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FILING RECEIPT

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Inventor(s)

Molly R. Perkins, Milton, MA;
Kevin M. Friedman, Melrose, MA;

Applicant(s)

ViraLogic Therapeutics, Inc., Boston, MA

Power of Attorney:
Eileen Sun--57270

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** **SMALL ENTITY** **

Title

LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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LYMPHOCYTE TARGETED LENTIVIRAL VECTORS

DETAILED DESCRIPTION

Engineered lentiviral vectors are described herein. The lentiviral vectors include a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein. In some embodiments, the targeting protein is selected to target an immune cell, including, for example a lymphocyte or a T cell. In certain such embodiments, the lentiviral vectors described herein are capable of selectively targeting and efficiently transducing resting lymphocytes, e.g., T cells.

Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors according to the present disclosure. In some embodiments, a lentiviral vector as described herein and/or cells transduced by such a vector may be used in treating a disease or disorder responsive to the presence of cells expressing the transgene delivered by the vector.

Definitions

Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term "about" means ± 20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of

the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

Terms understood by those in the art of antibody technology are each given the meaning acquired in the art, unless expressly defined differently herein. The term "antibody" is used in the broadest sense and includes polyclonal and monoclonal antibodies. An "antibody" may refer to an intact antibody comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as an antigen-binding portion (or antigen-binding domain) of an intact antibody that has or retains the capacity to bind a target molecule. An antibody may be naturally occurring, recombinantly produced, genetically engineered, or modified forms of immunoglobulins, for example intrabodies, peptibodies, nanobodies, single domain antibodies, SMIPs, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, triabodies, tetrabodies, tandem di-scFv, tandem tri-scFv, ADAPTIR). A monoclonal antibody or antigen-binding portion thereof may be non-human, chimeric, humanized, or human, preferably humanized or human. Immunoglobulin structure and function are reviewed, for example, in Harlow *et al.*, Eds., Antibodies: A Laboratory Manual, Chapter 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988). "Antigen-binding portion" or "antigen-binding domain" of an intact antibody is meant to encompass an "antibody fragment," which indicates a portion of an intact antibody and refers to the antigenic determining variable regions or complementary determining regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments, Fab'-SH, $F(ab')_2$, diabodies, linear antibodies, scFv antibodies, VH, and multispecific antibodies formed from antibody fragments. A "Fab" (fragment antigen binding) is a portion of an antibody that binds to antigens and includes the variable region and CH1 of the heavy chain linked to the light chain via an inter-chain disulfide bond. An antibody may be of any class or subclass, including IgG and subclasses thereof (IgG_1, IgG_2, IgG_3, IgG_4), IgM, IgE, IgA, and IgD.

The term "variable region" or "variable domain" in the context of an antibody refers to the domain of an antibody heavy or light chain that is involved in binding of the

antibody to antigen. The variable domains (or regions) of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three complementary determining regions (CDRs). (*See, e.g.*, Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991).

The terms "complementarity determining region" and "CDR," which are synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

As used herein, the terms “binding domain”, “binding region”, and “binding moiety" refer to a molecule, such as a peptide, oligopeptide, polypeptide, or protein that possesses the ability to specifically and non-covalently bind, associate, unite, recognize, or combine with a target molecule (*e.g.*, tumor antigen). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. In some embodiments, the binding domain is an antigen-binding domain, such as an antibody or functional binding domain or antigen-binding portion thereof. Exemplary binding domains include single chain antibody variable regions (*e.g.*, domain antibodies, sFv, scFv, Fab), receptor ectodomains (*e.g.*, TNF-α), ligands (*e.g.*, cytokines, chemokines), or synthetic polypeptides selected for the specific ability to bind to a biological molecule.

"Major histocompatibility complex molecule" (MHC molecule) refers to a glycoprotein that delivers a peptide antigen to a cell surface. MHC class I molecules are heterodimers composed of a membrane spanning α chain (with three α domains) and a non-covalently associated β2 microglobulin. MHC class II molecules are

composed of two transmembrane glycoproteins, α and β, both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where peptide:MHC complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. An MHC molecule may be from various animal species, including human, mouse, rat, or other mammals.

"Chimeric antigen receptor" (CAR) refers to a chimeric fusion protein comprising two or more distinct domains linked together in a way that does not occur naturally in a host cell and can function as a receptor when expressed on the surface of a cell. CARs are generally composed of an extracellular domain comprising a binding domain that binds a target antigen, an optional extracellular spacer domain, a transmembrane domain, and an intracellular signaling domain (*e.g.*, comprising an immunoreceptor tyrosine-based activation motif (ITAM)), and optionally an intracellular costimulatory domain). In certain embodiments, an intracellular signaling domain of a CAR has an ITAM (*e.g.*, CD3ζ) containing intracellular signaling domain and an intracellular costimulatory domain (*e.g.*, 4-1BB). In certain embodiments, a CAR is synthesized as a single polypeptide chain or is encoded by a nucleic acid molecule as a single chain polypeptide.

A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain affinities, such as Western blot, ELISA, analytical ultracentrifugation, spectroscopy, surface plasmon resonance (BIACORE®) analysis, and MHC tetramer analysis (*see also*, *e.g.*, Scatchard *et al.*, *Ann. N.Y. Acad. Sci. 51*:660, 1949; Wilson, *Science 295*:2103, 2002; Wolff *et al.*, *Cancer Res. 53*:2560, 1993; Altman *et al.*, Science 274:94-96, 1996; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent). As used herein, "specifically binds" refers to an association or union of a binding domain, or a fusion protein thereof, to a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1}, while not significantly associating or uniting with any other molecules or components in a sample.

The terms “antigen” and “Ag” refer to a molecule that is capable of inducing an immune response. The immune response that is induced may involve antibody production, the activation of specific immunologically-competent cells, or both. Macromolecules, including proteins, glycoproteins, and glycolipids, can serve as an antigen. Antigens can be derived from recombinant or genomic DNA. As contemplated herein, an antigen need not be encoded (i) solely by a full-length nucleotide sequence of a gene or (ii) by a “gene” at all. An antigen can be generated or synthesized, or an antigen can be derived from a biological sample. Such a biological sample can include, but is not limited, to a tissue sample, a tumor sample, a cell, or a biological fluid.

The term "epitope" or "antigenic epitope" includes any molecule, structure, amino acid sequence or protein determinant within an antigen that is specifically bound by a cognate immune binding molecule, such as an antibody or fragment thereof (*e.g.*, scFv), T cell receptor (TCR), CAR, or other binding molecule, domain or protein. Epitopic determinants generally contain chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be a linear epitope or a conformational epitope.

As used herein, an "effector domain" is an intracellular portion of a fusion protein or chimeric receptor that can directly or indirectly promote a biological or physiological response in a cell expressing the effector domain when receiving the appropriate signal. In certain embodiments, an effector domain is part of a protein or protein complex that receives a signal when bound. In other embodiments, the effector domain is part of a protein or protein complex that binds directly to a target molecule, which triggers a signal from the effector domain. For example, in response to binding of a CAR to a target molecule, the effector domain may transduce a signal to the interior of the host cell, eliciting an effector function. An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs. In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response.

"Junction amino acids" or "junction amino acid residues" refer to one or more (*e.g.*, about 2-20) amino acid residues between two adjacent motifs, regions or domains

of a polypeptide. Junction amino acids may result from the construct design of a chimeric protein (*e.g.*, amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a fusion protein).

A "disease" is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein, if the disease is not ameliorated, then the subject's health continues to deteriorate. In contrast, a "disorder" or "undesirable condition" in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject's state of health is less favorable than it would be in the absence of the disorder or undesirable condition. Left untreated, a disorder or undesirable condition does not necessarily result in a further decrease in the subject's state of health.

"Nucleic acid molecule" and "polynucleotide" can be in the form of RNA or DNA, which includes cDNA, genomic DNA, and synthetic DNA. A nucleic acid molecule may be composed of naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (*e.g.*, α-enantiomeric forms of naturally occurring nucleotides), or a combination of both. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. A nucleic acid molecule may be double stranded or single stranded, and if single stranded, may be the coding strand or non-coding (anti-sense strand). A coding molecule may have a coding sequence identical to a coding sequence known in the art or may have a different coding sequence, which, as the result of the redundancy or degeneracy of the genetic code, or by splicing, can encode the same polypeptide.

"Encoding" refers to the inherent property of specific polynucleotide sequences, such as DNA, cDNA, and mRNA sequences, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a polynucleotide encodes

a protein if transcription and translation of mRNA corresponding to that polynucleotide produces the protein in a cell or other biological system. Both a coding strand and a non-coding strand can be referred to as encoding a protein or other product of the polynucleotide. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence.

As used herein, the term "endogenous" or "native" refers to a gene, protein, compound, molecule or activity that is normally present in a host or host cell, including naturally occurring variants of the gene, protein, compound, molecule, or activity.

As used herein, "homologous" or "homolog" refers to a molecule or activity from a host cell that is related by ancestry to a second gene or activity, *e.g.*, from the same host cell, from a different host cell, from a different organism, from a different strain, from a different species. For example, a heterologous molecule or heterologous gene encoding the molecule may be homologous to a native host cell molecule or gene that encodes the molecule, respectively, and may optionally have an altered structure, sequence, expression level or any combination thereof.

As used herein, "heterologous" nucleic acid molecule, construct or sequence refers to a nucleic acid molecule or portion of a nucleic acid molecule that is not native to a host cell, but can be homologous to a nucleic acid molecule or portion of a nucleic acid molecule from the host cell. The source of the heterologous nucleic acid molecule, construct or sequence can be from a different genus or species. In some embodiments, the heterologous nucleic acid molecules are not naturally occurring. In certain embodiments, a heterologous nucleic acid molecule is added (*i.e.*, not endogenous or native) into a host cell or host genome by, for example, conjugation, transformation, transfection, transduction, electroporation, or the like, wherein the added molecule can integrate into the host cell genome or exist as extra-chromosomal genetic material (*e.g.*, as a plasmid or other form of self-replicating vector), and can be present in multiple copies. In addition, "heterologous" refers to a non-native enzyme, protein or other activity encoded by a non-endogenous nucleic acid molecule introduced into the host cell, even if the host cell encodes a homologous protein or activity.

As used herein, the term "engineered," "recombinant," "mutant," "modified" or "non-natural" refers to an organism, microorganism, cell, nucleic acid molecule, or vector that has been modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been genetically engineered by human intervention—that is, modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been altered such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated or constitutive, where such alterations or modifications can be introduced by genetic engineering. Human-generated genetic alterations can include, for example, modifications introducing nucleic acid molecules (which may include an expression control element, such as a promoter) encoding one or more proteins, chimeric receptors, or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof heterologous or homologous polypeptides from a reference or parent molecule. Additional exemplary modifications include, for example, modifications in non-coding regulatory regions in which the modifications alter expression of a gene or operon.

As used here, the term "transgene" refers to a gene or polynucleotide encoding a protein of interest (*e.g.*, a CAR) whose expression is desired in a host cell and that has been transferred by genetic engineering techniques into a cell. A transgene may encode proteins of therapeutic interest as well as proteins that are reporters, tags, markers, suicide proteins, etc. A transgene may be from a natural source, modification of a natural gene, or a recombinant or synthetic molecule. In certain embodiments, a transgene is a component of a vector.

The term "overexpressed" or "overexpression" of an antigen refers to an abnormally high level of antigen expression in a cell. Overexpressed antigen or overexpression of antigen is often associated with a disease state, such as in hematological malignancies and cells forming a solid tumor within a specific tissue or organ of a subject. Solid tumors or hematological malignancies characterized by overexpression of a tumor antigen can be determined by standard assays known in the art.

As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

As used herein, the term “mature polypeptide” or “mature protein” refers to a protein or polypeptide that is secreted or localized in the cell membrane or inside certain cell organelles (*e.g.*, the endoplasmic reticulum, golgi, or endosome) and includes a partially cleaved N-terminal signal sequence or does not include an N-terminal signal sequence (*i.e.,* the N-terminal signal sequence has been entirely removed, such as by an endogenous cleavage process, from the protein or polypeptide).

A “signal sequence”, also referred to as “signal peptide”, “leader sequence”, “leader peptide”, “localization signal” or “localization sequence”, is a short peptide (usually 13-36 amino acids in length) present at the N-terminus of newly synthesized proteins that are destined for the plasma membrane or a secretory pathway. A signal sequence typically comprises a short stretch of hydrophilic, positively charged amino acids at the N-terminus, a central hydrophobic domain of 5-15 residues, and a C-terminal region with a cleavage site for a signal sequence. In eukaryotes, a signal sequence prompts translocation of the newly synthesized protein to the endoplasmic reticulum where it is cleaved by the signal peptidase, creating a mature protein that then proceeds to its appropriate destination. The diversity of signal sequence length and amino acid composition makes it difficult to precisely predict the cleavage site. For

polypeptide sequences disclosures herein, where a signal sequence is noted, the polypeptide sequence absent the signal sequence or having a partial signal sequence is also contemplated.

The "percent identity" between two or more nucleic acid or amino acid sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical positions/total number of positions x 100), taking into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more sequences. The comparison of sequences and determination of percent identity between two or more sequences can be accomplished using a mathematical algorithm, such as BLAST and Gapped BLAST programs at their default parameters (*e.g.*, Altschul *et al.*, *J. Mol. Biol. 215*:403, 1990; see also BLASTN at www.ncbi.nlm.nih.gov/BLAST).

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (*see, e.g.*, WO 97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8).

The term "chimeric" refers to any nucleic acid molecule or protein that is not endogenous and comprises a combination of sequences joined or linked together that are not naturally found joined or linked together in nature. For example, a chimeric nucleic acid molecule may comprise nucleic acids encoding various domains from multiple different genes. In another example, a chimeric nucleic acid molecule may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences that are derived from the same source but arranged in a manner different than that found in nature.

The term "promoter" as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the

promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may include an enhancer sequence and other regulatory elements that are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one that expresses the gene product in a tissue specific manner.

A “constitutive” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

An “inducible” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A “tissue-specific” promoter is a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The phrase “under transcriptional control” or “operatively linked” as used herein means that a promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.

A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, for example, plasmids, cosmids, viruses, or phage. The term should also be construed to include non-plasmid and non-viral compounds that facilitate transfer of nucleic acid into cells. An "expression vector" is a vector that is capable of directing the expression of a protein encoded by one or more transgenes carried by the vector when it is present in the appropriate environment. The one or more transgenes to be expressed by a vector as described herein are encoded in an expression cassette.

A “lentiviral vector” is a vector derived from a lentivirus and includes one or more lentiviral packaging proteins and/or one or more lentiviral proteins necessary for expression of the one or more genes carried by the vector. The abbreviation “LVV” is

used herein to refer to a lentiviral vector (singular), as well as multiple lentiviral vectors (plural).

"Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Examples of lentiviruses include, but are not limited to HIV (human immunodeficiency virus, including HIV type 1 and HIV type 2, equine infectious anemia virus, feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus (SIV).

As used herein, the term “expression cassette” refers to a distinct component of a vector nucleic acid comprising at least one transgene and regulatory sequences controlling its expression (*e.g.*, promoter, 3’UTR) in a host cell. A tandem expression cassette refers to a component of a vector nucleic acid comprising at least two transgenes under the control of the same set of regulatory sequences for tandem expression of the at least two transgenes. In certain embodiments, the tandem expression cassette comprises at least two transgenes under the control of the same promoter. In certain embodiments, the first transgene and second transgene are separated by an internal ribosome entry site (IRES), furin cleavage site, or self-cleaving viral 2A peptide to allow for co-expression of two proteins from a single mRNA.

The term "immune system cell" or “immune cell” means any cell of the immune system that originates from a hematopoietic stem cell in the bone marrow. Hematopoietic stem cells give rise to two major lineages: myeloid progenitor cells (which give rise to myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) and lymphoid progenitor cells (which give rise to lymphoid cells such as T cells, B cells and natural killer (NK) cells). Exemplary immune system cells include a CD4+ T cell, a CD8+ T cell, a CD4- CD8- double negative T cell, a γδ T cell, a regulatory T cell, a natural killer cell, and a dendritic cell. Macrophages and dendritic cells may also be referred to as "antigen presenting cells" or "APCs," which are specialized cells that can activate T cells when a major histocompatibility complex (MHC) receptor on the surface of the APC complexed with a peptide interacts with a TCR on the surface of a T cell.

The term “lymphocyte” refers to immune cells of lymphoid origin, which are cells that show at least one phenotype characteristic of a lymphocyte or a precursor or

progenitor thereof that distinguishes the cells from cells of erythroid or myeloid lineages. The term "lymphocytes" encompasses T cells, B cells, and natural killer (NK) cells.

The term "T cells" refers to cells of T cell lineage. "Cells of T cell lineage" refer to cells that show at least one phenotypic characteristic of a T cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T cells (*e.g.* , $CD3^+$, $CD4^+$, $CD8^+$), or a physiological, morphological, functional, or immunological feature specific for a T cell. For example, cells of the T cell lineage may be progenitor or precursor cells committed to the T cell lineage; $CD25^+$ immature and inactivated T cells; cells that have undergone CD4 or CD8 linage commitment; thymocyte progenitor cells that are $CD4^+CD8^+$ double positive; single positive $CD4^+$ or $CD8^+$; TCRαβ or TCR γδ; or mature and functional or activated T cells. The term "T cells" encompasses naïve T cells (CD45RA+, CCR7+, CD62L+, CD27+, CD45RO-), central memory T cells (CD45RA-, $CD45RO^+$, $CD62L^+$, CCR7+, CD27+), effector memory T cells (CD45RA-, CD45RO+, CCR7-, CD62L-, CD27-), mucosal-associated invariant T (MAIT) cells, γδ T cells, Tregs, natural killer T cells, and tissue resident T cells.

The term "natural killer cells" or "NK cells" refers to large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor-generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph nodes, spleen, tonsils, and thymus, where they then enter into the circulation. NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector functions; often, NKT cell activity promotes NK cell activity by secreting IFNγ. In contrast to NKT cells, NK cells do not express T-cell antigen receptors (TCR) or pan T marker CD3 or surface immunoglobulins (Ig) B cell receptors, but they usually express the surface markers CD16 (FcγRIII) and CD56 in humans, NK1.1 or NK1.2 in C57BL/6 mice. Up to 80% of human NK cells also express CD8.

The term "cytotoxic activity," also referred to as "cytolytic activity," with respect to a cell (*e.g.*, T cell) that expresses an immune receptor (*e.g.*, a CAR) on its surface, means that upon antigen-specific signaling (*e.g.*, via the CAR) the cell induces a target

cell to undergo apoptosis. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via the release of cytotoxins, such as perforin, granzyme, and granulysin, from granules. Perforins insert into the target cell membrane and form pores that allow water and salts to rapidly enter the target cell. Granzymes are serine proteases that induce apoptosis in the target cell. Granulysin is also capable of forming pores in the target cell membrane and is a proinflammatory molecule. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via interaction of Fas ligand, which is upregulated on T cell following antigen-specific signaling, with Fas molecules expressed on the target cell. Fas is an apoptosis-signaling receptor molecule on the surface of a number of different cells.

A “disease” is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein, if the disease is not ameliorated, then the subject’s health continues to deteriorate. In contrast, a “disorder” or “undesirable condition” in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject’s state of health is less favorable than it would be in the absence of the disorder or undesirable condition. Left untreated, a disorder or undesirable condition does not necessarily result in a further decrease in the subject’s state of health.

The term “cancer” as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. The aberrant cells may form solid tumors or constitute a hematological malignancy. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include, but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

The term “subject,” “patient” and “individual” are used interchangeably herein and are intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals). Examples of subjects include humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, and transgenic species thereof.

"Adoptive cellular immunotherapy" or "adoptive immunotherapy" refers to the administration of naturally occurring or genetically engineered, disease antigen-specific immune cells (*e.g.*, T cells). Adoptive cellular immunotherapy may be autologous

(immune cells are from the recipient), allogeneic (immune cells are from a donor of the same species) or syngeneic (immune cells are from a donor genetically identical to the recipient).

"Autologous" refers to a graft (*e.g.*, organ, tissue, cells) derived from the same subject to which it is later to be re-introduced.

"Allogeneic" refers to a graft derived from a different subject of the same species.

A "therapeutically effective amount" or "effective amount" of a lentiviral vector or a cell transduced by a lentiviral vector as described herein (*e.g.*, a T cell expressing a CAR as encoded by the transgene of the lentiviral vector) refers to that amount of lentiviral particles or cells sufficient to result in amelioration of one or more symptoms of the disease, disorder, or undesired condition being treated.

"Treat" or "treatment" or "ameliorate" refers to medical management of a disease, disorder, or undesired condition of a subject. In general, an appropriate dose or treatment regimen comprising a lentiviral vector or a cell expressing a CAR of this disclosure is administered in an amount sufficient to elicit a therapeutic or prophylactic benefit. Therapeutic or prophylactic/preventive benefit includes improved clinical outcome; lessening or alleviation of symptoms associated with a disease, disorder, or undesired condition; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease, disorder, or undesired condition; stabilization of disease state; delay of disease progression; remission; survival; prolonged survival; or any combination thereof.

The term "anti-tumor effect" refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with a cancerous condition. An "anti-tumor effect" can also be manifested by prevention of a hematological malignancy or tumor formation.

Additional definitions are provided throughout the present disclosure.

Lentiviral Vector

Lentivirus is a genus of retroviruses that typically gives rise to slowly developing diseases due to their ability to incorporate into a host genome. Modified lentiviral genomes are useful as viral vectors for the delivery of a nucleic acids to a host cell.

The present disclosure provides self-inactivating lentiviral vectors ("LVV") that include a viral envelope comprising a mutated, heterologous envelope protein and a tropism defining molecule (also referred to as a "targeting protein"). The LVV described herein further includes a transgene, with the LVV being capable of specifically binding to a target immune cell and transducing the target immune cell such that the transgene is expressed by the immune cell. In some embodiments, the LVV may carry more than one transgene. In further specific embodiments, the transgene encodes a CAR.

Mutated Viral Envelope Protein

Lentiviral vectors as described herein are pseudotyped with a mutated heterologous viral envelope protein that, in the absence of mutation, mediates both cellular attachment and membrane fusion. In particular embodiments, the mutated envelope protein includes at least one mutation that inhibits the envelope protein's ability to bind its native target, while preserving the envelope protein's fusogenic properties. In specific embodiments, the heterologous envelope protein is a vesicular stomatitis virus G protein ("VSV-G", "VSV-G envelope protein" or "VSV-G protein") that includes one or more mutations that inhibit binding of VSV-G to the low-density lipid receptor ("LDL-R"), while preserving the VSV-G protein's fusogenic function.

When referring to the envelope protein's ability to bind its native target, the terms "inhibit" and "inhibits" encompass both complete elimination of binding by the envelope protein to its native target, as well as a significant reduction in binding by the envelope protein to its native target. In particular embodiments, "significant reduction" refers to a reduction selected from a reduction of at least 95%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, and at least 10% in binding to the native target.

The nucleic acid and amino acid sequences set forth in Table 8 provide references sequences for a wild type VSV-G envelope protein. SEQ ID NO: 77 is a reference nucleic acid sequence encoding the VSV-G envelope protein, and SEQ ID NO: 78 is an amino acid sequence of a reference VSV-G envelope protein. The position and nature of VSV-G mutations disclosed herein are described in reference to the nucleic acid sequence and the amino acid sequence provided by SEQ ID NO: 77 and SEQ ID NO: 78, respectively.

In some embodiments, the mutated VSV-G envelope protein includes a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, R354, an insertion of TT between N9 and Q10, an insertion of GGs between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, an insertion of GGS between N208 and Y209, and/or a deletion of residues 1-8. In certain embodiments, the VSV-G envelope protein includes two or more mutations selected from a mutation at one or more of H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354, an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In other embodiments, the VSV-G envelope protein includes three or more mutations selected from a mutation at one or more of H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354, an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In other embodiments, the mutated VSV-G envelope protein comprises a H8A, K47A, K47Q, Y209A, R354A, and/or R354Q mutation. In still other embodiments, the VSV-G envelope protein includes one or more mutations selected from a mutation at one or more of N9, Q10, K50, A51, S183, S179, N180, I182, M184, I347, T350, T352, and E353, an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208

and Y209, an insertion of GGS between P46 and K47, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In yet other embodiments, the VSV-G envelope protein includes two or more mutations selected from a mutation at one or more of N9, Q10, K50, A51, S183, S179, N180, I182, M184, I347, T350, T352, and E353, an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, an insertion of GGS between N208 and Y209, and a deletion of residues 1-8. In some embodiments, a mutated VSV-G envelope protein is selected from one or more of an H8A, K47A, K47Q, Y209A, R354A, and R354Q mutation. In other embodiments, the mutated VSV-G envelope protein is as described in Nikolic et al., "Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein." *Nature Comm.*, 2018, 9:1029, the relevant disclosures of which are incorporated by reference herein.

In specific embodiments, the mutant VSV-G envelope protein has an amino acid sequence according to SEQ ID NO: 74.

Targeting Protein

The targeting protein is a membrane-bound protein displayed on the viral envelope and includes an extracellular domain that includes a lymphocyte targeting domain and a transmembrane domain. A lymphocyte targeting domain is any protein or peptide that has an amino acid sequence and is a binding partner for a target molecule or ligand (*e.g.*, a cognate protein or ligand) on a target lymphocyte cell surface. In some embodiments, a lymphocyte targeting domain is a T cell targeting domain. In some embodiments, a lymphocyte targeting domain is a NK cell targeting domain. For example, in certain embodiments, the extracellular domain of the targeting protein includes a lymphocyte targeting domain that specifically binds a protein or ligand on the surface of a defined population of cells, such as a population of lymphocytes characterized by the presence of the targeted protein or ligand on the cell surface. In some embodiments, a lymphocyte targeting domain targets a specific type of lymphocyte, such as for example, T cells or natural killer (NK) cells. In some

embodiments, the targeting protein additionally includes a linker or hinge domain positioned between the transmembrane domain and the extracellular domain.

In some embodiments, the targeting protein is a full-length cell surface protein or receptor, wherein each component (*e.g.*, each of the extracellular domain, transmembrane domain, and intracellular domain) derived from a single protein or receptor. In other embodiments, the targeting protein is a chimeric protein, having at least two components derived from a different protein or receptor. In some such embodiments, a chimeric targeting protein may be a fully synthetic protein. In still other embodiments, a chimeric targeting protein may include one or more of an extracellular domain, an extracellular binding domain, a transmembrane domain, an intracellular domain, a hinge domain, or a linker that is a fully synthetic protein.

In some embodiments, the lymphocyte targeting domain includes an antibody. In such embodiments, the extracellular targeting domain may be a full-length antibody, an antibody fragment, a nanobody, or a single chain Fv fragment (scFv).

In particular embodiments, the targeting molecule includes a T cell targeting domain that specifically binds to a T cell surface marker, including, for example, a T cell antigen, a T cell surface receptor, or any other protein present on the surface of a targeted T cell. In some embodiments, the targeting protein specifically binds to a T cell marker selected from CD3, CD80, 4-1BB, AhR, CD3, CD2, CD7, CD4, CD8, CD25, CD44, CD45RA, CD47, CD62L, CD69, CD94, CD95, CD127, CD161, CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CCR10, PD-1, TCRa/b, CD5, CD27, CD45RO, CD45RB, CD57, CD103, CD122, P2RX7, TIGIT, LAG-3, TIM-3, and IL6ST.

In further embodiments, the targeting protein specifically binds to a γδ T cell marker selected from γδ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314).

In other embodiments, the targeting protein specifically binds to an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, and IL-21R.

In other embodiments, the targeting protein specifically binds to a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), and VLA4 (alpha4beta1 integrin).

In other embodiments, the targeting protein specifically binds to an NK cell marker selected from CD56, NKp46, CD16, KIR(s), NKG2 proteins (e.g., NKG2D (KLRK1, CD314)), KLRB1 (CD161), KLRD1 (cd94), IL2Rb (CD122), IL-21R, SLAMF6 (CD352), SLAMF7 (CD319), and IL-18R.

In some embodiments of the targeting protein, a linker is positioned between the transmembrane domain and the lymphocyte targeting domain. A linker is an amino acid linker and may be a rigid linker, a flexible linker, or an oligomerized linker. A rigid linker is an amino acid sequence that lacks flexibility (*e.g.*, may comprise at least one proline). In some embodiments, a rigid linker comprises a platelet-derived growth factor receptor (PDGFR) stalk or a CD8_ stalk. In some embodiments, a PDGFR stalk comprises an amino acid sequence comprising AVGQDTQEVIVVPHSLPFK. In some embodiments, a PDGFR stalk comprises an amino acid sequence comprising ASAKPTTTPAPRPPTPAPTIASQPLSLRPEAARPAAGGAVHTRGLDFAK.

A flexible linker is an amino acid sequence that has many degrees of freedom (*e.g.*, may comprise a plurality of amino acids with small side chains, *e.g.*, glycine or alanine). In some embodiments, a flexible linker comprises an amino acid sequence comprising GAPGAS. In some embodiments, a flexible linker comprises an amino acid sequence consisting of GAPGSGGGGSGGGGSAS. In some embodiments, a flexible linker comprises an amino acid sequence comprising GGGGS. In some embodiments, a flexible linker comprises an amino acid sequence comprising GGGS. In some embodiments, a flexible linker comprises an amino acid sequence comprising $(GAPGAS)_N$, $(G_3S)_N$, or $(G_4S)_N$, wherein N is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

An oligomerized linker is an amino acid that can oligomerize to another related amino acid. In some embodiments, an oligomerized linker is an amino acid sequence that can form a dimer, trimer, or tetramer. In some embodiments, an oligomerized linker comprises an IgG4 hinge domain (*e.g.*, ESKYGPPCPPCPAVGQDTQEVIVVPHSLPFK). In some embodiments, an oligomerized linker comprises an amino acid sequence that

can form a tetrameric coiled coil (*e.g.*, ASGGGGSGELAAIKQELAAIKKELAAIKWELAAIKQGAG). In some embodiments, an oligomerized linker comprises an amino acid sequence that can form a dimeric coiled coil (*e.g.*, ASESKYGPPCPPCP).

The targeting protein will typically include a signal sequence (also referred to as a signal peptide of localization sequence). The signal sequence can be located at the N- or C-terminal ends of the targeting protein. A signal sequence functions to translocate the targeting protein to the membrane that serves as the envelope of the lentiviral vector. Non-limiting examples of signal sequences that might be included in a targeting protein as described herein include an Ig Kappa leader sequence (*e.g.*, a murine Ig Kappa leader sequence comprising: METDTLLLWVLLLWVPGSTG) and a B2M signal peptide sequence (*e.g.*, a B2M signal peptide sequence comprising: MSRSVALAVLALLSLSGLEA). In certain embodiments, a mature targeting protein has a partially cleaved signal sequence that retains one or more amino acids of the full-length signal sequence. In other embodiments, cleavage by a signal peptidase results in complete removal of the signal sequence, resulting in a mature targeting protein that lacks a signal sequence entirely.

In some embodiments, the targeting protein is a CD80 protein. Where the targeting protein is a CD80 protein, it may include an extracellular domain according to SEQ ID NO: 6. In such embodiments, a CD80 protein may include a signal peptide according to SEQ ID NO: 4 and a transmembrane and intracellular domain according to SEQ ID NO: 8. In a specific embodiment, a CD80 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence according to SEQ ID NO: 2.

In some embodiments, where the targeting protein is a CD80 protein, it may include an extracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 6. In such embodiments, a CD80 protein may include a signal peptide that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 4 and a transmembrane and intracellular

domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 8. In a specific embodiment, a CD80 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 2.

TABLE 1: CD80 T cell targeting molecule

Full molecule	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG CAGTGGGGTCATTCACGTTACTAAAGAGGTCA AGGAGGTCGCAACATTGAGTTGTGGCCATAA CGTATCAGTTGAAGAACTCGCGCAGACACGG ATTTACTGGCAAAAGGAAAAGAAGATGGTGTT GACAATGATGAGCGGTGACATGAACATTTGG CCAGAGTACAAAAATCGAACGATATTCGATAT AACCAATAACTTGTCCATAGTAATACTTGCCTT GCGACCTTCTGACGAGGGAACGTATGAATGT GTAGTGCTTAAGTATGAAAAAGATGCCTTTAA GCGGGAACACTTGGCTGAGGTTACACTCTCC GTTAAGGCGGACTTTCCTACGCCGTCTATATC CGACTTCGAGATACCCACTTCTAACATTCGAC GCATCATTTGCTCAACCTCAGGTGGTTTCCCA GAGCCTCACTTGAGCTGGCTGGAGAATGGCG AAGAACTTAACGCAATCAATACCACGGTGTCC CAAGACCCGGAGACAGAGCTGTACGCCGTGT CATCCAAACTGGATTTTAACATGACGACAAAT CATAGTTTCATGTGTCTGATCAAATATGGGCA TCTCAGGGTGAATCAGACTTTTAATTGGAACA CTACCAAACAAGAGCACTTCCCAGATAATCTG TTGCCAAGCTGGGCGATAACTCTTATCTCCGT CAACGGTATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAGAA (SEQ ID NO: 1)	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GVIHVTKEVKEVATLSCGHNVSVEELAQTRIYW QKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNL SIVILALRPSDEGTYECVVLKYEKDAFKREHLAE VTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFP EPHLSWLENGEELNAINTTVSQDPETELYAVSS KLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTK QEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPR CRE (SEQ ID NO: 2)
CD80 signal peptide	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG CAGTGGGGTC (SEQ ID NO: 3)	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GV (SEQ ID NO: 4)
CD80 extracellular domain (ECD)	
Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAGGAGGTCGC AACATTGAGTTGTGGCCATAACGTATCAGTTG AAGAACTCGCGCAGACACGGATTTACTGGCA AAAGGAAAAGAAGATGGTGTTGACAATGATGA GCGGTGACATGAACATTTGGCCAGAGTACAA	IHVTKEVKEVATLSCGHNVSVEELAQTRIYWQK EKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIV ILALRPSDEGTYECVVLKYEKDAFKREHLAEVTL SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEP HLSWLENGEELNAINTTVSQDPETELYAVSSKL

AAATCGAACGATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCCTTGCGACCTTCTG ACGAGGGAACGTATGAATGTGTAGTGCTTAAG TATGAAAAAGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTTAAGGCGGAC TTTCCTACGCCGTCTATATCCGACTTCGAGAT ACCCACTTCTAACATTCGACGCATCATTTGCT CAACCTCAGGTGGTTTCCCAGAGCCTCACTTG AGCTGGCTGGAGAATGGCGAAGAACTTAACG CAATCAATACCACGGTGTCCCAAGACCCGGA GACAGAGCTGTACGCCGTGTCATCCAAACTG GATTTTAACATGACGACAAATCATAGTTTCATG TGTCTGATCAAATATGGGCATCTCAGGGTGAA TCAGACTTTTAATTGGAACACTACCAAACAAG AGCACTTCCCAGATAATCTGTTGCCAAGC (SEQ ID NO:5)	DFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQ EHFPDNLLPS (SEQ ID NO: 6)
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTCAACGGTAT CTTCGTAATTTGCTGCCTCACCTATTGTTTCG CGCCTCGATGCCGAGAA (SEQ ID NO: 7)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 8)

In some embodiments, the targeting protein is an anti-CD3 targeting protein. Where the targeting protein is an anti-CD3 targeting protein, it may include an anti-CD3 scFv having a light chain variable region according to SEQ ID NO: 14, a G_3S linker according to SEQ ID NO: 16, and a heavy chain variable region according to SEQ ID NO: 18. An anti-CD3 targeting protein as described may include a signal peptide according to SEQ ID NO: 12 and a hinge and transmembrane domain according to SEQ ID NO: 20. In a specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence according to SEQ ID NO: 10.

In some embodiments, where the targeting protein is an anti-CD3 targeting protein, it may include an anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 14, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 18. An anti-CD3 targeting protein as described may include a signal peptide that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 12 and a hinge and transmembrane domain that has at least about 75%, 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 20. In a specific embodiment, an anti-CD3 targeting protein included in a lentiviral vector as described herein includes an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 10.

TABLE 2: Anti-CD3 T cell targeting molecule

Full molecule	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC GGACATCCAGATGACCCAGACCACCTCCTCC CTGTCTGCCTCTCTGGGAGACAGAGTCACCA TCAGTTGCAGGGCAAGTCAGGACATTAGAAAT TATTTAAACTGGTATCAACAGAAACCAGATGG AACTGTTAAACTCCTGATCTACTACACATCAA GATTACACTCAGGAGTCCCATCAAAGTTCAGT GGCAGTGGGTCTGGAACAGATTATTCTCTCAC CATTAGCAACCTGGAGCAAGAGGATATTGCCA CTTACTTTTGCCAACAGGGTAATACGCTTCCG TGGACGTTCGCTGGAGGCACCAAGCTGGAAA TCAAACGGGCTGGAGGCGGTAGTGGCGGTG GATCAGGTGGAGGCAGCGGTGGCGGATCTG AGGTGCAGCTCCAGCAGTCTGGACCTGAGCT GGTGAAGCCTGGAGCTTCAATGAAGATATCCT GCAAGGCTTCTGGTTACTCATTCACTGGCTAC ACCATGAACTGGGTGAAGCAGAGTCATGGAA AGAACCTTGAGTGGATGGGACTTATTAATCCT TACAAAGGTGTTAGTACCTACAACCAGAAGTT CAAGGACAAGGCCACATTAACTGTAGACAAGT CATCCAGCACAGCCTACATGGAACTCCTCAGT CTGACATCTGAGGACTCTGCAGTCTATTACTG TGCAAGATCGGGGTACTACGGTGATAGTGAC TGGTACTTCGATGTCTGGGGCGCAGGGACCA CGGTCACCGTCTCCTCAACCACtACaCCAGCa CCtaGACCACCAACACCtGCGCCaACCATCGCa TCGCAGCCaCTGTCtCTGCGCCCAGAGGCaTG CCGGCCAGCaGCtGGGGGCGCAGTGCACACa AGGGGGCTGGACTTCGCaTGTGATATCTACAT CTGGGCaCCaTTGGCaGGGACTTGTGGGGTC CTTCTCCTGTCACTGGTTATCACCCTTTACTG C (SEQ ID NO: 9)	MALPVTALLLPLALLLHAARPDIQMTQTTSSLSA SLGDRVTISCRASQDIRNYLNWYQQKPDGTVKL LIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ EDIATYFCQQGNTLPWTFAGGTKLEIKRAGGGS GGGSGGGSGGGSEVQLQQSGPELVKPGASMK ISCKASGYSFTGYTMNWVKQSHGKNLEWMGLI NPYKGVSTYNQKFKDKATLTVDKSSSTAYMELL SLTSEDSAVYYCARSGYYGDSDWYFDVWGAG TTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLL LSLVITLYC (SEQ ID NO: 10)
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC G (SEQ ID NO: 11)	MALPVTALLLPLALLLHAARP (SEQ ID NO:12)
Anti-CD3 Light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACCCAGACCACCTCCTCCC TGTCTGCCTCTCTGGGAGACAGAGTCACCAT	DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN WYQQKPDGTVKLLIYYTSRLHSGVPSKFSGSGS

CAGTTGCAGGGCAAGTCAGGACATTAGAAATT ATTTAAACTGGTATCAACAGAAACCAGATGGA ACTGTTAAACTCCTGATCTACTACACATCAAG ATTACACTCAGGAGTCCCATCAAAGTTCAGTG GCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAGGATATTGCCAC TTACTTTTGCCAACAGGGTAATACGCTTCCGT GGACGTTCGCTGGAGGCACCAAGCTGGAAAT CAAACGGGCT (SEQ ID NO: 13)	GTDYSLTISNLEQEDIATYFCQQGNTLPWTFAG GTKLEIKRA (SEQ ID NO:14)
G_3S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCAGGTGGA GGCAGCGGTGGCGGATCT (SEQ ID NO: 15)	GGGSGGGSGGGSGGGS (SEQ ID NO:16)
Anti-CD3 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGACCTGAGC TGGTGAAGCCTGGAGCTTCAATGAAGATATCC TGCAAGGCTTCTGGTTACTCATTCACTGGCTA CACCATGAACTGGGTGAAGCAGAGTCATGGA AAGAACCTTGAGTGGATGGGACTTATTAATCC TTACAAAGGTGTTAGTACCTACAACCAGAAGT TCAAGGACAAGGCCACATTAACTGTAGACAAG TCATCCAGCACAGCCTACATGGAACTCCTCAG TCTGACATCTGAGGACTCTGCAGTCTATTACT GTGCAAGATCGGGGTACTACGGTGATAGTGA CTGGTACTTCGATGTCTGGGGCGCAGGGACC ACGGTCACCGTCTCCTCA (SEQ ID NO: 17)	EVQLQQSGPELVKPGASMKISCKASGYSFTGYT MNWVKQSHGKNLEWMGLINPYKGVSTYNQKF KDKATLTVDKSSSTAYMELLSLTSEDSAVYYCA RSGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 18)
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACtACaCCAGCaCCtaGACCACCAACACCt GCGCCaACCATCGCaTCGCAGCCaCTGTCtCT GCGCCCAGAGGCaTGCCGGCCAGCaGCtGGG GGCGCAGTGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCCaTTGGCa GGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCTTTACTGC (SEQ ID NO: 19)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 20)

In still further embodiments, a lentiviral vector according to the present description may include multiple targeting proteins or a single targeting protein having multiple different lymphocyte targeting domains. For example, the lentiviral vectors described herein can include at least two targeting proteins, with each of the targeting proteins having a NK cell targeting domain that specifically binds a different target. In another example, the lentiviral vectors described herein can include at least two targeting proteins, with each of the targeting proteins having a T cell targeting domain that specifically binds a different target. In yet another example, the lentiviral vectors described herein can include at least two targeting proteins, with a first targeting protein having a T cell targeting domain and a second targeting protein having a NK cell

targeting domain. In alternative embodiments, the lentiviral vectors described herein include a single targeting protein having two or more different lymphocyte targeting domains, two or more different T cell targeting domains, two or more different NK cell targeting domains (*e.g.*, a bi-specific or multi-specific targeting protein). In any such embodiments, the Tlymphocyte targeting domains included in the targeting proteins can be selected from any of those described herein.

In an exemplary embodiment, a lentiviral vector having multiple targeting proteins includes a CD80 targeting protein and an anti-CD3 targeting protein as detailed in Table 1 and Table 2, respectively. Where the lentiviral vector includes a CD80 targeting protein and an anti-CD3 targeting protein, the targeting proteins may be encoded on separate expression vectors used in producing the lentiviral vector. Alternatively, both a CD80 targeting protein and an anti-CD3 targeting protein may be encoded on a tandem expression cassette, providing a single expression cassette in a single expression vector that provides expression of both targeting proteins for production of the lentiviral vector. Exemplary embodiments of nucleic acid sequences and the corresponding amino acid sequences for a CD80 targeting protein and an anti-CD3 targeting protein expressed from the same expression cassette are provided in Table 3. In such an embodiment, the two targeting proteins may be linked by a P2A self-cleaving peptide. Table 3 provides an exemplary nucleic acid that may be used in a tandem expression cassette that includes a CD80 binding protein linked to an anti-CD3 binding protein via a P2A sequence. In a particular embodiment, the P2A sequence is encoded by a nucleic acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 29 and the amino acid sequence of the P2A sequence has an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 30.

TABLE 3: Co-expressed CD80 and anti-CD3 T cell targeting molecules

Full molecule	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GVIHVTKEVKEVATLSCGHNVSVEELAQTRIYW QKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNL

CAGTGGGGTCATTCACGTTACTAAAGAGGTCA AGGAGGTCGCAACATTGAGTTGTGGCCATAA CGTATCAGTTGAAGAACTCGCGCAGACACGG ATTTACTGGCAAAAGGAAAAGAAGATGGTGTT GACAATGATGAGCGGTGACATGAACATTTGG CCAGAGTACAAAAATCGAACGATATTCGATAT AACCAATAACTTGTCCATAGTAATACTTGCCTT GCGACCTTCTGACGAGGGAACGTATGAATGT GTAGTGCTTAAGTATGAAAAAGATGCCTTTAA GCGGGAACACTTGGCTGAGGTTACACTCTCC GTTAAGGCGGACTTTCCTACGCCGTCTATATC CGACTTCGAGATACCCACTTCTAACATTCGAC GCATCATTTGCTCAACCTCAGGTGGTTTCCCA GAGCCTCACTTGAGCTGGCTGGAGAATGGCG AAGAACTTAACGCAATCAATACCACGGTGTCC CAAGACCCGGAGACAGAGCTGTACGCCGTGT CATCCAAACTGGATTTTAACATGACGACAAAT CATAGTTTCATGTGTCTGATCAAATATGGGCA TCTCAGGGTGAATCAGACTTTTAATTGGAACA CTACCAAACAAGAGCACTTCCCAGATAATCTG TTGCCAAGCTGGGCGATAACTCTTATCTCCGT CAACGGTATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAGAAGGCAG CGGCGCCACCAACTTCTCCCTGCTGAAGCAG GCCGGCGACGTGGAAGAAAACCCTGGCCCCA TGGCCTTACCAGTGACCGCCTTGCTCCTGCC GCTGGCCTTGCTGCTCCACGCCGCCAGGCCG GACATCCAGATGACCCAGACCACCTCCTCCC TGTCTGCCTCTCTGGGAGACAGAGTCACCAT CAGTTGCAGGGCAAGTCAGGACATTAGAAATT ATTTAAACTGGTATCAACAGAAACCAGATGGA ACTGTTAAACTCCTGATCTACTACACATCAAG ATTACACTCAGGAGTCCCATCAAAGTTCAGTG GCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAGGATATTGCCAC TTACTTTTGCCAACAGGGTAATACGCTTCCGT GGACGTTCGCTGGAGGCACCAAGCTGGAAAT CAAACGGGCTGGAGGCGGTAGTGGCGGTGG ATCAGGTGGAGGCAGCGGTGGCGGATCTGA GGTGCAGCTCCAGCAGTCTGGACCTGAGCTG GTGAAGCCTGGAGCTTCAATGAAGATATCCTG CAAGGCTTCTGGTTACTCATTCACTGGCTACA CCATGAACTGGGTGAAGCAGAGTCATGGAAA GAACCTTGAGTGGATGGGACTTATTAATCCTT ACAAAGGTGTTAGTACCTACAACCAGAAGTTC AAGGACAAGGCCACATTAACTGTAGACAAGTC ATCCAGCACAGCCTACATGGAACTCCTCAGTC TGACATCTGAGGACTCTGCAGTCTATTACTGT GCAAGATCGGGGTACTACGGTGATAGTGACT GGTACTTCGATGTCTGGGGCGCAGGGACCAC GGTCACCGTCTCCTCAACCACtACaCCAGCaC CtaGACCACCAACACCtGCGCCaACCATCGCaT CGCAGCCaCTGTCtCTGCGCCCAGAGGCaTGC CGGCCAGCaGCtGGGGGCGCAGTGCACACaA GGGGGCTGGACTTCGCaTGTGATATCTACATC	SIVILALRPSDEGTYECVVLKYEKDAFKREHLAE VTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFP EPHLSWLENGEELNAINTTVSQDPETELYAVSS KLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTK QEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPR CREGSGATNFSLLKQAGDVEENPGPMALPVTA LLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTI SCRASQDIRNYLNWYQQKPDGTVKLLIYYTSRL HSGVPSKFSGSGSGTDYSLTISNLEQEDIATYFC QQGNTLPWTFAGGTKLEIKRAGGGSGGGSGG GSGGGSEVQLQQSGPELVKPGASMKISCKASG YSFTGYTMNWVKQSHGKNLEWMGLINPYKGVS TYNQKFKDKATLTVDKSSSTAYMELLSLTSEDS AVYYCARSGYYGDSDWYFDVWGAGTTVTVSS TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 22)

TGGGCaCCaTTGGCaGGGACTTGTGGGGTCC TTCTCCTGTCACTGGTTATCACCCTTTACTGC (SEQ ID NO: 21)	
CD80 signal peptide	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG CAGTGGGGTC (SEQ ID NO: 23)	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GV (SEQ ID NO: 24)
CD80 extracellular domain (ECD)	
Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAGGAGGTCGC AACATTGAGTTGTGGCCATAACGTATCAGTTG AAGAACTCGCGCAGACACGGATTTACTGGCA AAAGGAAAAGAAGATGGTGTTGACAATGATGA GCGGTGACATGAACATTTGGCCAGAGTACAA AAATCGAACGATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCCTTGCGACCTTCTG ACGAGGGAACGTATGAATGTGTAGTGCTTAAG TATGAAAAAGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTTAAGGCGGAC TTTCCTACGCCGTCTATATCCGACTTCGAGAT ACCCACTTCTAACATTCGACGCATCATTTGCT CAACCTCAGGTGGTTTCCCAGAGCCTCACTTG AGCTGGCTGGAGAATGGCGAAGAACTTAACG CAATCAATACCACGGTGTCCCAAGACCCGGA GACAGAGCTGTACGCCGTGTCATCCAAACTG GATTTTAACATGACGACAAATCATAGTTTCATG TGTCTGATCAAATATGGGCATCTCAGGGTGAA TCAGACTTTTAATTGGAACACTACCAAACAAG AGCACTTCCCAGATAATCTGTTGCCAAGC (SEQ ID NO: 25)	IHVTKEVKEVATLSCGHNVSVEELAQTRIYWQK EKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIV ILALRPSDEGTYECVVLKYEKDAFKREHLAEVTL SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEP HLSWLENGEELNAINTTVSQDPETELYAVSSKL DFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQ EHFPDNLLPS (SEQ ID NO: 26)
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTCAACGGTAT CTTCGTAATTTGCTGCCTCACCTATTGTTTCG CGCCTCGATGCCGAGAA (SEQ ID NO: 27)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 28)
P2A self-cleaving peptide	
Nucleotide	Amino acid
GGCAGCGGCGCCACCAACTTCTCCCTGCTGA AGCAGGCCGGCGACGTGGAAGAAAACCCTG GCCCC (SEQ ID NO: 29)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 30)
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC G (SEQ ID NO: 31)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 32)
Anti-CD3 Light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACCCAGACCACCTCCTCCC TGTCTGCCTCTCTGGGAGACAGAGTCACCAT CAGTTGCAGGGCAAGTCAGGACATTAGAAATT ATTTAAACTGGTATCAACAGAAACCAGATGGA ACTGTTAAACTCCTGATCTACTACACATCAAG ATTACACTCAGGAGTCCCATCAAAGTTCAGTG	DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN WYQQKPDGTVKLLIYYTSRLHSGVPSKFSGSGS GTDYSLTISNLEQEDIATYFCQQGNTLPWTFAG GTKLEIKRA (SEQ ID NO: 34)

GCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAGGATATTGCCAC TTACTTTTGCCAACAGGGTAATACGCTTCCGT GGACGTTCGCTGGAGGCACCAAGCTGGAAAT CAAACGGGCT (SEQ ID NO:33)	
G_3S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCAGGTGGA GGCAGCGGTGGCGGATCT (SEQ ID NO: 35)	GGGSGGGSGGGSGGGS (SEQ ID NO: 36)
Anti-CD3 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGACCTGAGC TGGTGAAGCCTGGAGCTTCAATGAAGATATCC TGCAAGGCTTCTGGTTACTCATTCACTGGCTA CACCATGAACTGGGTGAAGCAGAGTCATGGA AAGAACCTTGAGTGGATGGGACTTATTAATCC TTACAAAGGTGTTAGTACCTACAACCAGAAGT TCAAGGACAAGGCCACATTAACTGTAGACAAG TCATCCAGCACAGCCTACATGGAACTCCTCAG TCTGACATCTGAGGACTCTGCAGTCTATTACT GTGCAAGATCGGGGTACTACGGTGATAGTGA CTGGTACTTCGATGTCTGGGGCGCAGGGACC ACGGTCACCGTCTCCTCA (SEQ ID NO: 37)	EVQLQQSGPELVKPGASMKISCKASGYSFTGYT MNWVKQSHGKNLEWMGLINPYKGVSTYNQKF KDKATLTVDKSSSTAYMELLSLTSEDSAVYYCA RSGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 38)
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACtACaCCAGCaCCtaGACCACCAACACCt GCGCCaACCATCGCaTCGCAGCCaCTGTCtCT GCGCCCAGAGGCaTGCCGGCCAGCaGCtGGG GGCGCAGTGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCCaTTGGCa GGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCTTTACTGC (SEQ ID NO: 39)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 40)

Transgene

The lentiviral vectors described herein include a transgene encoding one or more proteins that is delivered to and expressed by a lymphocyte (e.g., T cell or NK cell) targeted by the vector. In particular embodiments, the transgene encodes a chimeric antigen receptor (CAR) that includes: an extracellular domain comprising a binding domain that specifically binds to a target molecule; an intracellular signaling domain, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM); and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

Binding domains suitable for use in CARs of the present disclosure include any antigen-binding polypeptide. A binding domain may comprise an antibody or antigen

binding fragment thereof, including for example, a full length heavy chain, Fab fragment, Fab', $F(ab')_2$, sFv, VH domain, VL domain, dAb, VHH, CDR, and scFv, specific for a target disease antigen. In certain embodiments, a CAR binding domain is murine, chimeric, human, or humanized. In further embodiments, CAR binding domain is an scFv having V_H and V_L regions that are human or humanized

A target molecule that is bound by an extracellular domain of a CAR of the present disclosure may be found on or in association with a target cell. Exemplary target cells include a cancer cell, a cell associated with an autoimmune disease or disorder, a neurodegenerative disease, or with an inflammatory disease or disorder, an infectious microbe (*e.g.*, bacteria, virus, or fungi), and an infected cell (*e.g.*, virus-infected cell). A cell of an infectious organism, such as a mammalian parasite, is also contemplated as a target cell.

The extracellular domain included in a CAR as described herein can include a binding domain that targets one or more of a variety of target molecules. In certain embodiments, the CAR includes a binding domain that specifically binds one or more of CD19, BCMA, alpha folate receptor, 5T4, Ab integrin, B7-H3, B7-H6, CAIX, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD52, CD70, CD79a, CD79b, CD80, CD123, CD138, CD171, CEA, CSPG4, EGFR, ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EpCAM, FAP, fetal AchR, FLT3, Fra, GD2, GD3, Glypican-3 (GPC3), HLA-A1 + MAGE1, HLA-A2 + MAGE1, HLA-A3 + MAGE1, HLA-A1 + NY-ESO-1, HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13 Ralpha2, Lambda, Lewis-Y, Kappa, mesothelin, Muc1, Muc16, NCAM, NKG2d ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, VEGFR2, BAFF-R, Claudin18.2, CD86, FcRL5, GPRC5, and TACI.

In certain embodiments, the extracellular domain of a CAR encoded by the transgene of the lentiviral vectors described the present disclosure optionally comprises an extracellular, non-signaling spacer or linker domain between the binding domain and the transmembrane domain. Where included, such a spacer or linker domain may position the binding domain away from the host cell surface to further enable proper cell-to-cell contact, binding, and activation. An extracellular spacer domain is generally

located between the extracellular binding domain and the transmembrane domain of the CAR. The length of the extracellular spacer may be varied to optimize target molecule binding based on the selected target molecule, selected binding epitope, binding domain size and affinity (*see*, *e.g.*, Guest *et al.*, *J. Immunother. 28*:203-11, 2005; PCT Publication No. WO 2014/031687). In certain embodiments, an extracellular spacer domain is an immunoglobulin hinge region (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, IgD). An immunoglobulin hinge region may be a wild type immunoglobulin hinge region or an altered wild type immunoglobulin hinge region. An altered IgG_4 hinge region is described in PCT Publication No. WO 2014/031687, which hinge region is incorporated herein by reference in its entirety. In some embodiments, an extracellular spacer domain comprises a modified IgG_4 hinge region having an amino acid sequence of ESKYGPPCPPCP.

Other examples of hinge regions that may be used in the CARs described herein include the hinge region from the extracellular regions of type 1 membrane proteins, such as CD8a, CD4, CD28 and CD7, which may be wild-type or variants thereof. In some embodiments, an extracellular spacer domain comprises all or a portion of an immunoglobulin Fc domain selected from: a CH1 domain, a CH2 domain, a CH3 domain, or combinations thereof (*see, e.g.*, PCT Publication WO2014/031687, which spacers are incorporated herein by reference in their entirety). In yet further embodiments, an extracellular spacer domain may comprise a stalk region of a type II C-lectin (the extracellular domain located between the C-type lectin domain and the transmembrane domain). Type II C-lectins include CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

CARs of the present disclosure comprise a transmembrane domain that connects and is positioned between the extracellular domain and the intracellular signaling domain. The transmembrane domain typically ranges in length from about 15 amino acids to about 30 amino acids. The transmembrane domain is a hydrophobic alpha helix that transverses the host cell membrane and anchors the CAR in the host cell membrane. The transmembrane domain may be directly fused to the binding domain or to the extracellular spacer domain if present. In certain embodiments, the transmembrane domain is derived from an integral membrane protein (*e.g.*, receptor,

cluster of differentiation (CD) molecule, enzyme, transporter, cell adhesion molecule, or the like). The transmembrane domain can be selected from the same molecule as the extracellular domain or the intracellular signaling domain. In some embodiments, the transmembrane domain and the extracellular domain are each selected from different molecules. In some embodiments, the transmembrane domain and the intracellular signaling domain are each selected from different molecules. In yet other embodiments, the transmembrane domain, the extracellular domain, and the intracellular signaling domain are each selected from different molecules.

Exemplary transmembrane domains for use in CARs of the present disclosure include a CD28, CD2, CD4, CD8a, CD5, CD3ε, CD3δ, CD3ζ, CD9, CD16, CD22, CD25, CD27, CD 28, CD33, CD37, CD40, CD45, CD64, CD79A, CD79B, CD80, CD86, CD95 (Fas), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD154 (CD40L), CD200R, CD223 (LAG3), CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcRα, FcRβ, FcRγ, Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRPα, pTα, TCRα, TCRβ, TIM3, TRIM, LPA5, and Zap70 transmembrane domain. An exemplary CD8a transmembrane domain with CD8a hinge region comprises an amino acid sequence of SEQ ID NO: 52.

The intracellular signaling domain of a CAR is an intracellular effector domain and is capable of transmitting functional signals to a cell in response to binding of the extracellular domain of the CAR to a target molecule and activates at least one of the normal effector functions or responses of the immune cell, *e.g.,* a T cell engineered to express the CAR. In some embodiments, the CAR induces a function of a T cell such as cytolytic activity or T helper activity, such as secretion of cytokines or other factors. The intracellular signaling domain may be any portion of an intracellular signaling molecule that retains sufficient signaling activity. In some embodiments, the intracellular signaling domain is obtained from an antigen receptor component (*e.g.*, TCR) or costimulatory molecule. In some embodiments, a full-length intracellular signaling domain of an antigen receptor or costimulatory molecule is used. In some

embodiments, a truncated portion of an intracellular signaling domain of an antigen receptor or costimulatory molecule is used, provided that the truncated portion retains sufficient signal transduction activity. In further embodiments, an intracellular signaling domain is a variant of a full length or truncated portion of an intracellular signaling domain of an antigen receptor co stimulatory molecule, provided that the variant retains sufficient signal transduction activity (*i.e.*, is a functional variant).

In certain embodiments, the intracellular signaling domain of a CAR comprises an immunoreceptor tyrosine-based activation motif (ITAM) containing signaling domain. An ITAM containing signaling domain generally contains at least one (one, two, three, four, or more) ITAMs, which refer to a conserved motif of YXXL/I-X_{6-8}-YXXL/I. An ITAM containing signaling domain may initiate T cell activation signaling following antigen binding or ligand engagement. ITAM-signaling domains include, for example, intracellular signaling domains of CD3γ, CD3δ, CD3ε, CD3ζ, CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcRγ, and CD66d. Exemplary CD3ζ signaling domains that may be used in CARs of the present disclosure comprise an amino acid sequence of SEQ ID NO: 56.

CAR intracellular signaling domains optionally comprise a costimulatory signaling domain, which, when activated in conjunction with a primary or classic (*e.g.*, ITAM-driven) activation signal, promotes or enhances T cell response, such as T cell activation, cytokine production, proliferation, differentiation, survival, effector function, or combinations thereof. Costimulatory signaling domains for use in CARs include, for example, CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, NKD2C, SLP76, TRIM, ZAP70, or any combination thereof. In a particular embodiment, the costimulatory signaling domain comprises a OX40, CD2, CD27, CD28, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), or 4-1BB (CD137) signaling domain. An exemplary 4-1BB costimulatory signaling domain comprises an amino acid sequence of SEQ ID NO:

54. In certain embodiments, a CAR comprises one, two, or more costimulatory signaling domains.

In some embodiments, a CAR of the present disclosure is a first generation CAR, a second generation CAR, or a third generation CAR. A first generation CAR generally has an intracellular signaling domain comprising an intracellular signaling domain of CD3ζ, FcγRI, or other ITAM-containing activating domain to provide a T cell activation signal. Second generation CARs further comprise a costimulatory signaling domain (*e.g.*, a costimulatory signaling domain from an endogenous T cell costimulatory receptor, such as CD28, 4-1BB, or ICOS). Third generation CARs comprise an ITAM-containing activating domain, a first costimulatory signaling domain and a second costimulatory signaling domain.

In some embodiments, one or more of the extracellular domain, the binding domain, the linker, the transmembrane domain, the intracellular signaling domain, or the costimulatory domain comprises junction amino acids. "Junction amino acids" or "junction amino acid residues" refer to one or more (*e.g.*, about 2-20) amino acid residues between two adjacent domains, motifs, regions, modules, or fragments of a protein, such as between a binding domain and an adjacent linker, between a transmembrane domain and an adjacent extracellular or intracellular domain, or on one or both ends of a linker that links two domains, motifs, regions, modules, or fragments (e.g., between a linker and an adjacent binding domain or between a linker and an adjacent hinge). Junction amino acids may result from the construct design of a fusion protein (e.g., amino acid residues resulting from the use of a restriction enzyme site or self-cleaving peptide sequences during the construction of a polynucleotide encoding a fusion protein). For example, a transmembrane domain of a fusion protein may have one or more junction amino acids at the amino-terminal end, carboxy -terminal end, or both.

In specific embodiments, the transgene encodes an anti-CD19 CAR molecule. In such embodiments, the extracellular binding domain may include an scFv as detailed in Table 4, having an anti-CD19 light chain variable region of SEQ ID NO: 46, a G4S linker of SEQ ID NO: 48, and an anti-CD19 heavy chain variable region of SEQ ID NO: 50.

An anti-CD19 CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain of SEQ ID NO: 52. In certain embodiments, the anti-CD19 CAR includes a 4-1BB costimulatory domain according to SEQ ID NO: 54 and a CD3ζ effector domain according to SEQ ID NO: 56. In a specific embodiment, an anti-CD19 CAR encoded by the transgene has an amino acid sequence according to SEQ ID NO: 42.

In other embodiments, the transgene encodes an anti-CD19 CAR molecule and extracellular binding domain may include an scFv having an anti-CD19 light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 46, and an anti-CD19 heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 50. An anti-CD19 CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52. In certain embodiments, the anti-CD19 CAR includes a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54 and a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56. In a specific embodiment, an anti-CD19 CAR encoded by the transgene has an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 42.

TABLE 4: Anti-CD19 CAR molecule

Full molecule	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC	MALPVTALLLPLALLLHAARPDIQMTQTTSSLSA
CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC	SLGDRVTISCRASQDISKYLNWYQQKPDGTVKL
GGACATCCAGATGACACAGACTACATCCTCCC	LIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQ
TGTCTGCCTCTCTGGGAGACAGAGTCACCAT	EDIATYFCQQGNTLPYTFGGGTKLEITGGGGSG
CAGTTGCAGGGCAAGTCAGGACATTAGTAAAT	GGGSGGGGSEVKLQESGPGLVAPSQSLSVTCT
ATTTAAATTGGTATCAGCAGAAACCAGATGGA	VSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSE
ACTGTTAAACTCCTGATCTACCATACATCAAG	TTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDD
ATTACACTCAGGAGTCCCATCAAGGTTCAGTG	TAIYYCAKHYYYGGSYAMDYWGQGTSVTVSST

GCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAAGATATTGCCAC TTACTTTTGCCAACAGGGTAATACGCTTCCGT ACACGTTCGGAGGGGGGACCAAGCTGGAGAT CACAGGTGGaGGTGGaTCGGGCGGTGGTGG GTCGGGTGGCGGCGGATCTGAGGTGAAACTG CAGGAGTCAGGACCTGGCCTGGTGGCGCCCT CACAGAGCCTGTCCGTCACATGCACTGTCTCA GGGGTCTCATTACCCGACTATGGTGTAAGCT GGATTCGCCAGCCTCCACGAAAGGGTCTGGA GTGGCTGGGAGTAATATGGGGTAGTGAAACC ACATACTATAATTCAGCTCTCAAATCCAGACT GACCATCATCAAGGACAACTCCAAGAGCCAA GTTTTCTTAAAAATGAACAGTCTGCAAACTGAT GACACAGCCATTTACTACTGTGCCAAACATTA TTACTACGGTGGTAGCTATGCTATGGACTACT GGGGCCAAGGAACCTCAGTCACCGTCTCCTC AACCACtACaCCAGCaCCtaGACCACCAACACCt GCGCCaACCATCGCaTCGCAGCCaCTGTCtCT GCGCCCAGAGGCaTGCCGGCCAGCaGCtGGG GGCGCAGTGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCCaTTGGCa GGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCTTTACTGCAAACGGGGCAGAAAG AAACTCCTGTATATATTCAAACAACCATTTATG AGACCAGTACAAACTACTCAAGAGGAAGATG GCTGTAGCTGCCGATTTCCAGAAGAAGAAGA AGGAGGATGTGAACTGAGAGTGAAGTTCAGC AGGAGCGCAGACGCCCCCGCGTACAAGCAG GGCCAGAACCAGCTCTATAACGAGCTCAATCT AGGACGAAGAGAGGAGTACGATGTTTTGGAC AAGAGACGTGGCCGGGACCCTGAGATGGGG GGAAAGCCGAGAAGGAAGAACCCTCAGGAAG GCCTGTACAATGAACTGCAGAAAGATAAGATG GCGGAGGCCTACAGTGAGATTGGGATGAAAG GCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTACCAGGGTCTCAGTACAGCCACCAA GGACACCTACGACGCCCTTCACATGCAGGCC CTGCCCCCTCGC (SEQ ID NO: 41)	TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCELRVKFSRSADAPAYKQGQNQLYNE LNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 42)
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC G (SEQ ID NO: 43)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 44)
Anti-CD19 light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACACAGACTACATCCTCCCT GTCTGCCTCTCTGGGAGACAGAGTCACCATC AGTTGCAGGGCAAGTCAGGACATTAGTAAATA TTTAAATTGGTATCAGCAGAAACCAGATGGAA CTGTTAAACTCCTGATCTACCATACATCAAGAT TACACTCAGGAGTCCCATCAAGGTTCAGTGG CAGTGGGTCTGGAACAGATTATTCTCTCACCA TTAGCAACCTGGAGCAAGAAGATATTGCCACT TACTTTTGCCAACAGGGTAATACGCTTCCGTA	DIQMTQTTSSLSASLGDRVTISCRASQDISKYLN WYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSG SGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGG GTKLEIT (SEQ ID NO: 46)

CACGTTCGGAGGGGGGACCAAGCTGGAGATC ACA (SEQ ID NO: 45)	
G_4S Linker	
Nucleotide	Amino acid
GGTGGaGGTGGaTCGGGCGGTGGTGGGTCG GGTGGCGGCGGATCT (SEQ ID NO: 47)	GGGGSGGGGSGGGGS (SEQ ID NO: 48)
Anti-CD19 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGAAACTGCAGGAGTCAGGACCTGGCC TGGTGGCGCCCTCACAGAGCCTGTCCGTCAC ATGCACTGTCTCAGGGGTCTCATTACCCGACT ATGGTGTAAGCTGGATTCGCCAGCCTCCACG AAAGGGTCTGGAGTGGCTGGGAGTAATATGG GGTAGTGAAACCACATACTATAATTCAGCTCT CAAATCCAGACTGACCATCATCAAGGACAACT CCAAGAGCCAAGTTTTCTTAAAAATGAACAGT CTGCAAACTGATGACACAGCCATTTACTACTG TGCCAAACATTATTACTACGGTGGTAGCTATG CTATGGACTACTGGGGCCAAGGAACCTCAGT CACCGTCTCCTCA (SEQ ID NO: 49)	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYG VSWIRQPPRKGLEWLGVIWGSETTYYNSALKSR LTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYY YGGSYAMDYWGQGTSVTVSS (SEQ ID NO: 50)
CD8a Hinge and Transmembrane	
Nucleotide	Amino acid
ACCACtACaCCAGCaCCtaGACCACCAACACCt GCGCCaACCATCGCaTCGCAGCCaCTGTCtCT GCGCCCAGAGGCaTGCCGGCCAGCaGCtGGG GGCGCAGTGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCCaTTGGCa GGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCTTTACTGC (SEQ ID NO: 51)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 52)
4-1BB costimulatory domain	
Nucleotide	Amino acid
AAACGGGGCAGAAAGAAACTCCTGTATATATT CAAACAACCATTTATGAGACCAGTACAAACTA CTCAAGAGGAAGATGGCTGTAGCTGCCGATT TCCAGAAGAAGAAGAAGGAGGATGTGAACTG (SEQ ID NO: 53)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCEL (SEQ ID NO: 54)
CD3 zeta effector domain	
Nucleotide	Amino acid
AGAGTGAAGTTCAGCAGGAGCGCAGACGCCC CCGCGTACAAGCAGGGCCAGAACCAGCTCTA TAACGAGCTCAATCTAGGACGAAGAGAGGAG TACGATGTTTTGGACAAGAGACGTGGCCGGG ACCCTGAGATGGGGGGAAAGCCGAGAAGGAA GAACCCTCAGGAAGGCCTGTACAATGAACTG CAGAAAGATAAGATGGCGGAGGCCTACAGTG AGATTGGGATGAAAGGCGAGCGCCGGAGGG GCAAGGGGCACGATGGCCTTTACCAGGGTCT CAGTACAGCCACCAAGGACACCTACGACGCC CTTCACATGCAGGCCCTGCCCCCTCGC (SEQ ID NO: 55)	RVKFSRSADAPAYKQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR (SEQ ID NO: 56)

In other embodiments, the transgene encodes an anti-BCMA CAR molecule. In such embodiments, the extracellular binding domain may include an scFv as detailed in Table 5, having an anti-BCMA light chain variable region of SEQ ID NO: 62, a linker of SEQ ID NO: 64, and an anti-BCMA heavy chain variable region of SEQ ID NO: 66. An anti-BCMA CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain of SEQ ID NO: 52. In certain embodiments, the anti-BCMA CAR includes a 4-1BB costimulatory domain according to SEQ ID NO: 54 and a CD3ζ effector domain according to SEQ ID NO: 56. In a specific embodiment, an anti-BCMA CAR encoded by the transgene has an amino acid sequence according to SEQ ID NO: 58.

In some embodiments, the transgene encodes an anti-BCMA CAR molecule. In such embodiments, the extracellular binding domain may include an scFv having an anti-BCMA light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 62, and an anti-BCMA heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 66. An anti-BCMA CAR useful in the context of the present description may include a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52. In certain embodiments, the anti-BCMA CAR includes a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54 and a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56. In a specific embodiment, an anti-BCMA CAR encoded by the transgene has an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 58.

TABLE 5: Anti-BCMA CAR molecule

Full molecule	
Nucleotide	Amino acid
ATGGCACTCCCCGTCACCGCCCTTCTCTTGC CCCTCGCCCTGCTGCTGCATGCTGCCAGGCC CGACATTGTGCTCACTCAGTCACCTCCCAGCC TGGCCATGAGCCTGGGAAAAAGGGCCACCAT CTCCTGTAGAGCCAGTGAGTCCGTCACAATCT TGGGGAGCCATCTTATTCACTGGTATCAGCAG AAGCCCGGGCAGCCTCCAACCCTTCTTATTCA GCTCGCGTCAAACGTCCAGACGGGTGTACCT GCCAGATTTTCTGGTAGCGGGTCCCGCACTG ATTTTACACTGACCATAGATCCAGTGGAAGAA GACGATGTGGCCGTGTATTATTGTCTGCAGAG CAGAACGATTCCTCGCACATTTGGTGGGGGT ACTAAGCTGGAGATTAAGGGAAGCACGTCCG GCTCAGGGAAGCCGGGCTCCGGCGAGGGAA GCACGAAGGGGCAAATTCAGCTGGTCCAGAG CGGACCTGAGCTGAAAAAACCCGGCGAGACT GTTAAGATCAGTTGTAAAGCATCTGGCTATAC CTTCACCGACTACAGCATAAATTGGGTGAAAC GAGCCCCTGGAAAGGGCCTCAAATGGATGGG TTGGATCAATACCGAAACTAGGGAGCCTGCTT ATGCATATGACTTCCGCGGGAGATTCGCCTTT TCACTCGAGACATCTGCCTCTACTGCTTACCT CCAAATAAACAACCTCAAGTATGAAGATACAG CCACTTACTTTTGCGCCCTCGACTATAGTTAC GCCATGGACTACTGGGGACAGGGAACCTCCG TTACCGTCAGTTCCGCGGCCGCAACCACAAC ACCTGCTCCAAGGCCCCCCACACCCGCTCCA ACTATAGCCAGCCAACCATTGAGCCTCAGACC TGAAGCTTGCAGGCCCGCAGCAGGAGGCGC CGTCCATACGCGAGGCCTGGACTTCGCGTGT GATATTTATATTTGGGCACCTTTGGCCGGAAC ATGTGGGGTGTTGCTTCTCTCCCTTGTGATCA CTCTGTATTGTAAGCGCGGGAGAAAGAAGCT CCTGTACATCTTCAAGCAGCCTTTTATGCGAC CTGTGCAAACCACTCAGGAAGAAGATGGGTG TTCATGCCGCTTCCCCGAGGAGGAAGAAGGA GGGTGTGAACTGAGGGTGAAATTTTCTAGAAG CGCCGATGCTCCCGCATATCAGCAGGGTCAG AATCAGCTCTACAATGAATTGAATCTCGGCAG GCGAGAAGAGTACGATGTTCTGGACAAGAGA CGGGGCAGGGATCCCGAGATGGGGGGAAAG CCCCGGAGAAAAAATCCTCAGGAGGGGTTGT ACAATGAGCTGCAGAAGGACAAGATGGCTGA AGCCTATAGCGAGATCGGAATGAAAGGCGAA AGACGCAGAGGCAAGGGGCATGACGGTCTGT ACCAGGGTCTCTCTACAGCCACCAAGGACAC TTATGATGCGTTGCATATGCAAGCCTTGCCAC CCCGC (SEQ ID NO: 57)	MALPVTALLLPLALLLHAARPDIVLTQSPPSLAM SLGKRATISCRASESVTILGSHLIHWYQQKPGQP PTLLIQLASNVQTGVPARFSGSGSRTDFTLTIDP VEEDDVAVYYCLQSRTIPRTFGGGTKLEIKGSTS GSGKPGSGEGSTKGQIQLVQSGPELKKPGETV KISCKASGYTFTDYSINWVKRAPGKGLKWMGWI NTETREPAYAYDFRGRFAFSLETSASTAYLQINN LKYEDTATYFCALDYSYAMDYWGQGTSVTVSS AAATTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 58)
CD8a signal peptide	
Nucleotide	Amino acid

ATGGCACTCCCCGTCACCGCCCTTCTCTTGC CCCTCGCCCTGCTGCTGCATGCTGCCAGGCC C (SEQ ID NO: 59)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 60)
Anti-BCMA light chain variable region	
Nucleotide	Amino acid
GACATTGTGCTCACTCAGTCACCTCCCAGCCT GGCCATGAGCCTGGGAAAAAGGGCCACCATC TCCTGTAGAGCCAGTGAGTCCGTCACAATCTT GGGGAGCCATCTTATTCACTGGTATCAGCAGA AGCCCGGGCAGCCTCCAACCCTTCTTATTCA GCTCGCGTCAAACGTCCAGACGGGTGTACCT GCCAGATTTTCTGGTAGCGGGTCCCGCACTG ATTTTACACTGACCATAGATCCAGTGGAAGAA GACGATGTGGCCGTGTATTATTGTCTGCAGAG CAGAACGATTCCTCGCACATTTGGTGGGGGT ACTAAGCTGGAGATTAAG (SEQ ID NO: 61)	DIVLTQSPPSLAMSLGKRATISCRASESVTILGS HLIHWYQQKPGQPPTLLIQLASNVQTGVPARFS GSGSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRT FGGGTKLEIK (SEQ ID NO: 62)
218 Linker	
Nucleotide	Amino acid
GGAAGCACGTCCGGCTCAGGGAAGCCGGGC TCCGGCGAGGGAAGCACGAAGGGG (SEQ ID NO: 63)	GSTSGSGKPGSGEGSTKG (SEQ ID NO: 64)
Anti-BCMA heavy chain variable region	
Nucleotide	Amino acid
CAAATTCAGCTGGTCCAGAGCGGACCTGAGC TGAAAAAACCCGGCGAGACTGTTAAGATCAGT TGTAAAGCATCTGGCTATACCTTCACCGACTA CAGCATAAATTGGGTGAAACGAGCCCCTGGA AAGGGCCTCAAATGGATGGGTTGGATCAATA CCGAAACTAGGGAGCCTGCTTATGCATATGAC TTCCGCGGGAGATTCGCCTTTTCACTCGAGAC ATCTGCCTCTACTGCTTACCTCCAAATAAACA ACCTCAAGTATGAAGATACAGCCACTTACTTT TGCGCCCTCGACTATAGTTACGCCATGGACTA CTGGGGACAGGGAACCTCCGTTACCGTCAGT TCC (SEQ ID NO: 65)	QIQLVQSGPELKKPGETVKISCKASGYTFTDYSI NWVKRAPGKGLKWMGWINTETREPAYAYDFR GRFAFSLETSASTAYLQINNLKYEDTATYFCALD YSYAMDYWGQGTSVTVSS (SEQ ID NO: 66)
CD8a Hinge and Transmembrane	
Nucleotide	Amino acid
ACCACAACACCTGCTCCAAGGCCCCCCACAC CCGCTCCAACTATAGCCAGCCAACCATTGAG CCTCAGACCTGAAGCTTGCAGGCCCGCAGCA GGAGGCGCCGTCCATACGCGAGGCCTGGAC TTCGCGTGTGATATTTATATTTGGGCACCTTT GGCCGGAACATGTGGGGTGTTGCTTCTCTCC CTTGTGATCACTCTGTATTGT (SEQ ID NO: 51)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 52)
4-1BB costimulatory domain	
Nucleotide	Amino acid
AAGCGCGGGAGAAAGAAGCTCCTGTACATCT TCAAGCAGCCTTTTATGCGACCTGTGCAAACC ACTCAGGAAGAAGATGGGTGTTCATGCCGCT TCCCCGAGGAGGAAGAAGGAGGGTGTGAACT G (SEQ ID NO: 53)	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP EEEEGGCEL (SEQ ID NO: 54)
CD3 zeta effector domain	
Nucleotide	Amino acid
AGGGTGAAATTTTCTAGAAGCGCCGATGCTCC CGCATATCAGCAGGGTCAGAATCAGCTCTACA	RVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ

ATGAATTGAATCTCGGCAGGCGAGAAGAGTA CGATGTTCTGGACAAGAGACGGGGCAGGGAT CCCGAGATGGGGGGAAAGCCCCGGAGAAAA AATCCTCAGGAGGGGTTGTACAATGAGCTGC AGAAGGACAAGATGGCTGAAGCCTATAGCGA GATCGGAATGAAAGGCGAAAGACGCAGAGGC AAGGGGCATGACGGTCTGTACCAGGGTCTCT CTACAGCCACCAAGGACACTTATGATGCGTTG CATATGCAAGCCTTGCCACCCCGC (SEQ ID NO: 55)	KDKMAEAYSEIGMKGERRRGKGHDGLYQGLST ATKDTYDALHMQALPPR (SEQ ID NO: 56)

CARs of the present disclosure may comprise polynucleotide sequences derived from any mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, transgenic species thereof, or any combination thereof. In some embodiments, the chimeric antigen receptor is murine, chimeric, human, or humanized.

Methods for Producing Lentiviral Vectors

Methods for producing the LVV disclosed are provided herein. In some embodiments, the methods described herein utilize a third generation vector system for producing LVV. Third generation vector production systems are typically four plasmid systems that include a transgene plasmid combined with three packaging plasmids, VSV-G, GagPol, and Rev. The transgene plasmid is combined at defined ratios with the three packaging plasmids and used to transfect producer cells, such as HEK293 producer cells. Productive viral particles are harvested from the producer cell culture media. Exemplary materials and methods for producing LVV particles are described in *Production of Lentiviral Vectors*, Merten et al., Molecular Therapy – Methods & Clinical Development (2016), 3, 16017, the contents of which are incorporated herein by reference. Third generation production systems are used for research and development and clinical purposes. Schematic representations of helper plasmids suitable for use in a third generation LVV production system are provided in FIG. 1.

The transgene plasmid encodes the only genetic materials that is transferred to the lymphocytes (e.g., T cells or NK cells) targeted by the resulting LVV and comprises a lentiviral backbone containing the transgene expression cassette flanked by cis-acting elements for encapsidation, reverse transcription and integration. In specific

embodiments, the transgene plasmid includes a deletion in the U3 element of the 3' LTR that causes loss of transcriptional capacity of the viral LTR once transferred into the target cells. The expression cassette included in the transgene plasmid may encode a single heterologous protein (*e.g.*, a single CAR as described herein) or multiple heterologous proteins (e.g., multiple CARs as described herein) for introduction into and expression by the target cell.

In a four plasmid LVV system the VSV-G plasmid includes a tandem expression cassette that encodes a mutated VSV-G envelope protein and a target protein as disclosed herein. In specific embodiments, the tandem expression cassette included in the VSV-G plasmid includes a polynucleotide that encodes a first signal peptide, a polynucleotide that encodes a target protein, a polynucleotide that encodes one of an internal ribosomal entry site (IRES), a furin cleavage site, or a viral 2A peptide, a polynucleotide that encodes a second signal peptide, and a polynucleotide that encodes a mutated VSV-G envelope protein. In certain embodiments, the polynucleotide encoding the mutant VSV-G envelope protein is positioned 5' to the polynucleotide encoding the targeting protein. In other embodiments, the polynucleotide encoding the mutant VSV-G envelope protein is positioned 3' to the polynucleotide encoding the targeting protein. The polynucleotide that encodes the targeting protein and the polynucleotide that encodes the mutated VSV-G are separated in the tandem cassette by the polynucleotide that encodes the IRES, furin cleavage site, or viral 2A peptide, which allows for co-expression of the two proteins from a single mRNA. In certain embodiments, a viral 2A peptide is a porcine teschovirus-1 (P2A), *Thosea asigna* virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or a variant thereof. Specific examples of amino acid and nucleotide sequences for the viral 2A peptides are provided in Table 8.

Table 8: 2A sequences

T2A peptide from Thosea asigna virus capsid protein	EGRGSLLTCGD VEENPGP (SEQ ID NO: 75)	GAGGGCAGGGGAAGTCTT CTAACATGCGGGGACGTG GAGGAAAATCCCGGCCCC (SEQ ID NO: 76)

P2A peptide from porcine teschovirus-1 polyprotein	ATNFSLLKQAG DVEENPGP (SEQ ID NO: 77)	GCCACGAACTTCTCTCTGT TAAAGCAAGCAGGAGACG TGGAAGAAAACCCCGGTC CT (SEQ ID NO: 78)
F2A peptide from foot-and-mouth disease virus polyprotein	VKQTLNFDLLKL AGDVESNPGP (SEQ ID NO: 79)	GTGAAACAGACTTTGAATT TTGACCTTCTCAAGTTGGC GGGAGACGTCGAGTCCAA CCCTGGGCCC (SEQ ID NO: 80)
E2A peptide from equine rhinitis A virus polyprotein	QCTNYALLKLA GDVESNPGP (SEQ ID NO: 81)	CAGTGTACTAATTATGCTC TCTTGAAATTGGCTGGAGA TGTTGAGAGCAACCCAGG TCCC (SEQ ID NO: 82)

In one embodiment, the tandem expression cassette of the VSV-G plasmid encodes an anti-CD3 targeting protein and a mutated VSV-G envelope protein. For example, in a specific embodiment, the tandem expression cassette may comprise a polynucleotide that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 67, which encodes an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 68. Such an embodiment is described in more detail in Table 6. As illustrated in Table 6, the anti-CD3 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide. In certain embodiments, that P2A peptide may be encoded by a nucleic acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 69, and the P2A peptide may have an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 70.

TABLE 6: Anti-CD3 targeting molecule co-expressed with mutant VSV-G

Full molecule	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC GGACATCCAGATGACCCAGACCACCTCCTCC CTGTCTGCCTCTCTGGGAGACAGAGTCACCA TCAGTTGCAGGGCAAGTCAGGACATTAGAAAT TATTTAAACTGGTATCAACAGAAACCAGATGG	MALPVTALLLPLALLLHAARPDIQMTQTTSSLSA SLGDRVTISCRASQDIRNYLNWYQQKPDGTVKL LIYYTSRLHSGVPSKFSGSGSGTDYSLTISNLEQ EDIATYFCQQGNTLPWTFAGGTKLEIKRAGGGS GGGSGGGSGGGSEVQLQQSGPELVKPGASMK ISCKASGYSFTGYTMNWVKQSHGKNLEWMGLI

AACTGTTAAACTCCTGATCTACTACACATCAA GATTACACTCAGGAGTCCCATCAAAGTTCAGT GGCAGTGGGTCTGGAACAGATTATTCTCTCAC CATTAGCAACCTGGAGCAAGAGGATATTGCCA CTTACTTTTGCCAACAGGGTAATACGCTTCCG TGGACGTTCGCTGGAGGCACCAAGCTGGAAA TCAAACGGGCTGGAGGCGGTAGTGGCGGTG GATCAGGTGGAGGCAGCGGTGGCGGATCTG AGGTGCAGCTCCAGCAGTCTGGACCTGAGCT GGTGAAGCCTGGAGCTTCAATGAAGATATCCT GCAAGGCTTCTGGTTACTCATTCACTGGCTAC ACCATGAACTGGGTGAAGCAGAGTCATGGAA AGAACCTTGAGTGGATGGGACTTATTAATCCT TACAAAGGTGTTAGTACCTACAACCAGAAGTT CAAGGACAAGGCCACATTAACTGTAGACAAGT CATCCAGCACAGCCTACATGGAACTCCTCAGT CTGACATCTGAGGACTCTGCAGTCTATTACTG TGCAAGATCGGGGTACTACGGTGATAGTGAC TGGTACTTCGATGTCTGGGGCGCAGGGACCA CGGTCACCGTCTCCTCAACCACtACaCCAGCa CCtaGACCACCAACACCtGCGCCaACCATCGCa TCGCAGCCaCTGTCtCTGCGCCCAGAGGCaTG CCGGCCAGCaGCtGGGGGCGCAGTGCACACa AGGGGGCTGGACTTCGCaTGTGATATCTACAT CTGGGCaCCaTTGGCaGGGACTTGTGGGGTC CTTCTCCTGTCACTGGTTATCACCCTTTACTG CGGCAGCGGCGCCACCAACTTCTCCCTGCTG AAGCAGGCCGGCGACGTGGAAGAAAACCCTG GCCCCATGAAGTGTCTGCTGTACCTGGCGTT CCTGTTTATCGGGGTGAACTGCAAGTTCACTA TCGTGTTTCCGCACAACCAAAAGGGCAACTG GAAAAACGTGCCTTCAAATTACCATTATTGCC CCAGCAGCTCGGACCTGAACTGGCACAATGA CCTCATTGGAACCGCGCTGCAGGTGAAGATG CCACAGAGCCACAAGGCTATCCAGGCTGACG GATGGATGTGCCACGCGTCAAAATGGGTGAC TACCTGCGATTTCCGCTGGTACGGACCAAAAT ACATCACGCACAGCATCAGATCATTCACCCCG TCAGTGGAACAATGCAAAGAATCCATCGAACA GACTAAGCAGGGAACCTGGCTGAACCCTGGA TTTCCGCCGCAGTCGTGTGGGTACGCAACCG TGACCGATGCAGAGGCCGTGATCGTGCAAGT CACGCCGCATCACGTGCTTGTGGACGAGTAC ACCGGAGAATGGGTCGATTCCCAGTTCATCAA CGGCAAGTGCTCCAACTACATTTGCCCAACC GTGCACAACAGCACTACTTGGCAtAGCGACTA CAAAGTGAAGGGTCTGTGTGATTCCAACCTGA TCTCCATGGATATCACTTTCTTCTCGGAAGAC GGCGAACTGTCCTCACTGGGCAAAGAAGGAA CTGGGTTTCGCTCAAATTACTTCGCCTACGAA ACTGGAGGAAAAGCCTGCAAGATGCAGTACT GCAAGCACTGGGGCGTGAGACTACCCAGCG GTGTCTGGTTCGAGATGGCCGATAAGGACCT GTTTGCAGCAGCGAGATTCCCGGAATGCCCT GAGGGATCGAGCATCTCCGCTCCAAGCCAAA CTTCAGTGGACGTGAGCCTGATCCAGGACGT	NPYKGVSTYNQKFKDKATLTVDKSSSTAYMELL SLTSEDSAVYYCARSGYYGDSDWYFDVWGAG TTVTVSSTTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLL LSLVITLYCGSGATNFSLLKQAGDVEENPGPMK CLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSN YHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQ ADGWMCHASKWVTTCDFRWYGPKYITHSIRSF TPSVEQCKESIEQTKQGTWLNPGFPPQSCGYA TVTDAEAVIVQVTPHHVLVDEYTGEWVDSQFIN GKCSNYICPTVHNSTTWHSDYKVKGLCDSNLIS MDITFFSEDGELSSLGKEGTGFRSNYFAYETGG KACKMQYCKHWGVRLPSGVWFEMADKDLFAA ARFPECPEGSSISAPSQTSVDVSLIQDVERILDY SLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPA FTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTT TEAELWDDWAPYEDVEIGPNGVLRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQL PDDESLFFGDTGLSKNPIELVEGWFSSWKSSIA SFFFIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDI EMNRLGK (SEQ ID NO: 68)

GGAACGGATTCTCGACTACTCGCTGTGCCAG GAGACCTGGTCGAAGATCAGAGCGGGACTGC CCATCTCACCGGTGGACCTGTCCTACCTGGC GCCAAAGAATCCGGGCACTGGACCGGCGTTC ACCATCATCAACGGCACCCTCAAATACTTCGA GACGCGGTACATCCGGGTGGACATCGCAGCT CCGATCCTCTCCCGGATGGTGGGAATGATCT CGGGGACTACTACCGAAGCCGAGCTCTGGGA CGACTGGGCACCTTACGAGGATGTCGAGATC GGACCTAACGGAGTGCTCCGGACCTCCTCCG GGTACAAGTTCCCTCTGTACATGATCGGCCAT GGCATGCTGGACTCGGATCTGCATCTGTCGT CCAAAGCACAGGTGTTTGAACACCCACACATT CAAGACGCCGCCAGCCAGCTGCCGGACGAT GAGTCGCTGTTCTTCGGAGACACGGGCTTGT CAAAGAATCCCATCGAGCTGGTGGAAGGATG GTTTTCATCCTGGAAAAGCAGCATCGCTTCAT TCTTCTTCATCATTGGCCTGATCATCGGCCTA TTTCTAGTCCTGCGGGTGGGAATTCATCTGTG CATCAAGCTCAAGCACACTAAGAAGCGGCAA ATCTACACTGATATCGAGATGAATCGCCTGGG CAAG (SEQ ID NO: 67)	
CD8a signal peptide	
Nucleotide	Amino acid
ATGGCCTTACCAGTGACCGCCTTGCTCCTGC CGCTGGCCTTGCTGCTCCACGCCGCCAGGCC G 9 (SEQ ID NO: 31)	MALPVTALLLPLALLLHAARP (SEQ ID NO: 32)
Anti-CD3 Light chain variable region	
Nucleotide	Amino acid
GACATCCAGATGACCCAGACCACCTCCTCCC TGTCTGCCTCTCTGGGAGACAGAGTCACCAT CAGTTGCAGGGCAAGTCAGGACATTAGAAATT ATTTAAACTGGTATCAACAGAAACCAGATGGA ACTGTTAAACTCCTGATCTACTACACATCAAG ATTACACTCAGGAGTCCCATCAAAGTTCAGTG GCAGTGGGTCTGGAACAGATTATTCTCTCACC ATTAGCAACCTGGAGCAAGAGGATATTGCCAC TTACTTTTGCCAACAGGGTAATACGCTTCCGT GGACGTTCGCTGGAGGCACCAAGCTGGAAAT CAAACGGGCT (SEQ ID NO: 33)	DIQMTQTTSSLSASLGDRVTISCRASQDIRNYLN WYQQKPDGTVKLLIYYTSRLHSGVPSKFSGSGS GTDYSLTISNLEQEDIATYFCQQGNTLPWTFAG GTKLEIKRA (SEQ ID NO: 34)
G_3S linker	
Nucleotide	Amino acid
GGAGGCGGTAGTGGCGGTGGATCAGGTGGA GGCAGCGGTGGCGGATCT (SEQ ID NO: 35)	GGGSGGGSGGGSGGGS (SEQ ID NO: 36)
Anti-CD3 heavy chain variable region	
Nucleotide	Amino acid
GAGGTGCAGCTCCAGCAGTCTGGACCTGAGC TGGTGAAGCCTGGAGCTTCAATGAAGATATCC TGCAAGGCTTCTGGTTACTCATTCACTGGCTA CACCATGAACTGGGTGAAGCAGAGTCATGGA AAGAACCTTGAGTGGATGGGACTTATTAATCC TTACAAAGGTGTTAGTACCTACAACCAGAAGT TCAAGGACAAGGCCACATTAACTGTAGACAAG TCATCCAGCACAGCCTACATGGAACTCCTCAG TCTGACATCTGAGGACTCTGCAGTCTATTACT	EVQLQQSGPELVKPGASMKISCKASGYSFTGYT MNWVKQSHGKNLEWMGLINPYKGVSTYNQKF KDKATLTVDKSSSTAYMELLSLTSEDSAVYYCA RSGYYGDSDWYFDVWGAGTTVTVSS (SEQ ID NO: 38)

GTGCAAGATCGGGGTACTACGGTGATAGTGA CTGGTACTTCGATGTCTGGGGCGCAGGGACC ACGGTCACCGTCTCCTCA (SEQ ID NO: 37)	
CD8a hinge and transmembrane	
Nucleotide	Amino acid
ACCACtACaCCAGCaCCtaGACCACCAACACCt GCGCCaACCATCGCaTCGCAGCCaCTGTCtCT GCGCCCAGAGGCaTGCCGGCCAGCaGCtGGG GGCGCAGTGCACACaAGGGGGCTGGACTTCG CaTGTGATATCTACATCTGGGCaCCaTTGGCa GGGACTTGTGGGGTCCTTCTCCTGTCACTGG TTATCACCCTTTACTGC (SEQ ID NO: 39)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLY C (SEQ ID NO: 40)
P2A self-cleaving peptide	
Nucleotide	Amino acid
GGCAGCGGCGCCACCAACTTCTCCCTGCTGA AGCAGGCCGGCGACGTGGAAGAAAACCCTG GCCCC (SEQ ID NO: 69)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 70)
VSV-G signal sequence	
Nucleotide	Amino acid
ATGAAGTGTCTGCTGTACCTGGCGTTCCTGTT TATCGGGGTGAACTGC (SEQ ID NO: 71)	MKCLLYLAFLFIGVNC (SEQ ID NO: 72)
Mutant VSV-G	
Nucleotide	Amino acid
AAGTTCACTATCGTGTTTCCGCACAACCAAAA GGGCAACTGGAAAAACGTGCCTTCAAATTACC ATTATTGCCCCAGCAGCTCGGACCTGAACTG GCACAATGACCTCATTGGAACCGCGCTGCAG GTGAAGATGCCACAGAGCCACAAGGCTATCC AGGCTGACGGATGGATGTGCCACGCGTCAAA ATGGGTGACTACCTGCGATTTCCGCTGGTAC GGACCAAAATACATCACGCACAGCATCAGATC ATTCACCCCGTCAGTGGAACAATGCAAAGAAT CCATCGAACAGACTAAGCAGGGAACCTGGCT GAACCCTGGATTTCCGCCGCAGTCGTGTGGG TACGCAACCGTGACCGATGCAGAGGCCGTGA TCGTGCAAGTCACGCCGCATCACGTGCTTGT GGACGAGTACACCGGAGAATGGGTCGATTCC CAGTTCATCAACGGCAAGTGCTCCAACTACAT TTGCCCAACCGTGCACAACAGCACTACTTGG CACAGCGACTACAAAGTGAAGGGTCTGTGTG ATTCCAACCTGATCTCCATGGATATCACTTTCT TCTCGGAAGACGGCGAACTGTCCTCACTGGG CAAAGAAGGAACTGGGTTTCGCTCAAATTACT TCGCCTACGAAACTGGAGGAAAAGCCTGCAA GATGCAGTACTGCAAGCACTGGGGCGTGAGA CTACCCAGCGGTGTCTGGTTCGAGATGGCCG ATAAGGACCTGTTTGCAGCAGCGAGATTCCC GGAATGCCCTGAGGGATCGAGCATCTCCGCT CCAAGCCAAACTTCAGTGGACGTGAGCCTGA TCCAGGACGTGGAACGGATTCTCGACTACTC GCTGTGCCAGGAGACCTGGTCGAAGATCAGA GCGGGACTGCCCATCTCACCGGTGGACCTGT CCTACCTGGCGCCAAAGAATCCGGGCACTGG ACCGGCGTTCACCATCATCAACGGCACCCTC AAATACTTCGAGACGCGGTACATCCGGGTGG	KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNW HNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQ TKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTP HHVLVDEYTGEWVDSQFINGKCSNYICPTVHNS TTWHSDYKVKGLCDSNLISMDITFFSEDGELSSL GKEGTGFRSNYFAYETGGKACKMQYCKHWGV RLPSGVWFEMADKDLFAAARFPECPEGSSISAP SQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPI SPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIR VDIAAPILSRMVGMISGTTTEAELWDDWAPYED VEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHL SSKAQVFEHPHIQDAASQLPDDESLFFGDTGLS KNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVLR VGIHLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 74)

ACATCGCAGCTCCGATCCTCTCCCGGATGGT GGGAATGATCTCGGGGACTACTACCGAAGCC GAGCTCTGGGACGACTGGGCACCTTACGAGG ATGTCGAGATCGGACCTAACGGAGTGCTCCG GACCTCCTCCGGGTACAAGTTCCCTCTGTACA TGATCGGCCATGGCATGCTGGACTCGGATCT GCATCTGTCGTCCAAAGCACAGGTGTTTGAAC ACCCACACATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTCGGAGAC ACGGGCTTGTCAAAGAATCCCATCGAGCTGG TGGAAGGATGGTTTTCATCCTGGAAAAGCAGC ATCGCTTCATTCTTCTTCATCATTGGCCTGATC ATCGGCCTATTTCTAGTCCTGCGGGTGGGAAT TCATCTGTGCATCAAGCTCAAGCACACTAAGA AGCGGCAAATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 73)	

In another embodiment, the tandem expression cassette of the VSV-G plasmid encodes a CD80 targeting protein and a mutated VSV-G envelope protein. For example, in a specific embodiment, the tandem expression cassette may comprise a polynucleotide according to SEQ ID NO: 75, which encodes an amino acid sequence of SEQ ID NO: 76. Such an embodiment is described in more detail in Table 7. As illustrated in Table 7, the CD80 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide encoded by SEQ ID NO: 69 and having an amino acid sequence according to SEQ ID NO: 70.

In some embodiments, where the tandem expression cassette of the VSV-G plasmid encodes a CD80 targeting protein and a mutated VSV-G envelope protein, the tandem expression cassette may include a polynucleotide that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 75, which encodes an amino acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 76. In certain embodiments, the CD80 targeting protein is separated from the mutated VSV-G envelope protein via a P2A self-cleaving peptide encoded a nucleic acid sequence that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 69, and the P2A self-cleaving peptide includes an amino acid that has at least

about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 70.

TABLE 7: CD80 T cell targeting molecule co-expressed with mutant VSV-G

Full molecule	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG CAGTGGGGTCATTCACGTTACTAAAGAGGTCA AGGAGGTCGCAACATTGAGTTGTGGCCATAA CGTATCAGTTGAAGAACTCGCGCAGACACGG ATTTACTGGCAAAAGGAAAAGAAGATGGTGTT GACAATGATGAGCGGTGACATGAACATTTGG CCAGAGTACAAAAATCGAACGATATTCGATAT AACCAATAACTTGTCCATAGTAATACTTGCCTT GCGACCTTCTGACGAGGGAACGTATGAATGT GTAGTGCTTAAGTATGAAAAAGATGCCTTTAA GCGGGAACACTTGGCTGAGGTTACACTCTCC GTTAAGGCGGACTTTCCTACGCCGTCTATATC CGACTTCGAGATACCCACTTCTAACATTCGAC GCATCATTTGCTCAACCTCAGGTGGTTTCCCA GAGCCTCACTTGAGCTGGCTGGAGAATGGCG AAGAACTTAACGCAATCAATACCACGGTGTCC CAAGACCCGGAGACAGAGCTGTACGCCGTGT CATCCAAACTGGATTTTAACATGACGACAAAT CATAGTTTCATGTGTCTGATCAAATATGGGCA TCTCAGGGTGAATCAGACTTTTAATTGGAACA CTACCAAACAAGAGCACTTCCCAGATAATCTG TTGCCAAGCTGGGCGATAACTCTTATCTCCGT CAACGGTATCTTCGTAATTTGCTGCCTCACCT ATTGTTTCGCGCCTCGATGCCGAGAAGGCAG CGGCGCCACCAACTTCTCCCTGCTGAAGCAG GCCGGCGACGTGGAAGAAAACCCTGGCCCCA TGAAGTGTCTGCTGTACCTGGCGTTCCTGTTT ATCGGGGTGAACTGCAAGTTCACTATCGTGTT TCCGCACAACCAAAAGGGCAACTGGAAAAAC GTGCCTTCAAATTACCATTATTGCCCCAGCAG CTCGGACCTGAACTGGCACAATGACCTCATTG GAACCGCGCTGCAGGTGAAGATGCCACAGAG CCACAAGGCTATCCAGGCTGACGGATGGATG TGCCACGCGTCAAAATGGGTGACTACCTGCG ATTTCCGCTGGTACGGACCAAAATACATCACG CACAGCATCAGATCATTCACCCCGTCAGTGGA ACAATGCAAAGAATCCATCGAACAGACTAAGC AGGGAACCTGGCTGAACCCTGGATTTCCGCC GCAGTCGTGTGGGTACGCAACCGTGACCGAT GCAGAGGCCGTGATCGTGCAAGTCACGCCGC ATCACGTGCTTGTGGACGAGTACACCGGAGA ATGGGTCGATTCCCAGTTCATCAACGGCAAGT	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GVIHVTKEVKEVATLSCGHNVSVEELAQTRIYW QKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNL SIVILALRPSDEGTYECVVLKYEKDAFKREHLAE VTLSVKADFPTPSISDFEIPTSNIRRIICSTSGGFP EPHLSWLENGEELNAINTTVSQDPETELYAVSS KLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTK QEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPR CREGSGATNFSLLKQAGDVEENPGPMKCLLYL AFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYC PSSSDLNWHNDLIGTALQVKMPQSHKAIQADG WMCHASKWVTTCDFRWYGPKYITHSIRSFTPS VEQCKESIEQTKQGTWLNPGFPPQSCGYATVT DAEAVIVQVTPHHVLVDEYTGEWVDSQFINGKC SNYICPTVHNSTTWHSDYKVKGLCDSNLISMDIT FFSEDGELSSLGKEGTGFRSNYFAYETGGKAC KMQYCKHWGVRLPSGVWFEMADKDLFAAARF PECPEGSSISAPSQTSVDVSLIQDVERILDYSLC QETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTII NGTLKYFETRYIRVDIAAPILSRMVGMISGTTTEA ELWDDWAPYEDVEIGPNGVLRTSSGYKFPLYMI GHGMLDSDLHLSSKAQVFEHPHIQDAASQLPD DESLFFGDTGLSKNPIELVEGWFSSWKSSIASFF FIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEM NRLGK (SEQ ID NO: 76)

GCTCCAACTACATTTGCCCAACCGTGCACAAC AGCACTACTTGGCACAGCGACTACAAAGTGAA GGGTCTGTGTGATTCCAACCTGATCTCCATGG ATATCACTTTCTTCTCGGAAGACGGCGAACTG TCCTCACTGGGCAAAGAAGGAACTGGGTTTC GCTCAAATTACTTCGCCTACGAAACTGGAGGA AAAGCCTGCAAGATGCAGTACTGCAAGCACT GGGGCGTGAGACTACCCAGCGGTGTCTGGTT CGAGATGGCCGATAAGGACCTGTTTGCAGCA GCGAGATTCCCGGAATGCCCTGAGGGATCGA GCATCTCCGCTCCAAGCCAAACTTCAGTGGA CGTGAGCCTGATCCAGGACGTGGAACGGATT CTCGACTACTCGCTGTGCCAGGAGACCTGGT CGAAGATCAGAGCGGGACTGCCCATCTCACC GGTGGACCTGTCCTACCTGGCGCCAAAGAAT CCGGGCACTGGACCGGCGTTCACCATCATCA ACGGCACCCTCAAATACTTCGAGACGCGGTA CATCCGGGTGGACATCGCAGCTCCGATCCTC TCCCGGATGGTGGGAATGATCTCGGGGACTA CTACCGAAGCCGAGCTCTGGGACGACTGGGC ACCTTACGAGGATGTCGAGATCGGACCTAAC GGAGTGCTCCGGACCTCCTCCGGGTACAAGT TCCCTCTGTACATGATCGGCCATGGCATGCTG GACTCGGATCTGCATCTGTCGTCCAAAGCACA GGTGTTTGAACACCCACACATTCAAGACGCC GCCAGCCAGCTGCCGGACGATGAGTCGCTGT TCTTCGGAGACACGGGCTTGTCAAAGAATCC CATCGAGCTGGTGGAAGGATGGTTTTCATCCT GGAAAAGCAGCATCGCTTCATTCTTCTTCATC ATTGGCCTGATCATCGGCCTATTTCTAGTCCT GCGGGTGGGAATTCATCTGTGCATCAAGCTC AAGCACACTAAGAAGCGGCAAATCTACACTGA TATCGAGATGAATCGCCTGGGCAAG (SEQ ID NO: 75)	
CD80 signal peptide	
Nucleotide	Amino acid
ATGGGTCATACACGCCGCCAAGGAACCTCAC CATCTAAGTGCCCATATCTGAATTTCTTTCAAC TTCTCGTGCTGGCGGGGCTCAGTCATTTCTG CAGTGGGGTC (SEQ ID NO: 3)	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCS GV (SEQ ID NO: 4)
CD80 extracellular domain (ECD)	
Nucleotide	Amino acid
ATTCACGTTACTAAAGAGGTCAAGGAGGTCGC AACATTGAGTTGTGGCCATAACGTATCAGTTG AAGAACTCGCGCAGACACGGATTTACTGGCA AAAGGAAAAGAAGATGGTGTTGACAATGATGA GCGGTGACATGAACATTTGGCCAGAGTACAA AAATCGAACGATATTCGATATAACCAATAACTT GTCCATAGTAATACTTGCCTTGCGACCTTCTG ACGAGGGAACGTATGAATGTGTAGTGCTTAAG TATGAAAAAGATGCCTTTAAGCGGGAACACTT GGCTGAGGTTACACTCTCCGTTAAGGCGGAC TTTCCTACGCCGTCTATATCCGACTTCGAGAT ACCCACTTCTAACATTCGACGCATCATTTGCT CAACCTCAGGTGGTTTCCCAGAGCCTCACTTG AGCTGGCTGGAGAATGGCGAAGAACTTAACG	IHVTKEVKEVATLSCGHNVSVEELAQTRIYWQK EKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIV ILALRPSDEGTYECVVLKYEKDAFKREHLAEVTL SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEP HLSWLENGEELNAINTTVSQDPETELYAVSSKL DFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQ EHFPDNLLPS (SEQ ID NO: 6)

CAATCAATACCACGGTGTCCCAAGACCCGGA GACAGAGCTGTACGCCGTGTCATCCAAACTG GATTTTAACATGACGACAAATCATAGTTTCATG TGTCTGATCAAATATGGGCATCTCAGGGTGAA TCAGACTTTTAATTGGAACACTACCAAACAAG AGCACTTCCCAGATAATCTGTTGCCAAGC (SEQ ID NO: 5)	
CD80 transmembrane and intracellular domain	
Nucleotide	Amino acid
TGGGCGATAACTCTTATCTCCGTCAACGGTAT CTTCGTAATTTGCTGCCTCACCTATTGTTTCG CGCCTCGATGCCGAGAA (SEQ ID NO: 7)	WAITLISVNGIFVICCLTYCFAPRCRE (SEQ ID NO: 8)
P2A self-cleaving	
Nucleotide	Amino acid
GGCAGCGGCGCCACCAACTTCTCCCTGCTGA AGCAGGCCGGCGACGTGGAAGAAAACCCTG GCCCC (SEQ ID NO: 69)	GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 70)
VSV-G signal sequence	
Nucleotide	Amino acid
ATGAAGTGTCTGCTGTACCTGGCGTTCCTGTT TATCGGGGTGAACTGC (SEQ ID NO: 71)	MKCLLYLAFLFIGVNC (SEQ ID NO: 72)
Mutant VSV-G	
Nucleotide	Amino acid
AAGTTCACTATCGTGTTTCCGCACAACCAAAA GGGCAACTGGAAAAACGTGCCTTCAAATTACC ATTATTGCCCCAGCAGCTCGGACCTGAACTG GCACAATGACCTCATTGGAACCGCGCTGCAG GTGAAGATGCCACAGAGCCACAAGGCTATCC AGGCTGACGGATGGATGTGCCACGCGTCAAA ATGGGTGACTACCTGCGATTTCCGCTGGTAC GGACCAAAATACATCACGCACAGCATCAGATC ATTCACCCCGTCAGTGGAACAATGCAAAGAAT CCATCGAACAGACTAAGCAGGGAACCTGGCT GAACCCTGGATTTCCGCCGCAGTCGTGTGGG TACGCAACCGTGACCGATGCAGAGGCCGTGA TCGTGCAAGTCACGCCGCATCACGTGCTTGT GGACGAGTACACCGGAGAATGGGTCGATTCC CAGTTCATCAACGGCAAGTGCTCCAACTACAT TTGCCCAACCGTGCACAACAGCACTACTTGG CACAGCGACTACAAAGTGAAGGGTCTGTGTG ATTCCAACCTGATCTCCATGGATATCACTTTCT TCTCGGAAGACGGCGAACTGTCCTCACTGGG CAAAGAAGGAACTGGGTTTCGCTCAAATTACT TCGCCTACGAAACTGGAGGAAAAGCCTGCAA GATGCAGTACTGCAAGCACTGGGGCGTGAGA CTACCCAGCGGTGTCTGGTTCGAGATGGCCG ATAAGGACCTGTTTGCAGCAGCGAGATTCCC GGAATGCCCTGAGGGATCGAGCATCTCCGCT CCAAGCCAAACTTCAGTGGACGTGAGCCTGA TCCAGGACGTGGAACGGATTCTCGACTACTC GCTGTGCCAGGAGACCTGGTCGAAGATCAGA GCGGGACTGCCCATCTCACCGGTGGACCTGT CCTACCTGGCGCCAAAGAATCCGGGCACTGG ACCGGCGTTCACCATCATCAACGGCACCCTC AAATACTTCGAGACGCGGTACATCCGGGTGG	KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNW HNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQ TKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTP HHVLVDEYTGEWVDSQFINGKCSNYICPTVHNS TTWHSDYKVKGLCDSNLISMDITFFSEDGELSSL GKEGTGFRSNYFAYETGGKACKMQYCKHWGV RLPSGVWFEMADKDLFAAARFPECPEGSSISAP SQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPI SPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIR VDIAAPILSRMVGMISGTTTEAELWDDWAPYED VEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHL SSKAQVFEHPHIQDAASQLPDDESLFFGDTGLS KNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVLR VGIHLCIKLKHTKKRQIYTDIEMNRLGK (SEQ ID NO: 74)

ACATCGCAGCTCCGATCCTCTCCCGGATGGT GGGAATGATCTCGGGGACTACTACCGAAGCC GAGCTCTGGGACGACTGGGCACCTTACGAGG ATGTCGAGATCGGACCTAACGGAGTGCTCCG GACCTCCTCCGGGTACAAGTTCCCTCTGTACA TGATCGGCCATGGCATGCTGGACTCGGATCT GCATCTGTCGTCCAAAGCACAGGTGTTTGAAC ACCCACACATTCAAGACGCCGCCAGCCAGCT GCCGGACGATGAGTCGCTGTTCTTCGGAGAC ACGGGCTTGTCAAAGAATCCCATCGAGCTGG TGGAAGGATGGTTTTCATCCTGGAAAAGCAGC ATCGCTTCATTCTTCTTCATCATTGGCCTGATC ATCGGCCTATTTCTAGTCCTGCGGGTGGGAAT TCATCTGTGCATCAAGCTCAAGCACACTAAGA AGCGGCAAATCTACACTGATATCGAGATGAAT CGCCTGGGCAAG (SEQ ID NO: 73)	

Table 8: WT VSV-G reference sequence

Nucleotide	Amino acid
ATGAAGTGTCTGCTGTACCTGGCGTTCCTGTT TATCGGGGTGAACTGCAAGTTCACTATCGTGT TTCCGCACAACCAAAAGGGCAACTGGAAAAA CGTGCCTTCAAATTACCATTATTGCCCCAGCA GCTCGGACCTGAACTGGCACAATGACCTCATT GGAACCGCGCTGCAGGTGAAGATGCCAAAGA GCCACAAGGCTATCCAGGCTGACGGATGGAT GTGCCACGCGTCAAAATGGGTGACTACCTGC GATTTCCGCTGGTACGGACCAAAATACATCAC GCACAGCATCAGATCATTCACCCCGTCAGTG GAACAATGCAAAGAATCCATCGAACAGACTAA GCAGGGAACCTGGCTGAACCCTGGATTTCCG CCGCAGTCGTGTGGGTACGCAACCGTGACCG ATGCAGAGGCCGTGATCGTGCAAGTCACGCC GCATCACGTGCTTGTGGACGAGTACACCGGA GAATGGGTCGATTCCCAGTTCATCAACGGCAA GTGCTCCAACTACATTTGCCCAACCGTGCACA ACAGCACTACTTGGCACAGCGACTACAAAGT GAAGGGTCTGTGTGATTCCAACCTGATCTCCA TGGATATCACTTTCTTCTCGGAAGACGGCGAA CTGTCCTCACTGGGCAAAGAAGGAACTGGGT TTCGCTCAAATTACTTCGCCTACGAAACTGGA GGAAAAGCCTGCAAGATGCAGTACTGCAAGC ACTGGGGCGTGAGACTACCCAGCGGTGTCTG GTTCGAGATGGCCGATAAGGACCTGTTTGCA GCAGCGAGATTCCCGGAATGCCCTGAGGGAT CGAGCATCTCCGCTCCAAGCCAAACTTCAGT GGACGTGAGCCTGATCCAGGACGTGGAACGG ATTCTCGACTACTCGCTGTGCCAGGAGACCT GGTCGAAGATCAGAGCGGGACTGCCCATCTC ACCGGTGGACCTGTCCTACCTGGCGCCAAAG AATCCGGGCACTGGACCGGCGTTCACCATCA TCAACGGCACCCTCAAATACTTCGAGACGCG	MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNV PSNYHYCPSSSDLNWHNDLIGTALQVKMPKSH KAIQADGWMCHASKWVTTCDFRWYGPKYITHS IRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSC GYATVTDAEAVIVQVTPHHVLVDEYTGEWVDSQ FINGKCSNYICPTVHNSTTWHSDYKVKGLCDSN LISMDITFFSEDGELSSLGKEGTGFRSNYFAYET GGKACKMQYCKHWGVRLPSGVWFEMADKDLF AAARFPECPEGSSISAPSQTSVDVSLIQDVERIL DYSLCQETWSKIRAGLPISPVDLSYLAPKNPGT GPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMIS GTTTERELWDDWAPYEDVEIGPNGVLRTSSGY KFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDA ASQLPDDESLFFGDTGLSKNPIELVEGWFSSWK SSIASFFFIIGLIIGLFLVLRVGIHLCIKLKHTKKRQI YTDIEMNRLGK (SEQ ID NO: 78)

GTACATCCGGGTGGACATCGCAGCTCCGATC CTCTCCCGGATGGTGGGAATGATCTCGGGGA CTACTACCGAACGCGAGCTCTGGGACGACTG GGCACCTTACGAGGATGTCGAGATCGGACCT AACGGAGTGCTCCGGACCTCCTCCGGGTACA AGTTCCCTCTGTACATGATCGGCCATGGCATG CTGGACTCGGATCTGCATCTGTCGTCCAAAG CACAGGTGTTTGAACACCCACACATTCAAGAC GCCGCCAGCCAGCTGCCGGACGATGAGTCG CTGTTCTTCGGAGACACGGGCTTGTCAAAGAA TCCCATCGAGCTGGTGGAAGGATGGTTTTCAT CCTGGAAAAGCAGCATCGCTTCATTCTTCTTC ATCATTGGCCTGATCATCGGCCTATTTCTAGT CCTGCGGGTGGGAATTCATCTGTGCATCAAG CTCAAGCACACTAAGAAGCGGCAAATCTACAC TGATATCGAGATGAATCGCCTGGGCAAG (SEQ ID NO: 77)	

In other embodiments, the methods for producing LVV according to the present disclosure may utilize a five plasmid system. Where a five plasmid system is used, the LVV production system utilizes four plasmids from a third generation vector system (i.e., a transgene plasmid, a GagPol plasmid, an Env plasmid, and a VSV-G plasmid). In addition, a five plasmid system as described herein includes a fifth plasmid that encodes a targeting protein (the "targeting protein plasmid"). The GagPol and Env packaging plasmids included in a five plasmid system may be standard packaging plasmids as described in *Production of Lentiviral Vectors, Merten et al., Molecular Therapy – Methods & Clinical Development* (2016), 3, 16017. The transgene plasmid includes an expression cassette that encodes one or more CAR transgenes according to the present disclosure, and the VSV-G plasmid includes an expression cassette that encodes a mutant VSV-G as described herein.

In embodiments of LVV production methods that utilize a combination of five plasmids, the fifth plasmid is a targeting protein plasmid and includes an expression cassette that encodes one or more targeting proteins. For example the targeting protein plasmid may include an expression cassette that includes a polynucleotide that encodes a CD80 targeting protein, an anti-CD3 targeting protein, or both a CD80 targeting protein and an anti-CD3 targeting protein. In certain embodiments, a targeting protein vector plasmid an expression cassette comprising a polynucleotide according to SEQ ID NO: 1, which encodes a CD80 targeting protein according to SEQ ID NO: 2. In other

embodiments, a targeting protein plasmid includes an expression cassette comprising a polynucleotide according to SEQ ID NO: 9, which encodes an anti-CD3 targeting protein according to SEQ ID NO: 10. In other embodiments, a targeting protein plasmid includes a tandem expression cassette that comprises a polynucleotide according to SEQ ID NO: 21, which encodes a polypeptide according to SEQ ID NO: 22.

Engineered Lymphocytes

The LVV described herein can be used to modify targeted lymphocytes (e.g., T cells or NK cells) to express a CAR encoded by a transgene carried by the LVV. In certain embodiments, the engineered lymphocytes have been transduced by an LVV according to the present disclosure. In such embodiments, the engineered lymphocytes (e.g., T cells or NK cells) express a CAR encoded by the transgene carried by the LVV. These lymphocytes are also referred to herein as "CAR modified lymphocytes." In specific embodiments, the engineered T cells are referred to herein as "CAR modified T cells." In other specific embodiments, the engineered NK cells are referred to herein as "CAR modified NK cells." In specific embodiments the CAR modified T cell expresses a CAR encoded by a transgene carried by an LVV described herein and is selected from naïve T cells (CD45RA+, CCR7+, CD62L+, CD27+, CD45RO-), central memory T cells (CD45RA-, CD45RO$^+$, CD62L$^+$, CCR7+, CD27+), effector memory T cells (CD45RA-, CD45RO+, CCR7-, CD62L-, CD27-), γδ T cells, mucosal-associated invariant T (MAIT) cells, Tregs, natural killer T cells, and tissue resident T cells.

In certain embodiments, the lymphocytes (e.g., T cells or NK cells) can be primary cells or cell lines derived from human, mouse, rat, or other mammals. If obtained from a mammal, a lymphocyte can be obtained from numerous sources, including blood, bone marrow, lymph node, thymus, or other tissues or fluids. A lymphocyte composition (e.g., T cell composition or NK cell composition) may be enriched or purified. T cell lines are well known in the art, some of which are described in Sandberg *et al.*, *Leukemia 21*:230, 2000. In certain embodiments, the T cells lack endogenous expression of a TCRα gene, TCRβ gene, or both. Such T cells may naturally lack endogenous expression of TCRα and β chains, or may have been modified to block expression (*e.g.*, T cells from a transgenic mouse that does not

express TCR α and β chains or cells that have been manipulated to inhibit expression of TCR α and β chains) or to knockout a TCRα chain, a TCRβ chain, or both genes.

In some embodiments, prior to genetic modification of the lymphocytes (e.g., T cells or NK cells) with a polynucleotide encoding a CAR molecule, a source of lymphocytes (e.g., T cells or NK cells) may be obtained from a subject (*e.g.*, whole blood, peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue). The lymphocytes, T cells, or NK cells can be enriched or isolated from a sample taken from the subject using methods known in the art. For example, T cells can be enriched in a whole blood sample taken from a subject using known methods for hypotonic lysis of red blood cells or T cells can be isolated from a sample taken from a subject using known Ficoll® reduction techniques. Specific host cell subsets can be collected in accordance with known techniques and enriched or depleted by known techniques, such as affinity binding to antibodies, flow cytometry and/or immunomagnetic selection. In some embodiments, after enrichment and/or depletion steps, the T cells are placed in contact with LVV as disclosed such that T cells obtained from the subject are transduced by the LVV and express the CAR encoded by the transgene carried by the LVV. In some embodiments, after enrichment and/or depletion steps, the NK cells are placed in contact with LVV as disclosed such that NK cells obtained from the subject are transduced by the LVV and express the CAR encoded by the transgene carried by the LVV.

Generally, resting T cells, such as resting CD4 and CD8 lymphocytes, and resting NK cells are refractory to genetic transduction by lentiviral vectors. As a result, in order to facilitate transduction of T cells or NK cells using a lentiviral vector, the T cells or NK cells are typically activated in vitro using stimulation reagents before genetic modification via a lentiviral vector can occur. Following stimulation and transduction, the genetically modified cells are then typically expanded in vitro and subsequently reintroduced into a patient. However, LVV described herein are capable of transducing resting T cells and/or resting NK cells. In certain embodiments, methods of transducing T cells with an LVV according to the present description include, placing the LVV in contact with a population of T cells, wherein in the T cells are not activated and the T

cells are not exposed to a stimulating agent during the transduction process. In certain embodiments, methods of transducing NK cells with an LVV according to the present description include, placing the LVV in contact with a population of NK cells, wherein in the NK cells are not activated and the NK cells are not exposed to a stimulating agent during the transduction process.

The expression of a CAR molecule on host cells may be functionally characterized according to any of a large number of art-accepted methodologies for assaying host T cell activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific. Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, CTL activity (*e.g.*, by detecting ^{51}Cr or Europium release from pre-loaded target cells, induction of caspase activity in target cells, extracellular release of lactate dehydrogenase by target cells), changes in T cell phenotypic marker expression, and other measures of T cell functions. Assaying host NK cell activity may also be assayed using similar methodologies. Procedures for performing these and similar assays are may be found, for example, in Lefkovits (*Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*, 1998). *See*, *also*, *Current Protocols in Immunology*; Weir, *Handbook of Experimental Immunology*, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) *Selected Methods in Cellular Immunology*, Freeman Publishing, San Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein. Cytokine levels may be determined according to methods known in the art, including for example, ELISA, ELISPOT, intracellular cytokine staining, flow cytometry, and any combination thereof (*e.g.,* intracellular cytokine staining and flow cytometry). Immune cell proliferation and clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as circulating lymphocytes in samples of peripheral blood cells or cells from lymph nodes, stimulating the cells with antigen, and measuring cytokine production, cell proliferation and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive assays, such as MTT assays and the like.

Methods of & Compositions for Treatment

In one aspect, the present disclosure provides methods of treating a disease in a subject comprising administering to the subject an effective amount of an LVV as described herein, a CAR modified lymphocyte (e.g., T cell or NK cell) as described herein, or pharmaceutical compositions thereof. In another aspect, the methods of treating a disease in a subject according to the present description include administering to the subject an effective amount of an LVV as described herein, a CAR modified lymphocyte (e.g., T cell or NK cell) as described herein, or pharmaceutical compositions thereof, in combination with one or more additional therapeutic agents.

Diseases that may be treated with the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) according to the present description include cancers. Adoptive immune and gene therapies are promising treatments for various types of cancer (Morgan *et al*., *Science 314*:126, 2006; Schmitt *et al*., *Hum. Gene Ther. 20*:1240, 2009; June, *J. Clin. Invest. 117*:1466, 2007) and infectious disease (Kitchen *et al*., *PLoS One 4*:38208, 2009; Rossi *et al*., *Nat. Biotechnol. 25*:1444, 2007; Zhang *et al*., *PLoS Pathog. 6*:e1001018, 2010; Luo *et al*., *J. Mol. Med. 89*:903, 2011).

A wide variety of cancers, including solid tumors and leukemias are amenable to the compositions and methods disclosed herein. Exemplary cancers that may be treated using the receptors, modified host cells, and composition described herein include adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid leukemia; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; and carcinoma (*e.g.*, Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell). Additional types of cancers that may be treated using the receptors, modified host cells, and composition described herein include histiocytic disorders; malignant histiocytosis; leukemia; Hodgkin's disease; immunoproliferative small; non-Hodgkin's lymphoma; plasmacytoma; multiple myeloma; chronic myeloid leukemia (CML); acute myeloid

leukemia (AML); plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; trophoblastic tumor. Further, the following types of cancers are also contemplated as amenable to treatment using the receptors, modified host cells, and composition described herein: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; sertoli cell tumor; theca cell tumor; leimyoma; leiomyosarcoma; myoblastoma; myomma; myosarcoma; rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin. The types of cancers that may be treated also include angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma; hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; hemangiosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms; nerofibromatosis; cervical dysplasia, and peritoneal cancer.

Examples of hyperproliferative disorders amenable to therapy using the receptors, modified host cells, and composition described herein include B-cell cancers (B-cell malignancies), including B-cell lymphomas (such as various forms of Hodgkin's disease, non-Hodgkin's lymphoma (NHL) or central nervous system lymphomas), leukemias (such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia, B cell blast transformation of chronic myeloid leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia, and myelomas (such as multiple myeloma). Additional B cell cancers that may be treated using the receptors, modified host cells, and composition described herein include small lymphocytic

lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

The CAR modified lymphocytes included in the compositions disclosed herein and administered to a subject may include, CAR modified T cells, *e.g.*, CD4+ T cells, CD8+ T cells, Natural Killer T cells, gamma delta T cells, or MAIT cells, or CAR modified NK cells. In certain embodiments, methods of treating a subject comprise administering an effective amount of an LVV as described herein or CAR modified lymphocytes (*i.e.,* recombinant cells that express one or more CARs) according to the present disclosure. In some embodiments, CAR modified T cells are administered to a subject. In some embodiments, CAR modified NK cells are administered to a subject. The CAR modified lymphocytes (e.g., T cells or NK cells) may be xenogeneic, syngeneic, allogeneic, or autologous to the subject.

Pharmaceutical compositions including the LVV or CAR engineered lymphocytes may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as the condition of the patient, size, weight, body surface area, age, sex, type and severity of the disease, particular therapy to be administered, particular form of the active ingredient, time and the method of administration, and other drugs being administered concurrently. The present disclosure provides pharmaceutical compositions comprising LVV or CAR modified lymphocytes and a pharmaceutically acceptable carrier, diluent, or excipient. Suitable excipients include water, saline, dextrose, glycerol, or the like and combinations thereof. Other suitable

infusion medium can be any isotonic medium formulation, including saline, Normosol R (Abbott), Plasma-Lyte A (Baxter), 5% dextrose in water, or Ringer's lactate.

A treatment effective amount of engineered lymphocytes (e.g., T cells or NK cells) in a pharmaceutical composition is at least one cell (for example, one CAR modified T cell) and is more typically greater than 10^2 cells, for example, up to 10^6, up to 10^7, up to 10^8 cells, up to 10^9 cells, up to 10^{10} cells, or up to 10^{11} cells or more. In certain embodiments, the cells are administered in a range from about 10^6 to about 10^{10} cells/m^2, preferably in a range of about 10^7 to about 10^9 cells/m^2. The number of cells will depend upon the ultimate use for which the composition is intended as well the type of cells included therein. For example, a composition comprising T cells modified to contain a CAR will comprise a T cell population containing from about 5% to about 95% or more of such cells. In certain embodiments, a composition comprising CAR modified T cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. In certain embodiments, a composition comprising CAR modified NK cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. For uses provided herein, the lymphocytes (e.g., T cells or NK cells) are generally in a volume of a liter or less, 500 mls or less, 250 mls or less, or 100 mls or less. Hence the density of the desired cells is typically greater than 10^4 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The lymphocytes (e.g., T cells or NK cells) may be administered as a single infusion or in multiple infusions over a range of time. Repeated infusions of CAR modified lymphocytes (e.g., T cells or NK cells) may be separated by days, weeks, months, or even years if relapses of disease or disease activity are present. A clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^6, 10^7, 10^8, 10^9, 10^{10}, or 10^{11} cells. A preferred dose for administration of a host cell comprising a recombinant expression vector as described herein is about 10^7 cells/m^2, about 5×10^7 cells/m^2, about 10^8 cells/m^2, about 5×10^8 cells/m^2, about 10^9 cells/m^2, about 5×10^9 cells/m^2, about 10^{10} cells/m^2, about 5×10^{10} cells/m^2, or about 10^{11} cells/m^2.

The LVV and CAR modified lymphocyte (e.g., T cell or NK cell) compositions as described herein may be administered to a subject intravenously, intraperitoneally, intratumorly, into the bone marrow (*e.g.*, intraosseous administration), into the lymph node (intranodally), and /or into cerebrospinal fluid.

The LVV vectors and CAR modified lymphocyte (e.g., T cell or NK cell) compositions may be administered to a subject in combination with one or more additional therapeutic agents. Examples of therapeutic agents that may be administered in combination with the LVV vectors or CAR modified lymphocyte (e.g., T cell or NK cell) according to the present description include radiation therapy, adoptive cellular immunotherapy agent (*e.g.*, recombinant TCR, enhanced affinity TCR, CAR, TCR-CAR, scTCR fusion protein, dendritic cell vaccine), antibody therapy, immune checkpoint molecule inhibitor therapy, UV light therapy, electric pulse therapy, high intensity focused ultrasound therapy, oncolytic virus therapy, or a pharmaceutical therapy, such as a chemotherapeutic agent, a therapeutic peptide, a hormone, an aptamer, antibiotic, anti-viral agent, anti-fungal agent, anti-inflammatory agent, a small molecule therapy, or any combination thereof.

Radiation therapy includes external beam radiation therapy (e.g., conventional external beam radiation therapy, stereotactic radiation, 3-dimensional conformal radiation therapy, intensity-modulated radiation therapy, volumetric modulated arc therapy, particle therapy, proton therapy, and auger therapy), brachytherapy, systemic radioisotope therapy, intraoperative radiotherapy, or any combination thereof.

Exemplary antibodies that may be used in conjunction with the LVV or CAR modified lymphocyte (e.g., T cell or NK cell) compositions described herein include rituxmab, pertuzumab, trastuzumab, alemtuzumab, Ibritumomab tiuxetan, Brentuximab vedotin, cetuximab, bevacizumab, abciximab, adalimumab, alefacept, basilizimab, belimumab, bezlotoxumab, canakinumab, certolizumab pegol, daclizumab, denosumab, efalizumab, golimumab, olaratumab, palivizumab, panitumumab, and tocilizumab.

Exemplary inhibitors of immune checkpoint molecules that that may be used in conjunction with the LVV or CAR modified lymphocyte (e.g., T cell or NK cell) compositions described herein include checkpoint inhibitors targeting PD-L1, PD-L2,

CD80, CD86, B7-H3, B7-H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, CEACAM-3, CEACAM-5, PVRL2, PD-1, CTLA-4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, PVRIG/CD112R, or any combination thereof. In certain embodiments, an immune checkpoint inhibitor may be an antibody, a peptide, an RNAi agent, or a small molecule. An antibody specific for CTLA-4 may be ipilimumab or tremelimumab. An antibody specific for PD-1 may be pidilizumab, nivolumab, or pembrolizumab. An antibody specific for PD-L1 may be durvalumab, atezolizumab, or avelumab.

Exemplary chemotherapeutics that may be used in conjunction with the LVV or CAR modified lymphocyte (e.g., T cell or NK cell) compositions described herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and a DNA repair inhibitor.

A chemotherapeutic includes non-specific cytotoxic agents that inhibit mitosis or cell division, as well as molecularly targeted therapy that blocks the growth and spread of cancer cells by targeting specific molecules that are involved in tumor growth, progression, and metastasis (*e.g.*, oncogenes). Exemplary non-specific chemotherapeutics for use in conjunction with the expression cassette compositions described herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), hypomethylating agent, and a DNA repair inhibitor.

Examples of chemotherapeutic agents considered for use in combination therapies contemplated herein include vemurafenib, dabrafenib, trametinib, cobimetinib, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-

pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®),fdabra tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), ibrutinib, venetoclax, crizotinib, alectinib, brigatinib, ceritinib, and vinorelbine (Navelbine®).

Exemplary alkylating agents for use in combination therapies contemplated herein include nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents for use in combination therapies contemplated herein include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D,

Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).

Exemplary platinum based agents for use in combination therapies contemplated herein include carboplatin, cisplatin, oxaliplatin, nedaplatin, picoplatin, satraplatin, phenanthriplatin, and triplatin tetranitrate.

Exemplary hypomethylating agents for use in combination therapies include azacitidine and decitabine.

Exemplary molecularly targeted inhibitors for use in combination therapies contemplated herein include small molecules that target molecules involved in cancer cell growth and survival, including for example, receptor tyrosine kinase inhibitors, RAF inhibitors, BCL-2 inhibitors, ABL inhibitors, TRK inhibitors, c-KIT inhibitors, c-MET inhibitors, CDK4/6 inhibitors, FAK inhibitors, FGFR inhibitors, FLT3 inhibitors, IDH1 inhibitors, IDH2 inhibitors, PDGFRA inhibitors, and RET inhibitors

Exemplary molecularly targeted therapy includes hormone antagonists, signal transduction inhibitors, gene expression inhibitors (*e.g.*, translation inhibitors), apoptosis inducers, angiogenesis inhibitors (*e.g.*, a VEGF pathway inhibitor), tyrosine kinase inhibitors (*e.g.*, an EGF/EGFR pathway inhibitor), growth factor inhibitors, GTPase inhibitors, serine/threonine kinase inhibitors, transcription factor inhibitors, inhibitors of driver mutations associated with cancer, B-Raf inhibitors, RAF inhibitors, a MEK

inhibitors, mTOR inhibitors, adenosine pathway inhibitors, EGFR inhibitors, PI3K inhibitors, BCL2 inhibitors, VEGFR inhibitors, MET inhibitors, MYC inhibitors, BCR-ABL inhibitors, ABL inhibitors, HER2 inhibitors, H-RAS inhibitors, K-RAS inhibitors, PDGFR inhibitors, ALK inhibitors, ROS1 inhibitors, BTK inhibitors, TRK inhibitors, c-KIT inhibitors, c-MET inhibitors, CDK4/6 inhibitors, FAK inhibitors, FGFR inhibitors, FLT3 inhibitors, IDH1 inhibitors, IDH2 inhibitors, PARP inhibitors, PARP inhibitors, PDGFRA inhibitors, and RET inhibitors.

Exemplary angiogenesis inhibitors include, without limitation A6 (Angstrom Pharmaceuticals), ABT-510 (Abbott Laboratories), ABT-627 (Atrasentan) (Abbott Laboratories/Xinlay), ABT-869 (Abbott Laboratories), Actimid (CC4047, Pomalidomide) (Celgene Corporation), AdGVPEDF.11D (GenVec), ADH-1 (Exherin) (Adherex Technologies), AEE788 (Novartis), AG-013736 (Axitinib) (Pfizer), AG3340 (Prinomastat) (Agouron Pharmaceuticals), AGX1053 (AngioGenex), AGX51 (AngioGenex), ALN-VSP (ALN-VSP O2) (Alnylam Pharmaceuticals), AMG 386 (Amgen), AMG706 (Amgen), Apatinib (YN968D1) (Jiangsu Hengrui Medicine), AP23573 (Ridaforolimus/MK8669) (Ariad Pharmaceuticals), AQ4N (Novavea), ARQ 197 (ArQule), ASA404 (Novartis/Antisoma), Atiprimod (Callisto Pharmaceuticals), ATN-161 (Attenuon), AV-412 (Aveo Pharmaceuticals), AV-951 (Aveo Pharmaceuticals), Avastin (Bevacizumab) (Genentech), AZD2171 (Cediranib/Recentin) (AstraZeneca), BAY 57-9352 (Telatinib) (Bayer), BEZ235 (Novartis), BIBF1120 (Boehringer Ingelheim Pharmaceuticals), BIBW 2992 (Boehringer Ingelheim Pharmaceuticals), BMS-275291 (Bristol-Myers Squibb), BMS-582664 (Brivanib) (Bristol-Myers Squibb), BMS-690514 (Bristol-Myers Squibb), Calcitriol, CCI-779 (Torisel) (Wyeth), CDP-791 (ImClone Systems), Ceflatonin (Homoharringtonine/HHT) (ChemGenex Therapeutics), Celebrex (Celecoxib) (Pfizer), CEP-7055 (Cephalon/Sanofi), CHIR-265 (Chiron Corporation), NGR-TNF, COL-3 (Metastat) (Collagenex Pharmaceuticals), Combretastatin (Oxigene), CP-751,871(Figitumumab) (Pfizer), CP-547,632 (Pfizer), CS-7017 (Daiichi Sankyo Pharma), CT-322 (Angiocept) (Adnexus), Curcumin, Dalteparin (Fragmin) (Pfizer), Disulfiram (Antabuse), E7820 (Eisai Limited), E7080 (Eisai Limited), EMD 121974 (Cilengitide) (EMD Pharmaceuticals), ENMD-1198 (EntreMed), ENMD-2076 (EntreMed), Endostar (Simcere), Erbitux (ImClone/Bristol-Myers Squibb), EZN-2208

(Enzon Pharmaceuticals), EZN-2968 (Enzon Pharmaceuticals), GC1008 (Genzyme), Genistein, GSK1363089 (Foretinib) (GlaxoSmithKline), GW786034 (Pazopanib) (GlaxoSmithKline), GT-111 (Vascular Biogenics Ltd.), IMC-1121B (Ramucirumab) (ImClone Systems), IMC-18F1 (ImClone Systems), IMC-3G3 (ImClone LLC), INCB007839 (Incyte Corporation), INGN 241 (Introgen Therapeutics), Iressa (ZD1839/Gefitinib), LBH589 (Faridak/Panobinostst) (Novartis), Lucentis (Ranibizumab) (Genentech/Novartis), LY317615 (Enzastaurin) (Eli Lilly and Company), Macugen (Pegaptanib) (Pfizer), MEDI522 (Abegrin) (MedImmune), MLN518 (Tandutinib) (Millennium), Neovastat (AE941/Benefin) (Aeterna Zentaris), Nexavar (Bayer/Onyx), NM-3 (Genzyme Corporation), Noscapine (Cougar Biotechnology), NPI-2358 (Nereus Pharmaceuticals), OSI-930 (OSI), Palomid 529 (Paloma Pharmaceuticals, Inc.), Panzem Capsules (2ME2) (EntreMed), Panzem NCD (2ME2) (EntreMed), PF-02341066 (Pfizer), PF-04554878 (Pfizer), PI-88 (Progen Industries/Medigen Biotechnology), PKC412 (Novartis), Polyphenon E (Green Tea Extract) (Polypheno E International, Inc.), PPI-2458 (Praecis Pharmaceuticals), PTC299 (PTC Therapeutics), PTK787 (Vatalanib) (Novartis), PXD101 (Belinostat) (CuraGen Corporation), RAD001 (Everolimus) (Novartis), RAF265 (Novartis), Regorafenib (BAY73-4506) (Bayer), Revlimid (Celgene), Retaane (Alcon Research), SN38 (Liposomal) (Neopharm), SNS-032 (BMS-387032) (Sunesis), SOM230 (Pasireotide) (Novartis), Squalamine (Genaera), Suramin, Sutent (Pfizer), Tarceva (Genentech), TB-403 (Thrombogenics), Tempostatin (Collard Biopharmaceuticals), Tetrathiomolybdate (Sigma-Aldrich), TG100801 (TargeGen), Thalidomide (Celgene Corporation), Tinzaparin Sodium, TKI258 (Novartis), TRC093 (Tracon Pharmaceuticals Inc.), VEGF Trap (Aflibercept) (Regeneron Pharmaceuticals), VEGF Trap-Eye (Regeneron Pharmaceuticals), Veglin (VasGene Therapeutics), Bortezomib (Millennium), XL184 (Exelixis), XL647 (Exelixis), XL784 (Exelixis), XL820 (Exelixis), XL999 (Exelixis), ZD6474 (AstraZeneca), Vorinostat (Merck), and ZSTK474.

Exemplary B-Raf inhibitors include vemurafenib, dabrafenib, and encorafenib.

Exemplary MEK inhibitors include binimetinib, cobimetinib, refametinib, selumetinib, and trametinib.

Exemplary BTK inhibitors include ibrutinib, Loxo-305, tirabrutinib, GDC-0853, acalabrutinib, ONO-4059, spebrutinib, BGB-3111, HM71224, and M7583.

Exemplary TRK inhibitors include entrectinib, larotrectinib, CH7057288, ONO-7579, LOXO-101, lestaurtinib, and LOXO-195.

Exemplary c-KIT inhibitors include imatinb, sunitinb, and ponatinib.

Exemplary c-MET inhibitors include capmatinib, crizotinib, tivantinib, onartuzumab, INCB28060, AMG-458, savolitinib, and tepotinib.

Exemplary CDK4/6 inhibitors include palbociclib, ribociclib, abemaciclib, and trilaciclib.

Exemplary FAK inhibitors include defactinib, GSK2256098, BI853520, and PF-00562271.

Exemplary FGFR inhibitors include erdafitinib, pemigatinib, infigratinib, rogaratinib, AZD4547, BGJ398, FP-1039, and ARQ 087.

Exemplary FLT-3 inhibitors include quizartinib, crenolanib, gilteritinib, midostaurin, and lestaurtinib.

Exemplary IDH1 inhibitors include ivosidenib, BAY-1436032, and AGI-5198.

An exemplary IDH2 inhibitor includes enasidenib.

Exemplary PARP inhibitors include talazoparib, niraparib, rucaparib, olaparib, veliparib, CEP 9722, E7016, AG014699, MK4827, BMN-673, and Pamiparib (BGB-290).

Exemplary PDGFRA inhibitors include imatinib, regorafenib, crenolanib, and olaratumab.

Exemplary pan-RAF inhibitors include belvarafenib, LXH254, LY3009120, INU-152, and HM95573.

Exemplary RET inhibitors include lenvatinib, alectinib, vandetanib, cabozantinib, BLU-667, and LOXO-292.

Exemplary ROS1 inhibitors include ceritinib, lorlatinib, entrectinib, crizotinib, TPX-0005, and DS-6051b.

Exemplary Vascular Endothelial Growth Factor (VEGF) receptor inhibitors include, but are not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)—((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino]-N-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); and Aflibercept (Eylea®).

Exemplary EGF pathway inhibitors include, without limitation tyrphostin 46, EKB-569, erlotinib (Tarceva®), gefitinib (Iressa®), erbitux, nimotuzumab, lapatinib (Tykerb®), cetuximab (anti-EGFR mAb), ^{188}Re-labeled nimotuzumab (anti-EGFR mAb), and those compounds that are generically and specifically disclosed in WO 97/02266, EP 0 564 409, WO 99/03854, EP 0 520 722, EP 0 566 226, EP 0 787 722, EP 0 837 063, U.S. Pat. No. 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and WO 96/33980. Exemplary EGFR antibodies include, but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Trastuzumab (Herceptin®); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS

339151-96-1); and ch806 (mAb-806, CAS 946414-09-1). Exemplary Epidermal growth factor receptor (EGFR) inhibitors include, but not limited to, Erlotinib hydrochloride (Tarceva®); ceritinib; brigatinib; osimeritinib; icotinib; Gefitnib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3″S″)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-[4-[[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS 187724-61-4); rocelitinib.

Exemplary mTOR inhibitors include, without limitation, rapamycin (Rapamune®), and analogs and derivatives thereof; SDZ-RAD; Temsirolimus (Torisel®; also known as CCI-779); Ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2[(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-d]pyrimidin-7(8H)-one (PF04691502, CAS 1013101-36-4); and N^2-[1,4-dioxo-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine-, inner salt (SF1126, CAS 936487-67-1).

Exemplary Phosphoinositide 3-kinase (PI3K) inhibitors include, but are not limited to, duvelisib, idelalisib, 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-

yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

Exemplary Protein Kinase B (PKB) or AKT inhibitors include, but are not limited to. 8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-f][1,6]naphthyridin-3(2H)-one (MK-2206, CAS 1032349-93-1); Perifosine (KRX0401); 4-Dodecyl-N-1,3,4-thiadiazol-2-yl -benzenesulfonamide (PHT-427, CAS 1191951-57-1); 4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(3S)-3-piperidinylmethoxy]-1H-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol (GSK690693, CAS 937174-76-0); 8-(1-Hydroxyethyl)-2-methoxy-3-[(4-methoxyphenyl)methoxy]-6H-dibenzo[b,d]pyran-6-one (palomid 529, P529, or SG-00529); Tricirbine (6-Amino-4-methyl-8-(β-D-ribofuranosyl)-4H,8H-pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine); (αS)-α-[[[5-(3-Methyl-1H-indazol-5-yl)-3-pyridinyl]oxy]methyl]-benzeneethanamine (A674563, CAS 552325-73-2); 4-[(4-Chlorophenyl)methyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-4-piperidinamine (CCT128930, CAS 885499-61-6); 4-(4-Chlorophenyl)-4-[4-(1H pyrazol-4-yl)phenyl]-piperidine (AT7867, CAS 857531-00-1); and Archexin (RX-0201, CAS 663232-27-7).

In certain embodiments, a tyrosine kinase inhibitor used in combination with the LVV or CAR modified T cells is an anaplastic lymphoma kinase (ALK) inhibitor. Exemplary ALK inhibitors include crizotinib, ceritinib, alectinib, brigatinib, dalantercept, entrectinib, and lorlatinib.

In certain embodiments where the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered in combination with one or more additional therapies, the LVV, CAR modified lymphocytes (e.g., T cells or NK cells), or one or more additional therapies may be administered at a dose that might otherwise be considered sub-therapeutic if administered as a monotherapy. Combination therapy includes administration of an LVV or CAR modified lymphocyte (e.g., T cell or NK cell) composition as described herein before an additional therapy (*e.g.*, 1 day to 30 days or more before the additional therapy), concurrently with an additional therapy (on the same day), or after an additional therapy (*e.g.*, 1 day – 30 days or more after the additional therapy). In certain embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered concurrently with the one or more additional therapies. In further embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days before or after administration of the one or more additional therapies. In still further embodiments, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) are administered within 4 weeks, within 3 weeks, within 2 weeks, or within 1 week before or after administration of the one or more additional therapies. Where the one or more additional therapies involves multiple doses, the LVV or CAR modified lymphocytes (e.g., T cells or NK cells) may be administered before or after the initial dose of the one or more additional therapies, after the final dose of the one or more additional therapies, or in between multiple doses of the one or more additional therapies.

Subjects that can be treated by the compositions and methods of the present disclosure include animals, such as humans, primates, cows, horses, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, or pigs. The subject may be male or female, and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

EXAMPLES

Example 1: Methods for generating lentiviral vectors with defined tropism

Self-inactivating lentiviral vectors (LVV) as described herein are produced using a third generation production system. An expression plasmid that contains the gene sequences desired for delivery by the LVV is combined at defined ratios with three packaging plasmids, VSV-G, GagPol, and Rev, and used to transfect HEK293 producer cells. Productive viral particles are harvested from the HEK293 culture media 2-3 days later.

The tropism of the resulting LVV is tested to determine that it can be redirected using a non-viral targeting protein as described herein expressed from one of the three packaging plasmids or by addition of a fifth plasmid to the transfection mix. The LVV vector includes a mutated VSV-G is envelope protein, where the mutated VSV-G includes mutations that abolish binding of the mutated VSV-G to its native receptor such that viral entry occurs via the non-viral, tropism defining targeting protein.

In a first method, a tropism-defining targeting protein derived from CD80 is expressed from the VSV-G packaging plasmid. CD80 is the natural ligand to T cell surface receptors, CD28 and CTLA4, and provides tropism to T cells. A modified CD80 molecule is cloned downstream of the mutated VSV-G, which contains mutations to abolish LDL receptor binding and is used to package a GFP-expressing LVV. CD80 and VSV-G are expressed at relatively equivalent levels on the surface of HEK293 producer cells, suggesting comparable decoration of the LVV particles. LVV generated with this approach are capable of transducing T cells as assessed by GFP expression.

In a second method, the tropism-defining receptor is cloned into the GagPol or the Rev packaging plasmid and transfected at higher or lower molar concentrations to the VSV-G plasmid, respectively. These modified plasmids are used to package a GFP-expressing LVV in separate experiments. Assessment of VSV-G and CD80 expression on the HEK-293 producer cells shows relative expression levels trended with the molar concentrations of the packaging plasmids. LVV generated with either of these packaging approaches are capable of transducing T cells as assessed by GFP expression.

In a third method, the tropism defining CD80 receptor is added as an additional plasmid during transfection of HEK293 cells intended to produce LVV. The amount of the CD80 plasmid used during LVV production is associated with the expression on HEK293 producer cells and is likely representative of decoration of the LVV particles. LVV generated with this packaging approach are capable of transducing T cells as assessed by GFP expression.

Example 2: Methods for ex vitro generation of tumor-reactive T cells engineered to express a chimeric antigen receptor (CAR) using a lentiviral vector containing T cell-specific tropism

Compared to standard lentiviral vectors (LVV) that use common third generation packaging systems, LVV packaged to redirect tropism as provided herein may have improved transduction efficiencies and specificity. The levels of available viral particles are likely increased when transducing a specific cell type as part of a heterogeneous mixture, as the LVV particles are not taken up by other, non-desired cell types. Here we test if improved transduction of T cells with a CAR molecule is possible in a heterogenous peripheral blood mononuclear cell (PBMC) mixture. We use a GFP reporter or one of two representative CAR molecules: an anti-CD19 CAR used to treat CD19-positive lymphomas and leukemias; or an anti-BCMA CAR used to treat Multiple Myeloma. The LVV is packaged to decorate the envelope with CD80 and redirect tropism toward the T cell surface molecules, CD28 and CTLA4.

In one study, we test the efficiency of T cell transduction in human whole blood from healthy donors obtained after informed consent. Standard LVV or LVV with T cell-redirected tropism is combined with whole blood from matched donors. T cells are purified from the whole blood using magnetically labelled beads followed by column separation. Transduction of T cells is assessed by quantitative PCR using primers specific for LVV, which demonstrate higher levels of T cell transduction are achieved using the T cell tropic LVV compared to standard LVV.

In a second study, we test the efficiency of T cell transduction of human T cells after gradient sedimentation of PBMC or after hypotonic red blood cell lysis. Healthy

donors provide cells by leukapheresis after informed consent. The PBMC are enriched using gradient sedimentation and matched donors are transduced with standard LVV or LVV with T cell redirected tropism. Transduction is assessed as before using quantitative PCR. T cells transduced using the T cell tropic LVV show higher levels of transduction compared to standard LVV.

Example 3: Tumor-specific T cells generated using a T cell targeted lentiviral vector

Using methods described herein, T cells expressing an anti-CD19 CAR or anti-BCMA CAR are generated by transducing PBMC from healthy donors. The T cell transductions are performed in the absence of any T cell stimulation typically required for efficient LVV-mediated gene transfer. Efficient transduction is only evident in T cells and relatively absent in non-T cells. Assessment of T cell lineages after transduction shows efficient gene transfer into naïve T cells. Taken together, these results demonstrate a unique capability of this T cell targeted virus to transduce resting T cells with high efficiency.

Anti-tumor activity of the CAR T cells is then assessed by flow cytometry after co-culture with CD19 or BCMA -positive cell lines and compared to culture with antigen negative cell lines. Anti-CD19 CAR T cells exhibit increased expression of T cell activation markers after culture with CD19-positive cell lines that is not observed with CD19-negative cell lines. Similar results are achieved with anti-BCMA CAR T cells after culture with BCMA-positive cells lines.

Example 4: Methods of T cell-specific in vivo delivery of genetic material using a T cell tropic lentiviral vector

The efficient and specific transduction of T cells with a T cell targeted LVV could permit safe and effective delivery of genetic material to T cells in vivo. We test this hypothesis using a humanized mouse model after engraftment of NSG mice with human PBMC. We assess in vivo transduction after infusion of a T cell targeted LVV expressing luciferase for in situ imaging. We detect luciferase-positive cells in animals

infused by intravenous, intraperitoneal, intraosseous injection but not in saline-treated animals.

In another set of experiments, humanized mice are infused intravenously with a GFP-expressing, T cell targeted LVV. Peripheral blood and splenic cells are harvested, and T cell specificity is assessed by flow cytometry. GFP positive cells are only detected in human T cells confirming T cell specificity of LVV transduction.

Specific T cell transduction has a key safety advantages to other in vivo CAR delivery approaches by minimizing risk of transducing tumor cells. We test this hypothesis in PBMC from chronic lymphocytic leukemia patients with detectable CD19 tumor cells. The PBMC are transduced with T cell targeted LVV and transduced cells are assessed using flow cytometry. We find efficient transduction of T cells without affecting tumor cells.

CLAIMS

1. A lentiviral vector, comprising:

a viral envelope comprising:

a heterologous lymphocyte targeting protein, the lymphocyte targeting protein comprising:

an extracellular domain comprising a lymphocyte targeting domain; and

a transmembrane domain; and

a mutated VSV-G envelope protein that exhibits inhibited binding to an LDL-R but mediates membrane fusion; and

an expression cassette that comprises a heterologous transgene.

2. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein comprising an extracellular domain comprising a T cell targeting domain.

3. The lentiviral vector according to claim 1, wherein the heterologous lymphocyte targeting protein is a heterologous natural killer (NK) cell targeting protein comprising an extracellular domain comprising a NK cell targeting domain.

4. A lentiviral vector according to claim 1 or 2, wherein the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein that specifically binds to:

(a) a T cell marker selected from CD3, CD80, 4-1BB, AhR, CD3, CD2, CD7, CD4, CD8, CD25, CD44, CD45RA, CD47, CD62L, CD69, CD94, CD95, CD127, CD161, CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CCR10, PD-1, TCRa/b, CD5, CD27, CD45RO, CD45RB, CD57, CD103, CD122, P2RX7, TIGIT, LAG-3, TIM-3, and IL6ST;

(b) a γδ T cell marker selected from γδ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314);

(c) an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, and IL-21R; or

(d) a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), and VLA4 (alpha4beta1 integrin).

5. A lentiviral vector according to claim 1, 2, or 4, wherein the heterologous lymphocyte targeting protein is a heterologous T cell targeting protein comprising an extracellular domain comprising a T cell targeting domain that specifically binds to a CD3 protein or a CD28 protein.

6. A lentiviral vector according to claim 1 or 3, wherein the heterologous lymphocyte targeting protein is a heterologous NK cell targeting protein that specifically binds to an NK cell marker selected from CD56, NKp46, CD16, KIR(s), NKG2 proteins (e.g., NKG2D (KLRK1, CD314)), KLRB1 (CD161), KLRD1 (cd94), IL2Rb (CD122), IL-21R, SLAMF6 (CD352), SLAMF7 (CD319), and IL-18R.

7. A lentiviral vector according to any one of claims 1, 2, or 4, wherein the targeting protein comprises a CD80 protein comprising an extracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 6.

8. A lentiviral vector according to any one of claims 1, 2, 4, and 7, wherein the targeting protein comprises a CD80 protein comprising a transmembrane and intracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 8

9. A lentiviral vector according to any one of claims 1, 2, 4, or 5, wherein the targeting protein comprises and anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 14, and a heavy chain variable region that has

at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 18.

10. A lentiviral vector according to any preceding claim, wherein the targeting protein as expressed in a host cell comprises signal sequence.

11. A lentiviral vector according to any preceding claim, wherein the mature targeting protein lacks a signal sequence.

12. A lentiviral vector according to any of claims 1-10, wherein the mature targeting protein includes at least one amino acid from a signal sequence.

13. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from one or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGs between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8.

14. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from two or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGs between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8.

15. A lentiviral vector according to any preceding claim, wherein the mutated VSV-G envelope protein comprises a mutation selected from one or more of H8A, K47A, K47Q, Y209A, R354A, and R354Q.

16. A lentiviral vector according to any preceding claim, wherein the heterologous transgene encodes a chimeric antigen receptor (CAR) comprising an extracellular domain comprising a binding domain that specifically binds to a target molecule; an intracellular signaling domain, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM); and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

17. A lentiviral vector according to claim 16, wherein the (CAR) encoded by the transgene comprises:

a binding domain that specifically binds one or more of CD19, BCMA, alpha folate receptor, 5T4, Ab integrin, B7-H3, B7-H6, CAIX, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD52, CD70, CD79a, CD79b, CD80, CD123, CD138, CD171, CEA, CSPG4, EGFR, ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EpCAM, FAP, fetal AchR, FLT3, Fra, GD2, GD3, Glypican-3 (GPC3), HLA-A1 + MAGE1, HLA-A2 + MAGE1, HLA-A3 + MAGE1, HLA-A1 + NY-ESO-1, HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13 Ralpha2, Lambda, Lewis-Y, Kappa, mesothelin, Muc1, Muc16, NCAM, NKG2d ligands, NY-ESO-1, PRAME, PSCA,

PSMA, ROR1, SSX, Survivin, TAG72, TEMs, VEGFR2, BAFF-R, Claudin18.2, CD86, FcRL5, GPRC5, and TACI;

a transmembrane domain selected from a CD28, CD2, CD4, CD8a, CD5, CD3ε, CD3δ, CD3ζ, CD9, CD16, CD22, CD25, CD27, CD 28, CD33, CD37, CD40, CD45, CD64, CD79A, CD79B, CD80, CD86, CD95 (Fas), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD154 (CD40L), CD200R, CD223 (LAG3), CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcRα, FcRβ, FcRγ, Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRPα, pTα, TCRα, TCRβ, TIM3, TRIM, LPA5, and Zap70 transmembrane domain;

an intracellular signaling domain selected from a CD3γ, CD3δ, CD3ε, CD3ζ, CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcRγ, and CD66d domain;

a costimulatory domain selected from one or more of a CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, NKD2C, SLP76, TRIM, and ZAP70 domain; and

optionally, an extracellular, non-signaling linker domain between the binding domain and the transmembrane domain, with the non-signaling linker domain being selected from:

an immunoglobulin hinge region selected from a wild type or modified IgG1, IgG2, IgG3, IgG4, IgA, and IgD hinge region;

a hinge region selected from a wild type or modified CD8a, CD4, CD28 and CD7 hinge region;

all or a portion of an Fc domain selected from one or more of a CH1 domain, a CH2 domain, and a CH3 domain; and

a stalk region of a type II C-lectin derived from a stalk region of CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

18. A lentiviral vector according to either of claims 16 or 17, wherein the (CAR) encoded by the transgene is an anti-CD19 CAR comprising:

a binding domain comprising anti-CD19 scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 46, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 50;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

19. A lentiviral vector according to either of claim 16 or 17, wherein the (CAR) encoded by the transgene is an anti-BCMA CAR comprising:

a binding domain comprising anti-BCMA scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 62, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 66;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

20. A method for producing a lymphocyte targeted lentiviral vector according to any one of the preceding claims, the method comprising:

transfecting a producer cell with a defined ratio of a GagPol plasmid, a Rev plasmid, a transgene plasmid, and a VSV-G plasmid, wherein;

the GagPol plasmid comprises a one or more polynucleotides encoding a lentiviral gag gene and a lentiviral pol gene and is capable of expressing a lentiviral gag protein and a lentiviral pol protein within the producer cell;

the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral rev protein within the producer cell;

the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

the VSV-G plasmid comprises a tandem expression cassette, wherein the tandem expression cassette comprises a polynucleotide encoding a mutated VSV-G envelope protein and a polynucleotide encoding a lymphocyte targeting protein and the VSV-G plasmid is capable of expressing the mutated VSV-G envelope protein and the lymphocyte targeting protein within the producer cell;

culturing the producer cell in a culture medium; and

harvesting lentiviral vector from the culture medium.

21. A method for producing a lymphocyte targeted lentiviral vector according to any one of the preceding claims, the method comprising:

transfecting a producer cell with a defined ratio of a GagPol plasmid, a Rev plasmid, a transgene plasmid, a VSV-G plasmid, and a lymphocyte targeting protein plasmid;

the GagPol plasmid comprises a one or more polynucleotides encoding a lentiviral gag gene and a lentiviral pol gene and is capable of expressing a lentiviral gag protein and a lentiviral pol protein within the producer cell;

the Rev plasmid comprises a polynucleotide encoding a lentiviral rev gene and is capable expressing a lentiviral rev protein within the producer cell;

the transgene plasmid comprises an expression cassette comprising a polynucleotide encoding a CAR; and

the VSV-G plasmid comprises a polynucleotide encoding a mutated VSV-G envelope protein and is capable of expressing the mutated VSV-G envelope protein within the producer cell; and

the lymphocyte targeting protein plasmid comprises a polynucleotide encoding a lymphocyte targeting protein and is capable of expressing the lymphocyte targeting protein within the producer cell;

culturing the producer cell in a culture medium; and

harvesting lentiviral vector from the culture medium.

22. The method according to either of claims 20 and 21, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein.

23. The method according to either of claims 20 and 21, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a NK cell targeting protein.

24. A method according to any one of claims 20-22, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a T cell targeting protein that specifically binds to:

(a) a T cell marker selected from CD3, CD80, 4-1BB, AhR, CD3, CD2, CD7, CD4, CD8, CD25, CD44, CD45RA, CD47, CD62L, CD69, CD94, CD95, CD127, CD161, CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CCR10, PD-1, TCRa/b, CD5, CD27, CD45RO, CD45RB, CD57, CD103, CD122, P2RX7, TIGIT, LAG-3, TIM-3, and IL6ST;

(b) a γδ T cell marker selected from γδ TCR, Vdelta1, Vdelta2, and NKG2D (KLRK1, CD314);

(c) an NK T cell marker selected from Invariant TCR (Va24-Ja18), CD185 (CXCR5), CXCR6, and IL-21R; or

(d) a MAIT cell marker selected from Va7.2, Ja33, CXCR6, IL-18R, KLRB1 (CD161), and VLA4 (alpha4beta1 integrin).

25. A method according to claim 24, wherein the targeting protein comprises a CD80 protein comprising an extracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 6.

26. A method according to either of claims 24 and 25, wherein the targeting protein comprises a CD80 protein comprising a transmembrane and intracellular domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 8

27. A method according any of claims 20-22 and 24, wherein the targeting protein comprises and anti-CD3 scFv having a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 14, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 18.28. The method according to any one of claims 20, 21, and 23, wherein the lymphocyte targeting protein encoded by the lymphocyte targeting protein plasmid is a NK cell targeting protein that specifically binds to an NK cell marker selected from CD56, NKp46, CD16, KIR(s), NKG2 proteins (e.g.,

NKG2D (KLRK1, CD314)), KLRB1 (CD161), KLRD1 (cd94), IL2Rb (CD122), IL-21R, SLAMF6 (CD352), SLAMF7 (CD319), and IL-18R;

28. A method according to any of claims 20-27, wherein the mutated VSV-G envelope protein encoded by the VSV-G plasmid comprises a mutation selected from one or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGs between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8.

29. A method according to any of claims 20-28, wherein the mutated VSV-G envelope protein encoded by the VSV-G plasmid comprises a mutation selected from two or more of:

a mutation at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, I347, T350, T352, E353, and R354;

an insertion of TT between N9 and Q10, an insertion of GGs between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; and

a deletion of residues 1-8.

30. A method according to any of claims 20-29, wherein the mutated VSV-G envelope protein encoded by the VSV-G plasmid comprises a mutation selected from one or more of H8A, K47A, K47Q, Y209A, R354A, and R354Q.

31. A method according to any of claims 20-30, wherein the CAR encoded by the transgene plasmid comprises an extracellular domain comprising a binding domain that specifically binds to a target molecule; an intracellular signaling domain, wherein the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM); and a transmembrane domain connecting the extracellular domain and intracellular signaling domain.

32. A method according to any of claims 20-31, wherein the CAR encoded by the transgene plasmid comprises:

a binding domain that specifically binds one or more of CD19, BCMA, alpha folate receptor, 5T4, Ab integrin, B7-H3, B7-H6, CAIX, CD20, CD22, CD23, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD52, CD70, CD79a, CD79b, CD80, CD123, CD138, CD171, CEA, CSPG4, EGFR, ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EpCAM, FAP, fetal AchR, FLT3, Fra, GD2, GD3, Glypican-3 (GPC3), HLA-A1 + MAGE1, HLA-A2 + MAGE1, HLA-A3 + MAGE1, HLA-A1 + NY-ESO-1, HLA-A2 + NY-ESO-1, HLA-A3 + NY-ESO-1, HLADR, IL-11Ralpha, IL-13 Ralpha2, Lambda, Lewis-Y, Kappa, mesothelin, Muc1, Muc16, NCAM, NKG2d ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, VEGFR2, BAFF-R, Claudin18.2, CD86, FcRL5, GPRC5, and TACI;

a transmembrane domain selected from a CD28, CD2, CD4, CD8a, CD5, CD3ε, CD3δ, CD3ζ, CD9, CD16, CD22, CD25, CD27, CD 28, CD33, CD37, CD40, CD45, CD64, CD79A, CD79B, CD80, CD86, CD95 (Fas), CD134 (OX40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD154 (CD40L), CD200R, CD223 (LAG3), CD270 (HVEM), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), CD279 (PD-1), CD300, CD357 (GITR), A2aR, DAP10, FcRα, FcRβ, FcRγ, Fyn, GAL9, KIR, Lck, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, PTCH2, ROR2, Ryk, Slp76, SIRPα, pTα, TCRα, TCRβ, TIM3, TRIM, LPA5, and Zap70 transmembrane domain;

an intracellular signaling domain selected from a CD3γ, CD3δ, CD3ε, CD3ζ, CD5, CD22, CD79a, CD278 (ICOS), DAP10, DAP12, FcRγ, and CD66d domain;

a costimulatory domain selected from one or more of a CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, NKD2C, SLP76, TRIM, and ZAP70 domain; and

optionally, an extracellular, non-signaling linker domain between the binding domain and the transmembrane domain, with the non-signaling linker domain being selected from:

an immunoglobulin hinge region selected from a wild type or modified IgG1, IgG2, IgG3, IgG4, IgA, and IgD hinge region;

a hinge region selected from a wild type or modified CD8a, CD4, CD28 and CD7 hinge region;

all or a portion of an Fc domain selected from one or more of a CH1 domain, a CH2 domain, and a CH3 domain; and

a stalk region of a type II C-lectin derived from a stalk region of CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

33. A method according to any of claims 20-32, wherein the CAR encoded by the transgene plasmid is an anti-CD19 CAR comprising:

a binding domain comprising anti-CD19 scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 46, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 50;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3ζ effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

34. A method according to any of claims 20-32, wherein the CAR encoded by the transgene plasmid is an anti-BCMA CAR comprising:

a binding domain comprising anti-BCMA scFv comprising a light chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 62, and a heavy chain variable region that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 66;

a CD8a hinge and transmembrane domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 52;

a CD3 effector domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 56; and

a 4-1BB costimulatory domain that has at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO: 54.

35. A method for producing a T cell expressing a heterologous transgene, the method comprising:

transducing the T cell with one or more LVVs according to any one of claims 1, 2, 4, and 6-19 wherein transduction of the T cell results in expression of the heterologous transgene by the T cell.

36. The method according to claim 35, wherein the T cell is a resting T cell.

37. The method of according to claim 35 or 36, wherein transducing the T cell is performed in vivo or ex vivo.

38. The method according to any one of claims 35-37, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorly, intraosseously, or intranodally.

39. The method according to any one of claims 35-37, wherein transducing the T cell comprises contacting T cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.

40. A method according to any one of claims 35-39, wherein transduction is accomplished without use of T cell stimulating agents.

41. A method for producing a T cell expressing a CAR, the method comprising:

transducing the T cell with one or more LVVs according to any one of claims 16-19 and transduction of the T cell results in expression of the CAR transgene by the T cell.

42. The method according to claim 41, wherein the T cell is a resting T cell.

43. The method of according to claim 41 or 42, wherein transducing the T cell is performed in vivo or ex vivo.

44. The method according to any one of claims 41-43, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorly, intraosseously, or intranodally.

45. The method according to any one of claims 41-43, wherein transducing the T cell comprises contacting T cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.

46. A method according to any one of claims 41-45, wherein transduction is accomplished without use of T cell stimulating agents.

47. A method for producing a NK cell expressing a heterologous transgene, the method comprising:

transducing the NK cell with one or more LVVs according to any one of claims 1, 3, 6, and 10-19, wherein transduction of the NK cell results in expression of the heterologous transgene by the NK cell.

48. The method according to claim 47, wherein the NK cell is a resting NK cell.

49. The method of according to claim 47 or 48, wherein transducing the NK cell is performed in vivo or ex vivo.

50. The method according to any one of claims 47-49, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorly, intraosseously, or intranodally.

51. The method according to any one of claims 47-49, wherein transducing the NK cell comprises contacting NK cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.

52. A method according to any one of claims 47-51, wherein transduction is accomplished without use of NK cell stimulating agents.

53. A method for producing a NK cell expressing a CAR, the method comprising:

transducing the NK cell with one or more LVVs according to any one of claims 16-19 and transduction of the NK cell results in expression of the CAR transgene by the NK cell.

54. The method according to claim 53, wherein the NK cell is a resting NK cell.

55. The method of according to claim 53 or 54, wherein transducing the NK cell is performed in vivo or ex vivo.

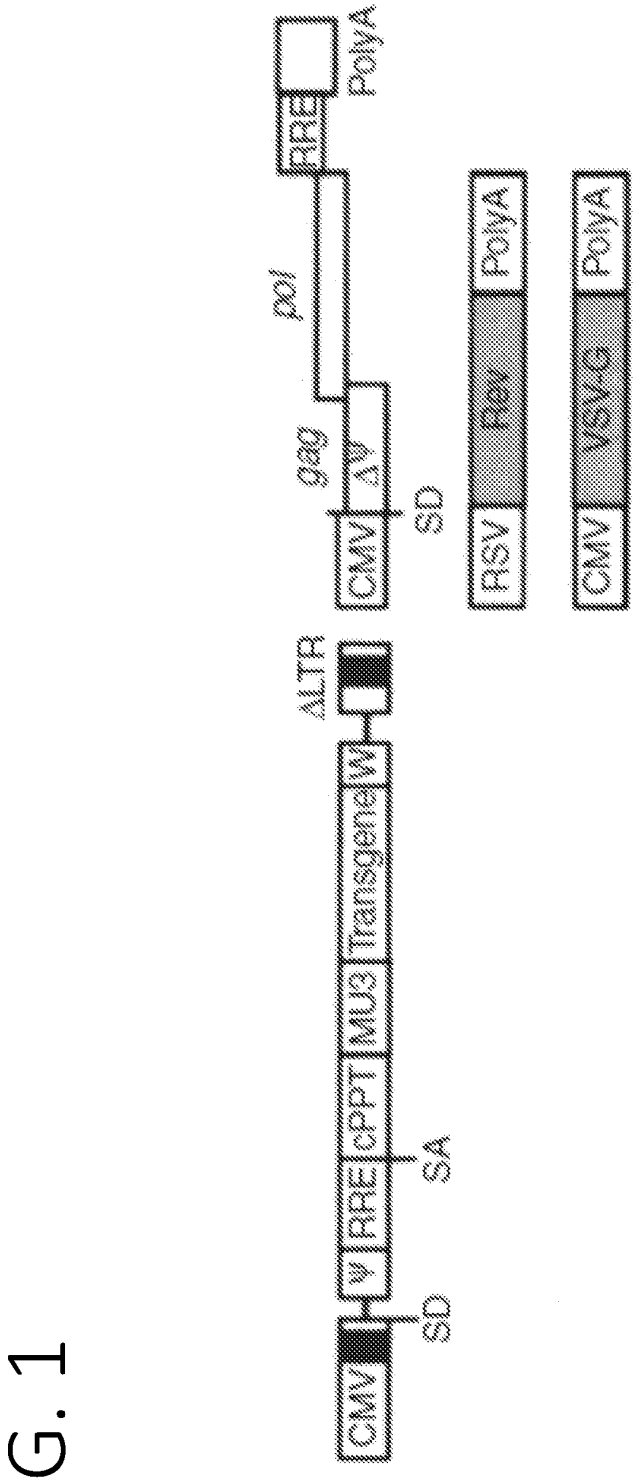
56. The method according to any one of claims 53-55, wherein the one or more LVVs is administered to a subject intravenously, intraperitoneally, intratumorly, intraosseously, or intranodally.

57. The method according to any one of claims 53-55, wherein transducing the NK cell comprises contacting NK cells within whole blood or peripheral blood mononuclear cells (PBMCs) obtained from a subject.

58. A method according to any one of claims 53-57, wherein transduction is accomplished without use of NK cell stimulating agents.

ABSTRACT

Provided herein are lentiviral vectors comprising a mutated, heterologous envelope protein, a targeting protein, and at least one transgene for delivery to and expression by a cell characterized by the targeting protein. Also provided are methods and materials for producing the lentiviral vectors described herein, methods for transducing target cells, and cells transduced by lentiviral vectors according to the present disclosure.


CMV
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RSV
Rev
PolyA
CMV
VSV-G
PolyA
SA

FIG. 1

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

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☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Molly	R.	Perkins	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	Milton	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	64 Bradlee Rd.
Address 2	

City	Milton	State/Province	MA
Postal Code	02186	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kevin	M.	Friedman	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

City	Melrose	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	4 Clover Circle
Address 2	

City	Melrose	State/Province	MA
Postal Code	02176	Country	US

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Attorney Docket Number	930207.401P1	**Small Entity Status Claimed**	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	1	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
● Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	ViraLogic Therapeutics, Inc.		
Mailing Address Information For Applicant:			
Address 1	34 Farnsworth St., 3rd Floor		
Address 2			
City	Boston	**State/Province**	MA
Country	US	Postal Code	02210
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Eileen S. Sun/			Date (YYYY-MM-DD)	2021-02-26
First Name	Eileen S.	Last Name	Sun, Ph.D.	Registration Number	57,270

Additional Signature may be generated within this form by selecting the Add button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	930207.401P1
	Application Number	
Title of Invention	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal	
Application Number:	
Filing Date:	
Title of Invention:	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS
First Named Inventor/Applicant Name:	Molly R. Perkins
Filer:	Eileen Soomie Sun/Jason Shen
Attorney Docket Number:	930207.401P1
Filed as Small Entity	
Filing Fees for Provisional	

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
PROVISIONAL APPLICATION FILING FEE	2005	1	150	150
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**150**

Electronic Acknowledgement Receipt	
EFS ID:	42043712
Application Number:	63154639
International Application Number:	
Confirmation Number:	6215
Title of Invention:	LYMPHOCYTE TARGETED LENTIVIRAL VECTORS
First Named Inventor/Applicant Name:	Molly R. Perkins
Customer Number:	500
Filer:	Eileen Soomie Sun/Jason Shen
Filer Authorized By:	Eileen Soomie Sun
Attorney Docket Number:	930207.401P1
Receipt Date:	26-FEB-2021
Filing Date:	
Time Stamp:	21:47:01
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$150
RAM confirmation Number	E20212PL47452081
Deposit Account	191090
Authorized User	Jason Shen
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		Application.PDF	412847 7ebd2a38469d35ee3914f758b5f29956cd9ef35c	yes	92
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Abstract		92	92	
	Claims		75	91	
	Specification		1	74	
Warnings:					
Information:					
2	Drawings-only black and white line drawings	Drawings.PDF	119626 52334e700caa7034705c5b5f02191ad5addd3042	no	1
Warnings:					
Information:					
3	Application Data Sheet	ADS.PDF	1255918 ec119650542e10fac4d23930e8af01d12e52a449	no	8
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	29585 b244091ecc3a7929511646a307ed40c77f052e5f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1817976		

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.