker.

[00316] Embodiment 61. The chimeric antigen receptor of embodiment 60, wherein said polypeptide linker consists of the sequence of SEQ ID NO:24.

[00317] Embodiment 62. The chimeric antigen receptor of any one of embodiments 56 to 61, wherein said spacer domain comprises an antibody domain.

[00318] Embodiment 63. The chimeric antigen receptor of embodiment 62, wherein said antibody domain comprises an immunoglobulin hinge domain, an immunoglobulin constant heavy chain 3 (CH3) domain, an immunoglobulin constant heavy chain 2 (CH2) domain, or any combination thereof.

[00319] Embodiment 64. The chimeric antigen receptor of embodiment 63, wherein said antibody domain consists of said immunoglobulin hinge domain.

[00320] Embodiment 65. The chimeric antigen receptor of embodiment 63, wherein said

antibody domain consists of an immunoglobulin hinge domain and said immunoglobulin constant heavy chain 3 (CH3) domain.

[00321] Embodiment 66. The chimeric antigen receptor of embodiment 63, wherein said antibody domain consists of said immunoglobulin hinge domain, said immunoglobulin constant heavy chain 3 (CH3) domain and said immunoglobulin constant heavy chain 2 (CH2) domain.

[00322] mbodiment 67. The chimeric antigen receptor of embodiment 63, wherein said spacer domain comprises the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00323] Embodiment 68. The chimeric antigen receptor of embodiment 63, wherein said spacer domain consists of the sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

[00324] Embodiment 69. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:21.

[00325] Embodiment 70. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain consists of the sequence of SEQ ID NO:21.

[00326] Embodiment 71. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:27.

[00327] Embodiment 72. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:27.

[00328] Embodiment 73. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:19.

[00329] Embodiment 74. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain consists of the sequence of SEQ ID NO:19.

[00330] Embodiment 75. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:20.

[00331] Embodiment 76. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said light chain variable domain consists of the sequence of SEQ ID NO:20.

[00332] Embodiment 77. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:25.

[00333] Embodiment 78. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:25.

[00334] Embodiment 79. The chimeric antigen receptor of any one of embodiments 56 to 68 wherein said heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:26.

[00335] Embodiment 80. The chimeric antigen receptor of any one of embodiments 56 to 68, wherein said heavy chain variable domain consists of the sequence of SEQ ID NO:26.

[00336] Embodiment 81. The chimeric antigen receptor of any one of embodiments 56 to 80, wherein said transmembrane domain comprises a CD8α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain, a CD3ζ transmembrane domain, or any combination thereof.

[00337] Embodiment 82. The chimeric antigen receptor of embodiment 81, wherein said transmembrane domain is a CD28 transmembrane domain.

[00338] Embodiment 83. The chimeric antigen receptor of embodiment 82, wherein said CD28 transmembrane domain comprises the sequence of SEQ ID NO:32.

[00339] Embodiment 84. The chimeric antigen receptor of embodiment 82, wherein said CD28 transmembrane domain consists of the sequence of SEQ ID NO:32.

[00340] Embodiment 85. The chimeric antigen receptor of any one of embodiments 56 to 84, wherein said intracellular domain comprises 4-1BB intracellular co-stimulatory signaling domain.

[00341] Embodiment 86. The chimeric antigen receptor of embodiment 85, wherein said 4-1BB intracellular co-stimulatory signaling domain comprises the sequence of SEQ ID NO:33.

[00342] Embodiment 87. The chimeric antigen receptor of embodiment 85, wherein said 4-1BB intracellular co-stimulatory signaling domain consists of the sequence of SEQ ID NO:33.

[00343] Embodiment 88. The chimeric antigen receptor of any one of embodiments 56 to 87, comprising a CD28 intracellular co-stimulatory signaling domain and a 4-1BB intracellular co-stimulatory signaling domain.

[00344] Embodiment 89.The chimeric antigen receptor of any one of embodiments 56 to 88, wherein said intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain.

[00345] Embodiment 90. The chimeric antigen receptor of embodiment 89, wherein said

CD3ζ intracellular T-cell signaling domain comprises the sequence of SEQ ID NO:34.

[00346] Embodiment 91. The chimeric antigen receptor of embodiment 89, wherein said CD3ζ intracellular T-cell signaling domain consists of the sequence of SEQ ID NO:34.

[00347] Embodiment 92. The chimeric antigen receptor of any one of embodiments 56 to 91 wherein said chimeric antigen receptor binds to a cell expressing ROR-1.

[00348] Embodiment 93. A nucleic acid encoding the chimeric antigen receptor of any one of embodiments 56 to 92.

[00349] Embodiment 94. The nucleic acid of embodiment 93, wherein the nucleic acid is a viral vector.

[00350] Embodiment 95. The nucleic acid of embodiment 94, wherein the viral vector is a lentiviral vector.

[00351] Embodiment 96. A cell comprising the nucleic acid of any one of embodiments 93 to 95.

[00352] Embodiment 97. A cell expressing the chimeric antigen receptor of any one of embodiments 56 to 92.

[00353] Embodiment 98. The cell of embodiment 96 or 97, wherein the cell is a T lymphocyte.

[00354] Embodiment 99. The cell of embodiment 98, wherein said T lymphocyte is a CD8 positive T lymphocyte.

[00355] Embodiment 100. The cell of embodiment 96 or 97, wherein the cell is a natural killer cell.

[00356] Embodiment 101. A pharmaceutical composition comprising a therapeutically effective amount of a population of cells of any one of embodiments 96 to 100, and a pharmaceutically acceptable diluent, carrier, or excipient.

[00357] Embodiment 102. The pharmaceutical composition of embodiment 101, formulated for intravenous injection.

[00358] Embodiment 103. A method of treating cancer in an individual in need thereof, said method comprising administering to said individual the pharmaceutical composition of embodim

[00359] Embodiment 104. The method of embodiment 103, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia (CLL), adult acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or

adrenal cancer.

[00360] Embodiment 105. The method of embodiment 103 or 104, wherein the method further comprises administering cirmtuzumab to the individual.

[00361] Embodiment 106. The method of embodiment 105, wherein said cirmtuzumab and said pharmaceutical composition of embodiment 101 or 102 are administered separately.

[00362] Embodiment 107. The pharmaceutical composition of embodiment 101 or 102 for use in a method of treating cancer in an individual.

[00363] Embodiment 108. The pharmaceutical composition for the use of embodiment 107, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia (CLL), adult acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

CLAIMS

WHAT IS CLAIMED IS:

1. A chimeric antigen receptor comprising:
   i. an antigen binding region, wherein the antigen binding region specifically binds ROR-1 and wherein the antigen binding region comprises a light chain variable domain and a heavy chain variable domain;
      (a) wherein the light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:43, a CDR L2 as set forth in SEQ ID NO:44 and a CDR L3 as set forth in SEQ ID NO:45; and the heavy chain variable domain comprises a CDR H1 as set forth in SEQ ID NO:46, a CDR H2 as set forth in SEQ ID NO:47, and a CDR H3 as set forth in SEQ ID NO:48; or
      (b) wherein the light chain variable domain comprises a CDR L1 as set forth in SEQ ID NO:49, a CDR L2 as set forth in SEQ ID NO:50 and a CDR L3 as set forth in SEQ ID NO:51; and the heavy chain variable domain comprising a CDR H1 as set forth in SEQ ID NO:52, a CDR H2 as set forth in SEQ ID NO:53, and a CDR H3 as set forth in SEQ ID NO:54;
   ii. a spacer domain, wherein the spacer domain comprises a spacer of between 10 and 240 amino acids in length;
   iii. a transmembrane domain; and
   iv. an intracellular domain.

2. The chimeric antigen receptor of claim 1, wherein the spacer domain is between 14 and 120 amino acids in length.

3. The chimeric antigen receptor of claim 1 or 2, wherein the light chain variable domain is coupled to the N-terminus or the C-terminus of the heavy chain variable domain.

4. The chimeric antigen receptor of claim 3, wherein the light chain variable domain is covalently coupled to the heavy chain variable domain through a polypeptide linker.

5. The chimeric antigen receptor of claim 4, wherein the polypeptide linker comprises an amino acid sequence of SEQ ID NO:24.

6. The chimeric antigen receptor of claim 1, wherein the spacer domain comprises an antibody domain.

7. The chimeric antigen receptor of claim 6, wherein the antibody domain comprises an immunoglobulin hinge domain, an immunoglobulin constant heavy chain 3 (CH3) domain, an immunoglobulin constant heavy chain 2 (CH2) domain, or a combination thereof.

8. The chimeric antigen receptor of claim 7, wherein the antibody domain consists of the immunoglobulin hinge domain.

9. The chimeric antigen receptor of claim 7, wherein the antibody domain consists of the immunoglobulin hinge domain and the immunoglobulin constant heavy chain 3 (CH3) domain.

10. The chimeric antigen receptor of claim 7, wherein the antibody domain consists of the immunoglobulin hinge domain, the immunoglobulin constant heavy chain 3 (CH3) domain and the immunoglobulin constant heavy chain 2 (CH2) domain.

11. The chimeric antigen receptor of claim 7, wherein the spacer domain comprises an amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

12. The chimeric antigen receptor of claim 7, wherein the spacer domain consists of an amino acid sequence of SEQ ID NO:29, SEQ ID NO:41 or SEQ ID NO:42.

13. The chimeric antigen receptor of claim 7, wherein the light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:21.

14. The chimeric antigen receptor of claim 7, wherein the light chain variable domain consists of an amino acid sequence of SEQ ID NO:21.

15. The chimeric antigen receptor of claim 7, wherein the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:27.

16. The chimeric antigen receptor of claim 1, wherein the heavy chain variable domain consists of an amino acid sequence of SEQ ID NO:27.

17. The chimeric antigen receptor of claim 1, wherein the light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:19.

18. The chimeric antigen receptor of claim 1, wherein the light chain variable domain consists of an amino acid sequence of SEQ ID NO:19.

19. The chimeric antigen receptor of claim 1, wherein the light chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:20.

20. The chimeric antigen receptor of claim 1, wherein the light chain variable domain consists of an amino acid sequence of SEQ ID NO:20.

21. The chimeric antigen receptor of claim 1, wherein the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:25.

22. The chimeric antigen receptor of claim 1, wherein the heavy chain variable domain consists of an amino acid sequence of SEQ ID NO:25.

23. The chimeric antigen receptor of claim 1, wherein the heavy chain variable domain comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO:26.

24. The chimeric antigen receptor of claim 1, wherein the heavy chain variable domain consists of an amino acid sequence of SEQ ID NO:26.

25. The chimeric antigen receptor of claim 1, wherein the transmembrane domain comprises a CD8α transmembrane domain, a CD28 transmembrane domain, a CD4 transmembrane domain, a CD3ζ transmembrane domain, or any combination thereof.

26. The chimeric antigen receptor of claim 25, wherein the transmembrane domain is a CD28 transmembrane domain.

27. The chimeric antigen receptor of claim 25, wherein the CD28 transmembrane domain comprises an amino acid sequence of SEQ ID NO:32.

28. The chimeric antigen receptor of claim 25, wherein the CD28 transmembrane domain consists of an amino acid sequence of SEQ ID NO:32.

29. The chimeric antigen receptor of claim 1, wherein the intracellular domain comprises an intracellular co-stimulatory signaling domain, an intracellular T-cell signaling domain, or a combination thereof.

30. The chimeric antigen receptor of claim 29, wherein the intracellular co-stimulatory signaling domain is a 4-1BB intracellular co-stimulatory signaling domain, a CD28 intracellular co-stimulatory signaling domain, a ICOS intracellular co-stimulatory signaling domain, an OX-40 intracellular co-stimulatory signaling domain, or any combination thereof.

31. The chimeric antigen receptor of claim 29, wherein the intracellular costimulatory signaling domain comprises a 4-1BB intracellular co-stimulatory signaling domain.

32. The chimeric antigen receptor of claim 31, wherein the 4-1BB intracellular co-stimulatory signaling domain comprises an amino acid sequence of SEQ ID NO:33.

33. The chimeric antigen receptor of claim 31, wherein the 4-1BB intracellular co-stimulatory signaling domain consists of an amino acid sequence of SEQ ID NO:33.

34. The chimeric antigen receptor of claim 29, the intracellular costimulatory signaling domain comprises a CD28 intracellular co-stimulatory signaling domain and a 4-1BB intracellular co-stimulatory signaling domain.

35. The chimeric antigen receptor of any one of claims 29-34, wherein the intracellular costimulatory signaling domain further comprises an intracellular T-cell signaling domain.

36. The chimeric antigen receptor of claim 35, wherein the intracellular T-cell signaling domain is a CD3ζ intracellular T-cell signaling domain.

37. The chimeric antigen receptor of claim 36, wherein the CD3ζ intracellular T-cell signaling domain comprises an amino acid sequence of SEQ ID NO:34.

38. The chimeric antigen receptor of claim 36, wherein the CD3ζ intracellular T-cell signaling domain consists of an amino acid sequence of SEQ ID NO:34.

39. The chimeric antigen receptor of claim 1, wherein the chimeric antigen receptor binds to a cell expressing ROR-1.

40. A nucleic acid encoding the chimeric antigen receptor of claim 1.

41. The nucleic acid of claim 40, wherein the nucleic acid is a viral vector.

42. The nucleic acid of claim 41, wherein the viral vector is a lentiviral vector.

43. A cell comprising the nucleic acid of claim 40.

44. A cell expressing the chimeric antigen receptor of claim 1.

45. The cell of claim 43 or 44, wherein the cell is a T lymphocyte.

46. The cell of claim 45, wherein the T lymphocyte is a CD4+ T lymphocyte or a CD8 + T lymphocyte.

47. The cell of claim 43 or 44, wherein the cell is a natural killer cell.

48. A pharmaceutical composition comprising a therapeutically effective amount of the cell of claim 43 and a pharmaceutically acceptable diluent, carrier, or excipient.

49. The pharmaceutical composition of claim 48, wherein the composition is formulated for intravenous injection.

50. A method of treating cancer in an individual in need thereof, the method comprising administering to the individual the pharmaceutical composition of claim 48.

51. The method of claim 50, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia, adult acute myeloid leukemia, acute lymphoblastic leukemia, a mantle cell lymphoma,ovarian cancer, colon cancer, lung cancer, skin

cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

52. The method of claim 50, wherein the cancer is a CD19-negative cancer or has reduced CD19 expression as a result of a prior treatement that targets CD19.

53. The method of claim 50, wherein the individual has previously been treated with a therapeutic that targets CD19.

54. The method of claim 53, wherein the therapeutic that targets CD19 is an antibody that binds to CD19.

55. The method of claim 53, wherein the therapeutic that targets CD19 is a chimeric antigen receptor T cell that targets CD19.

56. The method of claim 53, wherein the therapeutic that targets CD19 is a chimeric antigen receptor NK cell that targets CD19.

57. The method of any one of claims 50 to 56, wherein the cancer expresses ROR1.

58. The method of claim 50, further comprising administering cirmtuzumab to the individual.

59. The method of claim 58, wherein the cirmtuzumab and the pharmaceutical composition are administered separately.

60. The pharmaceutical composition of claim 48 for use in a method of treating cancer in an individual.

61. The pharmaceutical composition for the use of claim 60, wherein the cancer comprises a leukemia, a lymphoma, chronic lymphocytic leukemia, adult acute myeloid leukemia, acute lymphoblastic leukemia, a mantle cell lymphoma, ovarian cancer, colon cancer, lung cancer, skin cancer, pancreatic cancer, testicular cancer, bladder cancer, uterine cancer, prostate cancer, or adrenal cancer.

62. The pharmaceutical composition for the use of claim 60, wherein the cancer is a CD19 negative cancer or has reduced CD19 expression as a result of a prior treatment that targets CD19.

63. The pharmaceutical composition for the use of claim 60, wherein the individual has previously been treated with a therapeutic that targets CD19.

64. The pharmaceutical composition for the use of claim 63, wherein the therapeutic that targets CD19 is an antibody that binds to CD19.

65. The pharmaceutical composition for the use of claim 63, wherein the therapeutic that targets CD19 is a chimeric antigen receptor T cell that targets CD19.

66. The pharmaceutical composition for the use of claim 63, wherein the therapeutic that targets CD19 is a chimeric antigen receptor NK cell that targets CD19.

67. The pharmaceutical composition for the use of any one of claims 60 to 66, wherein the cancer expresses ROR1.

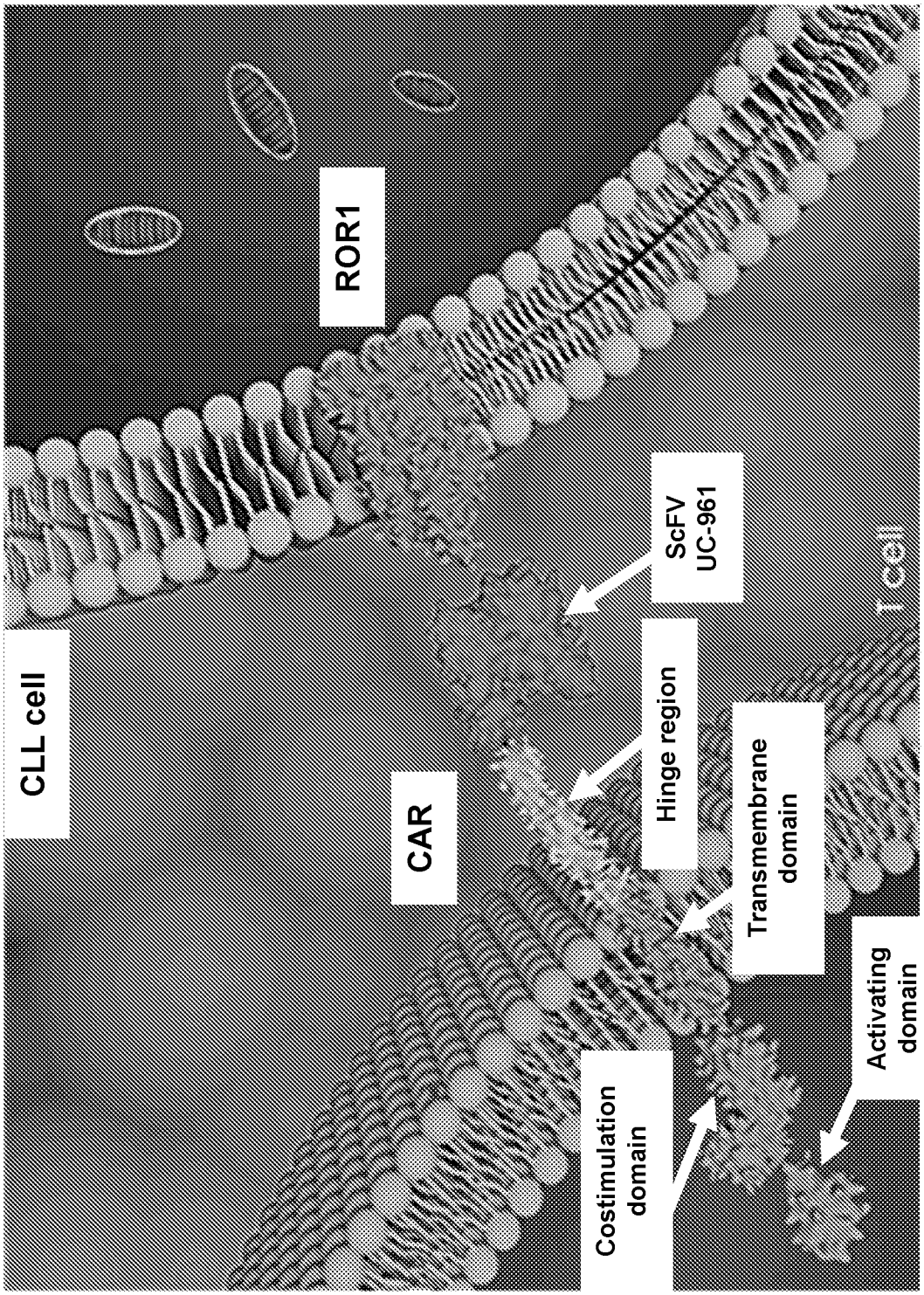

CLL cell
ROR1
CAR
ScFV
UC-961
Hinge region
Transmembrane
domain
T cell
Costimulation
domain
Activating
domain

FIG. 1A

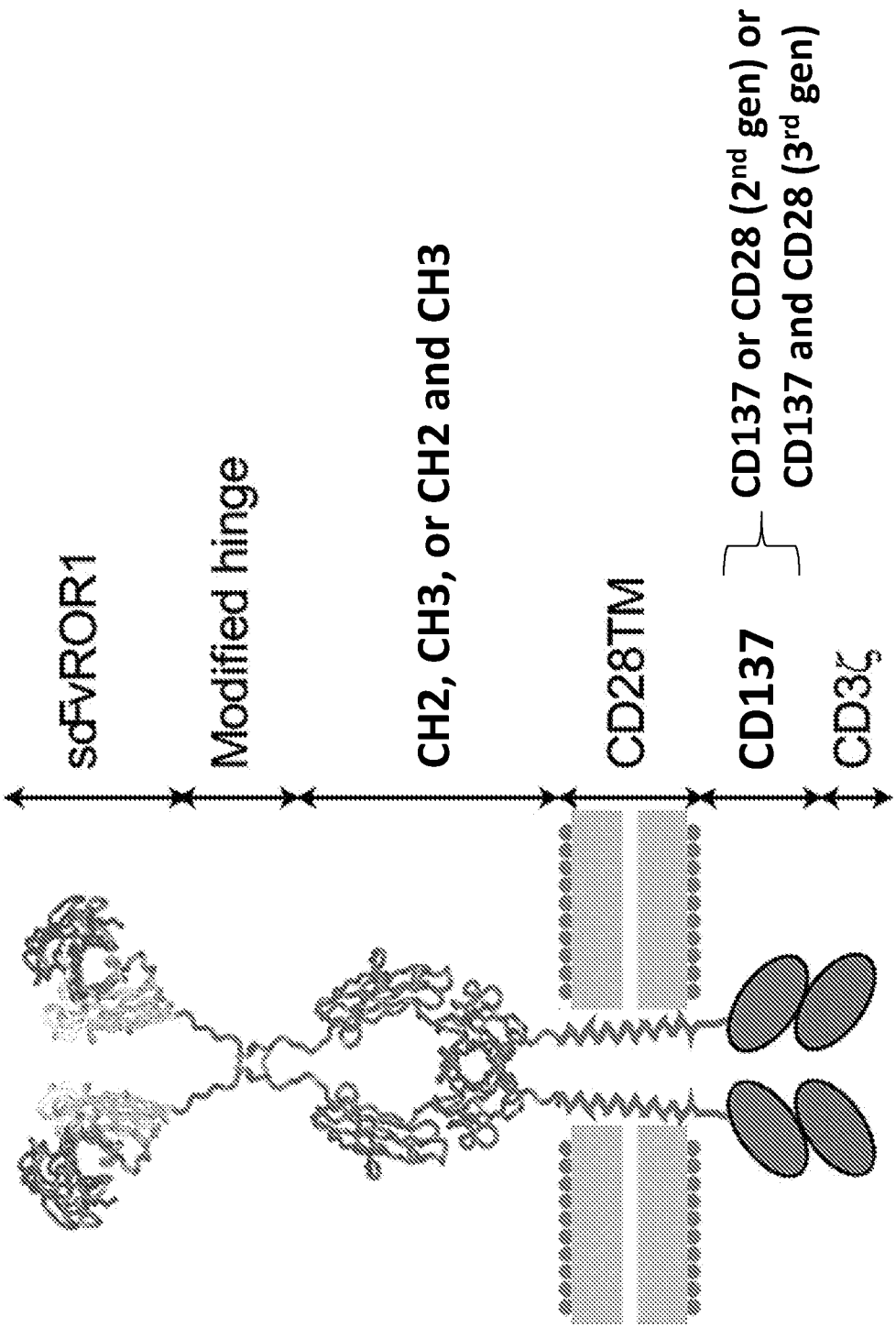

sdFvROR1
Modified hinge
CH2, CH3, or CH2 and CH3
CD28TM
CD137
CD3ζ
CD137 or CD28 (2nd gen) or
CD137 and CD28 (3rd gen)

FIG. 1B

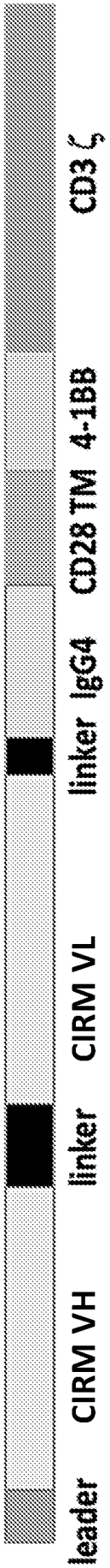


FIG. 1C

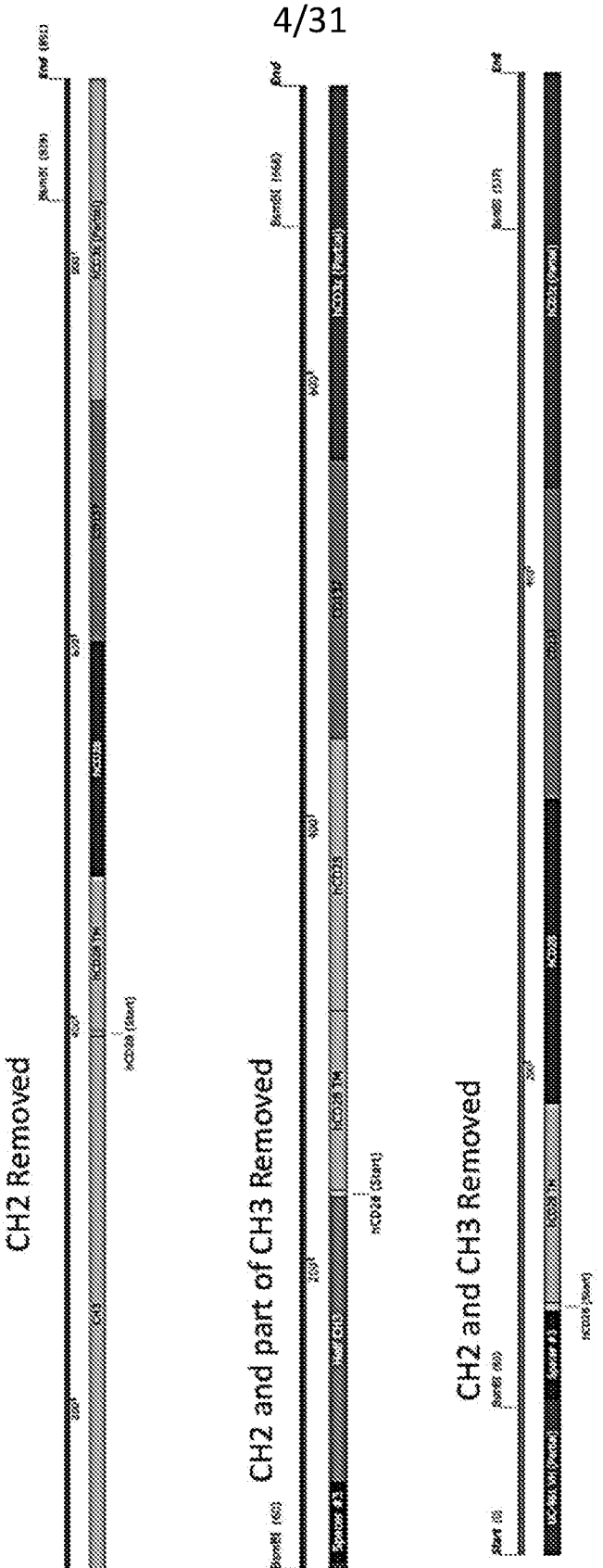


FIG. 2A

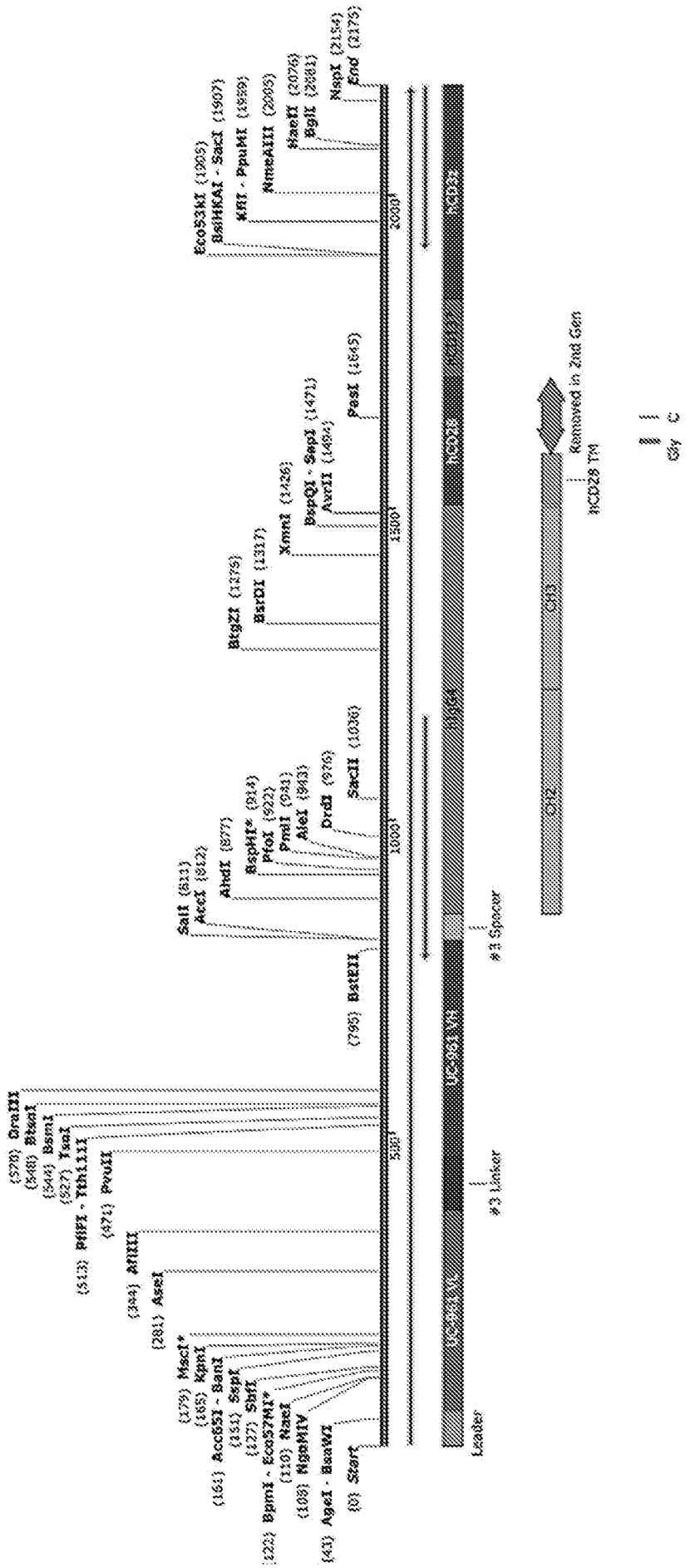

(578) DraIII
(548) BtsaI
(546) BsmI
(527) TsaI
(513) MfeI - Tth111I
(471) PvuII
(384) AflIII
(281) AseI
(179) MscI*
(168) KpnI
(164) Acc65I - BanI
(151) SspI
(127) SbfI
(122) BpmI - Eco57MI*
(110) NaeI
(108) NgoMIV
(43) AgeI - BsaWI
(0) Start
Leader
#3 Linker
5001
(795) BstEII
Sall (811)
AccI (812)
AhdI (877)
BspHI* (914)
PfoI (922)
PmlI (931)
AleI (943)
DrdI (976)
SacII (1036)
10001
BtgZI (1275)
BsrDI (1317)
XmnI (1426)
BspQI - SapI (1471)
AvrII (1484)
PasI (1545)
15001
Eco53kI (1905)
BsiWI - SacI (1907)
KflI - PpuMI (1939)
NmeAIII (2005)
HaeII (2026)
BglI (2081)
NspI (2154)
End (2175)
20001
1000t
CH1
CH2
CH3
Removed in 2nd Gen
hCD28 TM
#3 Spacer
Sig C

FIG. 2B

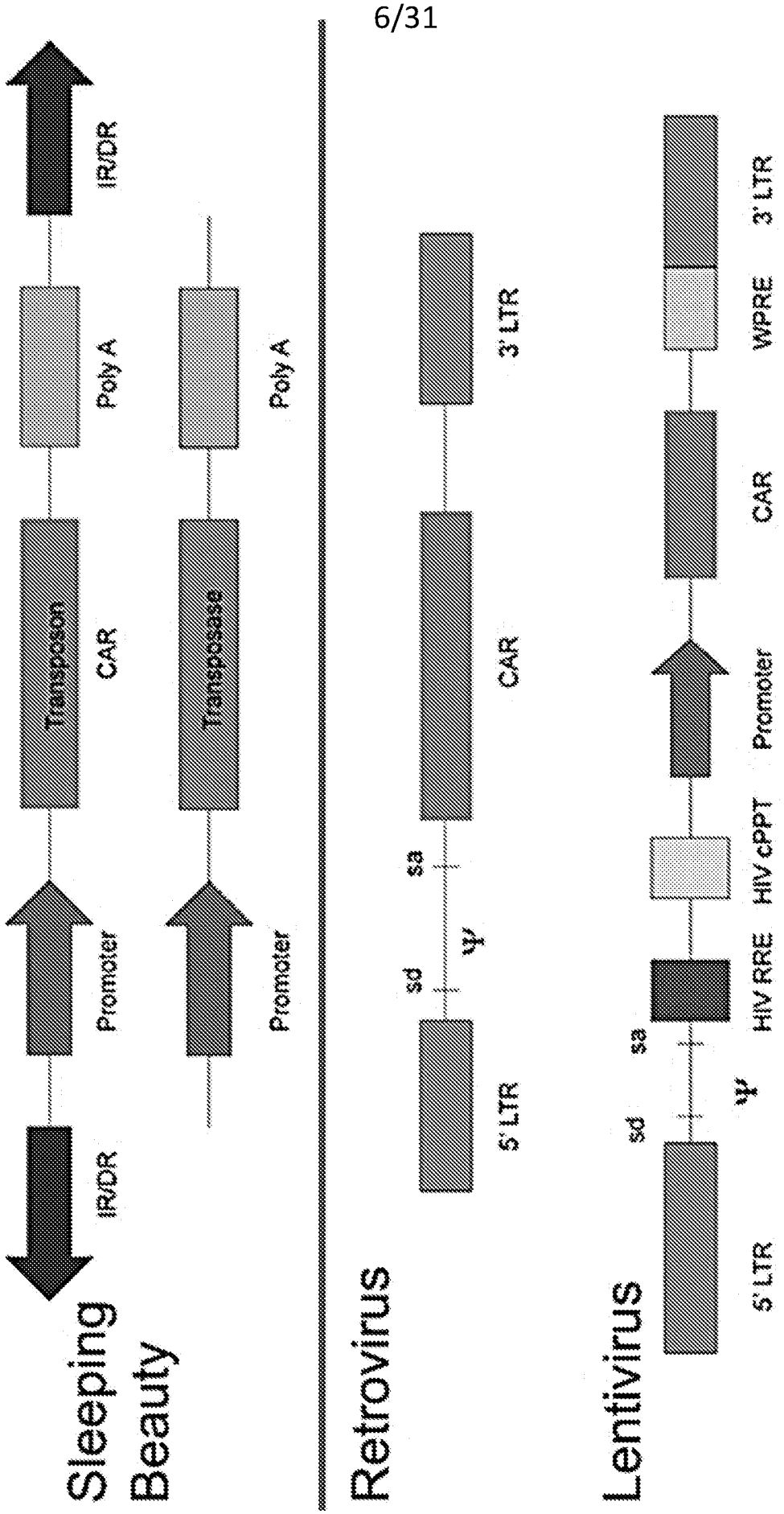

Sleeping Beauty
IR/DR
Promoter
Transposon
CAR
Poly A
IR/DR
Promoter
Transposase
Poly A
Retrovirus
5' LTR
sd
sa
ψ
CAR
3' LTR
Lentivirus
5' LTR
sd
sa
ψ
HIV RRE
HIV cPPT
Promoter
CAR
WPRE
3' LTR

FIG. 3

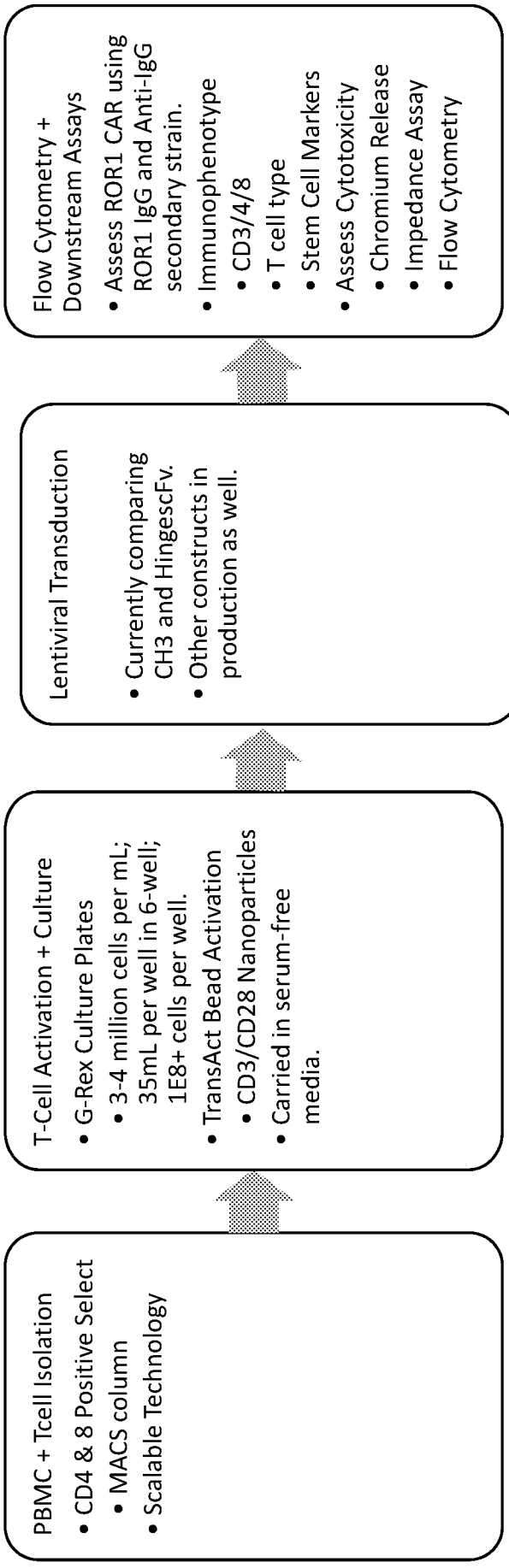

PBMC + Tcell Isolation
• CD4 & 8 Positive Select
• MACS column
• Scalable Technology
T-Cell Activation + Culture
• G-Rex Culture Plates
• 3-4 million cells per mL; 35mL per well in 6-well; 1E8+ cells per well.
• TransAct Bead Activation
• CD3/CD28 Nanoparticles
• Carried in serum-free media.
Lentiviral Transduction
• Currently comparing CH3 and HingescFv.
• Other constructs in production as well.
Flow Cytometry + Downstream Assays
• Assess ROR1 CAR using ROR1 IgG and Anti-IgG secondary strain.
• Immunophenotype
• CD3/4/8
• T cell type
• Stem Cell Markers
• Assess Cytotoxicity
• Chromium Release
• Impedance Assay
• Flow Cytometry

FIG. 4A

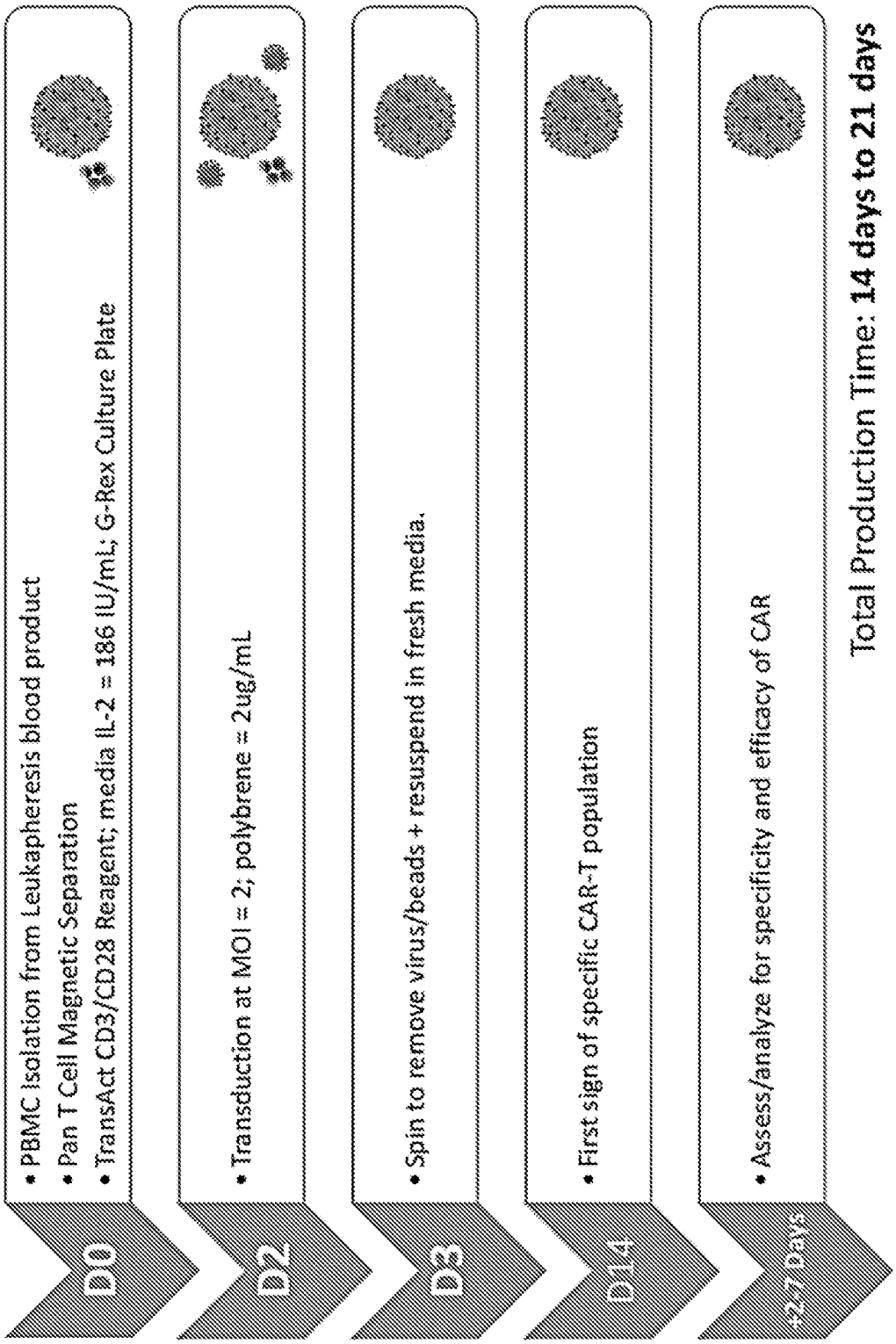

D0
• PBMC Isolation from Leukapheresis blood product
• Pan T Cell Magnetic Separation
• TransAct CD3/CD28 Reagent; media IL-2 = 186 IU/mL; G-Rex Culture Plate
D2
• Transduction at MOI = 2; polybrene = 2ug/mL
D3
• Spin to remove virus/beads + resuspend in fresh media.
D14
• First sign of specific CAR-T population
+2-7 Days
• Assess/analyze for specificity and efficacy of CAR
Total Production Time: 14 days to 21 days

FIG. 4B

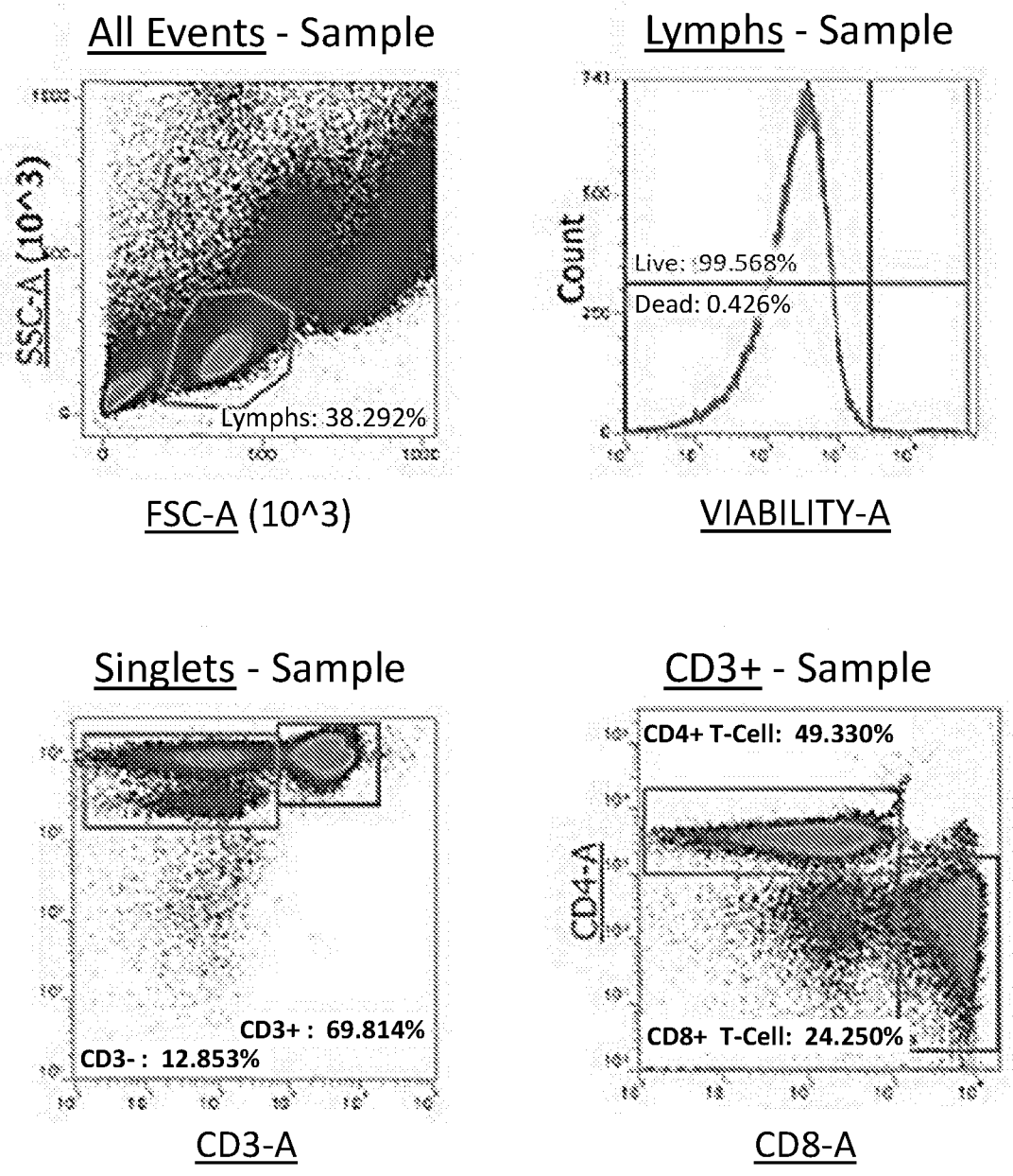

All Events - Sample
SSC-A (10^3)
Lymphs: 38.292%
FSC-A (10^3)
Lymphs - Sample
Count
Live: 99.568%
Dead: 0.426%
VIABILITY-A
Singlets - Sample
CD3+ : 69.814%
CD3- : 12.853%
CD3-A
CD3+ - Sample
CD4+ T-Cell: 49.330%
CD4-A
CD8+ T-Cell: 24.250%
CD8-A

FIG. 5A

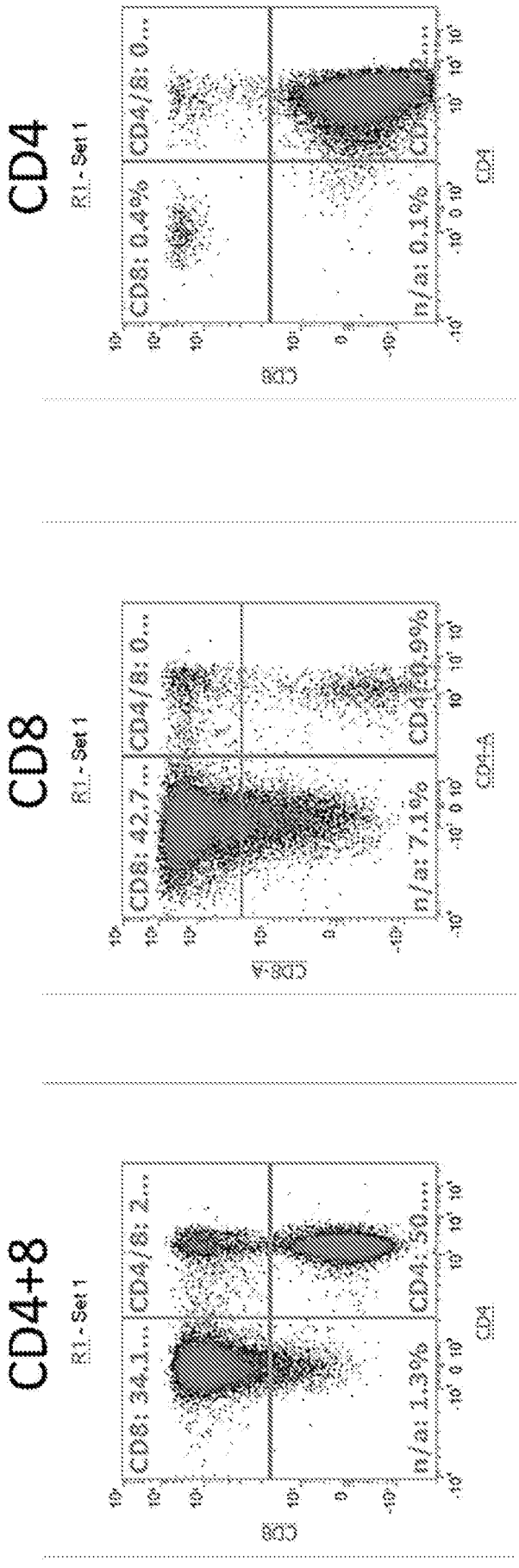

CD4
R1 - Set 1
CD8: 0.4%
CD4/8: 0...
n/a: 0.1%
CD8
CD4
CD8
R1 - Set 1
CD8: 42.7...
CD4/8: 0...
n/a: 7.1%
CD4: 49.9%
CD8-A
CD4-A
CD4+8
R1 - Set 1
CD8: 34.1...
CD4/8: 2...
n/a: 1.3%
CD4: 50....
CD8
CD4

FIG. 5B

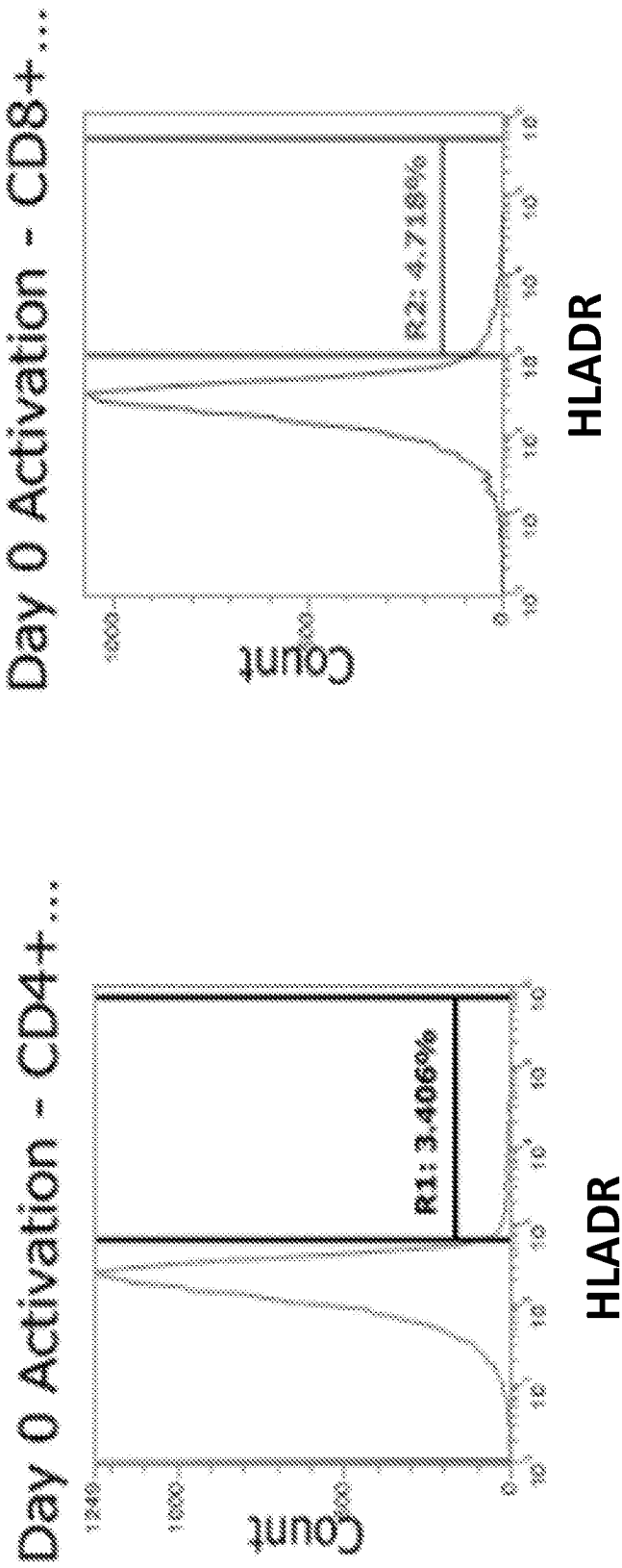

Day 0 Activation - CD8+...
R2: 4.718%
Count
HLADR
Day 0 Activation - CD4+...
R1: 3.466%
Count
HLADR

FIG. 6A

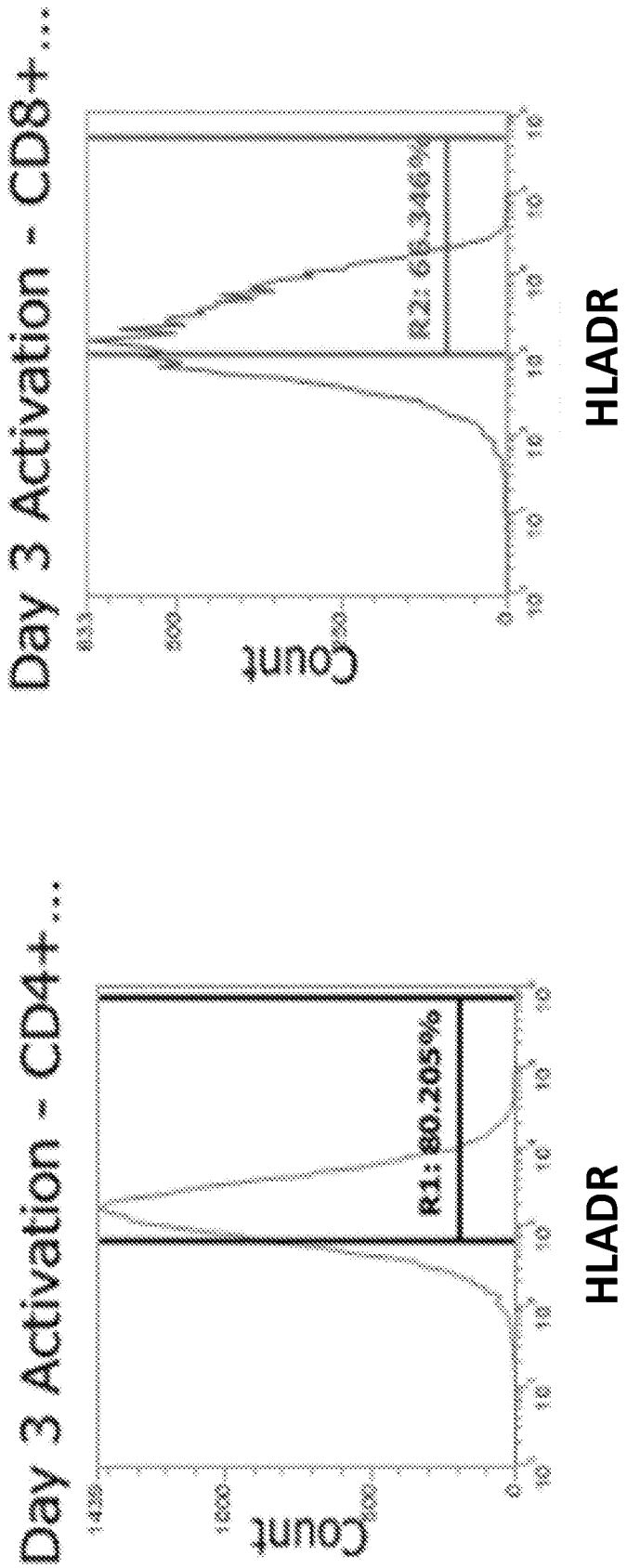


FIG. 6A (Continued)

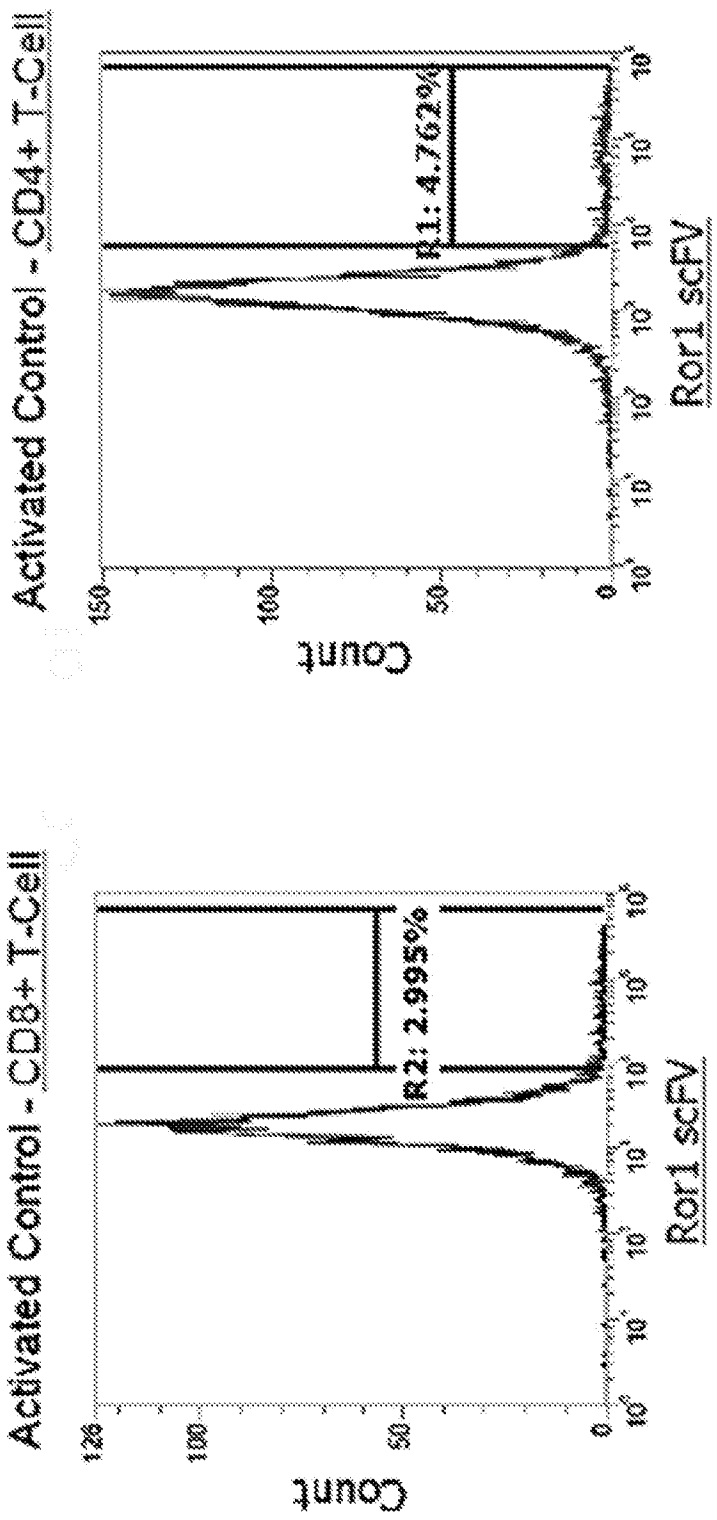

Activated Control - CD4+ T-Cell
R1: 4.762%
Count
Ror1 scFV
Activated Control - CD8+ T-Cell
R2: 2.995%
Count
Ror1 scFV

FIG. 6B

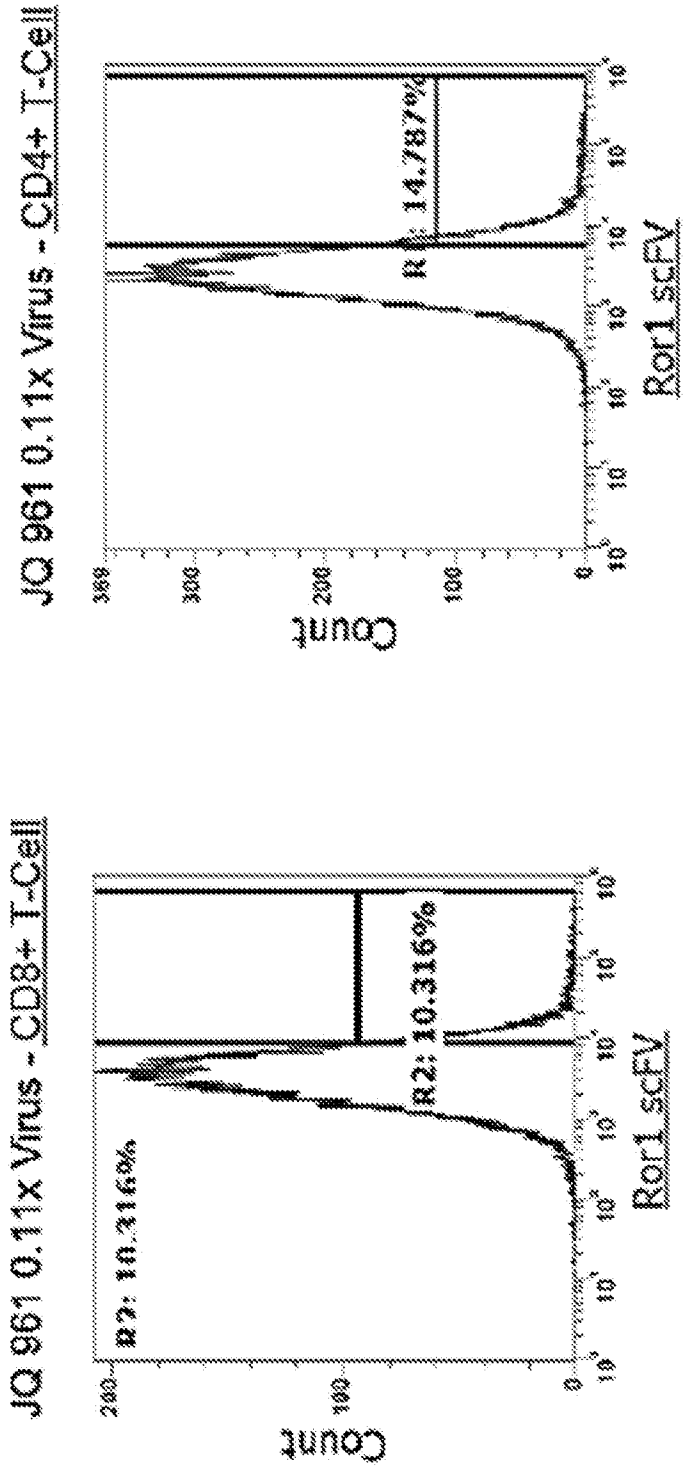


FIG. 6B (Continued)

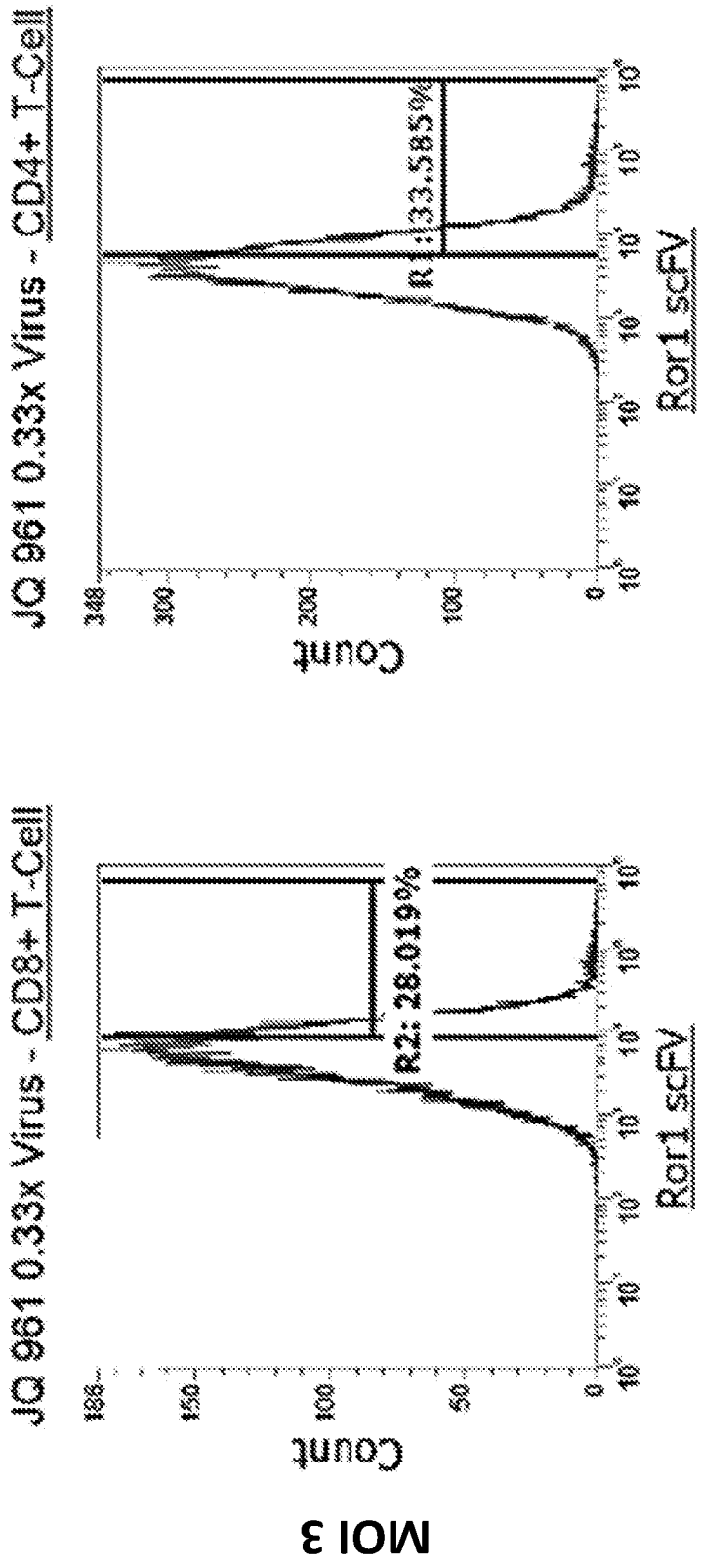


FIG. 6B (Continued)

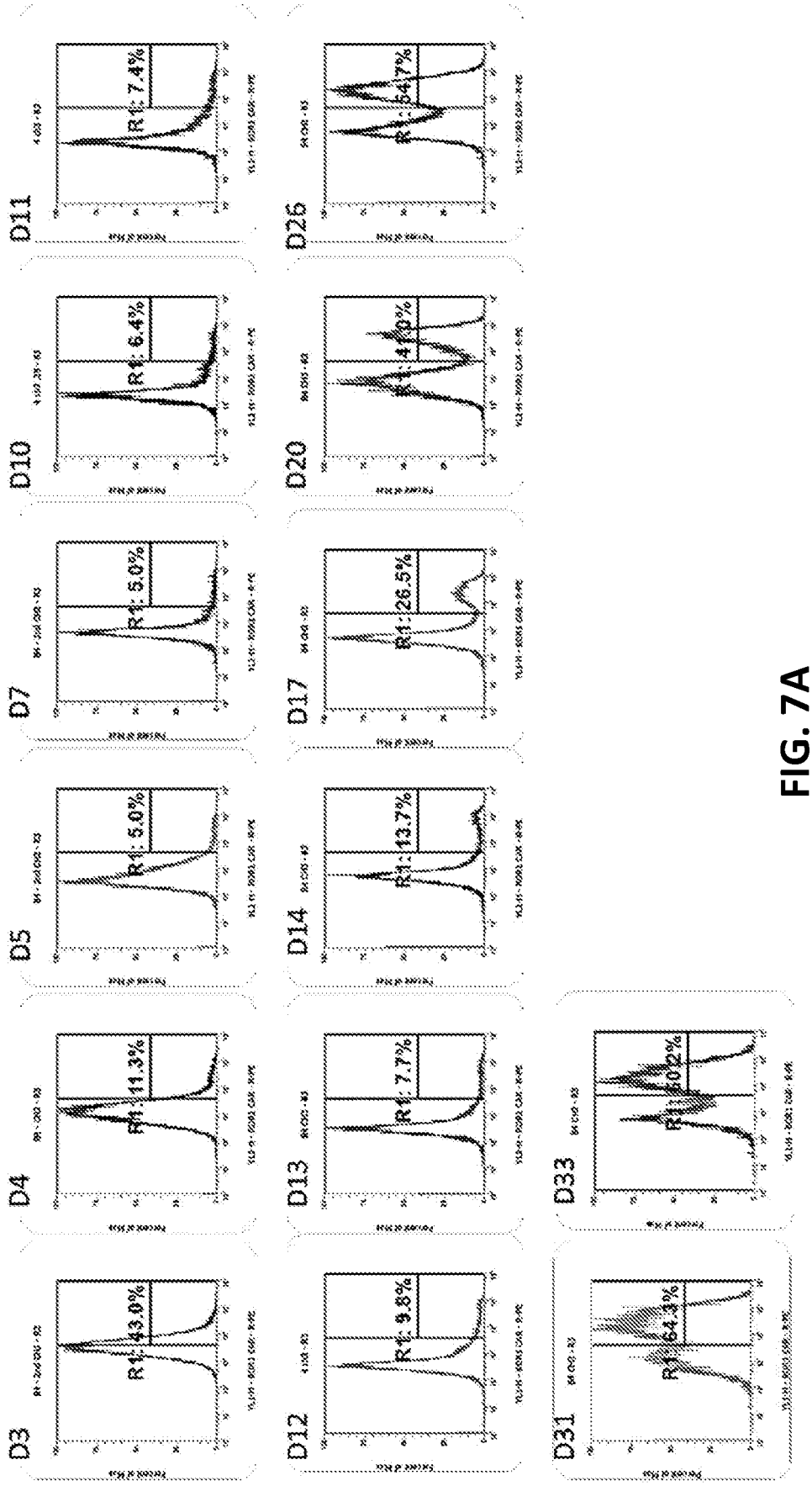

D3
R1: 43.0%
D4
R1: 11.3%
D5
R1: 5.0%
D7
R1: 5.0%
D10
R1: 6.4%
D11
R1: 7.4%
D12
R1: 9.8%
D13
R1: 7.7%
D14
R1: 13.7%
D17
R1: 26.5%
D20
R1: 47.0%
D26
R1: 54.7%
D31
R1: 64.3%
D33
R1: 60.2%

FIG. 7A

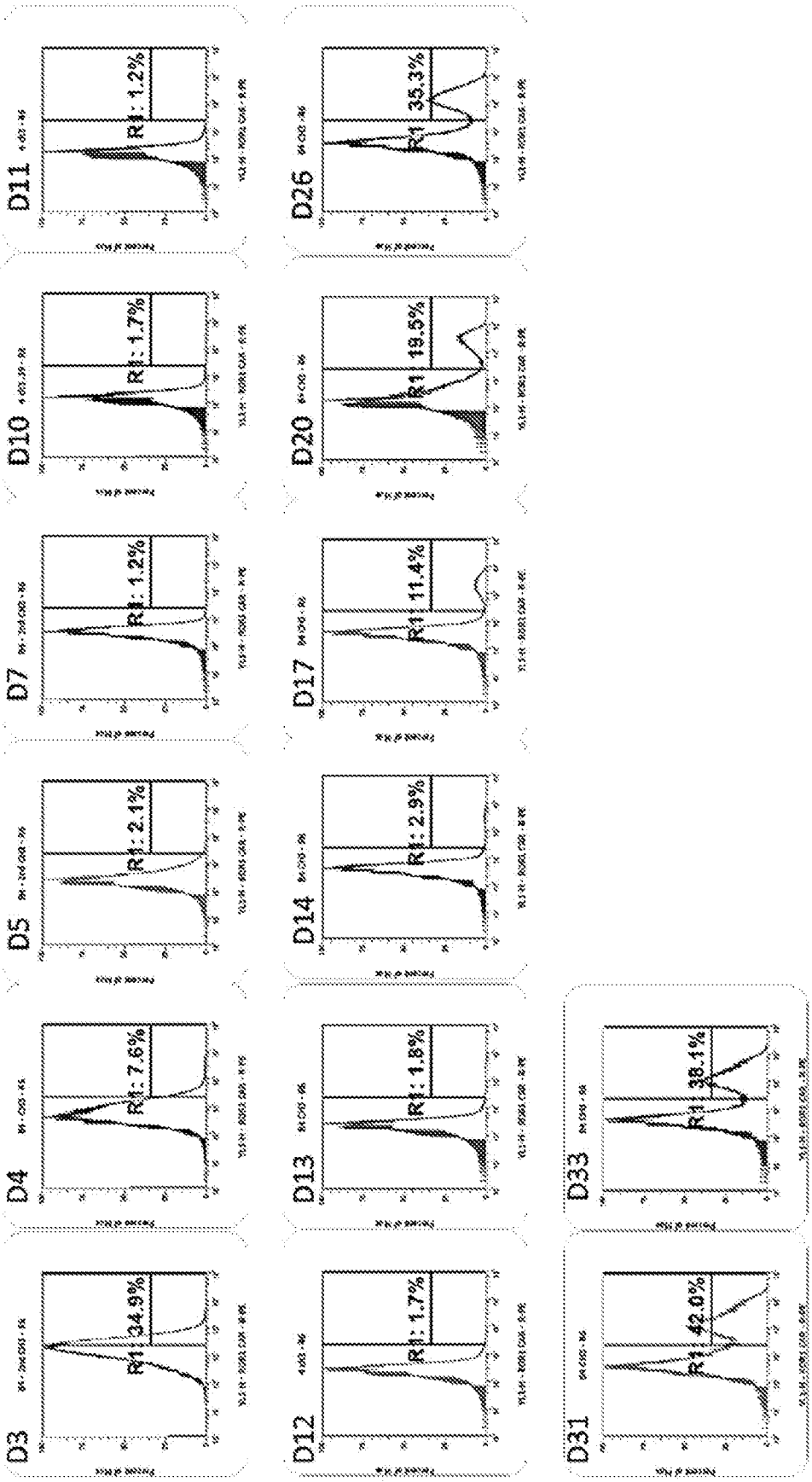

D11
R1: 1.2%
D26
R1: 35.3%
D10
R1: 1.7%
D20
R1: 19.5%
D7
R1: 1.2%
D17
R1: 11.4%
D5
R1: 2.1%
D14
R1: 2.9%
D4
R1: 7.6%
D13
R1: 1.8%
D33
R1: 38.1%
D3
R1: 34.9%
D12
R1: 1.7%
D31
R1: 42.0%

FIG. 7B

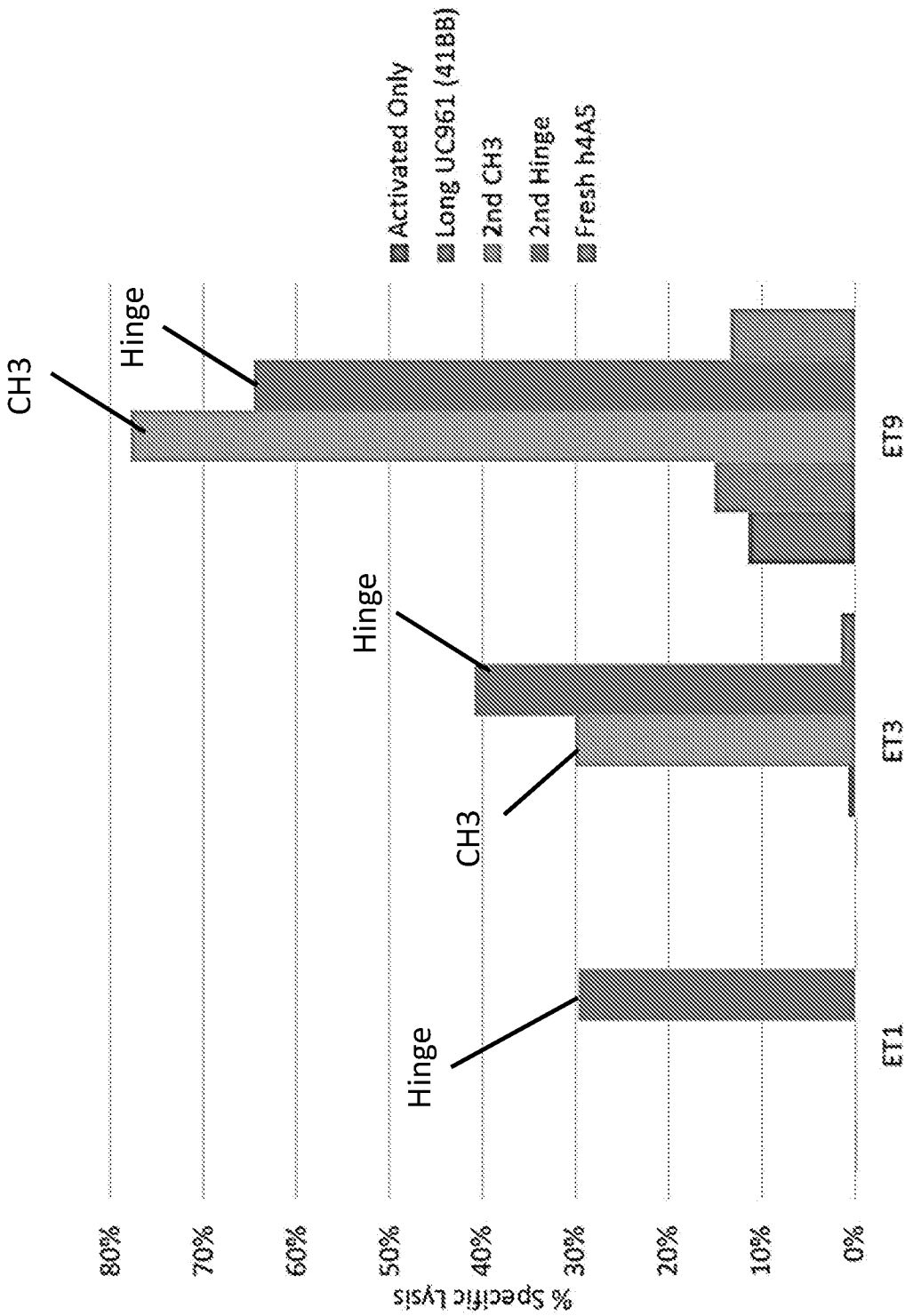

Activated Only
Long UC961 (41BB)
2nd CH3
2nd Hinge
Fresh h4A5
% Specific Lysis
80%
70%
60%
50%
40%
30%
20%
10%
0%
ET1
ET3
ET9
Hinge
CH3
Hinge
CH3
Hinge

FIG. 8A

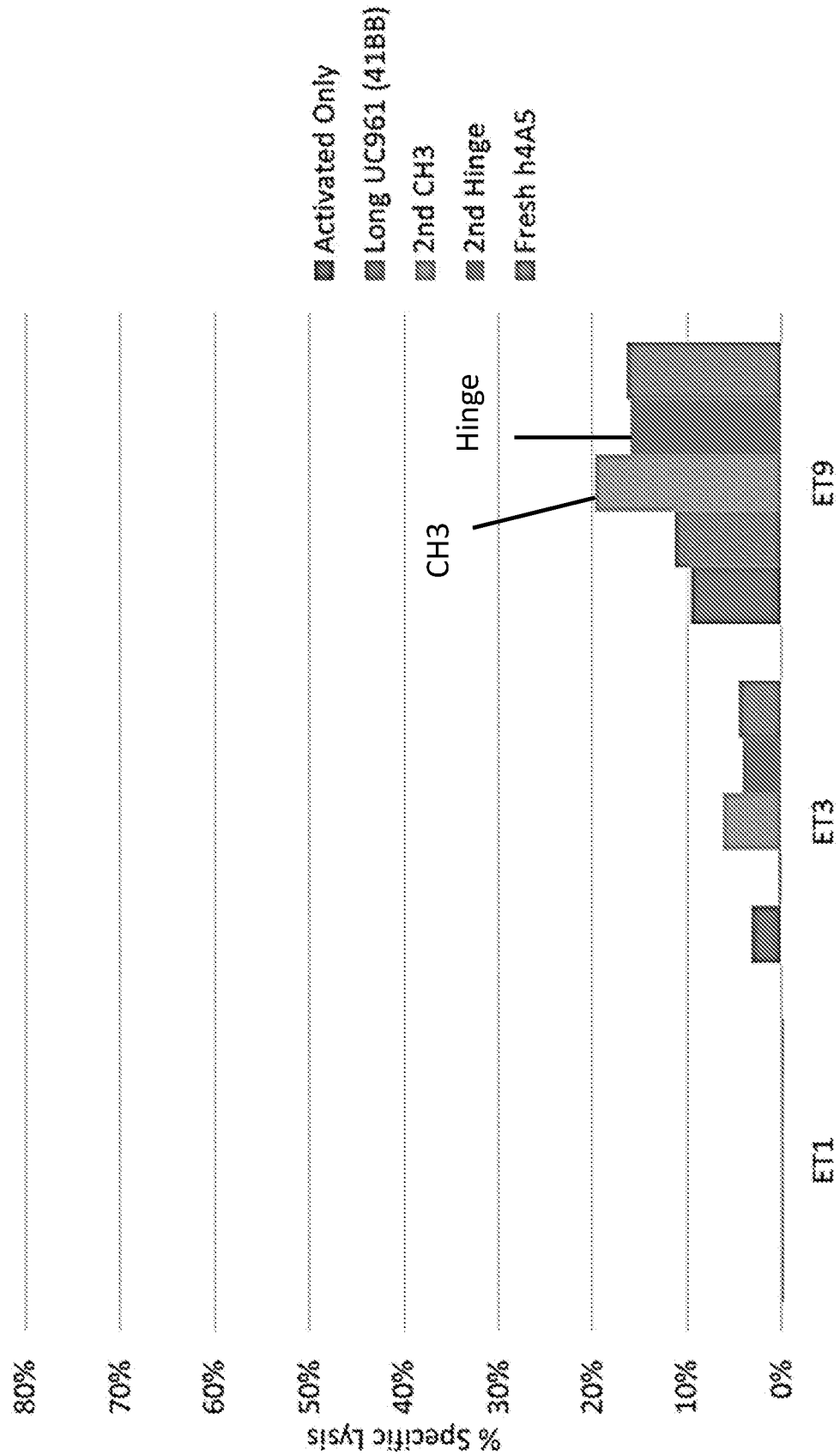

Activated Only
Long UC961 (41BB)
2nd CH3
2nd Hinge
Fresh h4A5
Hinge
CH3
ET9
ET3
ET1
% Specific lysis
80%
70%
60%
50%
40%
30%
20%
10%
0%

FIG. 8B

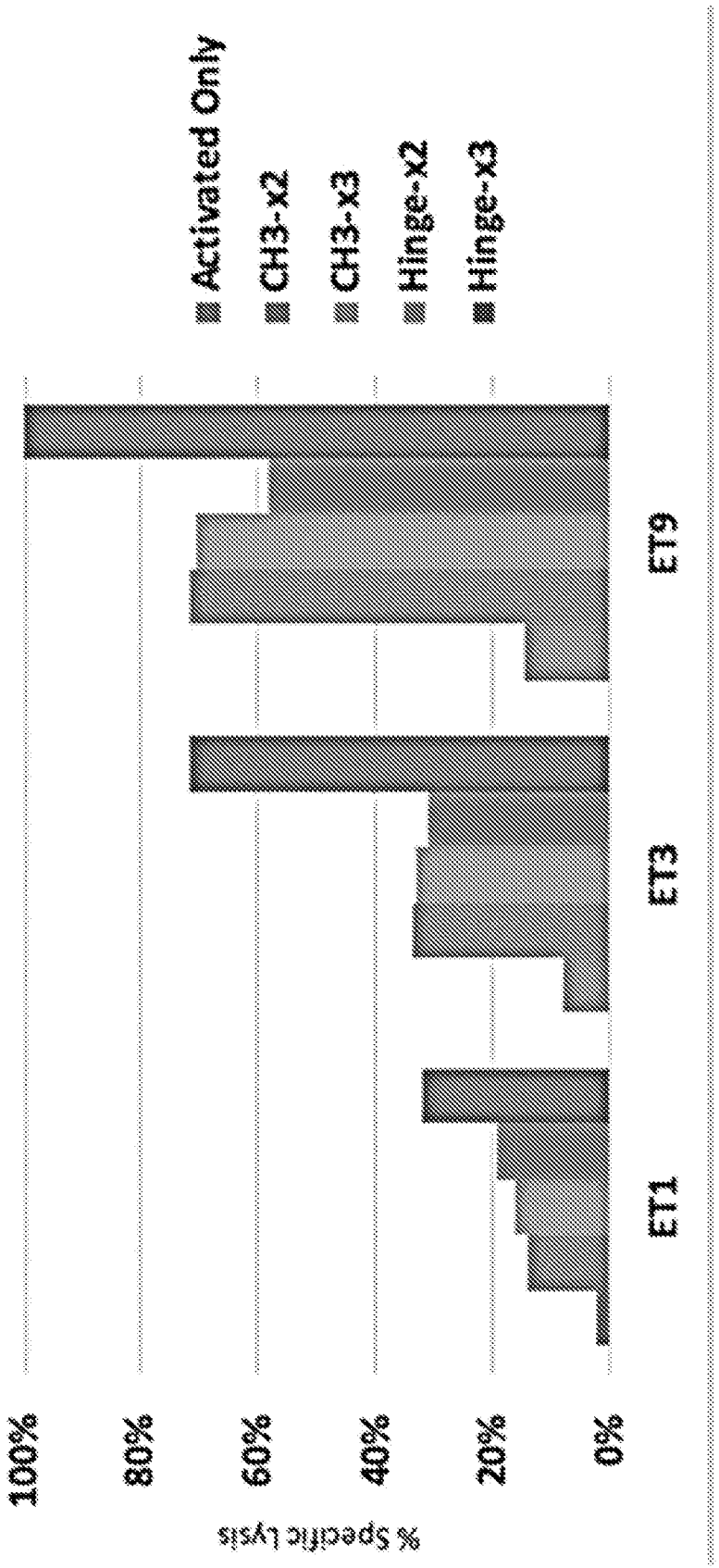

Activated Only
CH3-x2
CH3-x3
Hinge-x2
Hinge-x3
ET9
ET3
ET1
100%
80%
60%
40%
20%
0%
% Specific Lysis

FIG. 8C

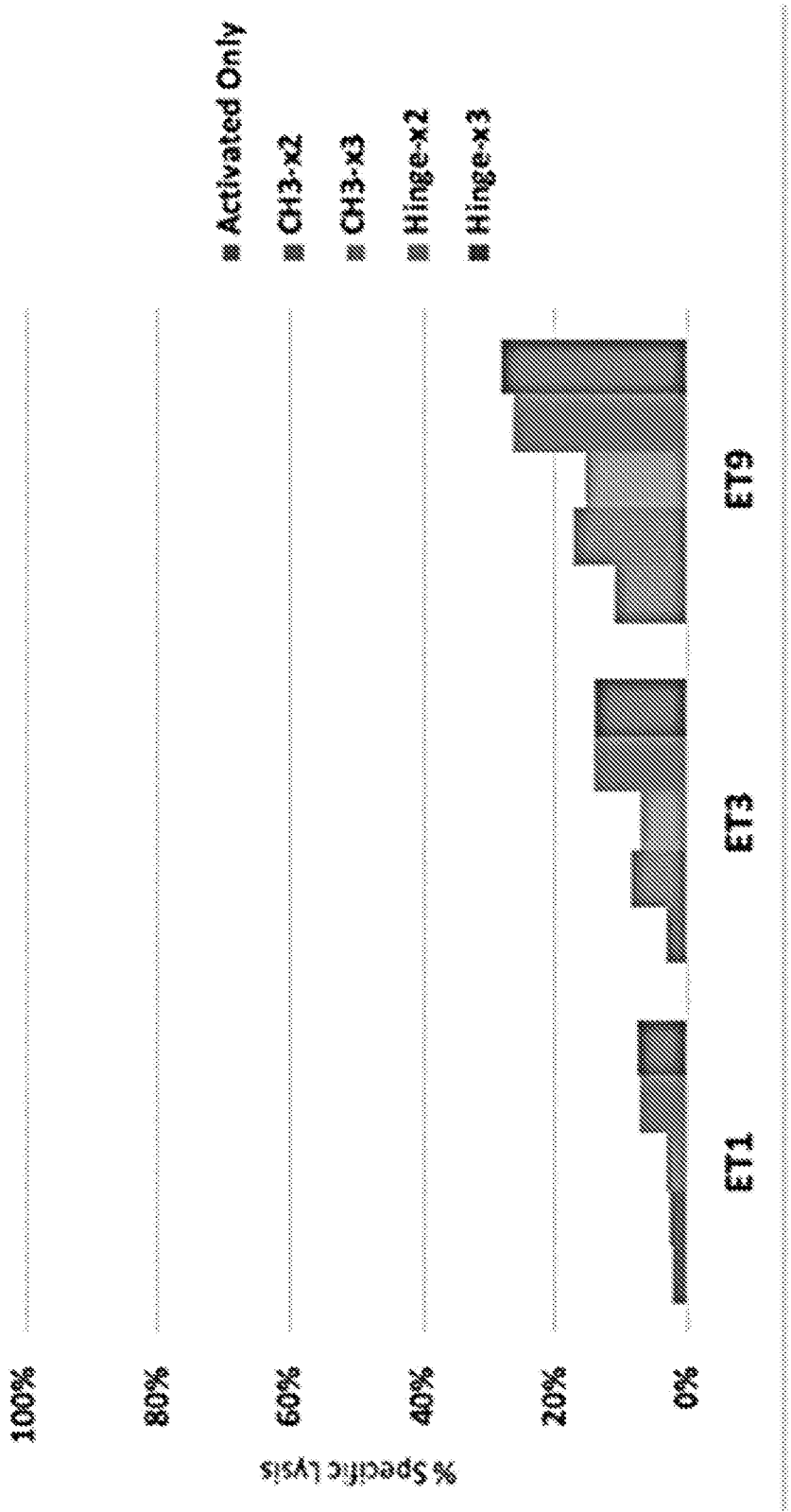

Activated Only
CH3-x2
CH3-x3
Hinge-x2
Hinge-x3
ET9
ET3
ET1
100%
80%
60%
40%
20%
0%
% Specific Lysis

FIG. 8D

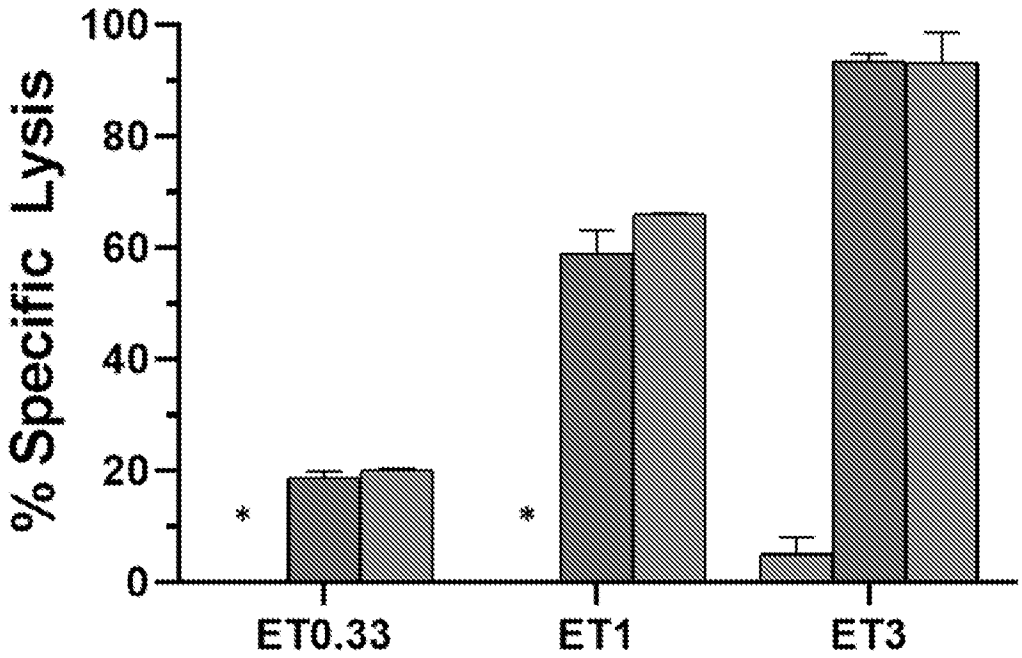


FIG. 8E

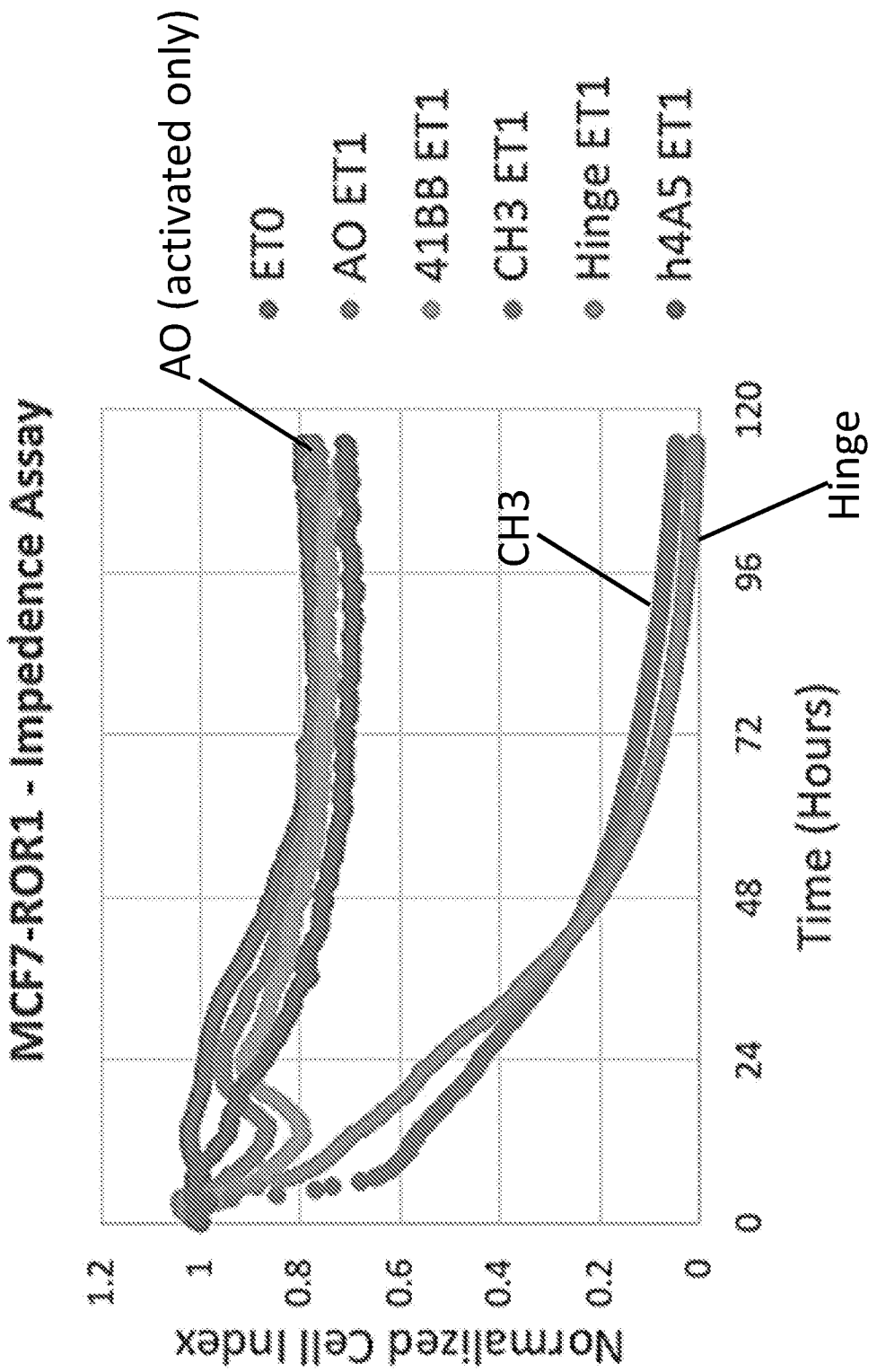

MCF7-ROR1 - Impedence Assay
AO (activated only)
ET0
AO ET1
41BB ET1
CH3 ET1
Hinge ET1
h4A5 ET1
CH3
Hinge
Normalized Cell Index
1.2
1
0.8
0.6
0.4
0.2
0
0
24
48
72
96
120
Time (Hours)

FIG. 9A

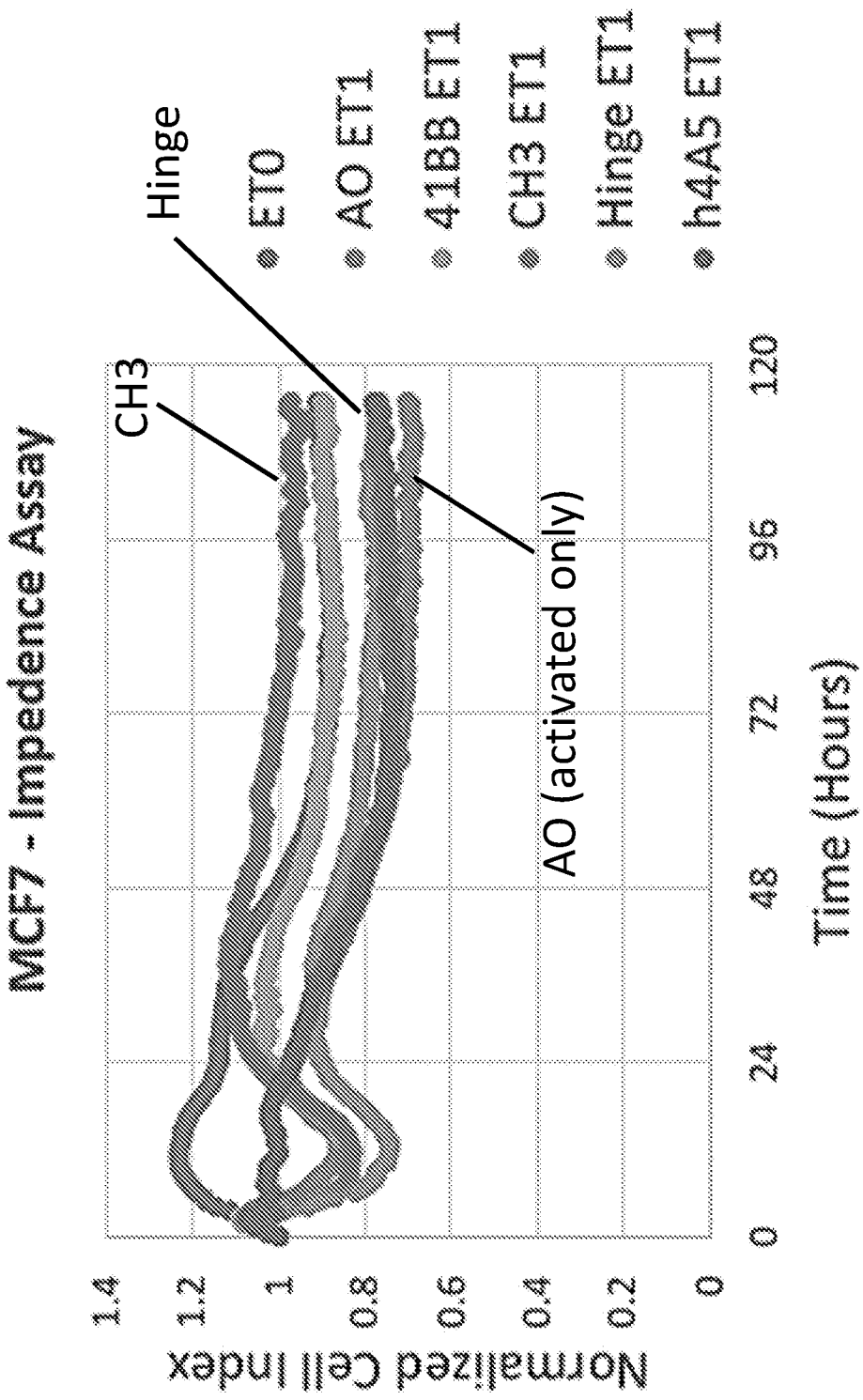

MCF7 - Impedence Assay
Normalized Cell Index
Time (Hours)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
24
48
72
96
120
CH3
Hinge
AO (activated only)
ET0
AO ET1
41BB ET1
CH3 ET1
Hinge ET1
h4A5 ET1

FIG. 9B

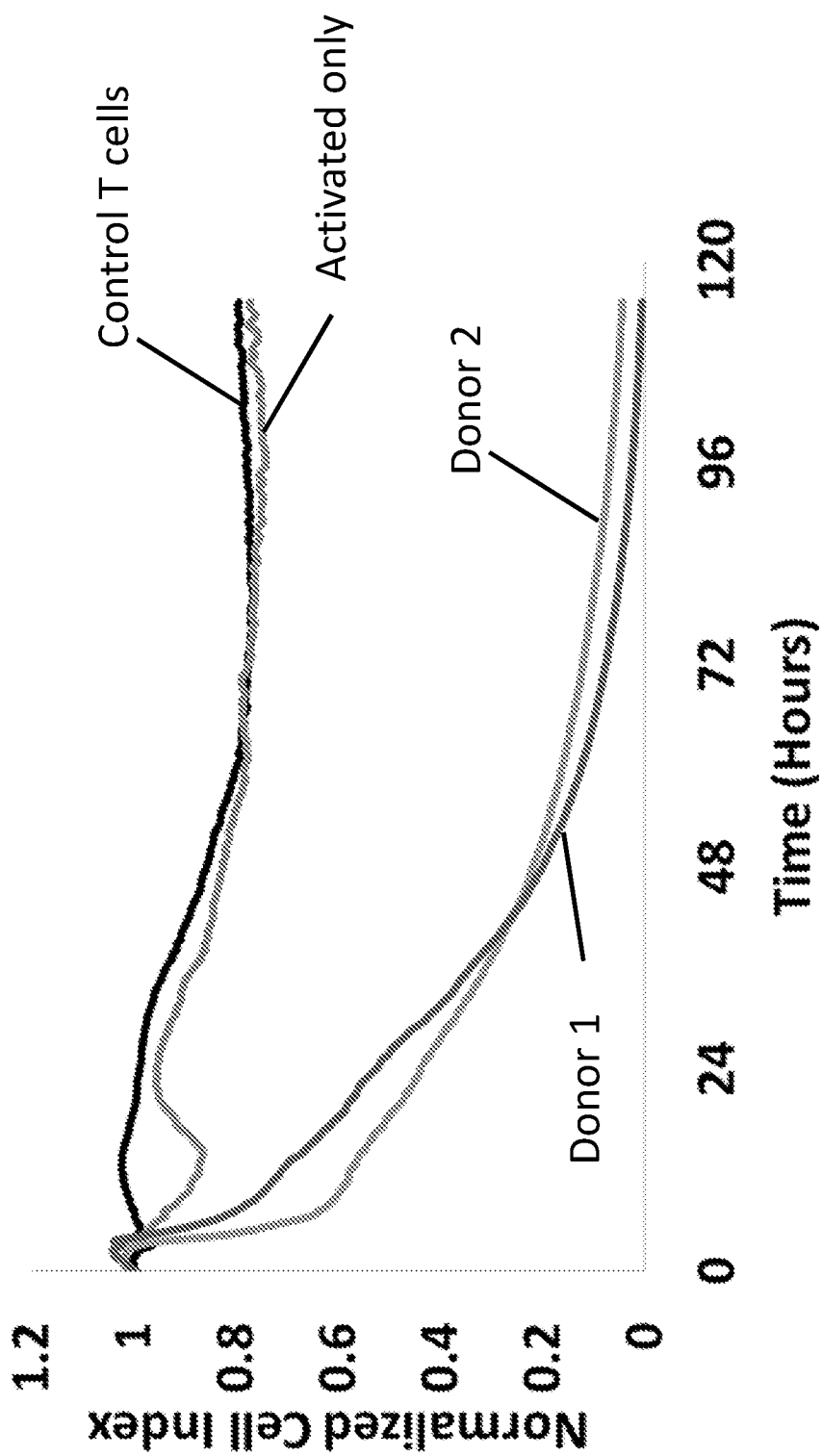

Control T cells
Activated only
Donor 2
Donor 1
Normalized Cell Index
1.2
1
0.8
0.6
0.4
0.2
0
0
24
48
72
96
120
Time (Hours)

FIG. 9C

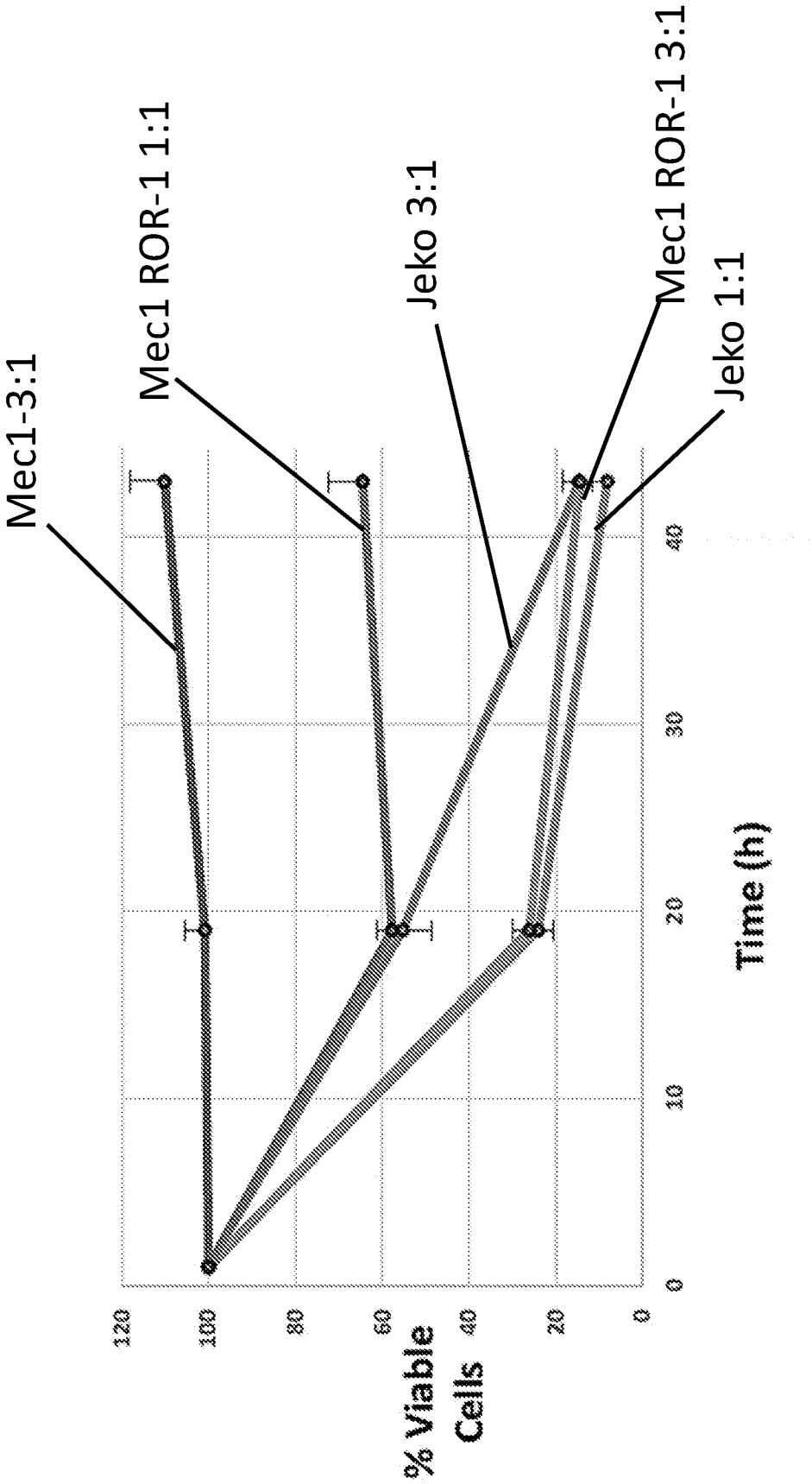
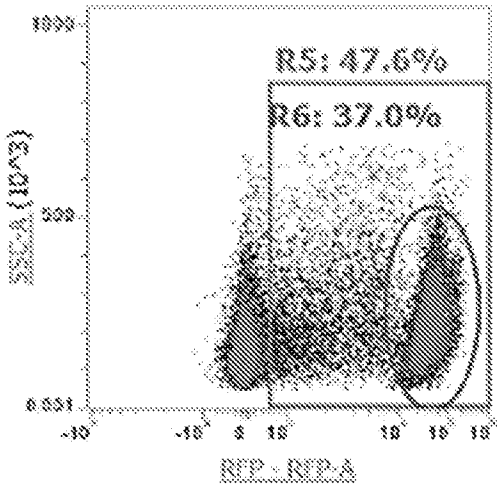
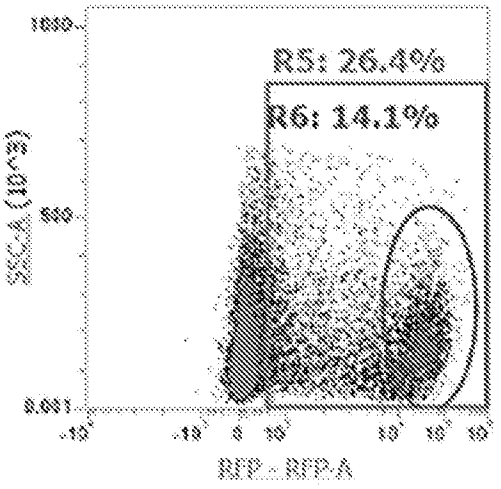

Mec1-3:1
Mec1 ROR-1 1:1
Jeko 3:1
Mec1 ROR-1 3:1
Jeko 1:1
% Viable Cells
120
100
80
60
40
20
0
0
10
20
30
40
Time (h)

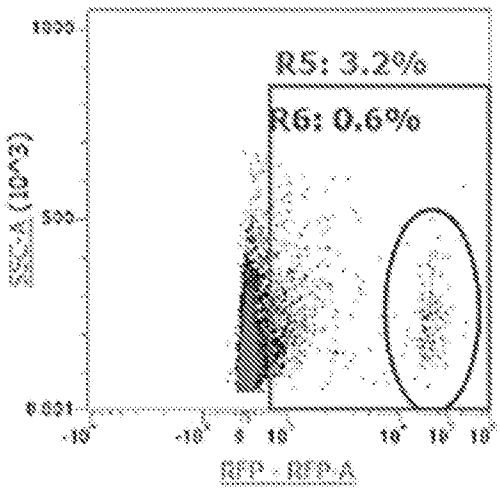
FIG. 10



Marrow/Femur



Kidney



Spleen

FIG. 11A

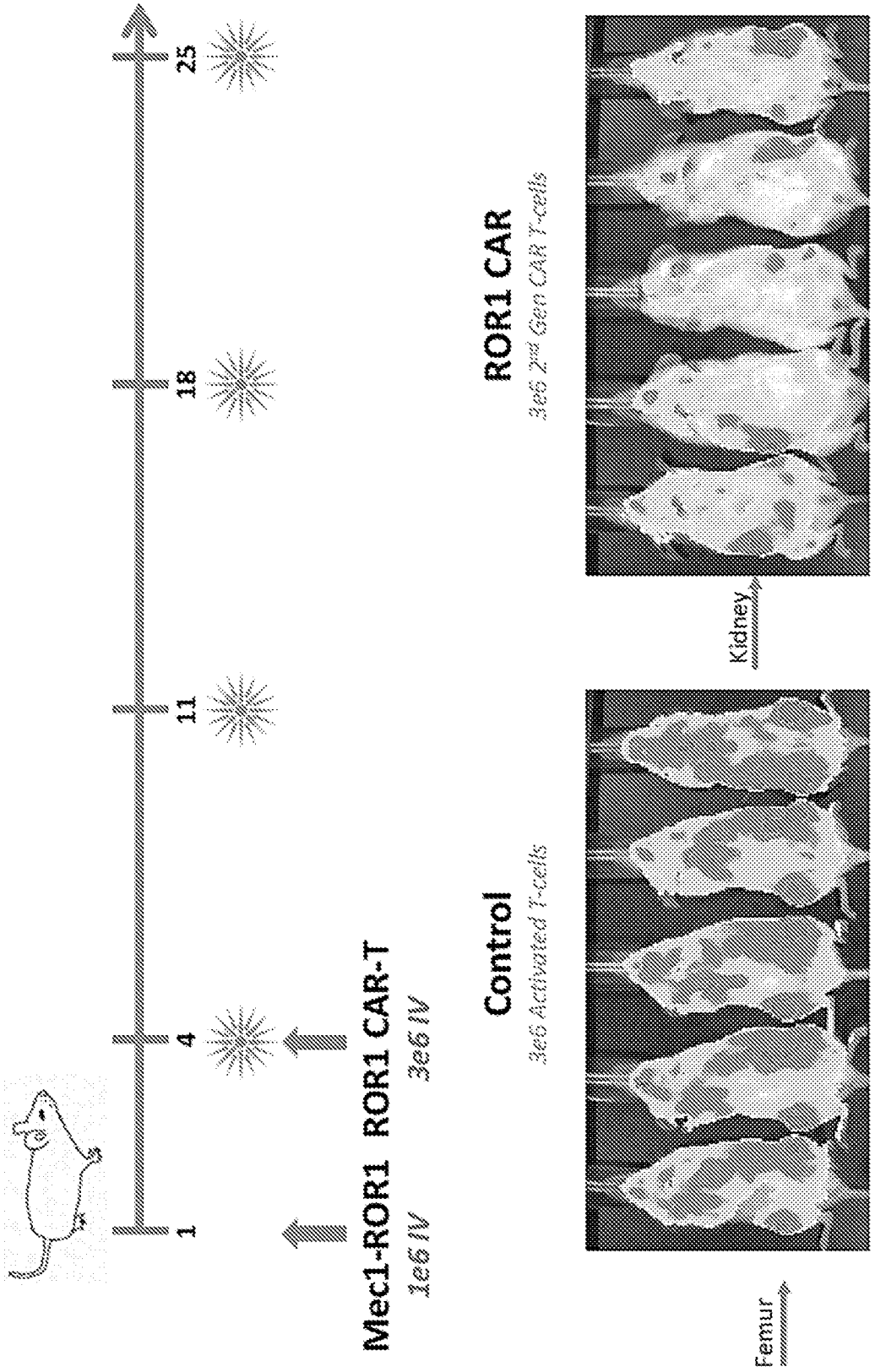

1
4
11
18
25
Mac1-ROR1 1e6 IV
ROR1 CAR-T 3e6 IV
Control
3e6 Activated T-cells
ROR1 CAR
3e6 2nd Gen CAR T-cells
Kidney
Femur

FIG. 11B

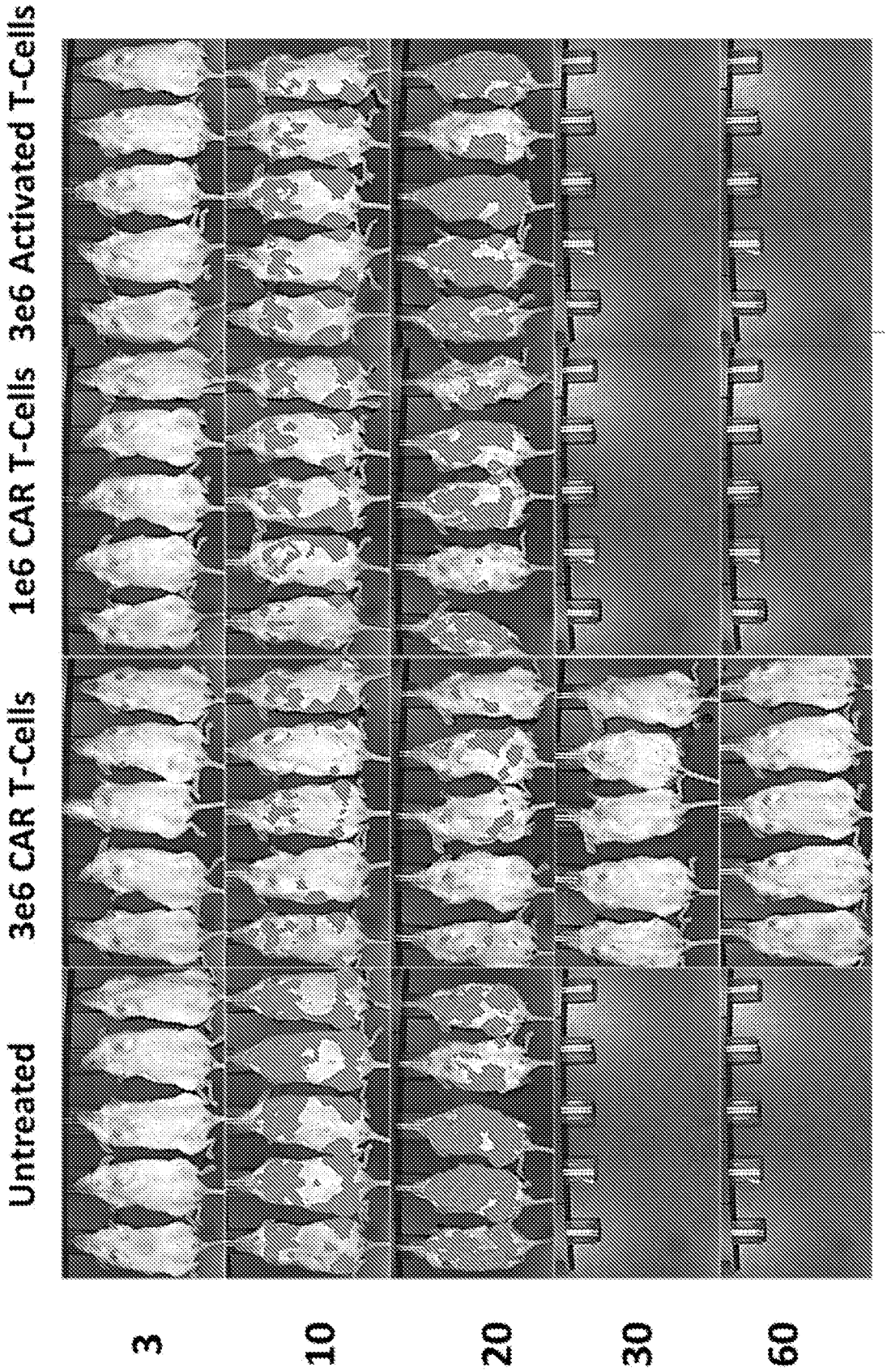

Untreated
3e6 CAR T-Cells
1e6 CAR T-Cells
3e6 Activated T-Cells
3
10
20
30
60

FIG. 12

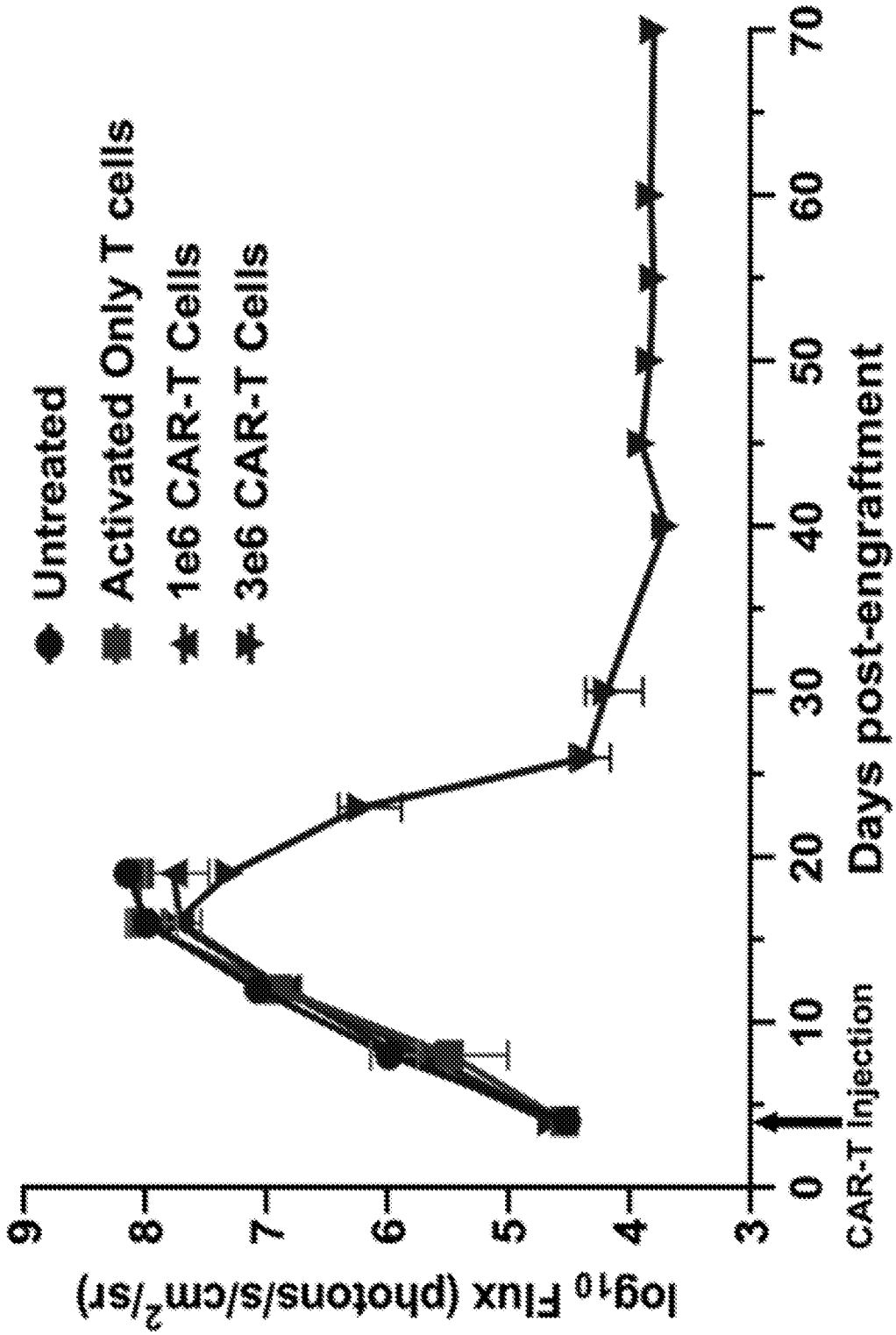

Untreated
Activated Only T cells
1e6 CAR-T Cells
3e6 CAR-T Cells
log10 Flux (photons/s/cm2/sr)
9
8
7
6
5
4
3
0
10
20
30
40
50
60
70
CAR-T injection
Days post-engraftment

FIG. 13

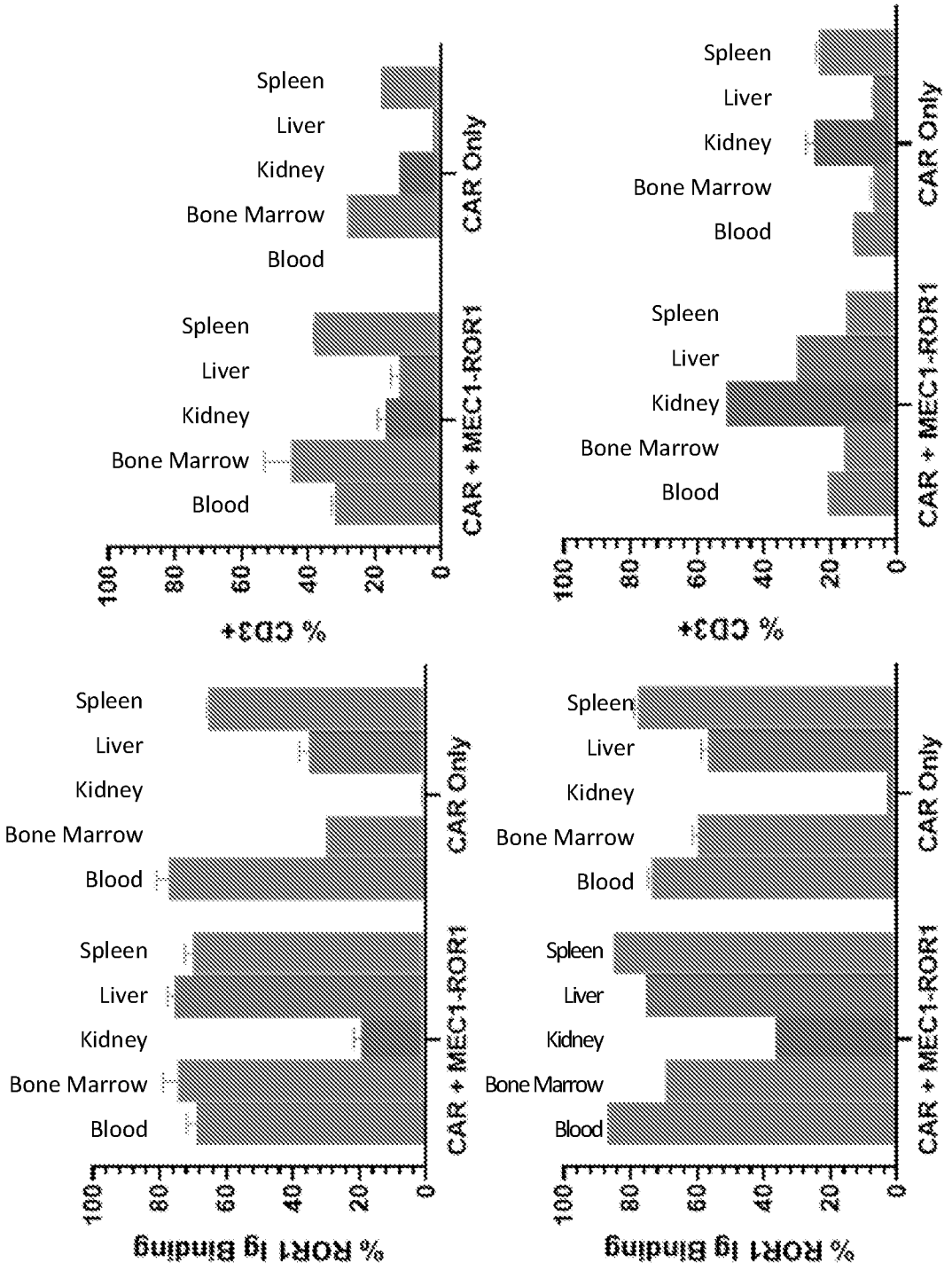

Spleen
Liver
Kidney
Bone Marrow
Blood
CAR Only
CAR + MEC1-ROR1
100
80
60
40
20
0
% CD3+
% ROR1 Ig Binding

FIG. 14