# LENTIVIRAL PARTICLES DISPLAYING FUSION MOLECULES AND USES THEREOF

## CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 63/422,678, filed November 4, 2022, U.S. Provisional Application No. 63/422,920, filed November 4, 2022, U.S. Provisional Application No. 63/487,784, filed March 1, 2023, U.S. Provisional Application No. 63/466,471, filed May 15, 2023, and U.S. Provisional Application No. 63/579,188, filed Aug 28, 2023, which applications are incorporated herein by reference in their entireties.

## INCORPORATION BY REFERENCE OF SEQUENCE LISTING

**[0002]** The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled 061479-508001WO_SeqList_ST26.xml, created November 3, 2023, which is 236 kilobytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

## FIELD OF THE INVENTION

**[0003]** This disclosure relates generally to cell biology, immunology, and medicine—more particularly to lentiviral particles for use as medical treatments.

## BACKGROUND

**[0004]** Cellular therapy generally employs the transduction of immune cells ex vivo to generate a population of therapeutic cells to be introduced into the patient. T cells may be genetically engineered for use as therapeutic agents. For example, T cells from an autologous or allogenic source can be transduced ex vivo with a vector encoding a chimeric antigen receptor. The resulting CAR T-cells are then infused into the patient. Some chimeric antigen receptor (CAR) T cells have been approved as treatments for liquid tumors. Improved methods and compositions for enhancing T cells are needed. Genetic engineering of T cells may require delivery of polynucleotides into the T cells selected for engineering, a procedure termed transduction. Transduction of T cells may be achieved using various viral and non-viral delivery vehicles. In one example, a recombinant lentivirus is used for transduction. The lentiviral particle may be engineered to display, on its surface, molecules that enhance transduction. An antibody or antibody fragment against a component of a T cell receptor, such as CD3, may be surface-displayed on the lentivirus to target the virus to T cells. Surface

display of one or more ligands for CD28, a molecule expressed by T cells, may cause the T cells to activate, which makes them more susceptible to transduction. Ligands for CD28 may include, for example, CD80 and CD86.

**[0005]** Currently, patients with aggressive B-cell malignancies that have failed standard therapies, including chemotherapy and often hematopoietic stem cell transplantation (HSCT), have the option to receive an autologous CAR-T cell product that redirects their T cells against the antigen CD 19 by means of an ex-vivo manufacturing process. However, manufacture of these products requires a complex series of steps, starting with collection of the patient's peripheral blood mononuclear cells via a leukapheresis procedure, followed by genetic modification of the patient's T cells in a cGMP facility that introduces delays, risks, and complex logistics into patient care. This is followed by the administration of lymphodepleting chemotherapy prior to infusion of the final drug product. There is an unmet medical need for patients with relap sed/refractory B-cell malignancies, both in terms of their untreated disease as well as inability to manufacture or tolerate the timing of logistics of newer cellular products.

**[0006]** The present disclosure provides compositions and methods related to in vivo transduction of immune cells to treat cancer and/or B-cell malignancies.

## SUMMARY

**[0007]** The present disclosure relates, in part, to the recognition by the present inventors that, by engineering a particle used as a delivery vehicle to display an adhesion molecule on its surface, transduction of target cells (such as T cells) by the particle may be enhanced, resulting in in vivo generation of T cells expressing a chimeric antigen receptor (CAR). In one embodiment, the present disclosure provides a viral particle comprising a vector genome comprising a polynucleotide sequence encoding an anti-CD19 chimeric antigen receptor, wherein the viral particle transduces immune cells in vivo.

**[0008]** The present inventors have further recognized that engineered particles comprising a polynucleotide encoding a payload, such as a CAR, may be enhanced by fusing an adhesion molecule to a costimulatory molecule, an activation molecule, or both.

**[0009]** Accordingly, in one embodiment, the disclosure provides a lentiviral particle for transduction of target cells, comprising, displayed on the surface of the lentiviral particle, a fusion molecule comprising an adhesion molecule linked to a costimulatory molecule, an activation molecule, or both. The particle may be a viral particle, such as a lentiviral particle. The adhesion molecule, costimulatory molecule, and activation molecule may each be proteins and may collectively be fused into one (or more) fusion proteins. In one

embodiment, the present disclosure provides a lentiviral particle comprising a polycistronic construct comprising a polynucleotide sequence encoding an anti-CD19 chimeric antigen receptor.

**[0010]** In other embodiments, the disclosure provides *ex vivo* and *in vivo* uses of the lentiviral particles (such as for cell manufacturing and medical treatments), pharmaceutical compositions, and kits, as well as methods of making the particles, polynucleotides, and host cells.

**[0011]** In some embodiments, the adhesion molecule comprises CD58, a CD58 extracellular domain, or a functional fragment of CD58; optionally wherein the fusion molecule comprises a CD58 extracellular domain, or a functional fragment thereof, a CD80 or CD86 extracellular domain, or a functional fragment thereof, and an activation domain, for example, an antigen-binding fragment of an anti-CD3 antibody.

**[0012]** In some embodiments, the present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD80 or a CD86 extracellular domain, or a functional fragment thereof.

**[0013]** In some embodiments, the present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding gragment of an anti-CD3 antibody, and

c) a CD80 extracellular domain, or a functional fragment thereof.

**[0014]** In some embodiments, the present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD86 extracellular domain, or a functional fragment thereof.

**[0015]** In all such embodiments, the lentiviral particle may further comprise a viral glycoprotein (G protein). In an exemplary embodiment, the present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle:

(1) a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD80 extracellular domain, or a functional fragment thereof; and

(2) a viral glycoprotein.

**[0016]** In an exemplary embodiment, the present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle:

(1) a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD86 extracellular domain, or a functional fragment thereof; and

(2) a viral glycoprotein. In some embodiments, the G protein is a cocal glycoprotein.

**[0017]** In some embodiments, the G protein is a VSV-G protein. In additional embodiments, the lentiviral particle may further comprise a payload comprising a polynucleotide encoding a protein, such as a chimeric antigen receptor.

**[0018]** The present disclosure provides a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising:

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody,

c) a CD80 or CD86 extracellular domain, or a functional fragment thereof; and a viral glycoprotein (G protein), wherein the lentiviral particle comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19.

**[0018a]** Disclosed herein is a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising: a) a CD58 extracellular domain, or a functional fragment thereof, b) a CD80 extracellular domain, or a functional fragment thereof, c) an antigen-binding fragment of an anti-CD3 antibody; and a viral glycoprotein (G protein), wherein the lentiviral particle comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19.

**[0019]** In some embodiments, the lentiviral particle comprises a polynucleotide encoding a free FKBP12-rapamycin binding (FRB).

**[0020]** In some embodiments, the lentiviral particle comprises a polynucleotide encoding a synthetic cytokine gamma chain polypeptide and a synthetic cytokine beta chain polypeptide.

**[0021]** In some embodiments, the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv domain, wherein the scFv further comprises a VL comprising

the polypeptide sequence of SEQ ID NO: 206 and a VH comprising the polypeptide sequence of SEQ ID NO: 208.

**[0022]** In some embodiments, the scFv comprises a spacer comprising a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 207.

**[0023]** In some embodiments, the scFv spacer comprises the polypeptide sequence of SEQ ID NO: 207.

**[0024]** In some embodiments, the scFv comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 195.

**[0025]** In some embodiments, the scFv comprises the polypeptide sequence of SEQ ID NO: 195.

**[0026]** In some embodiments, the scFv is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 194.

**[0027]** In some embodiments, the scFv is encoded by the polynucleotide sequence of SEQ ID NO: 194.

**[0028]** In some embodiments, the chimeric antigen receptor comprises a CD8 hinge domain.

**[0029]** In some embodiments, the CD8 hinge domain is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 196.

**[0030]** In some embodiments, the CD8 hinge domain is encoded by the polynucleotide sequence of SEQ ID NO: 196.

**[0031]** In some embodiments, the CD8 hinge domain comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 197.

**[0032]** In some embodiments, the CD8 hinge domain comprises the polypeptide sequence of SEQ ID NO: 197.

**[0033]** In some embodiments, the chimeric antigen receptor comprises a CD28 transmembrane domain.

**[0034]** In some embodiments, the CD28 transmembrane domain is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 198.

**[0035]** In some embodiments, the CD28 transmembrane domain is encoded by the polynucleotide sequence of SEQ ID NO: 198.

**[0036]** In some embodiments, the CD28 transmembrane domain comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 199.

**[0037]** In some embodiments, the CD28 transmembrane domain comprises the polypeptide sequence of SEQ ID NO: 199.

**[0038]** In some embodiments, the chimeric antigen receptor comprises a 4-1BB endodomain.

**[0039]** In some embodiments, the 4-1BB endodomain is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 200.

**[0040]** In some embodiments, the 4-1BB endodomain is encoded by the polynucleotide sequence of SEQ ID NO: 200.

**[0041]** In some embodiments, the 4-1BB endodomain comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 201.

**[0042]** In some embodiments, the 4-1BB endodomain comprises the polypeptide sequence of SEQ ID NO: 201.

**[0043]** In some embodiments, the chimeric antigen receptor comprises a CD3ζ endodomain.

**[0044]** In some embodiments, the CD3ζ endodomain is encoded by a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 202.

**[0045]** In some embodiments, the CD3ζ endodomain is encoded by the polynucleotide sequence of SEQ ID NO: 202.

**[0046]** In some embodiments, the CD3ζ endodomain comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 203.

**[0047]** In some embodiments, the CD3ζ endodomain comprises the polypeptide sequence of SEQ ID NO: 203.

**[0048]** In some embodiments, the polynucleotide encoding the chimeric antigen receptor comprises a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 204.

**[0049]** In some embodiments, the polynucleotide encoding the chimeric antigen receptor comprises the polynucleotide sequence of SEQ ID NO: 204.

**[0050]** In some embodiments, the chimeric antigen receptor comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 205.

**[0051]** In some embodiments, the chimeric antigen receptor comprises the polypeptide sequence of SEQ ID NO: 205.

**[0052]** In some embodiments, the CD58 extracellular domain, or a functional fragment thereof, comprises a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 10.

**[0053]** In some embodiments, the antigen-binding fragment of an anti-CD3 antibody is an scFv domain comprising a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 31.

**[0054]** The lentiviral particle of any one of claims 1-36, wherein the CD80 extracellular domain, or a functional fragment thereof, comprises a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 12.

**[0055]** In some embodiments, the CD86 extracellular domain, or a functional fragment thereof, comprises a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 13.

**[0056]** In some embodiments, the fusion molecule comprises, in N to C-terminal order, the CD58 extracellular domain, the antigen-binding fragment of an anti-CD3 antibody, and the CD86 extracellular domain.

**[0057]** In some embodiments, the fusion molecule comprises a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 33.

**[0058]** In some embodiments, the fusion molecule comprises, in N to C-terminal order, the CD58 extracellular domain, the antigen-binding fragment of an anti-CD3 antibody, and the CD80 extracellular domain.

**[0059]** In some embodiments, the viral glycoprotein (G protein) comprises a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 74.

**[0060]** In some embodiments, the lentiviral particle comprises a polycistronic construct comprising in 5′ to 3′ order:

a. a first expression cassette comprising a nucleotide sequence encoding the free FRB,
b. a second expression cassette comprising a nucleotide sequence encoding the synthetic cytokine gamma chain polypeptide,
c. a third expression cassette comprising a nucleotide sequence encoding the synthetic cytokine beta chain polypeptide, and
d. a fourth expression cassette comprising a nucleotide sequence encoding the chimeric antigen receptor (CAR); wherein each of the expression cassettes are separated by a nucleotide sequence encoding a cleavage site sequence.

**[0061]** In some embodiments, the polynucleotide sequence encoding FRB that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NOs: 256, 257, or 258.

**[0062]** In some embodiments, the polynucleotide sequence encoding the free FRB comprises the polynucleotide sequence of SEQ ID NOs: 256, 257, or 258.

**[0063]** In some embodiments, the free FRB polynucleotide sequence encodes a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NOs: 251, 252, or 260.

**[0064]** In some embodiments, the FRB polynucleotide sequence encodes the polypeptide sequence of SEQ ID NOs: 251, 252, or 260.

**[0065]** In some embodiments, the polynucleotide sequence encoding a synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NOs: 261, 262, or 263.

**[0066]** In some embodiments, the polynucleotide encoding the synthetic cytokine gamma chain polypeptide comprises the polynucleotide sequence of SEQ ID NOs: 261, 262, or 263.

**[0067]** In some embodiments, the synthetic cytokine gamma chain polypeptide comprises interleukin 2 receptor subunit γ (IL2RG) comprising a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 264 or 265.

**[0068]** In some embodiments, the IL2RG comprises the polypeptide sequence of SEQ ID NOs: 264 or 265.

**[0069]** In some embodiments, the second expression cassette comprises a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 266.

**[0070]** In some embodiments, the second expression cassette comprises the polynucleotide sequence of SEQ ID NO: 266.

**[0071]** In some embodiments, the second expression cassette encodes a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 267.

**[0072]** In some embodiments, the second expression cassette encodes a polypeptide sequence comprising the sequence of SEQ ID NO: 267.

**[0073]** In some embodiments, the second expression cassette further comprises a polynucleotide sequence encoding FKBP12 that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NOs: 268 or 269.

**[0074]** In some embodiments, the polynucleotide sequence encoding the FKBP12 comprises the polynucleotide sequence of SEQ ID NOs: 268 or 269.

**[0075]** In some embodiments, the FKBP12 comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 253

**[0076]** In some embodiments, the FKBP12 comprises the polypeptide sequence of SEQ ID NO: 253.

**[0077]** In some embodiments, the polynucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NOs: 270 or 271.

**[0078]** In some embodiments, wherein the polynucleotide encoding the synthetic cytokine beta chain polypeptide comprises the polynucleotide sequence of SEQ ID NOs: 270 or 271.

**[0079]** In some embodiments, the synthetic cytokine beta chain polypeptide comprises interleukin 2 receptor subunit β (IL2RB) comprising a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NOs: 272 or 273.

**[0080]** In some embodiments, the IL2RB comprises the polypeptide sequence of SEQ ID NOs: 272 or 273.

**[0081]** In some embodiments, the third expression cassette further comprises a polynucleotide sequence encoding FKBP12 that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 274.

**[0082]** In some embodiments, the polynucleotide sequence encoding the FKBP12 comprises the polynucleotide sequence of SEQ ID NO: 274.

**[0083]** In some embodiments, the FKBP12 comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 275.

**[0084]** In some embodiments, the FKBP12 comprises the polypeptide sequence of SEQ ID NO: 275.

**[0085]** In some embodiments, the third expression cassette comprises a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence of SEQ ID NO: 276.

**[0086]** In some embodiments, the third expression cassette comprises the polynucleotide sequence of SEQ ID NO: 276.

**[0087]** In some embodiments, the third expression cassette encodes a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 277.

**[0088]** In some embodiments, the third expression cassette encodes the polypeptide sequence comprising the sequence of SEQ ID NO: 277.

**[0089]** The present disclosure provides a method of treating a CD19+ cancer in a subject in need thereof, the method comprising administering to the subject the lentiviral particle of any preceding claim.

**[0090]** In some embodiments, the lentiviral particle is administered by intranodal, intravenous, or subcutaneous injection.

**[0091]** In some embodiments, the lentiviral particle is administered by intranodal injection via an inguinal lymph node.

**[0092]** The present disclosure provides a method of treating a CD19+ cancer in a subject in need thereof, the method comprising providing immune cells of a subject, contacting the immune cells of a subject by extracorporeal incubation with a lentiviral particle of the present disclosure, and administering the immune cell to the subject by transfusion.

**[0093]** In some embodiments, the subject suffers from or is at risk for a B-cell malignancy, relapsed/refractory CD19-expressing malignancy, diffuse large B-cell lymphoma (DLBCL), Burkitt's type large B-cell lymphoma (B-LBL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), mantle cell lymphoma (MCL), hematological malignancy, colon cancer, lung cancer, liver cancer, breast cancer, renal cancer, prostate cancer, ovarian cancer, skin cancer, melanoma, bone cancer, brain cancer, squamous cell carcinoma, leukemia, myeloma, B cell lymphoma, kidney cancer, uterine cancer, adenocarcinoma, pancreatic cancer, chronic myelogenous leukemia, glioblastoma, neuroblastoma, medulloblastoma, or sarcoma.

**[0094]** In some embodiments, the method comprises administering a non-physiological ligand.

**[0095]** In some embodiments, the non-physiological ligand comprises rapamycin or a rapamycin analog.

**[0096]** The present disclosure provides a pharmaceutical composition comprising a lentiviral particle of the present disclosure, and a pharmaceutically acceptable carrier.

**[0096a]** Disclosed herein is a phamaceutical composition for use in treating a CD19+ cancer in a subject in need thereof, wherein the lentiviral particle as efined herein is to be administered to the subject.

**[0097]** Disclosed herein is a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD80 extracellular domain, or a functional fragment thereof; and a viral glycoprotein (G protein); wherein the lentiviral particle further comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19, a free FRB, a synthetic cytokine gamma chain polypeptide, and a synthetic cytokine beta chain polypeptide, wherein the chimeric antigen receptor comprises a ligand-binding domain comprising an

scFv, a hinge domain, a transmembrane domain, a 41BB endodomain, and a CD3ζ endodomain, and wherein the scFv comprises a VL comprising SEQ ID NO: 206 and a VH comprising SEQ ID NO: 208, the hinge domain comprises SEQ ID NO: 197, the transmembrane domain comprises SEQ ID NO: 199, the 41BB endodomain comprises SEQ ID NO: 201, and the CD3ζ endodomain comprises SEQ ID NO: 203.

**[0098]** In some embodiments, the lentiviral particle comprises a polycistronic construct comprising in 5′ to 3′ order:

a. a first expression cassette comprising a nucleotide sequence encoding the free FRB,
b. a second expression cassette comprising a nucleotide sequence encoding the synthetic cytokine gamma chain polypeptide,
c. a third expression cassette comprising a nucleotide sequence encoding the synthetic cytokine beta chain polypeptide, and
d. a fourth expression cassette comprising a nucleotide sequence encoding the chimeric antigen receptor (CAR), wherein each of the expression cassettes are separated by a nucleotide sequence encoding a cleavage site sequence.

**[0099]** In some embodiments, the polynucleotide sequence encoding FRB that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NOs: 256, 257, or 258.

**[0100]** In some embodiments, the free FRB polynucleotide sequence encodes a polypeptide sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NOs: 251, 252, or 260.

**[0101]** In some embodiments, the polynucleotide sequence encoding a synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NOs: 261, 262, or 263.

**[0102]** In some embodiments, the synthetic cytokine gamma chain polypeptide comprises interleukin 2 receptor subunit γ (IL2RG) comprising a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 264 or 265.

**[0103]** In some embodiments, the second expression cassette comprises a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 266.

**[0104]** In some embodiments, the second expression cassette encodes a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polypeptide sequence of SEQ ID NO: 267.

**[0105]** In some embodiments, the second expression cassette further comprises a polynucleotide sequence encoding FKBP12 that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NOs: 268 or 269.

**[0106]** In some embodiments, the FKBP12 comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polypeptide sequence of SEQ ID NO: 253

**[0107]** In some embodiments, the polynucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NOs: 270 or 271.

**[0108]** In some embodiments, the synthetic cytokine beta chain polypeptide comprises interleukin 2 receptor subunit β (IL2RB) comprising a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NOs: 272 or 273.

**[0109]** In some embodiments, the third expression cassette further comprises a polynucleotide sequence encoding FKBP12 that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 274.

**[0110]** In some embodiments, the FKBP12 comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polypeptide sequence of SEQ ID NO: 275.

**[0111]** In some embodiments, the third expression cassette comprises a polynucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 276.

**[0112]** In some embodiments, the third expression cassette encodes a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polypeptide sequence of SEQ ID NO: 277.

**[0113]** In some embodiments, the fusion molecule comprises a polypeptide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the polypeptide sequence of SEQ ID NO: 72.

**[0114]** In some embodiments, the fusion molecule comprises the polypeptide sequence of SEQ ID NO: 72.

**[0115]** In some embodiments, the fusion molecule comprises in 5′ to 3′ order:

a. a CD58 extracellular domain, or a functional fragment thereof,
b. an antigen-binding fragment of an anti-CD3 antibody, and
c. a CD80 extracellular domain, or a functional fragment thereof.

**[0116]** In some embodiments, the lentiviral particle comprises a polycistronic construct comprising in 5′ to 3′ order:

a. a first expression cassette comprising a nucleotide sequence encoding the free FRB,
b. a second expression cassette comprising a nucleotide sequence encoding the synthetic cytokine gamma chain polypeptide,
c. a third expression cassette comprising a nucleotide sequence encoding the synthetic cytokine beta chain polypeptide, and
d. a fourth expression cassette comprising a nucleotide sequence encoding the chimeric antigen receptor (CAR); wherein the CAR specifically binds CD19.

**[0117]** In some embodiments, the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv comprising a VL comprising SEQ ID NO: 206 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto and a VH comprising SEQ ID NO: 208 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0118]** In some embodiments, the chimeric antigen receptor comprises a hinge domain comprising SEQ ID NO: 197 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0119]** In some embodiments, the chimeric antigen receptor comprises a transmembrane domain comprising SEQ ID NO: 199 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0120]** In some embodiments, the chimeric antigen receptor comprises a 41BB endodomain domain comprising SEQ ID NO: 201 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0121]** In some embodiments, the chimeric antigen receptor comprises a CD3ζ endodomain comprising SEQ ID NO: 203 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0122]** In some embodiments, the fourth expression cassette encodes a polypeptide comprising SEQ ID NO: 205 or a sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical thereto.

**[0123]** Disclosed herein is a lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD80 extracellular domain, or a functional fragment thereof; and

a viral glycoprotein (G protein); wherein the lentiviral particle further comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19, wherein the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv, a hinge domain, a transmembrane domain, a 41BB endodomain, and a CD3ζ endodomain, and wherein the scFv comprises a VL comprising SEQ ID NO: 206 and a VH comprising SEQ ID NO: 208, the hinge domain comprises SEQ ID NO: 197, the transmembrane domain comprises SEQ ID NO: 199, the 41BB endodomain comprises SEQ ID NO: 201, and the CD3ζ endodomain comprises SEQ ID NO: 203.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0124] FIG. 1A** depicts a schematic of an embodiment of the present disclosure wherein a lentiviral particle is modified with a fusion molecule and a glycoprotein on its surface and the particle comprises a payload encoding for an anti-CD19 chimeric antigen receptor (CAR). In the depicted embodiment, the CAR comprises an anti-CD19 single chain antibody fragment binding domain, a hinge domain, a transmembrane domain derived from CD28, and 41BB and CD3z intracellular signaling domains.

**[0125] FIG. 1B** depicts T-cell activation by a lentiviral particle displaying a single-chain variable fragment specific for CD3, a viral envelope protein (Cocal G), and two costimulatory molecules.

**[0126] FIG. 2A** shows activation of CD8+ T cells as measured by % CD25+ cells with a lentiviral particle displaying CD3scfv or CD3scfv+CD80.

**[0127] FIG. 2B** shows activation of CD8+ T cells as measured by % CD25+ cells with a lentiviral particle displaying CD3scfv only, CD3scfv+CD80 or CD3scfv+CD58.

**[0128] FIGs. 2C-2D** show levels of CAR expression in CD8+ T cells as determined by %CAR expression (**FIG. 2C**) or total CAR+ CD8+ T cells (**FIG. 2D**) generated using lentiviral particles with CD3scfv only or CD3scfv+CD80.

**[0129] FIGs. 2E-2F** show levels of CAR expression in CD3+ T cells as determined by %CAR expression (**FIG. 2E**) or total CAR+ CD3+ T cells (**FIG. 2F**) generated using lentiviral particles with CD3scfv only, CD3scfv+CD80 or CD3scfv+CD58.

**[0130] FIGs. 2G-2H** show fold expansion of CAR+CD8+ T cells generated with lentiviral particles with CD3scfv only or CD3scfv+CD80 stimulated with IL-2 (**FIG. 2G**) or rapamycin (**FIG. 2H**).

**[0131] FIG. 3A** shows percentages of CD25(+) CD8 T cells after incubation with a lentiviral particle displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58.

**[0132] FIG. 3B** shows the geometric mean fluorescent intensity (gMFI) of CD25(+) CD8 T cells after incubation with a lentiviral particle displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58.

**[0133] FIGs. 3C-3E** show production of cytokines 3 days after incubation with particles displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58. IFN-γ (**FIG. 3C**), IL-2 (**FIG. 3D**), and TNF-α (**FIG. 3E**) levels were measured.

**[0134] FIGs. 3F-3G** show CAR expression in CD3+ T cells generated with lentiviral particles displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 (mixed particles). Percentage (%) CAR expression (**FIG. 3F**) and total CAR+ T cells (**FIG. 3G**) were measured.

**[0135] FIGs. 3H-3I** show CAR expression in CD8+ T cells generated with lentiviral particles displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 (same particle). Percentage (%) CAR expression (**FIG. 3H**) and total CAR+ T cells (**FIG. 3I**) were measured.

**[0136] FIGs. 3J-3L** show staining of Cocal (**FIG. 3J**), CD80 (**FIG. 3K**) or CD58 (**FIG. 3L**) on CD8+ T cells incubated with lentiviral particles displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58.

**[0137] FIG. 3M** shows a principal components analysis with three main clusters of differentiation based on particle costimulatory-molecule makeup using CCR7, CD45RO, CD45RA, CD27, CD25, CAR+, CD4, and CD8 markers and total cells.

**[0138] FIG. 3N** shows CD3scfv+CD80 particles generate CAR+ T cells with a predominantly central memory ($T_{cm}$) phenotype compared to CD3scfv only, which produced effector T cells ($T_{eff}$).

**[0139] FIG. 3O** shows CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles generate CAR+ T cells with a predominantly central memory ($T_{cm}$) phenotype compared to CD3scfv only, which produced effector T cells ($T_{eff}$) central memory T cells ($T_{cm}$).

**[0140] FIG. 4A** shows the number of K562.CD19 cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles. The particles were added to PBMCs at an MOI of 10 along with Tumor cells at PBMC:Tumor ratio of 5:1 and put directly on the Incucyte® live-cell imaging system. CD3scfv+CD80+CD58 CAR T cells were generated using a mixture of individual particles.

**[0141] FIG. 4B** shows the number of Raji cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles. The particles were added to PBMCs at an MOI of 10 along with Tumor cells at PBMC:Tumor ratio of 5:1 and put directly on the incucyte. CD3scfv+CD80+CD58 CAR T cells were generated using a mixture of individual particles.

**[0142] FIG. 4C** shows the number of K562.CD19 cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles. 7 days after transduction at an MOI of 10, the total CAR+ cells were calculated and incubated with either K562.CD19 at E:T ratios of 0.5 and 1, respectively. CD3scfv+CD80+CD58 CAR T cells were generated using a mixture of individual particles.

**[0143] FIG. 4D** shows the number of Raji cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles. 7 days after transduction at an MOI of 10, the total CAR+ cells were calculated and incubated with either Raji cells at E:T ratios of 0.5 and 1, respectively. CD3scfv+CD80+CD58 CAR T cells were generated using a mixture of individual particles.

**[0144] FIG. 4E** shows the number of K562.CD19 cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or

CD3scfv+CD80+CD58 particles. 7 days after transduction at an MOI of 10, the total CAR+ cells were calculated and incubated with K562.CD19 cells at E:T ratios of 1:1, respectively. CD3scfv+CD80+CD58 CAR T cells were generated using a single particle with both costimulatory and adhesion molecules.

**[0145] FIG. 4F** shows the number of Nalm6 cells over several days after incubation with anti-CD19 CAR+ T cells generated with lentiviral particles encoding an anti-CD19 CAR and displaying CD3scfv only, CD3scfv+CD80, CD3scfv+CD58, or CD3scfv+CD80+CD58 particles. 7 days after transduction at an MOI of 10, the total CAR+ cells were calculated and incubated with Nalm6 cells at E:T ratios of 1:1, respectively. CD3scfv+CD80+CD58 CAR T cells were generated using a single particle with both costimulatory and adhesion molecules. **FIG. 4A-4F** are labeled with a key to the right of each plot with labels that correspond (in order) to the right end of each line in the plot.

**[0146] FIG. 5A** shows the number of CAR T cells in blood samples of NSG MHCI/II KO mice 11 days after injection of PMBCs and lentiviral particles displaying CD3scfv only or CD3scfv+CD80 particles.

**[0147] FIGs. 5B-5C** show the tumor burden in NSG MHCI/II KO mice over 100 days after administration with lentiviral particles displaying CD3scfv only (**FIG. 5B**) or CD3scfv+CD80 (**FIG. 5C**).

**[0148] FIGs. 6A-6B** show number of cells expressing a CAR 3 days (**FIG. 6A**) or 7 days (**FIG. 6B**) after transduction of PBMCs from three healthy donors with lentiviral particles displaying CD3scfv only or CD3scfv+CD80+CD58 particles.

**[0149] FIGs. 7A-7C** show expression of CAR in cells transduced with lentiviral particles pseudotyped with mutant VSV-G envelope proteins. SupT1 cells (**FIG. 7A**) or PBMCs from two healthy donors (**FIGs. 7B-7C**) were cultured with lentiviral particles having an anti-CD19 CAR payload and displaying mutant VSV-G envelope proteins with or without CD3scfv+CD80+CD58. CAR expression was assessed in CD4+ T cells (**FIG. 7B**) and CD8+ T cells (**FIG. 7C**) after transduction of the PBMCs.

**[0150] FIG. 8** shows the number of CAR negative T cells in the blood of mice after administration of particles at indicated doses encoding an anti-CD19 CAR and displaying CD3scfv only or CD3scfv+CD80+CD58.

**[0151] FIG. 9A** is a schematic that shows an illustrative fusion protein comprising a CD58 extracellular region and α-CD3 scFv fused to the N-terminus of a CD80 via a linker. The construct is termed "498."

**[0152] FIG. 9B** is a schematic that shows an illustrative fusion protein comprising a CD58 extracellular region fused to the N-terminus of a CD80 via a linker. The construct is termed "455." α-CD3 scFv is expressed as a separate polypeptide in the producer cells.

**[0153] FIG. 10** shows staining of Cocal in CD8+ T cells generated with lentiviral particles displaying α-CD3 scFv, CD80, and CD58 which were expressed by the lentiviral particle producer cells as separate polypeptides ("Separate"); lentiviral particles displaying α-CD3 scFv, CD80, and CD58 which were expressed by the lentiviral particle producer cells as fusion polypeptide comprising CD58 fused to CD80, with α-CD3 scFv expressed as a separate polypeptide ("455"); and lentiviral particles displaying a fusion protein comparing CD58, α-CD3 scFv, and CD80 ("498"), or control without lentiviral particles ("MOI 0").

**[0154] FIG. 11A** shows the percent CD25(+) CD4+ T cells after incubation with lentiviral particles, labelled as in FIG. 10.

**[0155] FIG. 11B** shows the percent of CD25(+) CD8+ T cells after incubation with lentiviral particles, labelled as in FIG. 10.

**[0156] FIG. 11C** shows the geometric mean fluorescent intensity (gMFI) CD25(+) CD4+ T cells after incubation with lentiviral particles, labelled as in FIG. 10.

**[0157] FIG. 11D** shows the geometric mean fluorescent intensity (gMFI) of CD25(+) CD8+ T cells after incubation with lentiviral particles, labelled as in FIG. 10.

**[0158] FIGs. 12A-12C** show production of cytokines 3 days after incubation with lentiviral particles, labelled as in FIG. 10. IFN-γ (**FIG. 12A**), IL-2 (**FIG. 12B**), and TNF-α (**FIG. 12C**) levels were measured. Particles comprised an anti-CD19-FRB-RACR payload. FRB =FKBP-rapamycin complex binding domain; RACR = rapamycin-activated cell-surface receptor.

**[0159] FIGs. 13A-13D** show "#498" displaying particles generate CAR+ T cells with a larger proportion of memory-like CD4+ CAR T cells, CAR T cells expressing senescence marker CD57, (**FIG. 13A** and **FIG. 13B**) or memory-like CD8+ (**FIG. 13C and FIG. 13D**) CAR T cells compared to "#455" or "Separate" displaying particles.

**[0160] FIG. 14A** is a schematic that shows an illustrative experimental timeline.

**[0161] FIG. 14B** shows the percent CD25(+) CD3+ T cells in blood after incubation with lentiviral particles, labelled as in **FIG. 10**.

**[0162] FIG. 14C** shows the percent CD71(+) CD3+ T cells in blood after incubation with lentiviral particles, labelled as in **FIG. 10**.

**[0163] FIG. 14D** shows level of IFN-γ cytokine measured 4 days after incubation with lentiviral particles, labelled as in **FIG. 10**.

**[0164] FIG. 15A-15C** is a panel of graphs showing geometric mean fluorescent intensity (gMFI) of Cocal in cells generated with lentiviral particles via extracorporeal *in vivo* incubation. Cells were stained prior to incubation with lentiviral particles "pre-particle", following lentiviral particle incubation with cell but before washing ("particle, pre-wash"), or following lentiviral particle incubation with cell and after washing ("Final"). Lentiviral particles display CD58 and CD80 expressed as a fusion polypeptide are labeled as in FIG. 10. Lentiviral particle incubation with CD4+ T cells, CD8+ T cells, NK T cells, NK cells, CD56+ NK cells, monocytes, B cells, and other mean fluorescent intensity (MFI) was evaluated.

**[0165] FIG. 16A** shows CAR+ T cells in the blood of mice injected with PBMCs from Donor 1 either after Lupagen™ wash or after incubation with lentiviral particles labeled as in FIG. 10.

**[0166] FIG. 16B** shows CAR+ T cells in the blood of mice injected with PBMCs from Donor 2 either after Lupagen™ wash or after incubation with lentiviral particles labeled as in FIG. 10.

**[0167] FIG. 16C** shows total tumor burden (Total flux) over the course of 21 days of the study in the blood of mice injected with PBMCs from Donor 1, either after Lupagen™ wash or after incubation labeled as in FIG. 10.

**[0168] FIG. 16D** shows total tumor burden (Total flux) over the course of 21 days of the study in the blood of mice injected with PBMCs from Donor 2 either after Lupagen™ wash or after incubation labeled as in FIG. 10.

**[0169] FIG. 16E** shows Bioluminescence imaging using the IVIS™ spectrum system depicting total tumor burden quantitated in **FIG. 16C** and **FIG. 16D**.

**[0170] FIGs. 17A-17B** show expression of CD25 in CD4+ (**FIG. 17A**) cells transduced with lentiviral particles produced using the indicated surface plasmids encoding variations of CD58 and CD80 fusion polypeptide expression or expression of CD25 in CD8+ cells (**FIG. 17B**).

**[0171] FIG. 17C** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured and labeled as in **FIG. 10**. T cell early activation measured by CD25 expression level on Day 3 post PBMC

culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 2 and 5.

**[0172] FIGs. 18A-18B** show CAR expression in CD4+ (**FIG. 18A**) cells transduced with lentiviral particles produced using the indicated surface plasmids encoding variations of CD58 and CD80 fusion polypeptide expression or expression of CD25 in CD8+ cells (**FIG. 18B**). CAR expression was measured by FMC63 expression level on Day 7 post PBMC culturing and analyzed by flow cytometry.

**[0173] FIG. 18C** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured with lentiviral particles displaying variations of CD58 and CD80 fusion polypeptide. CAR expression as measured by FMC63 expression level on Day 7 post PBMC culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 2 and 5.

**[0174] FIGs. 19A-19D** show the effects of indicated lentiviral surface proteins on FMC63 CAR-T induced cytotoxicity in the presence of NucLight™ Red-labeled Nalm6 target cells expressing hCD19 antigen. The IncuCyte™ kinetic killing curves for each CAR-T variant transduced using lentivirus displaying variations of CD58 and CD80 fusion polypeptides at MOI 2. **FIG. 19A** shows a CAR-T to target cell ratio of 0.25:1. **FIG. 19B** shows killing curves when CAR-T to target cell ratio is 0.5:1. **FIG. 19C** shows killing curves when CAR-T to target cell ratio is 1:1. **FIG. 19D** shows target-cell lytic capabilities of CAR-T cells by integrating the area under the normalized target cell killing curves (AUC) when CAR-T cells to target cell ratio range from 0.25 to 4. Percent (%) Antigen specific, CAR mediated Killing = $(1-(AUC/AUC_{Mock}))*100$.

**FIG. 20** is a bar graph showing target-dependent IFN-γ, IL-2, and TNFα secretion in FMC63 CAR-T cells, which were transduced lentiviral particles displaying indicated variations of CD58 and CD80 fusion polypeptides at MOI 2. The transduced T cells were co-cultured with Nalm6 target cells at CAR-T cell to target cell ratio of 1:1."Mock" denotes FMC63 CAR-T cells cultured without Nalm6 target cells; "Target Only" denotes Nalm6 cells cultured without T cells.

**[0175] FIGs. 21A-21B** show CD25 expression in CD4+ (**FIG. 21A**) cells transduced with lentiviral particles produced using the indicated surface plasmids encoding variations of CD58 and CD80 fusion polypeptide expression or expression of CD25 in CD8+ cells (**FIG. 21B**).

**[0176] FIG. 21C** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured with lentiviral particles displaying variations of CD58 and CD80 fusion polypeptide. T cell early activation measured by CD25 expression level on Day 3 post PBMC culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 0.5 and 1.

**[0177] FIGs. 22A-22B** show CAR expression in CD4+ (**FIG. 22A**) cells transduced with lentiviral particles produced using the indicated surface plasmids encoding variations of CD58 and CD80 fusion polypeptide expression or expression of CD25 in CD8+ cells (**FIG. 22B**).

**[0178] FIG. 22C** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured with lentiviral particles displaying variations of CD58 and CD80 fusion polypeptide. CAR expression as measured by FMC63 expression level on Day 7 post PBMC culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 0.5 and 1.

**[0179] FIG. 23** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured with lentiviral particles displaying variations of CD58, CD80, and anti-CD3 scFv fusion polypeptides. T cell early activation measured by CD25 expression level on Day 3 post PBMC culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 1 and 10.

**[0180] FIG. 24** shows a bar graph of the effects of lentivirus particles produced using the indicated surface plasmids. Non-stimulated human PBMCs were cultured with lentiviral particles displaying variations of CD58, CD80, and anti-CD3 scFv fusion polypeptides. CAR expression as measured by FMC63 expression level on Day 7 post PBMC culturing was analyzed by flow cytometry. NTC (non-transduced cells) are included for comparison. Lentiviral particles were added at multiplicity of infection (MOI) 1 and 10.

**[0181] FIG. 25** shows a graph of CAR+ T cell expansion over 11 days post transduction. The CAR+ T cells were transduced with lentiviral particles displaying variations of CD58, CD80, and anti-CD3 scFv fusion polypeptides. On Day 3 post transduction, PBMCs are washed to remove lentiviral particles, and seeded in fresh culture media at 0.5E6 cells per

well. CAR+ cells were determined by staining for surface expression of anti-FMC63 scFv, and analyzed by flow cytometry.

**[0182] FIG. 26** is a schematic of the fusion polypeptide screening approach depicted in **FIGs. 17-25**.

**[0183] FIG. 27A** shows diagrams of illustrative fusion proteins.

**[0184] FIG. 27B** shows diagrams of illustrative fusion proteins. The 21aa linker may have the polypeptide sequence GSSGGSGGGGSGGGGSGGGGS (SEQ ID NO: 34). The 23aa linker may have the polypeptide sequence GSSGGSGGGGSGGGGSGGGGSSG (SEQ ID NO: 35).

**[0185] FIG. 28A** shows a study design and timeline.

**[0186] FIG. 28B** is a graph showing staining of Cocal on CD3+ T cells incubated with engineered particles displaying CD58, CD80, and anti-CD3 scFv tri-fusion polypeptide.

**[0187] FIG. 28C** is a graph showing staining of Cocal on engineered particle bound T cells The left peak shows CD3- T cells and the right peak shows CD3+ T cells. The engineered particles display a CD58, CD80, and anti-CD3 scFv tri-fusion polypeptide "Engineered particle".

**[0188] FIG. 28D** shows CD25 expression in CD8+ T cells on day 3 after transduction with lentiviral particles displaying CD58, CD80, and anti-CD3 scFv tri-fusion polypeptide "Engineered particle".

**[0189] FIG. 28E** shows CAR expression in CD8+ T cells on day 7 after transduction with lentiviral particles displaying CD58, CD80, and anti-CD3 scFv tri-fusion polypeptide "Engineered particle".

**[0190] FIG. 29** shows the number of Nalm6 cells after anti-CD19 CAR+ T cells were serial-stimulated with Nalm6 tumor cells every 2-3 days. anti-CD19 CAR+ T cells were generated with lentiviral particles encoding an anti-CD19 CAR transgene and displaying CD3scfv-CD80-CD58 tri-fusion polypeptide particles "Engineered particle". Arrows denote stimulation with Nalm6 tumor cells. Error bars denote mean±SEM.

**[0191] FIG. 30A** shows the study design and timeline. **FIG. 30B** shows the number of cells expressing activation marker CD25 in circulation four days after transduction with lentiviral particles displaying CD3scfv-CD80-CD58- tri-fusion polypeptide. **FIG. 30C** shows the number of cells expressing activation marker CD71 in circulation four days after transduction with lentiviral particles displaying CD3scfv-CD80-CD58 tri-fusion polypeptide. **FIG. 30D** shows production of IFN-γ 4 days after incubation with particles

displaying CD3scfv-CD80-CD58 tri-fusion polypeptide "Engineered particle". **FIG. 30E** shows the number of T cells expressing an anti-CD19 CAR in the blood 11 days after transduction with lentiviral particles displaying CD3scfv-CD80-CD58- tri-fusion polypeptide at a lentiviral dose of 10 Million or 50 Million transducing units (TU). **FIG. 30F** shows the tumor burden in NSG MHCI/II KO mice after administration of lentiviral particles displaying CD3scfv-CD80-CD58 tri-fusion polypeptide at a lentiviral dose of 10 Million or 50 Million transducing units (TU).

**[0192] FIG. 31A** shows the study design and timeline. **FIG. 31B** shows the number of T cells from Donor 1 and Donor 2 expressing an anti-CD19 CAR in the blood 14 days after extracorporeal incubation with lentiviral particles. **FIG. 31C** shows the tumor burden in Donor 1 and Donor 2 NSG MHCI/II KO mice after administration of T cells produced by via extracorporeal incubation with lentiviral particles. N=7 animals; error bars denote mean±SEM. **FIG. 31D** shows the study design and timeline for re-challenge study. **FIG. 31E** shows the tumor burden in NSG MHCI/II KO mice after administration of T cells produced via extracorporeal incubation of PBMCs from Donor 1 or Donor 2 incubated with lentiviral particles following tumor cell re-challenge at Day 49. Error bars denote mean±SEM.

**[0193] FIG. 32A** shows the study design and timeline. **FIG. 32B** shows %CAR+ T cells (left panel) and total CAR+ T cells (right panel) in the blood of mice injected with PBMCs from Donor 1 after Lupagen™ incubation with lentiviral particles displaying α-CD3 scFv, CD80, and CD58 which were expressed by the lentiviral particle producer cells as a bi-fusion polypeptide comprising CD58 fused to CD80, with α-CD3 scFv expressed as a separate polypeptide ("#455"); and lentiviral particles displaying a tri-fusion protein comparing CD58, α-CD3 scFv, and CD80 ("#498"), or control PBMCs not incubated with lentiviral particles. **FIG. 32C** shows %CAR+ T cells (left panel) and total CAR+ T cells (right panel) in the blood of mice injected with PBMCs from Donor 2 after Lupagen™ incubation with lentiviral particles displaying α-CD3 scFv, CD80, and CD58 which were expressed by the lentiviral particle producer cells as a bi-fusion polypeptide comprising CD58 fused to CD80, with α-CD3 scFv expressed as a separate polypeptide("#455"); and lentiviral particles displaying a tri-fusion protein comparing CD58, α-CD3 scFv, and CD80("#498"), or control PBMCs not incubated with lentiviral particles. **FIG. 32D** shows Bioluminescence imaging using the IVIS™ spectrum system depicting total tumor burden. Images show mice injected with PBMCs from Donor 1 after Lupagen™ incubation with

lentiviral particles displaying "#455" a dual fusion or "#498" a triple fusion (left panel), and mice injected with PBMCs from Donor 2 after Lupagen™ incubation with lentiviral particles displaying "#455" a dual fusion or "#498" a triple fusion (right panel). **FIGs. 32E-32G** show total tumor burden (Total flux) over the course of 45 days of the study in the blood of mice injected with PBMCs from Donor 1 (top row of panels) or Donor 2 (bottom row of panels) after Lupagen™ incubation with untreated PBMCs (**FIG. 32E),** lentiviral particles displaying "#455" a dual fusion (**FIGs. 32F),** or "#498" a triple fusion (**FIGs. 32G**). **FIGs. 32H-32I** show total tumor burden (Total flux) over the course of 28 days of the study in the blood of mice injected with PBMCs from Donor 1 (**FIG. 32H**) or Donor 2 (**FIG. 32I**) after Lupagen™ incubation with untreated PBMC control, lentiviral particles displaying "#455", or "#498" a dual fusion or a triple fusion polypeptide, respectively.

**[0194] FIG. 33A** shows the study design and timeline for re-challenge study. **FIG. 33B** shows the tumor burden in NSG MHCI/II KO mice after administration of T cells produced via extracorporeal incubation of PBMCs from Donor 1 (D1) or Donor 2 (D2) incubated with lentiviral particles displaying "#455" a dual fusion construct, or "#498" a triple fusion construct following tumor cell re-challenge at Day 49. **FIG. 33C** shows Bioluminescence imaging using the IVIS™ spectrum system depicting total tumor burden. Images show mice injected with PBMCs from Donor 1 after Lupagen™ incubation with lentiviral particles displaying "#455" a dual fusion or "#498" a triple fusion (left panel), and mice injected with PBMCs from Donor 2 after Lupagen™ incubation with lentiviral particles displaying "#455" a dual fusion or "#498" a triple fusion (right panel) following tumor cell rechallenge at Day 49.

**[0195] FIG. 34A-34C** include examples of CD58, CD80 and CD3 scFV triple fusion sequences.

**[0196] FIG. 35A-35C** include examples of CD58, CD80 and CD3 scFV triple fusion sequences.

**[0197] FIG. 36** depicts studies comparing the function of engineered lentiviral particles comprising an anti-CD3 scFv and cocal glycoprotein ("anti-CD3scFv") with engineered lentiviral particles comprising an anti-CD3scFv, a CD58 protein, and a CD80 protein in addition to a cocal glycoprotein ("Tri protein"). **FIG. 36A** includes plots illustrating comparative activation data showing the dose-dependent activation of CD4 and CD8 T cells in response to incubation with each particle type. **FIG. 36B** includes plots illustrating comparative particle-T cell binding data. **FIG. 36C** includes plots illustrating comparative

transduction data showing the dose-dependent transduction efficiency and total number of transduced CD4 and CD8 T cells after incubation with each particle type.. **FIG. 36D** includes plots illustrating comparative cytokine production data showing dose-dependent stimulation of IFN-y, IL-2, and TNF-α after incubation with each particle type. **FIG. 36E** includes a plot illustrating comparative serial stimulation data. **FIG. 36F** includes plots demonstrating that cells incubated with particles comprising an anti-CD3scFv, a CD58 protein, and a CD80 protein ("Tri protein") produced more inflammatory cytokines than cells incubated with particles comprising an anti-CD3 scFv ("anti-CD3scFv"), but no costimulatory or adhesion molecules. **FIG. 36G** includes plots demonstrating that particles comprising an anti-CD3scFv, a CD58 protein, and a CD80 protein were able to generate a higher proportion of CCR7+ and CD27+ CD4 and CD8 T cells compared with particles comprising an anti-CD3 scFv, but no costimulatory or adhesion molecules.

**[0198] FIG. 37** describes an *in vivo* mouse study to evaluate the function of particles comprising an anti-CD3 scFv, but no costimulatory or adhesion molecules and particles comprising an anti-CD3scFv, a CD58 protein, and a CD80 protein. **FIG. 37A** depicts the study design. **FIG. 37B** includes plots illustrating *in vivo* activation data at various dose levels of particles. **FIG. 37C** includes plots illustrating in vivo transduction of T cells at varying dose levels of particles. **FIG. 37D** includes plots demonstrating tumor growth and control across the study and in particular showing that the tri protein particles controlled tumor growth to a greater extent than the anti-CD3 scFv particles.

**[0199] FIG. 38** includes data comparing engineered particles comprising CD58, CD80, and an anti-CD3 scFv separately expressed with engineered particles comprising a fusion protein comprising CD58, anti-CD3 scFv, and CD80 expressed together. **FIG. 38A** includes plots illustrating particle-T cell binding data. **FIG. 38B** includes plots illustrating comparative activation data across varying MOIs. **FIG. 38C** includes plots illustrating transduction data across varying MOIs. **FIG. 38D** includes plots demonstrating cytokine production by cells following incubation with the two variations of engineered particles.

**[0200] FIG. 39** describes an *in vivo* mouse study to evaluate the function of particles comprising CD58, CD80, and an anti-CD3 scFv separately expressed with engineered particles comprising a fusion protein comprising CD58, anti-CD3 scFv, and CD80 expressed together. **FIG. 39A** includes plots illustrating *in vivo* activation data at various dose levels of particles. **FIG. 39B** includes plots illustrating *in vivo* transduction of T cells at varying dose levels of particles. **FIG. 39C** includes plots demonstrating tumor growth

and control across the study and in particular showing that the fusion protein particles controlled tumor growth to a greater extent than the particles comprising CD58, CD80, and an anti-CD3 scFv separately expressed.

## DETAILED DESCRIPTION

**[0201]** The disclosure relates generally to a surface-engineered viral particle comprising a vector genome comprising a polynucleotide sequence encoding an anti-CD19 chimeric antigen receptor, wherein the viral particle transduces immune cells in vivo. In particular, the present disclosure relates to particles comprising fusion molecules for use in transduction of target cells, such as immune cells, or specifically T cells. In one aspect, the disclosure provides, a particle for in vivo generation of CAR-T cells, comprising, displayed on the surface of the particle, a fusion molecule comprising an adhesion molecule linked to a costimulatory molecule, an activation molecule, or both.

**[0202]** The term "transduction" is used in its broadest sense to mean delivery of an agent to a cell, such as a therapeutic agent. The agent may be a small molecule, polynucleotide, or polypeptide. A combination of agents may be delivered, such as several polynucleotides or a protein-nucleic acid complex (e.g., a gene-editing nuclease in complex with guide nucleic acid).

**[0203]** The fusion molecules of the disclosure combine an adhesion molecule with a costimulatory molecule, an activation molecule, or both. Without being bound by theory, it is believed that the inclusion of two or more of these types of molecules in a fusion molecule may cause such a particle, when it encounters a target cell, to form a macromolecular complex at the interface of the particle and cell that acts as artificial supramolecular activation cluster (SMAC).

**[0204]** T cells that encounter an antigen-presenting cell (APC) form an immune synapse known as a SMAC. In natural SMACs, the APC presents an antigen in complex with a major histocompatibility complex (MHC) molecule to the T cell receptor (TCR) on a T cell; CD80 or CD86 interact with CD28 to provide a costimulatory signal; and CD58 interacts with CD2 to adhere the APC to the T cell. The interactions between CD58 and CD2 may also provide an activatory or costimulatory signal. The adhesion molecule displayed on a particle may be CD58. SMACs may further present costimulatory molecules. Costimulatory molecules that may be displayed on a particle include CD80 and CD86.

**[0205]** As contemplated by the present disclosure, a particle may be engineered to display on its surface any of the foregoing adhesion molecules or costimulatory molecules;

extracellular fragments thereof; or functional fragments thereof. Extracellular portions of these molecules may be identified in databases such as UniProt, which is available at www.uniprot.org, or may be predicted using methods, such as a method implemented by the TMHMM 2.0 program available at services.healthtech.dtu.dk. Furthermore, in some cases, functional fragments of each are identified in scientific literature or they may be identified using laboratory methods. For example, one may predict the identify fragments of a protein likely to form well-folded domains. Fragments may be tested in binding assays against a cognate molecule, or used in pull-down assays compared to the full molecule. Functional assays, such as expression of a fluorescence reporter under the control of a promoter activated by T-cell signaling (*e.g.*, the NKkB promoter) when a T cell is contacted with a cell or particle expressing a putative functional fragment. The sequence of the adhesion molecule, costimulatory molecule, or activation molecule may be varied to identify and use variants that retain function. For example, conservative mutations may be made to a molecule or a molecule may be randomly mutated with the function of the variant confirmed experimentally.

**[0206]** An adhesion molecule, costimulatory molecule, and activation molecule may be linked in any order with only the most N-terminal or C-terminal of the molecules connected to a transmembrane region or anchor. In a variant, the fusion molecule comprises or is associated with another membrane-associated molecule, thereby displaying the fusion molecule on the particle. The term "display" is used, in a broad sense, to mean the position on the surface of the particle such that the molecule may contact cognate molecules on the target cell.

***Chimeric Antigen Receptors (CARs)***

**[0207]** In some embodiments, a particle such as a lentiviral particle described herein is used to transduce a nucleic acid sequence (polynucleotide) encoding one or more chimeric antigen receptor (CARs) into a cell (*e.g.*, a T lymphocyte). In some embodiments, the transduction of the lentiviral particle results in expression of one or more CARs in the transduced cells.

**[0208]** CARs are artificial membrane-bound proteins that direct a T lymphocyte to an antigen and stimulate the T lymphocyte to kill cells displaying the antigen. See, *e.g.*, Eshhar, U.S. Pat. No. 7,741,465. Generally, CARs are genetically engineered receptors comprising an extracellular domain that binds to an antigen, *e.g.*, an antigen on a cell, an optional linker, a transmembrane domain, and an intracellular (cytoplasmic) domain comprising a

costimulatory domain and/or a signaling domain that transmits an activation signal to an immune cell. With a CAR, a single receptor can be programmed to both recognize a specific antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR can target and kill the tumor cell. All other conditions being satisfied, when a CAR is expressed on the surface of, *e.g.*, a T lymphocyte, and the extracellular domain of the CAR binds to an antigen, the intracellular signaling domain transmits a signal to the T lymphocyte to activate and/or proliferate, and, if the antigen is present on a cell surface, to kill the cell expressing the antigen. Because T lymphocytes may require two signals, a primary activation signal and a costimulatory signal, in order to maximally activate, CARs can comprise a stimulatory and a costimulatory domain such that binding of the antigen to the extracellular domain results in transmission of both a primary activation signal and a costimulatory signal. Some illustrative CARs are known in the art and may designed in a modular fashion, *e.g.* as described in (see, *e.g.*, Guedan S, Calderon H, Posey AD, Maus MV, *Molecular Therapy - Methods & Clinical Development*. 2019; 12: 145-156), incorporated by reference.

**[0209]** In some embodiments, a lentiviral particle disclosed herein comprises a polynucleotide encoding a CAR comprising an extracellular domain that binds to CD19, a hinge domain, a transmembrane domain, and an intracellular signaling domain. In some embodiments, the intracellular signaling domain comprises a costimulatory domain and an activation domain. In some embodiments, the costimulatory and activation domains are a single domain, for example a single intracellular domain that provides both costimulation and activation signals to a cell. In other embodiments, the intracellular signaling domain comprises either a costimulatory domain or an activation domain. In some embodiments, the CAR comprises an extracellular domain, a CD8a hinge domain, a CD8a transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a lentiviral particle disclosed herein comprises a polynucleotide encoding a CAR comprising an extracellular domain, a CD8a hinge, a CD28 transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain.

*CAR Intracellular Domain*

**[0210]** In some embodiments, the intracellular domain of the CAR is or comprises an intracellular domain or motif of a protein that is expressed on the surface of T lymphocytes and triggers activation and/or proliferation of said T lymphocytes. In some embodiments,

such a domain or motif is able to transmit a signal for activation of a T lymphocyte in response to antigen binding to the CAR's extracellular portion. In some embodiments, this domain or motif comprises, or is, an ITAM (immunoreceptor tyrosine-based activation motif). ITAM-containing polypeptides suitable for CARs include, for example, the zeta CD3 chain (CD3ζ) or ITAM-containing portions thereof. In some embodiments, the intracellular domain is a CD3ζ intracellular signaling domain. In some embodiments, the intracellular domain is from a lymphocyte receptor chain, a TCR/CD3 complex protein, an Fc receptor subunit or an IL-2 receptor subunit. In some embodiments, the intracellular signaling domain of CAR may be the signaling domains of for example CD3ζ, CD3ε, CD22, CD79a, CD66d or CD39. “Intracellular signaling domain” refers to the part of a CAR polypeptide that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit effector cell function, *e.g.*, activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited following antigen binding to the extracellular CAR domain.

**[0211]** In some embodiments, the intracellular domain of the CAR is the zeta CD3 chain (CD3zeta).

**[0212]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose intracellular domain comprises a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 82.

**[0213]** RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD ALHMQALPPR (SEQ ID NO: 82)

**[0214]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the intracellular domain of a CAR comprising a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 83.

**[0215]** CGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTGCCTACCAGCAGGGC CAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACG TGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCAAGCCTCGGA GAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGC CGAGGCCTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGA

CACGATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGGACACATATGATG
CCCTGCACATGCAGGCCCTGCCACCTAGG (SEQ ID NO: 83)

**[0216]** In some embodiments, the CAR additionally comprises one or more co-stimulatory domains or motifs, *e.g.*, as part of the intracellular domain of the polypeptide.

**[0217]** Co-stimulatory molecules may include cell surface molecules other than antigen receptors or Fc receptors that provide a second signal useful for efficient activation and function of T lymphocytes upon binding to antigen. The one or more co-stimulatory domains or motifs can, for example, be, or comprise, one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD134) polypeptide sequence, a co-stimulatory 4-1BB (CD137) polypeptide sequence, or a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence, or other costimulatory domain or motif, or any combination thereof. In some embodiments, the one or more co-stimulatory domains are selected from the group consisting of intracellular domains of 4-1BB, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, and ZAP70.

**[0218]** In some embodiments, the co-stimulatory domain is an intracellular domain of 4-1BB, CD28, or OX40. Illustrative CAR constructs comprising a CD28 signaling domain are disclosed in US Patent No. 7,446,190, incorporated by reference. Illustrative CAR constructs comprising a 4-1BB signaling domain are disclosed in US Patent No. 9,856,322 and US Patent No. 8,399,964, each incorporated by reference.

**[0219]** In some embodiments, the lentiviral particle comprises a polynucleotide or a polypeptide that encodes a CAR comprising an IgG4 linker operatively linked to a CD28 transmembrane domain operatively linked to a 4-1BB co-stimulatory domain operatively linked to a CD3zeta signaling domain.

**[0220]** In some embodiments, the lentiviral particle comprises a polynucleotide or a polypeptide that encodes a CAR comprising an IgG4 linker operatively linked to a CD8a transmembrane domain operatively linked to a 4-1BB co-stimulatory domain operatively linked to a CD3zeta signaling domain.

**[0221]** In some embodiments, the lentiviral particle comprises a polynucleotide or a polypeptide that encodes a CAR comprising an IgG4 linker operatively linked to a CD8a transmembrane domain operatively linked to a CD28 co-stimulatory domain operatively linked to a CD3zeta signaling domain.

**[0222]** In some embodiments, the lentiviral particle comprises a polynucleotide or a polypeptide that encodes a CAR comprising a CD8a linker operatively linked to a CD8a transmembrane domain operatively linked to a 4-1BB co-stimulatory domain operatively linked to a CD3zeta signaling domain.

**[0223]** In some embodiments, the lentiviral particle comprises a polynucleotide or a polypeptide that encodes a CAR comprising a CD28 linker operatively linked to a CD28 transmembrane domain operatively linked to a CD28 co-stimulatory domain operatively linked to a CD3zeta signaling domain.

**[0224]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose intracellular domain comprises a co-stimulatory 4-1BB polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 84.

**[0225]** KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 84)

**[0226]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the intracellular domain of a CAR comprising a co-stimulatory 4-1BB sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 85.

**[0227]** CGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTGCCTACCAGCAGGGC
CAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACG
TGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCAAGCCTCGGA
GAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGC
CGAGGCCTATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGA
CACGATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGGACACATATGATG
CCCTGCACATGCAGGCCCTGCCACCTAGG (SEQ ID NO: 85)

**[0228]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose intracellular domain comprises an IgG4 linker operatively linked to a CD28 transmembrane domain operatively linked to a co-stimulatory 4-1BB polypeptide operatively linked to a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 86.

**[0229]** ESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL

NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 86)

**[0230]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the intracellular domain of a CAR comprising an IgG4 linker operatively linked to a CD28 transmembrane domain operatively linked to a co-stimulatory 4-1BB polypeptide operatively linked to a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 87.

**[0231]** GAGTCTAAGTATGGCCCTCCATGCCCCCCTTGTCCTATGTTCTGGGTGC
TGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGTGGCC
TTTATCATCTTCTGGGTGAAGCGCGGCCGGAAGAAGCTGCTGTATATCTTTAA
GCAGCCCTTCATGAGACCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGC
TGTAGGTTTCCAGAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCT
CTCGGAGCGCCGATGCCCCTGCCTACCAGCAGGGACAGAACCAGCTGTATAA
CGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGG
GGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAG
GGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTATTCCGAGA
TCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTACCA
GGGCCTGAGCACCGCCACAAAGGACACCTATGATGCCCTGCACATGCAGGCC
CTGCCACCCAGG (SEQ ID NO: 87)

**[0232]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose intracellular domain comprises an IgG4 linker operatively linked to a CD28 transmembrane domain operatively linked to a co-stimulatory 4-1BB polypeptide operatively linked to a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 88.

**[0233]** ESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEE
NPGP (SEQ ID NO: 88)

**[0234]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the intracellular domain of a CAR comprising an IgG4 linker operatively linked to a CD28 transmembrane domain operatively linked to a co-stimulatory 4-1BB polypeptide

operatively linked to a CD3zeta domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 89.

**[0235]** GAGTCCAAGTACGGCCCACCCTGCCCTCCATGTCCCATGTTTTGGGTGC TGGTGGTGGTGGGAGGCGTGCTGGCCTGTTATTCCCTGCTGGTGACCGTGGCC TTCATCATCTTTTGGGTGAAGCGCGGCCGGAAGAAGCTGCTGTACATCTTCAA GCAGCCCTTCATGAGACCCGTGCAGACCACACAGGAGGAGGACGGCTGCAGC TGTAGGTTCCCAGAGGAGGAGGAGGGAGGATGCGAGCTGAGGGTGAAGTTTT CCCGGTCTGCCGATGCCCCTGCCTATCAGCAGGGCCAGAATCAGCTGTACAAC GAGCTGAATCTGGGCAGGCGCGAGGAGTACGACGTGCTGGATAAGAGGAGAG GAAGGGACCCTGAGATGGGAGGCAAGCCAAGGCGCAAGAACCCTCAGGAGG GCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTACTCCGAGAT CGGCATGAAGGGAGAGCGGAGAAGGGGCAAGGGACACGATGGCCTGTATCA GGGCCTGAGCACCGCCACAAAGGACACCTACGATGCACTGCACATGCAGGCC CTGCCACCTAGAGGATCTGGAGCCACAAACTTCAGCCTGCTGAAGCAGGCCG GCGATGTGGAGGAGAATCCTGGACCA (SEQ ID NO: 89)

**[0236]** In some embodiments, the intracellular domain can be further modified to encode a detectable, for example, a fluorescent, protein (*e.g.*, green fluorescent protein) or any variants thereof.

*CAR Transmembrane Region*

**[0237]** The transmembrane region can be any transmembrane region that can be incorporated into a functional CAR, *e.g.*, a transmembrane region from a CD28, CD4, or a CD8 molecule.

**[0238]** In some embodiments, the transmembrane domain of CAR may be the transmembrane domain of CD8, an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1 BB (CD137), 4-1 BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME

(SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, NKG2D "in reverse orientation", and/or NKG2C. In some embodiments, the transmembrane domain of the CAR may be the transmembrane domain of CD28. In some embodiments, the transmembrane domain of a CAR may be the transmembrane domain of CD8, for example, CD8α.

*CAR Linker Region*

**[0239]** The optional linker or hinge of CAR positioned between the extracellular domain and the transmembrane domain may be a polypeptide of about 2 to over 100 amino acids in length. The linker can include or be composed of flexible residues such as glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used, *e.g.*, when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Longer linkers may also be advantageous when the target antigen is closer to the cell surface.

**[0240]** In some embodiments, the linker is from a hinge region or portion of the hinge region of any immunoglobulin or other transmembrane protein. For example, the hinge region may be from IgG1, IgG2, IgG3, IgG4, PD1, CD8, or CD28, or a portion thereof. In some embodiments, the linker is from a portion of an immunoglobulin, for example IgG4. In some embodiments, the linker is a portion of an immunoglobulin, for example IgG1. In some embodiments, the linker is a portion of the extracellular domain of CD28. In other embodiments, the linker is a portion of the extracellular domain of CD8. In other embodiments, the linker is a portion of the extracellular domain of PD1.

**[0241]** In some embodiments, the linker is an IgG4 linker operably linked to a CD28 transmembrane domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 90.

**[0242]** ESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWV (SEQ ID NO: 90)

**[0243]** In some embodiments, the linker is an IgG4 linker operably linked to a CD28 transmembrane domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 91.

**[0244]** GAGTCTAAGTATGGCCCACCCTGCCCTCCATGTCCAATGTTCTGGGTGC TGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGTGGCC TTTATCATCTTCTGGGTG (SEQ ID NO: 91)

*CAR Extracellular Domain*

**[0245]** In some embodiments, the nucleic acid transduced into cells using the methods described herein comprises a sequence that encodes a polypeptide, wherein the extracellular domain of the polypeptide binds to an antigen of interest. In some embodiments, the extracellular domain comprises a receptor, or a portion of a receptor, that binds to said antigen. In some embodiments, the extracellular domain comprises, or is, an antibody or an antigen-binding portion thereof. In some embodiments, the extracellular domain comprises, or is, a single-chain Fv domain. The single-chain Fv domain can comprise, for example, a VL linked to VH by a flexible linker, wherein said VL and VH are from an antibody that binds said antigen.

**[0246]** In some embodiments, the extracellular domain of CAR may contain any polypeptide that binds the desired antigen (*e.g.* prostate neoantigen or antigen expressed on a tumor of interest). The extracellular domain may comprise a scFv, a portion of an antibody or an alternative scaffold. CARs may also be engineered to bind two or more desired antigens that may be arranged in tandem and separated by linker sequences. For example, one or more domain antibodies, scFvs, llama VHH antibodies or other VH only antibody fragments may be organized in tandem via a linker to provide bispecificity or multispecificity to the CAR.

**[0247]** In some embodiments, the antigen is expressed on a B-cell malignancy cell, relapsed/refractory CD19-expressing malignancy cell, diffuse large B-cell lymphoma (DLBCL) cell, Burkitt's type large B-cell lymphoma (B-LBL) cell, follicular lymphoma (FL) cell, chronic lymphocytic leukemia (CLL) cell, acute lymphocytic leukemia (ALL) cell, mantle cell lymphoma (MCL) cell, hematological malignancy cell, colon cancer cell, lung cancer cell, liver cancer cell, breast cancer cell, renal cancer cell, prostate cancer cell, ovarian cancer cell, skin cancer cell, melanoma cell, bone cancer cell, brain cancer cell, squamous cell carcinoma cell, leukemia cell, myeloma cell, B cell lymphoma cell, kidney cancer cell, uterine cancer cell, adenocarcinoma cell, pancreatic cancer cell, chronic myelogenous leukemia cell, glioblastoma cell, neuroblastoma cell, medulloblastoma cell, or a sarcoma cell.

**[0248]** In some embodiments, the CAR is a second-generation CAR comprising an anti-fluorescein scFv linked to the 4-1BB costimulatory domain and the CD3zeta intracellular signaling domain.

**[0249]** In some embodiments, the antigen is CD19. CAR T therapies targeting CD19 have been approved by the FDA and include Yescarta, Tecartus, Kymriah and Breyanzi. CARs targeting CD19 are described, for example, in US Publication No. 20160152723, US Patent No. 10,736,918, US Patent No. 10,357,514, and US Patent No. 7,446,190, each incorporated by reference.

**[0250]** In some embodiments, a CAR comprises an extracellular domain comprising a FMC63 scFv binding domain for CD19 binding. In some embodiments, the CAR is a second-generation CAR comprising the FMC63 mouse anti-human CD19 scFv linked to the 4-1BB costimulatory domain and the CD3zeta intracellular signaling domain. In some embodiments, a CAR comprises a binding domain for CD19, a CD8a hinge, a CD8a transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a CAR comprises a binding domain for CD19, an IgG4 hinge, a CD28 transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a CAR comprises a binding domain for CD19, a CD28 hinge, a CD28 transmembrane domain, a CD28 costimulatory domain, and CD3zeta signaling domain. In some embodiments, a CAR comprises an extracellular domain comprising a FMC63 scFv binding domain for CD19 binding, a CD8a hinge, a CD8a transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a CAR comprises an extracellular domain comprising a FMC63 scFv binding domain for CD19 binding, an IgG4 hinge, a CD28 transmembrane domain, a 4-1BB costimulatory domain, and a CD3zeta signaling domain. In some embodiments, a CAR comprises an extracellular domain comprising a FMC63 scFv binding domain for CD19 binding, a CD28 hinge, a CD28 transmembrane domain, a CD28 costimulatory domain, and CD3zeta signaling domain.

**[0251]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose extracellular domain comprises a hCSF2R (human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α-chain) signal sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 92.

**[0252]** MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 92)

**[0253]** In some embodiments, the lentiviral particle comprises a polynucleotide encoding a CAR whose extracellular domain comprises a αCD19 scFv (CD19 VL linked to a CD19

VH) that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 93.

**[0254]** DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITG STSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQ PPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYC AKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO: 93)

**[0255]** The complementary determining regions (CDR) of this anti-CD19 scFv of SEQ ID NO: 93 are RASQDISKYLN, (CDR-L1; SEQ ID NO: 94), HTSRLHS (CDR-L2; SEQ ID NO: 95), QQGNTLPYT (CDR-L3; SEQ ID NO: 96), DYGV (CDR-H1; SEQ ID NO: 97), VIWGSETTYYNSALKS (CDR-H2; SEQ ID NO: 98), HYYYGGSYAMDY (CDR-H3; SEQ ID NO: 99). In some embodiments, the lentiviral particle comprises a polynucleotide encoding a CAR whose extracellular domain comprises a αCD19 scFv having these CDRs, wherein optionally the αCD19 scFv shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 93.

**[0256]** In some embodiments, the lentiviral particle comprises a polynucleotide encoding a CAR whose extracellular domain comprises a αCD19 scFv having these CDRs, wherein optionally the αCD19 scFv shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 93 or 100.

**[0257]** MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISK YLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYF CQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNS KSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO: 100)

**[0258]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding a hCSF2R (human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α-chain) signal sequence for the extracellular domain of CAR that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 101.

**[0259]** ATGCTGCTGCTGGTGACCTCCCTGCTGCTGTGCGAGCTGCCTCACCCAG CCTTTCTGCTGATCCCC (SEQ ID NO: 101)

**[0260]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the extracellular domain of a CAR comprising a αCD19 scFv that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 102.

**[0261]** GACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGC GACAGAGTGACCATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTACCTGAA CTGGTATCAGCAGAAGCCAGATGGCACAGTGAAGCTGCTGATCTACCACACCT CCAGGCTGCACTCTGGAGTGCCAAGCCGGTTCTCCGGATCTGGAAGCGGCACC GACTATTCCCTGACAATCTCTAACCTGGAGCAGGAGGATATCGCCACATACTT TTGCCAGCAGGGCAATACCCTGCCATATACATTCGGCGGAGGAACCAAGCTG GAGATCACCGGATCCACATCTGGAAGCGGCAAGCCAGGAAGCGGAGAGGGAT CCACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGCAC CATCCCAGTCTCTGAGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACT ACGGCGTGTCCTGGATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGG CGTGATCTGGGGCTCTGAGACCACATACTATAATTCTGCCCTGAAGAGCCGCC TGACCATCATCAAGGACAACTCCAAGTCTCAGGTGTTTCTGAAGATGAATAGC CTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGCACTACTATTACGG CGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCTCCGTGACAGTGTCTA GC (SEQ ID NO: 102)

**[0262]** In some embodiments, the lentiviral particle comprises a polypeptide comprising a CAR whose extracellular domain comprises a αCD19 scFv that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 103.

**[0263]** MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISK YLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYF CQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNS KSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS (SEQ ID NO: 103)

**[0264]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the extracellular domain of a CAR comprising a αCD19 scFv that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 104.

**[0265]** ATGCTGCTGCTGGTGACCAGCCTGCTGCTGTGCGAGCTGCCACACCCTG
CCTTCCTGCTGATCCCAGATATCCAGATGACACAGACCACATCCTCTCTGTCCG
CCTCTCTGGGCGACAGAGTGACCATCTCTTGTAGGGCCAGCCAGGATATCTCC
AAGTACCTGAACTGGTATCAGCAGAAGCCTGACGGCACAGTGAAGCTGCTGA
TCTACCACACCTCTAGGCTGCACAGCGGAGTGCCATCCCGGTTCAGCGGATCC
GGATCTGGAACAGACTATTCTCTGACCATCAGCAACCTGGAGCAGGAGGATAT
CGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATATACATTCGGCGGAG
GAACCAAGCTGGAGATCACCGGAAGCACATCCGGATCTGGCAAGCCAGGATC
CGGAGAGGGATCTACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGG
ACTGGTGGCACCCAGCCAGTCCCTGTCTGTGACCTGTACAGTGTCTGGCGTGA
GCCTGCCCGATTACGGCGTGTCCTGGATCAGACAGCCACCAAGGAAGGGACT
GGAGTGGCTGGGCGTGATCTGGGGCTCTGAGACCACATACTATAATAGCGCCC
TGAAGTCCCGGCTGACCATCATCAAGGACAACAGCAAGTCCCAGGTGTTTCTG
AAGATGAATAGCCTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGC
ACTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCTCC
GTGACAGTGAGCTCC (SEQ ID NO: 104)

**[0266]** In some embodiments, the lentiviral particle comprises a polynucleotide encoding a CAR whose extracellular domain comprises a αCD19 scFv that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 100.

**[0267]** The complementary determining regions (CDR) of this scFv are RASQDISKYLN, (CDR-L1; SEQ ID NO: 94), HTSRLHS (CDR-L2; SEQ ID NO: 95), QQGNTLPYT (CDR-L3; SEQ ID NO: 96), DYGV (CDR-H1; SEQ ID NO: 97), VIWGSETTYYNSALKS (CDR-H2; SEQ ID NO: 98), HYYYGGSYAMDY (CDR-H3; SEQ ID NO: 99). In some embodiments, the lentiviral particle comprises a polynucleotide encoding a CAR whose extracellular domain comprises a αCD19 scFv having these CDRs, wherein optionally the αCD19 scFv shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 100.

**[0268]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the extracellular domain of a CAR comprising a αCD19 scFv that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 105.

**[0269]** ATGCTGCTGCTGGTGACATCCCTGCTGCTGTGCGAGCTGCCACACCCAG
CCTTCCTGCTGATCCCCGATATCCAGATGACCCAGACCACAAGCTCCCTGAGC
GCCTCCCTGGGCGACAGGGTGACAATCTCTTGTCGGGCCAGCCAGGATATCTC
CAAGTATCTGAATTGGTACCAGCAGAAGCCCGACGGCACCGTGAAGCTGCTG
ATCTATCACACATCTAGACTGCACAGCGGCGTGCCTTCCAGGTTTTCTGGCAG
CGGCTCCGGCACCGACTACTCTCTGACAATCAGCAACCTGGAGCAGGAGGAT
ATCGCCACCTATTTCTGCCAGCAGGGCAATACCCTGCCTTACACATTTGGCGG
CGGCACAAAGCTGGAGATCACCGGCTCTACAAGCGGATCCGGCAAGCCAGGA
TCCGGAGAGGGATCTACCAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCTG
GACTGGTGGCACCATCTCAGAGCCTGTCCGTGACCTGTACAGTGTCTGGCGTG
AGCCTGCCAGATTATGGCGTGAGCTGGATCAGGCAGCCACCTAGGAAGGGAC
TGGAGTGGCTGGGCGTGATCTGGGGCTCCGAGACCACATACTATAACAGCGCC
CTGAAGTCCCGCCTGACCATCATCAAGGACAACTCTAAGAGCCAGGTGTTCCT
GAAGATGAATTCCCTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGC
ACTACTATTACGGCGGCTCTTATGCCATGGATTACTGGGGCCAGGGCACCAGC
GTGACAGTGTCTAGC (SEQ ID NO: 105)

**[0270]** In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a modified IgG4 hinge domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 187, 188, or 189. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a PD1 hinge domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 190. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising an IgG1 hinge domain that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 191. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD8 hinge domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 192. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD28 hinge domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 193.

**[0271]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding the extracellular domain of a CAR comprising an anti-CD19 scFv comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 194. In some embodiments, the lentiviral particle comprises a nucleic acid encoding the extracellular domain of a CAR comprising an anti-CD19 scFv comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 195. In some embodiments, the αCD19 scFv VL comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 206.

**[0272]** In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD8 hinge domain comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 196. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD8 hinge domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 197.

**[0273]** In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD28 transmembrane domain comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 198. In some embodiments, the lentiviral particle disclosed herein comprises a nucleic acid encoding a CAR comprising a CD28 transmembrane domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 199.

**[0274]** In some embodiments, the lentiviral particle disclosed herein comprises a polynucleotide that encodes a CAR comprising a 4-1BB co-stimulatory domain comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 200. In some embodiments, the lentiviral particle disclosed herein encodes a CAR comprising a 4-1BB co-stimulatory domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 201. In some embodiments, the lentiviral particle disclosed herein comprises a polynucleotide that

encodes a CAR comprising a CD3zeta signaling domain comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 202. In some embodiments, the lentiviral particle disclosed herein encodes a CAR comprising a CD3zeta signaling domain comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 203.

**[0275]** In some embodiments, the lentiviral particle comprises a nucleic acid encoding an anti-CD19 CAR comprising a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 204. In some embodiments, the lentiviral particle comprises a nucleic acid encoding an anti-CD19 CAR comprising an amino acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 205.

**Table 1**

| SEQ ID NO: | SEQUENCE | Description |
|---|---|---|
| 187 | ESKYGPPCPPCPM | modified IgG4 hinge |
| 188 | ESKYGPPSPPSPA | modified IgG4 hinge |
| 189 | ESKYGPPSPPSP | modified IgG4 hinge |
| 190 | PSPRPAGQFQTLV | PD1 hinge |
| 191 | EPKSCDKTHTCP | IgG1 hinge |
| 192 | GAVHTRGLDFACD | CD8 hinge |
| 193 | LCPSPLFPGPSKP | CD28 hinge |
| 194 | GACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGC GACAGAGTGACCATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTAC CTGAACTGGTATCAGCAGAAGCCAGATGGCACAGTGAAGCTGCTGATC TACCACACCTCCAGGCTGCACTCTGGAGTGCCAAGCCGGTTCTCCGGA TCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGAGCAG GAGGATATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATAT ACATTCGGCGGAGGAACCAAGCTGGAGATCACCGGATCCACATCTGG AAGCGGCAAGCCAGGAAGCGGAGAGGGATCCACAAAGGGAGAGGTG AAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCATCCCAGTCTCTG AGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACGGCGTG TCCTGGATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGT GATCTGGGGCTCTGAGACCACATACTATAATTCTGCCCTGAAGAGCCG CCTGACCATCATCAAGGACAACTCCAAGTCTCAGGTGTTTCTGAAGAT GAATAGCCTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGCA CTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCAC CTCCGTGACAGTGTCTAGC | Anti-CD19 CAR scFv |
| 195 | DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHT SRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTK LEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLP DYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFL KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS | Anti-CD19 CAR scFv |
| 196 | GGCGCTGTGCACACCAGAGGACTGGATTTCGCCTGCGAC | Anti-CD19 CAR CD8 hinge |
| 197 | GAVHTRGLDFACD | Anti-CD19 CAR CD8 hinge |
| 198 | TTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGC TGGTGACCGTGGCCTTTATCATCTTCTGGGTG | Anti-CD19 CAR CD28 transmembrane domain |
| 199 | FWVLVVVGGVLACYSLLVTVAFIIFWV | Anti-CD19 CAR CD28 transmembrane domain |
| 200 | AAGAGAGGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGCCCTTCATG CGCCCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTT CCAGAGGAGGAGGAGGGAGGATGCGAGCTG | Anti-CD19 CAR 41BB |
| 201 | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL | Anti-CD19 CAR 41BB polypeptide |

| | | |
|---|---|---|
| 202 | CGCGTGAAGTTCAGCCGGTCCGCCGATGCCCCTGCCTACCAGCAGGGC<br>CAGAACCAGCTGTATAACGAGCTGAATCTGGGCCGGAGAGAGGAGTA<br>CGACGTGCTGGATAAGAGGAGGGGAAGGGACCCAGAGATGGGAGGCA<br>AGCCTCGGAGAAAGAACCCACAGGAGGGCCTGTACAATGAGCTGCAG<br>AAGGACAAGATGGCCGAGGCCTATTCTGAGATCGGCATGAAGGGAGA<br>GAGGCGCCGGGGCAAGGGACACGATGGCCTGTACCAGGGCCTGAGCA<br>CCGCCACAAAGGACACATATGATGCCCTGCACATGCAGGCCCTGCCAC<br>CTAGG | Anti-CD19 CAR<br>CD3z |
| 203 | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK<br>PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA<br>TKDTYDALHMQALPPR | Anti-CD19 CAR<br>CD3z polypeptide |
| 204 | GACATCCAGATGACACAGACCACAAGCTCCCTGTCTGCCAGCCTGGGC<br>GACAGAGTGACCATCTCCTGTAGGGCCTCTCAGGATATCAGCAAGTAC<br>CTGAACTGGTATCAGCAGAAGCCAGATGGCACAGTGAAGCTGCTGATC<br>TACCACACCTCCAGGCTGCACTCTGGAGTGCCAAGCCGGTTCTCCGGA<br>TCTGGAAGCGGCACCGACTATTCCCTGACAATCTCTAACCTGGAGCAG<br>GAGGATATCGCCACATACTTTTGCCAGCAGGGCAATACCCTGCCATAT<br>ACATTCGGCGGAGGAACCAAGCTGGAGATCACCGGATCCACATCTGG<br>AAGCGGCAAGCCAGGAAGCGGAGAGGGATCCACAAAGGGAGAGGTG<br>AAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCATCCCAGTCTCTG<br>AGCGTGACCTGTACAGTGTCCGGCGTGTCTCTGCCTGACTACGGCGTG<br>TCCTGGATCAGGCAGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGT<br>GATCTGGGGCTCTGAGACCACATACTATAATTCTGCCCTGAAGAGCCG<br>CCTGACCATCATCAAGGACAACTCCAAGTCTCAGGTGTTTCTGAAGAT<br>GAATAGCCTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGCA<br>CTACTATTACGGCGGCTCCTACGCCATGGATTATTGGGGCCAGGGCAC<br>CTCCGTGACAGTGTCTAGCGGCGCTGTGCACACCAGAGGACTGGATTT<br>CGCCTGCGACTTCTGGGTGCTGGTGGTGGTGGGAGGCGTGCTGGCCTG<br>TTACTCCCTGCTGGTGACCGTGGCCTTTATCATCTTCTGGGTGAAGAGA<br>GGCAGGAAGAAGCTGCTGTATATCTTTAAGCAGCCCTTCATGCGCCCT<br>GTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGTCGGTTTCCAGA<br>GGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCAGCCGGTCCG<br>CCGATGCCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTATAACGAGC<br>TGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGG<br>GGAAGGGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACA<br>GGAGGGCCTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCT<br>ATTCTGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACAC<br>GATGGCCTGTACCAGGGCCTGAGCACCGCCACAAAGGACACATATGAT<br>GCCCTGCACATGCAGGCCCTGCCACCTAGG | Full-length anti-<br>CD19 CAR |
| 205 | DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHT<br>SRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTK<br>LEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSLSVTCTVSGVSLP<br>DYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFL<br>KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSGAVHTRGL<br>DFACDFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRP<br>VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL<br>GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI<br>GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR | Full-length anti-<br>CD19 CAR<br>polypeptide |
| 206 | DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHT<br>SRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTK<br>LEIT | Anti-CD19 CAR<br>scFv VL |
| 207 | GSTSGSGKPGSGEGSTKG | Anti-CD19 CAR<br>scFv spacer |
| 208 | EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVI<br>WGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYY<br>GGSYAMDYWGQGTSVTVSS | Anti-CD19 CAR<br>scFv VH |

**[0276]** In some embodiments, the CAR is a second-generation CAR comprising the FMC63 mouse anti-human CD19 scFv linked to the CD28 costimulatory domain and the CD3zeta intracellular signaling domain. In some embodiments, the CAR is a second-generation CAR comprising the FMC63 mouse anti-human CD19 scFv linked to a CD8 transmembrane domain, 4-1BB costimulatory domain, and the CD3zeta intracellular signaling domain.

**[0277]** In some embodiments of the polypeptides described herein, the extracellular domain is joined to said transmembrane domain directly or by a linker, spacer or hinge polypeptide sequence, *e.g.*, a sequence from CD28 or a sequence from CTLA4.

**[0278]** In some embodiments, the extracellular domain that binds the desired antigen may be from antibodies or their antigen binding fragments generated using the technologies described herein.

***Adhesion Molecules***

**[0279]** Disclosed herein, in some embodiments, are adhesion molecules. The adhesion molecule may be included as part of a fusion molecule. The adhesion molecule may be included as part of a particle (e.g. on a particle surface).

**[0280]** As used herein, the term "adhesion molecule" refers, in a broad sense, to a molecular component of a SMAC or other immune synapse, other than an activation molecule (e.g. TCR-binding agent) or a costimulatory molecule, which in turn contributes to adhesion of a particle to target cells. Adhesion molecules from natural sources may be molecules expressed, natively, on antigen-presenting cells and adapted for use here on particles. Both naturally occurring adhesion molecules, and their variants, and artificial adhesion molecules, such as antibodies, or fragments thereof, are contemplated. An adhesion molecule, as the term is used herein, specifically binds a conjugate molecule with affinity sufficient to cause increased adhesion between the particle and the target cell compared to the adhesion of a reference particle lacking the adhesion molecule to the same or similar target cell. The term adhesion molecule includes but is not limited to CD58, a CD58 extracellular portion, and functional fragments of CD58. As described above, the term "functional fragment" is used herein to describe a fragment of a polypeptide, or other molecule, that retains the desired function of the polypeptide. For example, a functional fragment of CD58 is a fragment of CD58 that specifically binds CD2. The adhesion molecule may be a protein, termed herein an "adhesion protein."

**[0281]** In some embodiments, the costimulatory and/or adhesion molecule comprises an amino acid sequence 100% identical to a sequence in Table 2 or Table 3. In some embodiments, the costimulatory and/or adhesion molecule shares at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a sequence in Table 2 or Table 3. In some embodiments, the costimulatory and/or adhesion molecule shares less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 96%, less than 97%, less than 98%, less than 99%, or less than 100% identity to a sequence in Table 2 or Table 3.

**[0282]** Polypeptide sequences of illustrative adhesion molecules are provided in Table 2, with the "start" and "end" positions of the extracellular portion of each. In each case, the adhesion molecule may comprise a polypeptide at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity to any sequence in Table 2, or functional fragments thereof. Functional fragments may be or include any 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, or 600 (or any range thereof) amino acid portion that retains binding affinity to its cognate molecule, when measured using affinity assays such as biolayer interferometry or other assays that may be known in the art.

**Table 2**

| Adhesion Molecule | SEQ ID NO: | Sequence | Start of the extra-cellular portion | End of the extra-cellular portion |
|---|---|---|---|---|
| CD58 | 1 | MVAGSDAGRALGVLSVVCLLHCFGFISCFSQQIYGVVY GNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFS SFKNRVYLDTVSGSLTIYNLTSSDEDEYEMESPNITDTM KFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHRG LIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSN PLFNTTSSIILTTCIPSSGHSRHRYALIPIPLAVITTCIVLY MNGILKCDRKPDRTNSN | 29 | 215 |

**Table 3**

| **Name** | **Sequence** | **SEQ ID NO:** | **Region** |
|---|---|---|---|
| CD80 Extracellular Domain | VIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKEKK MVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRPS DEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPSI SDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAINTT VSQDPETELYAVSSKLDFNMTTNHSFMCLIKYGHLRV NQTFNWNTTKQEHFPDN | 134 | Val35 - Asn242 |
| CD86 Extracellular Domain | APLKIQAYFNETADLPCQFANSQNQSLSELVVFWQDQ ENLVLNEVYLGKEKFDSVHSKYMGRTSFDSDSWTLRL HNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELSVLAN FSQPEIVPISNITENVYINLTCSSIHGYPEPKKMSVLLRT | 135 | Ala24 - Pro247 |

| | | | |
|---|---|---|---|
| | KNSTIEYDGVMQKSQDNVTELYDVSISLSVSFPDVTSN MTIFCILETDKTRLLSSPFSIELEDPQPPPDHIP | | |
| CD58 Extracellular Domain | FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVA ELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEY EMESPNITDTMKFFLYV | 136 | Phe29 - Val120 |

**[0283]** In some embodiments, the costimulatory and/or adhesion molecule is linked to a transmembrane domain. In some embodiments, the transmembrane domain may be the transmembrane domain of CD8, an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1 BB (CD137), 4-1 BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

**[0284]** Without wishing to be bound by theory, reducing foreign junctions (i.e., between an adhesion molecule and a transmembrane domain) in the foreign nucleic acid incorporated into a lentiviral particle may reduce the immunogenicity of a subject to a lentiviral particle. Accordingly, in some embodiments, the transmembrane domain of multi-domain fusion polypeptide is derived from the same protein as the membrane proximal domain. For example, the MDF may include a fragment of CD56 that includes both the CD56 extracellular domain and CD56 transmembrane domain, e.g., as a one contiguous polypeptide sequence. In a variation, this CD56 fragment includes a linker or other insertion between the CD56 extracellular domain and the CD56 transmembrane domain. As another example, the MDF may include a CD80 or CD86 fragment that includes the CD80 or CD86 transmembrane domain, respectively, as a contiguous polypeptide sequence, or in a variation, a sequence with a linker or other insertion between said two domains.

**[0285]** For example, in some embodiments, the costimulatory and transmembrane domains are derived from CD80 and domains appear in series as they would in the endogenous protein (i.e. as a single sequence). In some embodiments wherein the costimulatory and/or

adhesion domains are comprised within a fusion molecule, there may be multiple extracellular domains. For example, a fusion molecule as described herein may comprise a binding domain from CD58 and a binding domain from CD80. In such an example, the CD80 domain may be the most membrane proximal and therefore the fusion molecule would comprise both the binding and transmembrane domains from CD80 as they appear in the endogenous protein.

**[0286]** In some embodiments, the adhesion molecule is CD58. CD58 is also known as lymphocyte function-associated antigen 3 (LFA-3). CD58 binds to CD2 (LFA-2) on T cells. The extracellular portion of CD58 is residues 29-215 of SEQ ID NO: 1 (SEQ ID NO: 10):
FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFSSFKNRVY
LDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFLYVLESLPSPTLTCALTNGSIEV
QCMIPEHYNSHRGLIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFN
TTSSIILTTCIPSSGHSRHR (SEQ ID NO: 10)

**[0287]** In some embodiments, the polypeptide sequence of CD58 shares at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 248:
FSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQKDKVAELENSEFRAFSSFKNRVY
LDTVSGSLTIYNLTSSDEDEYEMESPNITDTMKFFLYVLESL (SEQ ID NO: 248)

**[0288]** A crystal structure of CD58 is described in Ikemizu et al. *PNAS USA* 96(8):4289-94 (1999). The extracellular portion of CD58 has a ligand-binding domain and a second extracellular domain. In embodiments, the ligand-binding domain may be used as the functional fragment of CD58—*i.e.*, without the second extracellular domain.

**[0289]** In some embodiments, the adhesion molecule (or the fusion protein) comprises the polypeptide sequence of SEQ ID NO: 1 or 10, or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 1 or 10. In some embodiments, the adhesion molecule (or the fusion protein) comprises a sequence having less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 96%, less than 97%, less than 98%, less than 99%, or less than 100% identity to SEQ ID NO: 1 or 10. The adhesion molecule may encoded by a polynucleotide (e.g. a DNA or RNA polynucleotide).

**[0290]** The adhesion molecule may encoded by the polynucleotide sequence of CD58, SEQ ID NO: 11, or by a subsequence encoding the extracellular portion or a functional fragment. SEQ ID NO: 11 (5′ to 3′):

ATGGTTGCTGGGAGCGACGCGGGGCGGGCCCTGGGGGTCCTCAGCGTGGTCT
GCCTGCTGCACTGCTTTGGTTTCATCAGCTGTTTTTCCCAACAAATATATGGTG
TTGTGTATGGGAATGTAACTTTCCATGTACCAAGCAATGTGCCTTTAAAAGAG
GTCCTATGGAAAAAACAAAAGGATAAAGTTGCAGAACTGGAAAATTCTGAGT
TCAGAGCTTTCTCATCTTTTAAAAATAGGGTTTATTTAGACACTGTGTCAGGTA
GCCTCACTATCTACAACTTAACATCATCAGATGAAGATGAGTATGAAATGGAA
TCGCCAAATATTACTGATACCATGAAGTTCTTTCTTTATGTGCTTGAGTCTCTT
CCATCTCCCACACTAACTTGTGCATTGACTAATGGAAGCATTGAAGTCCAATG
CATGATACCAGAGCATTACAACAGCCATCGAGGACTTATAATGTACTCATGGG
ATTGTCCTATGGAGCAATGTAAACGTAACTCAACCAGTATATATTTTAAGATG
GAAAATGATCTTCCACAAAAAATACAGTGTACTCTTAGCAATCCATTATTTAA
TACAACATCATCAATCATTTTGACAACCTGTATCCCAAGCAGCGGTCATTCAA
GACACAGATATGCACTTATACCCATACCATTAGCAGTAATTACAACATGTATT
GTGCTGTATATGAATGGTATTCTGAAATGTGACAGAAAACCAGACAGAACCA
ACTCCAAT.

**[0291]** The polynucleotide sequence may be varied by codon-optimization or other methods to generate polynucleotide sequences having at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 11, or a suitable subsequence, which may be used to express the adhesion molecule.

**[0292]** It will be appreciated that further variants of CD58 may be used. For example, homologs of CD58 from other species (mice, ape, horse, etc.) may be identified and tested for use in transducing human, or non-human, target cells. It is expected that at least some non-human homologs will retain adhesion molecule function when used with human target cells.

**[0293]** Further adhesion molecules useful in the practice of the present invention may include any molecule that specifically binds CD2, LFA-1, or DNAM-1. For example, the adhesion molecule may be a molecule that comprises an antibody, or antigen-binding fragment thereof, specific to CD2, LFA-1, or DNAM-1.

**[0294]** In some embodiments, the adhesion molecule binds to CD2. CD2 is also known as T11, LFA-2, and the erythrocyte rosette receptor. In its native state, CD2 is a surface protein expressed on T lymphocytes and NK cells. CD2 is a natural ligand for CD58. In addition to performing adhesion functions, engagement of CD2 by CD58 provides a costimulatory signal that may enhance activation and effector functions. In some embodiments, the particle comprises an adhesion molecule that binds to CD2, which may be CD58 or a fragment thereof. In some embodiments, the lentiviral particle comprises an antibody, single domain antibody, antibody fragment, and/or nanobody specific for CD2.

**[0295]** The foregoing description of CD58 and its derivatives as the adhesion molecule and CD2 as the cognate molecule may be extrapolated to the other adhesion molecules described herein. The adhesion molecule (or the fusion protein) may comprise any polypeptide sequence of in Table 2, to an extracellular portion thereof, or to a functional fragment thereof, or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to a sequence in Table 2, to an extracellular portion thereof, or to a functional fragment thereof.

***Costimulatory Molecules***

**[0296]** Disclosed herein, in some embodiments, are costimulatory molecules. The costimulatory molecule may be included as part of a fusion molecule. The costimulatory molecule may be included as part of a particle (e.g., displayed on a particle surface).

**[0297]** The fusion molecule displayed on the particle may include a costimulatory molecule. However, in some embodiments, the fusion molecule does not include a costimulatory molecule. The particle may display a costimulatory molecule as a separate molecule on the surface of the particle, or the particle may lack any costimulatory molecule. The costimulatory molecule may be a protein, termed herein a "costimulatory protein."

**[0298]** As used herein, the term "costimulatory molecule" refers to a molecule capable of providing a costimulatory signal to target cells, other than an adhesion molecule as defined herein. In a non-limiting example, interactions between CD58 and CD2 may also provide an activatory or costimulatory signal. In T cell biology, the binding of the T cell receptor by an antigen can provide the primary stimulatory signal to the cell. So-called costimulatory signals are provided by accessory molecules. An example costimulatory signal is the signal provided by binding of CD28 on T cells by a ligand. Some examples of ligands of CD28 include CD80 and CD86.

**[0299]** Illustrative costimulatory molecules include, but are not limited to, CD80 or CD86. Each of the foregoing may be employed as a costimulatory molecules as a full-length protein, an extracellular domain, or functional fragment.

**[0300]** Polypeptide sequences of illustrative costimulatory molecules are provided in Table 4, with the "start" and "end" positions of the extracellular portion of each. In each case, the costimulatory molecule may comprise a polypeptide at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any sequence in Table 4, or functional fragments thereof. In some embodiments, the costimulatory molecule comprises a polypeptide having less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 96%, less than 97%, less than 98%, less than 99%, or 100% sequence identity to any sequence in Table 4, or a functional fragment thereof. Functional fragments may be or include any 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, or 600 amino acid portion that retains binding affinity to its cognate molecule, when measured using affinity assays such as biolayer interferometry or other assays known in the art.

**Table 4**

| Costimulatory Molecule | SEQ ID NO: | Sequence | start | end |
|---|---|---|---|---|
| CD80 | 12 | MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSGVIHVTKE VKEVATLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGD MNIWPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYE KDAFKREHLAEVTLSVKADFPTPSISDFEIPTSNIRRIICSTSG GFPEPHLSWLENGEELNAINTTVSQDPETELYAVSSKLDFN MTTNHSFMCLIKYGHLRVNQTFNWNTTKQEHFPDNLLPSW AITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV | 35 | 242 |
| CD80 | 250 | VIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKEKK MVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILALRP SDEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTP SISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEEL NAINTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIK YGHLRVNQTFNWNTTKQEHFPDNLLPSWAITLISVNG IFVICCLTYCFAPRCRERRRNERLRRESVRPV | | |
| CD86 | 13 | MDPQCTMGLSNILFVMAFLLSGAAPLKIQAYFNETADLPCQ FANSQNQSLSELVVFWQDQENLVLNEVYLGKEKFDSVHSK YMGRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRI HQMNSELSVLANFSQPEIVPISNITENVYINLTCSSIHGYPEPK KMSVLLRTKNSTIEYDGVMQKSQDNVTELYDVSISLSVSFP DVTSNMTIFCILETDKTRLLSSPFSIELEDPQPPPDHIPWITAV LPTVIICVMVFCLILWKWKKKRPRNSYKCGTNTMEREESE QTKKREKIHIPERSDEAQRVFKSSKTSSCDKSDTCF | 24 | 247 |

**[0301]** In some embodiments, the costimulatory molecule is or includes CD80. In some embodiments, the costimulatory molecule is or includes a molecule that binds CD28. CD80 binds to CD28. The extracellular portion of CD80 includes residues 35-230 of SEQ ID NO: 12, which includes an Ig-like V-type domain (SEQ ID NO: 25) and an Ig-like C2-type domain (SEQ ID NO: 26), either or both of which may be included to form the costimulatory molecule.

**[0302]** VIHVTKEVKEVATLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGDMNI WPEYKNRTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVT (SEQ ID NO: 25)

**[0303]** PSISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETELYA VSSKLDFNMTTNHSFMCLIKYGHLRVNQTFN (SEQ ID NO: 26)

**[0304]** The crystal structure of CD80 (also known as B7-1) is described in Ikemizu et al. *Immunity* 12:51-60 (2000). The extracellular portion of CD80 has two domains, described above. In embodiments, one or both of the domains may be used as the functional fragment of CD80.

**[0305]** In some embodiments, the costimulatory molecule is or includes CD86. CD86 binds to CD28. The extracellular portion of CD86 includes residues 33-225 of SEQ ID NO: 13, which includes an Ig-like V-type domain (SEQ ID NO: 27) and an Ig-like C2-type domain (SEQ ID NO: 28), either or both of which may be included to form the costimulatory molecule.

**[0306]** NETADLPCQFANSQNQSLSELVVFWQDQENLVLNEVYLGKEKFDSVHSK YMGRTSFDSDSWTLRLHNLQIKDKGLYQCIIHHKKPTGMIRIHQMNSELS (SEQ ID NO: 27)

**[0307]** NVYINLTCSSIHGYPEPKKMSVLLRTKNSTIEYDGVMQKSQDNVTELYDVS ISLSVSFPDVTSNMTIFCILETDKT (SEQ ID NO: 28)

**[0308]** The crystal structure of CD86 (also known as B7-1) is described in Schwartz et al. *Nature* 410: 604-608 (2001). The extracellular portion of CD86 has two domains, described above. In embodiments, one or both of the domains may be used as the functional fragment of CD86.

**[0309]** It will be appreciated that further variants of CD80 or CD86 may be used. For example, homologs of CD80 or CD86 from other species (mice, ape, horse, etc.) may be identified and tested for use in transducing human, or non-human, target cells. It is expected

that at least some non-human homologs will retain costimulatory molecule function when used with human target cells.

**[0310]** In some embodiments, the costimulatory molecule (or the fusion protein) comprises the polypeptide sequence of one or more of SEQ ID NO: 12-13 and 25-28, or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to one or more of SEQ ID NO: 12-13 and 25-28.

**[0311]** In some embodiments, the costimulatory molecule CD80 comprises the polypeptide sequence of SEQ ID NO: 250, or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 250.

**[0312]** In some embodiments, the costimulatory molecule (or the fusion protein) comprises a polypeptide sequence having less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 96%, less than 97%, less than 98%, less than 99%, or less than 100% identity to one or more of SEQ ID NO: 12-13 and 25-28. The costimulatory molecule may encoded by a polynucleotide (e.g. a DNA or RNA polynucleotide). The costimulatory molecule may encoded by the polynucleotide sequence of CD80 (SEQ ID NO: 29) or CD86 (SEQ ID NO: 30), or by a subsequence encoding the extracellular portion or a functional fragment.

**[0313]** ATGGGCCACACACGGAGGCAGGGAACATCACCATCCAAGTGTCCATAC
CTCAATTTCTTTCAGCTCTTGGTGCTGGCTGGTCTTTCTCACTTCTGTTCAGGTG
TTATCCACGTGACCAAGGAAGTGAAAGAAGTGGCAACGCTGTCCTGTGGTCAC
AATGTTTCTGTTGAAGAGCTGGCACAAACTCGCATCTACTGGCAAAAGGAGAA
GAAAATGGTGCTGACTATGATGTCTGGGGACATGAATATATGGCCCGAGTACA
AGAACCGGACCATCTTTGATATCACTAATAACCTCTCCATTGTGATCCTGGCTC
TGCGCCCATCTGACGAGGGCACATACGAGTGTGTTGTTCTGAAGTATGAAAAA
GACGCTTTCAAGCGGGAACACCTGGCTGAAGTGACGTTATCAGTCAAAGCTGA
CTTCCCTACACCTAGTATATCTGACTTTGAAATTCCAACTTCTAATATTAGAAG
GATAATTTGCTCAACCTCTGGAGGTTTTCCAGAGCCTCACCTCTCCTGGTTGGA
AAATGGAGAAGAATTAAATGCCATCAACACAACAGTTTCCCAAGATCCTGAA
ACTGAGCTCTATGCTGTTAGCAGCAAACTGGATTTCAATATGACAACCAACCA
CAGCTTCATGTGTCTCATCAAGTATGGACATTTAAGAGTGAATCAGACCTTCA
ACTGGAATACAACCAAGCAAGAGCATTTTCCTGATAACCTGCTCCCATCCTGG

GCCATTACCTTAATCTCAGTAAATGGAATTTTTGTGATATGCTGCCTGACCTAC
TGCTTTGCCCCAAGATGCAGAGAGAGAAGGAGGAATGAGAGATTGAGAAGGG
AAAGTGTACGCCCTGTA (SEQ ID NO: 29)

**[0314]** ATGGATCCCCAGTGCACTATGGGACTGAGTAACATTCTCTTTGTGATGG
CCTTCCTGCTCTCTGGTGCTGCTCCTCTGAAGATTCAAGCTTATTTCAATGAGA
CTGCAGACCTGCCATGCCAATTTGCAAACTCTCAAAACCAAAGCCTGAGTGAG
CTAGTAGTATTTTGGCAGGACCAGGAAAACTTGGTTCTGAATGAGGTATACTT
AGGCAAAGAGAAATTTGACAGTGTTCATTCCAAGTATATGGGCCGCACAAGTT
TTGATTCGGACAGTTGGACCCTGAGACTTCACAATCTTCAGATCAAGGACAAG
GGCTTGTATCAATGTATCATCCATCACAAAAAGCCCACAGGAATGATTCGCAT
CCACCAGATGAACTCTGAACTGTCAGTGCTTGCTAACTTCAGTCAACCTGAAA
TAGTACCAATTTCTAATATAACAGAAAATGTGTACATAAATTTGACCTGCTCA
TCTATACACGGTTACCCAGAACCTAAGAAGATGAGTGTTTTGCTAAGAACCAA
GAACTCAACTATCGAGTATGATGGTGTTATGCAGAAATCTCAAGATAATGTCA
CAGAACTGTACGACGTTTCCATCAGCTTGTCTGTTTCATTCCCTGATGTTACGA
GCAATATGACCATCTTCTGTATTCTGGAAACTGACAAGACGCGGCTTTTATCTT
CACCTTTCTCTATAGAGCTTGAGGACCCTCAGCCTCCCCCAGACCACATTCCTT
GGATTACAGCTGTACTTCCAACAGTTATTATATGTGTGATGGTTTTCTGTCTAA
TTCTATGGAAATGGAAGAAGAAGAAGCGGCCTCGCAACTCTTATAAATGTGG
AACCAACACAATGGAGAGGGAAGAGAGTGAACAGACCAAGAAAAGAGAAAA
AATCCATATACCTGAAAGGTCTGATGAAGCCCAGCGTGTTTTTAAAAGTTCGA
AGACATCTTCATGCGACAAAAGTGATACATGTTTT (SEQ ID NO: 30)

**[0315]** The polynucleotide sequence may be varied by codon-optimization or other methods to generate polynucleotide sequences having at least 50%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 29 or 30, or a suitable subsequence, which may be used to express the costimulatory molecule.

**[0316]** The foregoing description of CD80, CD86, and their derivatives as the costimulatory molecule and CD28 as the cognate molecule may be extrapolated to the other costimulatory molecules described herein, including but not limited to those listed in Table 4. The costimulatory molecule (or the fusion protein) may comprise any polypeptide sequence in Table 4, or an extracellular portion thereof, or a functional fragment thereof, or a sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at

least 97%, at least 98% or at least 99% identity to a sequence in Table 4, or an extracellular portion thereof, or a functional fragment thereof.

***Activation Molecules***

**[0317]** Disclosed herein, in some embodiments, are activation molecules. The activation molecule may be included as part of a fusion molecule. The activation molecule may be included as part of a particle (e.g. displayed on a particle surface). An example of an activation molecule may include a TCR-binding molecule. An activation molecule is generally a molecule that activates an immune cell with a primary immune activation signal.

**[0318]** The fusion molecule displayed on the particle may include an activation molecule (e.g. a TCR-binding molecule) or other subunit that provides an activation signal to a target cell. However, in some embodiments, the fusion molecule does not include a TCR-binding molecule or other activation molecule. The particle may display a TCR-binding molecule as a separate molecule on the surface of the particle, or the particle may lack any TCR-binding molecule. The TCR-binding molecule may be a protein, termed herein a "TCR-binding protein." The activation molecule may be or include an activation protein.

**[0319]** As used herein, the term "TCR-binding molecule" refers to a molecule capable of directly binding the extracellular portion of the T cell receptor (TCR) by contacting one or more components of the TCR or otherwise providing a primary or "signal 1" activation signal to a target cell (e.g. a T cell or NK cell). The structure of the TCR, its components, and function is described in Sušac et al. *Cell* 185(17):3201-3213.e19 (2022). Some examples of TCR-binding molecules may include an antibody, or antigen binding fragment, that specifically binds CD3 (an anti-CD3 monoclonal antibody, or antigen binding fragment thereof). In some embodiments, the activation molecule comprises an antibody, single domain antibody, antibody fragment, nanobody, or other binding protein specific for CD3. Illustrative antibodies include OKT3 (also known as Muromonab-CD3), otelixizumab, teplizumab and visilizumab. The complementarity determining regions of OKT3 are as follows:

**[0320]** CDRH1: GYTFTRY (SEQ ID NO. 48)

**[0321]** CDRH2: NPSRGY (SEQ ID NO. 49)

**[0322]** CDRH3: YYDDHYCLDY (SEQ ID NO. 50)

**[0323]** CDRL1: SASSSVSYMN (SEQ ID NO. 51)

**[0324]** CDRL2: DTSKLAS (SEQ ID NO. 52)

**[0325]** CDRL3: QQWSSNPFT (SEQ ID NO. 53)

**[0326]** The activation molecule (e.g. TCR-binding molecule) may be a single chain variable fragment (scFv) displayed on the particle as linked to a transmembrane region or an anchor. OKT3 in scFv format may be used.

**[0327]** In some embodiments, the activation molecule (e.g. TCR-binding molecule) is or includes an scFv comprising a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the anti-CD3 scFv of SEQ ID NO: 31, which includes a variable light (VL) and variable heavy (VH) domain with a 3 × GGGS linker:

**[0328]** DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTS KLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRT SGGGGSGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWV RQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDTG VYFCARYYDDHYCLDYWGQGTPVTVSSAAAKP (SEQ ID NO: 31)

**[0329]** In some embodiments, the activation molecule (e.g. TCR-binding molecule) is or includes an scFv comprising a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the anti-CD3 scFv of SEQ ID NO: 249, which includes a variable light (VL) and variable heavy (VH) domain with a 3 × GGGS linker:

**[0330]** DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTS KLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRT SGGGGSGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWV RQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMDSLRPEDTG VYFCARYYDDHYCLDYWGQGTPVTVSSA (SEQ ID NO: 249)

**[0331]** The complementarity determining regions of the anti-CD3 scFv of SEQ ID NO: 249 scFv are as follows:

**[0332]** CDRH1: RYTMH (SEQ ID NO: 54)

**[0333]** CDRH2: YINPSRGYTNYNQKVKD (SEQ ID NO: 55)

**[0334]** CDRH3: YYDDHYCLDY (SEQ ID NO: 56)

**[0335]** CDRL1: SASSSVSYMN (SEQ ID NO: 57)

**[0336]** CDRL2: DTSKLASG (SEQ ID NO: 58)

**[0337]** CDRL3: QQWSSNPFT (SEQ ID NO: 59)

**[0338]** Other activation molecules and/or domains may comprise the binding regions of other proteins commonly found in the supramolecular activation complex (SMAC) between T lymphocytes and antigen presenting cells. For example, CD3, CD2, CD4, CD8, CD28, LFA-1, CD45, CD43, CD40, ICAM-1, CTLA-4, CD80, CD86, MHC, LFA-3, AND CD40L are proteins that may be present within the SMAC. The fusion proteins disclosed herein may comprise portions of these proteins or domains that bind to these proteins. For example, without wishing to be bound by theory, T cells may express one or both of CD4 and/or CD8 and fusion molecules disclosed herein may comprise domains that engage with either or both of CD4 and/or CD8.

**[0339]** When cells other than T cells are the intended target of the particles comprising a fusion molecule as disclosed herein, other binding domains may be more appropriate. For example, particles targeting NK cells may comprise domains that engage with proteins found on NK cells. In some embodiments, these proteins include CD2, CD16, NKp46, NKp30, and NKG2D. In some such embodiments, fusion proteins intended to target and/or activate NK cells may comprise domains that bind to CD2, CD16, NKp46, NKG2D, etc. Domains that bind to NKG2D may be derived from NKG2D ligands including, but not limited to: MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, and ULBP6. In some embodiments, the fusion proteins described herein comprise a CD58 domain, a domain that binds NKG2D, and optionally a third domain which enhances activation of the target NK cell.

**[0340]** The activation molecule may be encoded by a polynucleotide (e.g. a DNA or RNA polynucleotide).

***Fusion Molecule***

**[0341]** Disclosed herein, in some embodiments, are fusion molecules. The fusion molecule may include an adhesion molecule, a costimulatory molecule, or an activation molecule. The fusion molecule may include an adhesion molecule. The fusion molecule may include a costimulatory molecule. The fusion molecule may include an activation molecule. The fusion molecule may include an adhesion molecule, a costimulatory molecule, and an activation molecule. The fusion molecule may include an adhesion molecule and an activation molecule. The fusion molecule may include a costimulatory molecule and an activation molecule. The fusion molecule may be or include a fusion protein. The fusion molecule may be included as part of a particle. The fusion molecule may be used in a method described herein.

**[0342]** In some embodiments, the disclosure provides a fusion molecule comprising a combination of an adhesion molecule, a costimulatory molecule, and an activation molecule (e.g. a TCR-binding molecule), thereof each component linked directly or indirectly to the other components. In some embodiments, the fusion molecule comprises adhesion molecule, a costimulatory molecule, and an activation molecule (e.g. a TCR-binding molecule). In some embodiments, the fusion molecule comprises adhesion molecule and a costimulatory molecule, but not a TCR-binding molecule. In some embodiments, the fusion molecule comprises adhesion molecule and an activation molecule (e.g. a TCR-binding molecule), but not a costimulatory molecule. The fusion molecule may further comprise one or more additional adhesion molecules, costimulatory molecules, or activation molecules (e.g. TCR-binding molecules).

**[0343]** As used herein, the term "fusion molecule" refers to any molecule having multiple components link together, directly or indirectly, covalently or non-covalently. The fusion molecule may be made up of several proteins. When those proteins are linked together into a single molecule by peptide bonds, the fusion molecule is termed a "fusion protein."

**[0344]** The fusion molecule may be made using various linkers, including chemical (covalent) bonds (*e.g.*, by click chemistry) or by peptide bounds. When the fusion molecule is a fusion protein, the linker between each component of the fusion protein may be a single peptide bound (*i.e.*, a direct C- to N- peptide bound in a polypeptide chain) or via a polypeptide linker. Illustrative polypeptide linkers may include, but are not limited to, the glycine-serine linkers, such as GGSGGS, GSSGSS, or others.

**[0345]** In some embodiments, the fusion molecule is or includes a fusion protein. The fusion protein may comprise an adhesion protein, one or more polypeptide linkers, and a costimulatory portion. In some embodiments, the fusion protein comprises an adhesion molecule, a costimulatory molecule, and an activation molecule.

**[0346]** In some embodiments of the fusion protein, the adhesion molecule is N-terminal to the costimulatory molecule. In some embodiments, the adhesion molecule is N-terminal to the activation molecule. In some embodiments, the adhesion molecule is C-terminal to the costimulatory molecule. In some embodiments, the adhesion molecule is C-terminal to the activation molecule.

**[0347]** In some embodiments of the fusion protein, the activation molecule is N-terminal to the costimulatory molecule. In some embodiments, the activation molecule is N-terminal to the adhesion molecule. In some embodiments, the activation molecule is C-terminal to the

costimulatory molecule. In some embodiments, the activation molecule is C-terminal to the adhesion molecule.

**[0348]** In some embodiments of the fusion protein, the costimulatory molecule is N-terminal to the activation molecule. In some embodiments, the costimulatory molecule is N-terminal to the adhesion molecule. In some embodiments, the costimulatory molecule is C-terminal to the activation molecule. In some embodiments, the costimulatory molecule is C-terminal to the adhesion molecule.

**[0349]** Some embodiments of the fusion protein includes a linker. Some embodiments include multiple linkers. In some embodiments, a linker directly connects the costimulatory molecule with the adhesion molecule. In some embodiments, a linker directly connects the costimulatory molecule with the activation molecule. In some embodiments, a linker directly connects the adhesion molecule with the activation molecule.

**[0350]** In some embodiments of the fusion protein, an N terminal end of the costimulatory molecule is juxtaposed (directly or via a linker) with an end of the adhesion molecule. In some embodiments of the fusion protein, a C terminal end of the costimulatory molecule is juxtaposed (directly or via a linker) with an end of the adhesion molecule. In some embodiments of the fusion protein, an N terminal end of the costimulatory molecule is juxtaposed (directly or via a linker) with an end of the activation molecule. In some embodiments of the fusion protein, a C terminal end of the costimulatory molecule is juxtaposed (directly or via a linker) with an end of the activation molecule.

**[0351]** In some embodiments of the fusion protein, an N terminal end of the activation molecule is juxtaposed (directly or via a linker) with an end of the adhesion molecule. In some embodiments of the fusion protein, a C terminal end of the activation molecule is juxtaposed (directly or via a linker) with an end of the adhesion molecule. In some embodiments of the fusion protein, an N terminal end of the activation molecule is juxtaposed (directly or via a linker) with an end of the costimulatory molecule. In some embodiments of the fusion protein, a C terminal end of the activation molecule is juxtaposed (directly or via a linker) with an end of the costimulatory molecule.

**[0352]** In some embodiments of the fusion protein, an N terminal end of the adhesion molecule is juxtaposed (directly or via a linker) with an end of the costimulatory molecule. In some embodiments of the fusion protein, a C terminal end of the adhesion molecule is juxtaposed (directly or via a linker) with an end of the costimulatory molecule. In some embodiments of the fusion protein, an N terminal end of the adhesion molecule is juxtaposed

(directly or via a linker) with an end of the activation molecule. In some embodiments of the fusion protein, a C terminal end of the adhesion molecule is juxtaposed (directly or via a linker) with an end of the activation molecule.The fusion protein may comprise, in any order, a CD80, a CD80 extracellular portion, or a functional fragment of CD80; a CD58, a CD58 extracellular portion; or a functional fragment of CD58; an activation molecule (e.g. a TCR-binding molecule); and polypeptide linkers.

**[0353]** The fusion protein may comprise, in N- to C-terminal order, CD80, a CD80 extracellular portion, or a functional fragment of CD80; a polypeptide linker; and CD58, a CD58 extracellular portion; or a functional fragment of CD58.

**[0354]** The fusion protein may comprise, in N- to C-terminal order, CD58, a CD58 extracellular portion; or a functional fragment of CD58; a polypeptide linker; and CD80, a CD80 extracellular portion, or a functional fragment of CD80.

**[0355]** The fusion protein may comprise, in N- to C-terminal order, an activation molecule (e.g. a TCR-binding protein); a polypeptide linker; CD80, a CD80 extracellular portion, or a functional fragment of CD80; a polypeptide linker; and CD58, a CD58 extracellular portion; or a functional fragment of CD58.

**[0356]** The fusion protein may comprise, in N- to C-terminal order, CD80, a CD80 extracellular portion, or a functional fragment of CD80; a polypeptide linker; CD58, a CD58 extracellular portion; or a functional fragment of CD58; a polypeptide linker; and an activation molecule (e.g. a TCR-binding protein).

**[0357]** The fusion protein may comprise, in N- to C-terminal order, an activation molecule (e.g. a TCR-binding protein); a polypeptide linker; CD58, a CD58 extracellular portion; or a functional fragment of CD58; a polypeptide linker; and CD80, a CD80 extracellular portion, or a functional fragment of CD80.

**[0358]** The fusion protein may comprise, in N- to C-terminal order, CD58, a CD58 extracellular portion; or a functional fragment of CD58; a polypeptide linker; CD80, a CD80 extracellular portion, or a functional fragment of CD80; a polypeptide linker; and an activation molecule (e.g. a TCR-binding protein).

**[0359]** An illustrative fusion protein comprises a CD58 extracellular region and α-CD3 scFv fused to the N-terminus of a CD80 via a linker; this construct is termed a tri-fusion polypepetide and/or termed “498.”

**[0360]** An illustrative fusion protein comprises a CD58 extracellular region fused to the N-terminus of a CD80 via a linker; this construct is termed a bi-fusion polypeptide and/or

termed "455." In this construct, an αCD3 scFv is expressed as a separate polypeptide in the producer cells.

**[0361]** In each case, the polypeptide linker may be optional. It may be omitted by directly linking protein molecule to the next via a peptide bound. Although one may generate fusion proteins through chemical synthesis, fusion proteins are made by expressing the fusion protein from a single polynucleotide comprising a polynucleotide sequence encoding the entire fusion protein. Methods for designing and cloning polynucleotides are known in the art.

**[0362]** The fusion molecule may be encoded by a polynucleotide (e.g. a DNA or RNA polynucleotide). In some embodiments, the disclosure provides polynucleotides encoding such fusion proteins. The polynucleotide may be an isolated polynucleotide, or it may be part of a vector (*e.g.*, a plasmid) or it may be introduced into and propagated in a host cell.

**[0363]** Polypeptide sequences of illustrative triple CD58+CD80+αCD3scFv fusion proteins are provided in Table 5. In each case, the fusion protein may comprise a polypeptide at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% sequence identity to any sequence in Table 5. In some embodiments, the fusion protein may comprise a polypeptide less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 99%, or less than 100% sequence identity to any sequence in Table 5. In each case, an optional signal peptide is shown in parentheses. The signal peptide is cleaved during expression of the sequence. Sequence identity to a reference sequence is determined without the optional residues. Diagrams of each fusion are provided in **FIG. 27B**.

**Table 5**

| Fusion Protein | SEQ ID NO: | Sequence |
|---|---|---|
| #497 | 73 | (MGVKVLFALICIAVAEA)DIQMTQSPSSLSASVGDRVTITCSASSSV SYMNWYQQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTI SSLQPEDIATYYCQQWSSNPFTFGQGTKLQITRTSGGGGSGGGGSG GGGSQVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQ APGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNSKNTAFLQMD SLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSAGSSGGSGG GGSGGGGSGGGGSFSQQIYGVVYGNVTFHVPSNVPLKEVLWKKQ KDKVAELENSEFRAFSSFKNRVYLDTVSGSLTIYNLTSSDEDEYEM ESPNITDTMKFFLYVLESLPSPTLTCALTNGSIEVQCMIPEHYNSHR GLIMYSWDCPMEQCKRNSTSIYFKMENDLPQKIQCTLSNPLFNTTS SIILTTCIPSSGHSRHRGSGVIHVTKEVKEVATLSCGHNVSVEELAQ TRIYWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNNLSIVILAL RPSDEGTYECVVLKYEKDAFKREHLAEVTLSVKADFPTPSISDFEIP TSNIRRIICSTSGGFPEPHLSWLENGEELNAINTTVSQDPETELYAVS |

| | | |
|---|---|---|
| | | SKLDFNMTTNHSFMCLIKYGHLRVNQTFNWNTTKQEHFPDNLLPS<br>WAITLISVNGIFVICCLTYCFAPRCRERRRNERLRRESVRPV |
| #498 | 33 | (MVAGSDAGRALGVLSVVCLLHCFGFISC)FSQQIYGVVYGNVTFH<br>VPSNVPLKEVLWKKQKDKVAELENSEFRAFSSFKNRVYLDTVSGS<br>LTIYNLTSSDEDEYEMESPNITDTMKFFLYVLESLGSGDIQMTQSPS<br>SLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLA<br>SGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGT<br>KLQITRTSGGGGSGGGGSGGGGSQVQLVQSGGGVVQPGRSLRLSC<br>KASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRGYTNYNQKVKD<br>RFTISRDNSKNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWG<br>QGTPVTVSSAGSSGGSGGGGSGGGGSGGGGSSGVIHVTKEVKEVA<br>TLSCGHNVSVEELAQTRIYWQKEKKMVLTMMSGDMNIWPEYKN<br>RTIFDITNNLSIVILALRPSDEGTYECVVLKYEKDAFKREHLAEVTL<br>SVKADFPTPSISDFEIPTSNIRRIICSTSGGFPEPHLSWLENGEELNAI<br>NTTVSQDPETELYAVSSKLDFNMTTNHSFMCLIKYGHLRVNQTFN<br>WNTTKQEHFPDNLLPSWAITLISVNGIFVICCLTYCFAPRCRERRRN<br>ERLRRESVRPV |

**[0364] FIGs. 34A-34C and 35A-35C** include examples of CD58, CD80 and CD3 scFV triple fusion sequences. Some embodiments include a nucleic acid sequence having at least at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% sequence identity to a nucleic acid sequence in any of **FIGs. 34A-34C and 35A-35C** or any of SEQ ID NOs: 235-246, or to a fragment or portion thereof such as may be identified in the figure keys. Some embodiments include a nucleic acid sequence having less than less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 99%, or less than 100% sequence identity to a nucleic acid sequence in any of **FIGs. 34A-34C and 35A-35C** or any of SEQ ID NOs: 235-246, or to a fragment or portion thereof such as may be identified in the figure keys. Some embodiments include an amino acid sequence having at least at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% sequence identity to an amino acid sequence in any of **FIGs. 34A-34C and 35A-35C** or any of SEQ ID NOs: 235-246, or to a fragment or portion thereof such as may be identified in the figure keys. Some embodiments include an amino acid sequence having less than less than 75%, less than 80%, less than 85%, less than 90%, less than 91%, less than 92%, less than 93%, less than 94%, less than 95%, less than 99%, or less than 100% sequence identity to an amino acid sequence in any of **FIGs. 34A-34C and 35A-35C** or any of SEQ ID NOs: 235-246, or to a fragment or portion thereof such as may be identified in the figure keys.

***Particles***

**[0365]** In some embodiments, the disclosure provides particles of various types, including but not limited to lentiviral particles (*i.e.*, a virion), lipid nanoparticles (LNPs), lipoplexes,

liposomes, and nanocarriers. The particle may include an adhesion molecule, a costimulatory molecule, an activation molecule, a combination thereof. Any of the adhesion molecule, costimulatory molecule, or activation may be included in a fusion molecule. The adhesion molecule, costimulatory molecule, activation molecule, or combination thereof may be included at a surface of the particle. The fusion molecule may be included at a surface of the particle.

**[0366]** The particle may be a lipid nanoparticle (LNP) or a poly(beta-amino) esters (PBAE) nanocarriers, both of which have been shown to transduce T cells when administered to a subject *in vivo* or contacted with T cells *ex vivo*. Using the compositions and methods described herein, transduction of T cells with LNPs and PBAE-based nanocarriers may be increased.

**[0367]** In some embodiments, the particle is a viral particle. Methods for generating viral vectors from various virus types are known in the art. Exemplary types of viral particles that may be recombinantly engineered as delivery vehicles include retroviruses, lentivirus (e.g. HIV and its derivatives and SIV), adeno-associated virus, adenovirus, MMLV retrovirus, MSCV retrovirus, baculovirus, vesicular stomatitis virus, herpes simplex virus, and vaccinia virus. Examples include adeno-associated virus (AAV) particles used for gene therapy. In a preferred embodiment, the particle is a retroviral particle. In a particularly preferred embodiment, the particle is a lentiviral particle.

**[0368]** Lentiviral particles may be made using packaging cell lines as described in WO 2016/139463 or by using a polycistronic vector as described in Int'l Pat. Pub. No. WO 2020/106992 A1. Each of the foregoing specifically describes methods for making lentiviral particles. Their disclosures are incorporated by reference herein. Numerous other methods for making viral particles, including lentiviral particles, may be useful.

**[0369]** Retroviruses, a group that includes lentiviruses, are enveloped viruses. The fusion molecules described herein may be displayed on such enveloped viruses by expressing the fusion molecule, or its various components, in a host cell under the control of a suitable promotor (or promoters). Each component may include a signal sequence for secretion. At least one component should include a transmembrane region or anchor sequence (such as the C-terminal signal sequence that directs the attachment of a GPI anchor). The other components may associate with the first component either during the secretion process or after secretion. In the case of single fusion proteins, only one transmembrane region or anchor sequence may be required, although those of skill in the art may envision and employ

multiple transmembrane regions or anchor sequences. In some embodiments, the fusion molecule is a fusion protein comprising a C-terminal transmembrane region, expressed from a polynucleotide that encodes an N-terminal signal peptide. The signal peptide may be cleaved during expression of the protein at the cell surface of the producer cell, leaving a membrane-tethered fusion protein without the signal sequence. The lentiviral particle may then be made when a virion buds from the surface of the producer cell, incorporating as its envelope portions of the cell membrane that include one or more copies of the fusion protein.

**[0370]** Lentiviral particles generally package a vector genome and, incidentally or intentionally, may package other molecules present in the producer cell. The vector genome may be an artificial vector genome engineered to encode a heterologous protein or polynucleotide.

**[0371]** Lentiviral particles may contain structural and/or functional genetic elements that are primarily from a virus. Lentiviral particles are characterized by the predominant source of genetic or structural material in the lentiviral particle. Thus, the term "retroviral particle" refers to a viral particle containing structural proteins and vector genome elements, primarily from a retrovirus. Likewise, the term "lentiviral particle" refers to a viral particle containing structural proteins and vector genome elements, primarily from a lentivirus. To package its vector genome, a lentiviral particle may generally require at least one copy of the long-terminal repeats (LTRs) that flank a native lentiviral vector genome, or functional variants thereof.

**[0372]** In some embodiments, a viral particle comprises a viral glycoprotein. In some embodiments, the viral particle comprises a viral glycoprotein different from the native viral glycoprotein. When the viral glycoprotein is heterologous to the vector genome, the viral particle is termed a "pseudotyped" viral particle. For example, in some embodiments, the viral particle is derived from HIV, which typically includes the glycoprotein gp120. However, such HIV-based particles may be "pseudotyped" and, instead of expressing their native glycoprotein, express a glycoprotein from a different virus. For example, the viral glycoprotein may be a portion of RD114 or one of its variants, VSV-G, Gibbon-ape leukemia virus (GALV), the Amphotropic envelope glycoprotein, Measles envelope glycoprotein, or baboon retroviral envelope glycoprotein. In some embodiments, the viral envelope glycoprotein is a G protein from the Cocal strain (Cocal G), or a functional variant thereof. Illustrative viral glycoproteins include the VSV G protein, the Cocal G protein, and variants thereof. Illustrative viral glycoproteins may be expressed as a single protein or in

multiple subunits or parts. The viral glycoprotein may serve as ligand for cell-surface receptors on a target cell, and thereby promote transduction or the target cell. The viral glycoprotein may be engineered to lack LDLR binding affinity—for example, by mutation at positions 47 (*e.g.*, K47Q) and/or 354 (*e.g.*, R354A). This may be termed a "blinded" viral glycoprotein. Illustrative envelope variants are provided in, *e.g.*, US 2020/0216502 A1, which is incorporated herein by reference in its entirety. Surprisingly, in some embodiments, the fusion molecules as described herein may permit use of a viral glycoprotein that does not, by itself, cause transduction of target cell. Without being bound by theory, it is believed that the fusion protein may serve as a ligand for cell-surface receptor while the viral glycoprotein retains a structural function, but not a function as a ligand for a cell-surface receptor.

**[0373]** In some embodiments, the viral glycoprotein is a VSV-G glycoprotein that comprises a mutation at position 47. In some embodiments, the viral glycoprotein is a VSV-G glycoprotein that comprises a mutation at position 354. In some embodiments, the viral glycoprotein is a VSV-G glycoprotein that comprises a K47Q mutation. In some embodiments, the viral glycoprotein is a VSV-G glycoprotein that comprises a R354A mutation. In some embodiments, the viral glycoprotein is a VSV-G glycoprotein that comprises a K47Q and a R354A mutation. In some embodiments, the viral glycoprotein is a cocal glycoprotein that comprises a mutation at position 47. In some embodiments, the viral glycoprotein is a cocal glycoprotein that comprises a mutation at position 354. In some embodiments, the viral glycoprotein is a cocal glycoprotein that comprises a K47Q mutation. In some embodiments, the viral glycoprotein is a cocal glycoprotein that comprises a R354A mutation. In some embodiments, the viral glycoprotein is a cocal glycoprotein that comprises a K47Q and a R354A mutation.

**[0374]** The Cocal G protein may have a polypeptide sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity to the following sequence:

**[0375]** NFLLLTFIVLPLCSHAKFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHND
LLGITMKVKMPKTHKAIQADGWMCHAAKWITTCDFRWYGPKYITHSIHSIQPTSE
QCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQATPHHVLVDEYTGEWI
DSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSEDGKKESIG
KPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLP
ECPVGATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPK

NPGTGPAFTIINGTLKYFETRYIRIDIDNPIISKMVGKISGSQTERELWTEWFPYEGV
EIGPNGILKTPTGYKFPLFMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEET
LFFGDTGISKNPVELIEGWFSSWKSTVVTFFFAIGVFILLYVVARIVIAVRYRYQGS
NNKRIYNDIEMSRFRK (SEQ ID NO: 74).

**[0376]** The Cocal G protein may have a polypeptide sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity to the following sequence:

**[0377]** MNFLLLTFIVLPLCSHAKFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHN
DLLGITMKVKMPKTHKAIQADGWMCHAAKWITTCDFRWYGPKYITHSIHSIQPTS
EQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQATPHHVLVDEYTGE
WIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSEDGKKESI
GKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKL
PECPVGATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAP
KNPGTGPAFTIINGTLKYFETRYIRIDIDNPIISKMVGKISGSQTERELWTEWFPYEG
VEIGPNGILKTPTGYKFPLFMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEE
TLFFGDTGISKNPVELIEGWFSSWKSTVVTFFFAIGVFILLYVVARIVIAVRYRYQG
SNNKRIYNDIEMSRFRK (SEQ ID NO: 247)

**[0378]** Illustrative lentiviral particles and methods for making them are described in Naldini et al. *Science* 272:263-7 (1996); Zufferey et al. *J. Virol.* 72:9873-9880 (1998); Dull et al. *J. Virol.* 72:8463-8471 (1998); Miyoshi et al. J. Virol. 72:8150-57 (1998); U.S. Pat. No. 6,013,516; and U.S. Pat. No. 5,994,136.

**[0379]** Protocols for producing replication-defective recombinant viruses are provided in W095/14785, W096/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278,056, and W094/19478.

**[0380]** Viral particles may be assessed in various ways, including, for example, measuring the vector copy number (VCN) or vector genomes (vg) in a sample of viral particle by quantitative polymerase chain reaction (qPCR) or digital droplet PCR (ddPCR), or testing to the viral particles on target cells to measure a "titer" of the virus in, *e.g.*, infectious units per milliliter (IU/mL). For example, the titer may be assessed using a functional assay performed on the cultured tumor cell line HT1080 as described in Humbert et al. *Molecular Therapy* 24:1237–1246 (2016). When titer is assessed on a cultured cell line that is continually dividing, stimulation may be unnecessary, and hence the measured titer may be uninfluenced by surface engineering of the retroviral particle. Other methods for assessing

the efficiency of retroviral vector systems are provided in Gaererts et al. *BMC Biotechnol.* 6:34 (2006).

***Payloads***

**[0381]** The particle may be used to deliver a payload. The term "payload" refers to any molecule or combination of molecules whose delivery to a target cell is desired. Various payloads may be delivered using the particles desired herein, including but not limited to small molecules, polynucleotide and proteins. When the selected target cell is a T cell, the particles of the disclosure may be used to deliver a therapeutic agent targeting T cells, to genetically modified T cells, or to deliver a polynucleotide encoding a protein of interest to the T cell. Similarly, particles disclosed herein may be used to delivery payloads to NK cells.

**[0382]** The payload may be a polynucleotide, such as a polynucleotide whose sequence encodes a protein or a non-coding nucleic acid (*e.g.*, shRNA, microRNA, or siRNA). The polynucleotide may be an RNA, such as a messenger RNA (mRNA) or the vector genome of an RNA virus. It may be a DNA, such as the vector genome of a DNA virus.

**[0383]** The payload may be a polynucleotide comprising a polynucleotide encoding a chimeric antigen receptor (CAR). Illustrative CARs, and polynucleotide encoding them, are described herein. CARs useful in the present disclosure are also provided in in U.S. Pat. Nos. 7,741,465; 9,856,322 and 8,399,964.

**[0384]** In some embodiments, the CAR is a CAR that specifically binds CD19. CAR T therapies targeting CD19 have been approved by the FDA and include YESCARTA, TECARTUS, KYMRIAH AND BREYANZI. CARs targeting CD19 are described, for example, in U.S. Pat. Pub. No. 20160152723; and U.S. Pat. Nos. 10,736,918; 10,357,514; and 7,446,190.

**[0385]** The payload may comprise a polynucleotide whose sequence encodes small molecule-inducible cytokine receptor, such as a rapamycin-activated cell-surface receptor (RACR). Small molecule-inducible cytokine receptors are described in, *e.g.*, U.S. Pat. Pub. No. 2020/0123224.

**[0386]** An illustrative polynucleotide insert for a particle is SEQ ID NO: 76:

GCCACCATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTC
CACGCCGCCAGGCCGGATGTCGTGATGACCCAGACCCCCCTCAGCCTCCCAGT
GTCCCTCGGTGACCAGGCTTCTATTAGTTGCAGATCCAGCCAGTCCCTCGTGC
ACTCTAACGGTAATACCTACCTGAGATGGTATCTCCAGAAGCCCGGACAGAGC

CCTAAGGTGCTGATCTACAAAGTCTCCAACCGGGTGTCTGGAGTCCCTGACCG
CTTCTCAGGGAGCGGTTCCGGCACCGACTTCACCCTGAAGATCAACCGGGTGG
AGGCCGAAGACCTCGGCGTCTATTTCTGCTCTCAGAGTACACATGTGCCCTGG
ACCTTCGGCGGAGGGACCAAGCTGGAGATCAAAAGCTCCGCAGACGATGCCA
AGAAAGATGCCGCTAAGAAAGACGATGCTAAGAAAGACGATGCAAAGAAAG
ACGGTGGCGTGAAGCTGGATGAAACCGGAGGAGGTCTCGTCCAGCCAGGAGG
AGCCATGAAGCTGAGTTGCGTGACCAGCGGATTCACCTTTGGGCACTACTGGA
TGAACTGGGTGCGACAGTCCCCAGAGAAGGGGCTCGAATGGGTCGCTCAGTT
CAGGAACAAACCCTACAATTATGAGACATACTATTCAGACAGCGTGAAGGGC
AGGTTTACTATCAGTAGAGACGATTCCAAATCTAGCGTGTACCTGCAGATGAA
CAATCTCAGGGTCGAAGATACAGGCATCTACTATTGCACAGGGGCATCCTATG
GTATGGAGTATCTCGGTCAGGGGACAAGCGTCACAGTCAGTTTCGTGCCGGTC
TTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGCGACCACCAACACCGG
CGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCA
GCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCT
ACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTA
TCACCCTTTACTGCAACCACAGGAACCGTTTCTCTGTTGTTAAACGGGGCAGA
AAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTAC
TCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGA
TGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGC
AGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTA
CGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCG
AGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGA
TGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAA
GGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTAC
GACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA (SEQ ID NO: 76)

**[0387]** In some embodiments, the CAR may be encoded by a polynucleotide sequence that encodes a signal peptide to signal transport of the CAR in the cell. It is understood that typically the signal peptide is removed from the protein.

**[0388]** An illustrative CAR amino acid sequence without a signal peptide may comprise SEQ ID NO: 77:

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIYK
VSNRVSGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFCSQSTHVPWTFGGGTKLEI

KSSADDAKKDAAKKDDAKKDDAKKDGGVKLDETGGGLVQPGGAMKLSCVTSG
FTFGHYWMNWVRQSPEKGLEWVAQFRNKPYNYETYYSDSVKGRFTISRDDSKSS
VYLQMNNLRVEDTGIYYCTGASYGMEYLGQGTSVTVSFVPVFLPAKPTTTPAPRP
PTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI
TLYCNHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR (SEQ ID NO: 77)

**[0389]** An illustrative CAR amino acid sequence signal peptide may comprise SEQ ID NO: 78:

MALPVTALLLPLALLLHAARP (SEQ ID NO: 78)

**[0390]**

**[0391]** An illustrative polynucleotide insert for a particle is SEQ ID NO: 81:

**[0392]** CTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCT
TTCTGCTGATCCCCTCCGTGCTGACCCAGCCTAGCTCCGTGTCTGCCGCACCAG
GACAGAAGGTGACAATCAGCTGTTCCGGCTCTACCAGCAACATCGGCAACAA
TTACGTGAGCTGGTACCAGCAGCACCCTGGCAAGGCCCCAAAGCTGATGATCT
ACGACGTGTCCAAGAGGCCATCTGGAGTGCCTGATCGGTTCTCCGGCTCTAAG
AGCGGCAATTCCGCCTCTCTGGACATCAGCGGACTGCAGTCCGAGGACGAGG
CAGATTACTATTGCGCCGCCTGGGACGATAGCCTGTCCGAGTTTCTGTTCGGC
ACCGGCACAAAGCTGACCGTGCTGGGCTCTACAAGCGGATCCGGCAAGCCAG
GATCTGGAGAGGGCAGCACAAAGGGACAGGTGCAGCTGGTGGAGAGCGGAG
GAAACCTGGTGCAGCCAGGAGGCTCCCTGCGCCTGTCTTGTGCCGCCAGCGGC
TTTACCTTCGGCTCTTTTAGCATGTCCTGGGTGCGCCAGGCACCTGGAGGAGG
ACTGGAGTGGGTGGCCGGCCTGAGCGCCCGGTCTAGCCTGACACACTATGCCG
ACTCCGTGAAGGGCCGCTTCACCATCTCCCGGGATAACGCCAAGAATAGCGTG
TACCTGCAGATGAATAGCCTGCGGGTGGAGGACACAGCCGTGTACTATTGCGC
CAGGCGCTCCTATGATTCCTCTGGCTACTGGGGCCACTTTTACTCTTATATGGA
CGTGTGGGGACAGGGCACCCTGGTGACAGTGAGCTCCACCACCACACCTGCTC
CTAGACCACCTACACCCGCTCCTACCATCGCCAGCCAGCCTCTGTCTCTGAGA
CCTGAGGCCTGTAGACCTGCCGCTGGAGGCGCTGTGCACACCAGAGGACTGG
ATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCTGGAACATGCGGCGTG
CTGCTCCTGAGCCTGGTGATCACACTGTACTGCAAGCGCGGCCGGAAGAAGCT

GCTCTACATCTTTAAGCAGCCATTCATGCGCCCCGTGCAGACCACACAGGAGG
AGGACGGCTGCTCCTGTCGGTTTCCAGAGGAGGAGGAGGGAGGATGTGAGCT
GAGAGTGAAGTTCTCTAGGAGCGCCGATGCCCCTGCCTATCAGCAGGGACAG
AACCAGCTGTACAACGAGCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGC
TGGATAAGAGGAGGGGAAGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAA
AGAACCCACAGGAGGGCCTGTATAATGAGCTGCAGAAGGACAAGATGGCCGA
GGCCTACTCCGAGATCGGCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACAC
GATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGGACACATACGATGCCC
TGCACATGCAGGCCCTGCCTCCAAGGTGA

**[0393]** In various embodiments, the particle comprises a polynucleotide having a polynucleotide sequence according to one or more of SEQ ID NOs. 75-76 or 80-81, or polynucleotide sequence similar to it. The polynucleotide sequence may encode, and cells transduced by the particle may express, a CAR having a polypeptide sequence according to one or more of SEQ ID NOs. 14, 77, 79, or polynucleotide sequence similar to it. The polypeptide sequence may comprise a humanized immunoglobulin variable domain.

**[0394]** As used herein, the term "similar" may refer to a polynucleotide or polypeptide sequence at least about 75%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% to a reference sequence.

**[0395]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in any order, on a polycistronic transcript: a promoter, a therapeutic protein (*e.g.* CAR), optionally a cytosolic FRB domain or a portion thereof, and optionally a synthetic cytokine polypeptide (*e.g.* RACR). In some embodiments, the polycistronic transcript comprises a promoter and a CAR. Illustrative promoters include, without limitation, a cytomegalovirus (CMV) promoter, a CAG promoter, an SV40 promoter, an SV40/CD43 promoter, and a MND promoter.

**[0396]** In some embodiments, the MND promoter comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 118.

GAACAGAGAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGT
TCCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAA
ACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGAT
GGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTT

TCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCA
ATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCTATATA
AGCAGAGCTCGTTTAGTGAACCGTCAGATCGCTAGC (SEQ ID NO: 118)

**[0397]** In some embodiments, the MND promoter comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 172.

AATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGGATCAAGGTCAGGAACA
GAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTG
CCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGG
ATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCC
CCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAG
TTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGAGC
CCACAACCCCTCACTCGGC (SEQ ID NO: 172)

**[0398]** In some embodiments, the CSF2RA signal sequence comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 173.

ATGCTGCTGCTGGTGACAAGCCTGCTGCTGTGCGAGCTGCCTCACCCAGCCTT
TCTGCTGATCCCC (SEQ ID NO: 173)

**[0399]** The disclosure provides a polynucleotide construct comprising a contiguous polynucleotide sequence encoding at least two synthetic receptors and methods for uses thereof. In some embodiments, the polynucleotide construct is a polycistronic construct encoding a synthetic cytokine receptor, a synthetic chimeric antigen receptor (CAR), and a freely diffusible FRB, in which the cytokine receptor is responsive to rapamycin binding. Advantageously, FRB reduces the inhibitory effects of rapamycin on mTOR in cells engineered to express the polycistronic constructs provided herein. Expression of the freely diffusible FRB can promote consistent activation and proliferation of engineered cells.

**[0400]** In some aspects, provided herein is a lentiviral vector comprising any one of the polycistronic constructs disclosed herein. In some aspects, provided herein is a cell comprising any of the lentiviral vectors disclosed herein.

**[0401]** In some aspects, provided herein is a method of transducing a cell comprising contacting a target cell with any of the polycistronic constructs disclosed herein.

**[0402]** In some aspects, provided herein is a method of expressing a chimeric antigen receptor and/or a synthetic cytokine receptor in a target cell. In some aspects, provided herein is a cell produced by any of the methods disclosed herein.

**[0403]** In some aspects, provided herein is a method of administering to a subject any of the cells disclosed herein. In some aspects, provided herein is a method of administering to a subject any of the lentiviral vectors disclosed herein.

**[0404]** All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference. If a definition set forth herein is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth herein prevails over the definition that is incorporated herein by reference.

**[0405]** The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

*Polycistronic Constructs*

**[0406]** Provided herein are polycistronic constructs encoding one or more separate proteins. In some embodiments, the polycistronic constructs comprise one, two, three, or four expression cassettes each encoding a separate protein. In some embodiments, the polycistronic constructs comprise four expression cassettes each encoding a separate protein. In some embodiments, the expression cassettes are separated by cleavable linkers.

**[0407]** In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding an FRB. In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding a chimeric antigen receptor (CAR). In some embodiments, the polycistronic constructs provided herein comprise a nucleotide sequence encoding a synthetic cytokine polypeptide. In some embodiments, the synthetic cytokine polypeptide comprises a synthetic cytokine gamma chain polypeptide and a synthetic cytokine beta chain polypeptide. In some embodiments, the synthetic cytokine gamma chain comprises interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the synthetic cytokine gamma chain further comprises FRB. In some embodiments, the synthetic cytokine beta chain comprises interleukin 2 receptor subunit β (IL2RB). In some embodiments, the synthetic cytokine gamma chain comprises further FKBP12. In other embodiments, the synthetic cytokine gamma chain comprises interleukin

2 receptor subunit γ (IL2RG). In some embodiments, the synthetic cytokine gamma chain further comprises FKBP12. In some embodiments, the synthetic cytokine beta chain comprises interleukin 2 receptor subunit β (IL2RB). In some embodiments, the synthetic cytokine beta chain further comprises FRB.

**[0408]** In some embodiments, the polycistronic construct provided herein comprises nucleotide sequences encoding an FRB, a synthetic cytokine polypeptide, and a CAR.

**[0409]** In some embodiments, the polycistronic construct comprises a nucleotide sequence encoding FRB, a nucleotide sequence encoding a synthetic cytokine polypeptide, and a nucleotide sequence encoding a CAR. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises a first nucleotide sequence encoding FRB:IL2RG and a second nucleotide sequence encoding FKBP12:IL2RB. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises a first nucleotide sequence encoding FKBP12:IL2RG and a second nucleotide sequence encoding FRB:IL2RB.

*Cytosolic FRB*

**[0410]** In some embodiments, an expression cassette of the polycistronic construct encodes an FRB domain. The FRB domain is an approximately 270 base pair (bp) domain from the mTOR protein kinase. It may be expressed in the cytosol as a freely diffusible soluble protein.

**[0411]** In some embodiments, the first expression cassette in the polycistronic construct comprises a nucleotide sequence encoding an FRB. In some embodiments, when the FRB is expressed, it is a freely diffusible soluble protein ("free FRB").

**[0412]** In some of any embodiments, the method further includes administering a non-physiological ligand to the subject. In some embodiments, the non-physiological ligand is able to bind to the synthetic cytokine receptor and induce gamma cytokine signaling in the cell. In some embodiments, the nonphysiological ligand includes rapamycin or a rapamycin analog.

**[0413]** In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 256, 257, or 258. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NOs: 256, 257, or 258. In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID Nos: 256, 257, or 258.

**[0414]** In some embodiments, the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs: 251, 252, or 260. In some embodiments, the FRB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs: 251, 252, or 260. In some embodiments, the FRB comprises the amino acid sequence of SEQ ID NOs: 251, 252, or 260.

**[0415]** In some embodiments, synthetic cytokine receptor complex comprises a cytosolic polypeptide that binds to the ligand or a complex comprising the ligand.

**[0416]** Advantageously, the cytosolic FRB confers resistance to the immunosuppressive effect of the non-physiological ligand (*e.g.*, rapamycin or rapalog).

*Synthetic Cytokine Receptor*

**[0417]** In some embodiments, an expression cassette of the polycistronic construct encodes a synthetic cytokine receptor. The synthetic cytokine receptors of the present disclosure comprise a synthetic gamma chain and a synthetic beta chain, each comprising a dimerization domain. The dimerization domains controllably dimerize in the presence of a non-physiological ligand, thereby activating signaling of the synthetic cytokine receptor.

**[0418]** The synthetic gamma chain polypeptide comprises a first dimerization domain, a first transmembrane domain, and an interleukin-2 receptor subunit gamma (IL-2RG) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2G intracellular domain.

**[0419]** The synthetic beta chain polypeptide comprises a second dimerization domain, a second transmembrane domain, and an intracellular domain selected from an interleukin-2 receptor subunit beta (IL-2RB) intracellular domain, an interleukin-7 receptor subunit beta (IL-7RB) intracellular domain, or an interleukin-21 receptor subunit beta (IL-21RB) intracellular domain. The dimerization domain may be extracellular (N-terminal to the transmembrane domain) or intracellular (C-terminal to the transmembrane domain and N- or C-terminal to the IL-2RB or IL-7RB intracellular domain).

**[0420]** In some embodiments, the polycistronic construct provided herein comprises one or more nucleotide sequences encoding a synthetic cytokine receptor. In some embodiments, the one or more nucleotide sequences correspond to one or more expression cassettes. In some embodiments, the polynucleotide construct provided herein comprises one expression cassette encoding IL2RG and a second expression cassette encoding IL2RB.

**[0421]** In some embodiments, the synthetic gamma chain polypeptide is encoded by a nucleic acid sequence that encodes a signal peptide. In some embodiments, the synthetic beta chain polypeptide is encoded by a nucleic acid sequence that encodes a signal peptide. A skilled artisan is readily familiar with signal peptides that can provide a signal to transport a nascent protein in the cells. Any of a variety of signal peptides can be employed.

**[0422]** In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 261, 262, or 263. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide is at least 100% identical to the nucleotide sequence of SEQ ID NOs: 261, 262, or 263. In some embodiments, the nucleotide encoding the synthetic cytokine gamma chain polypeptide comprises the nucleotide sequence of SEQ ID NOs: 261, 262, or 263.

**[0423]** In some embodiments, the synthetic cytokine gamma chain polypeptide comprises interleukin 2 receptor subunit γ (IL2RG). In some embodiments, the IL2RG comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs: 264 or 265. In some embodiments, the IL2RG comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs: 264 or 265. In some embodiments, the IL2RG comprises the amino acid sequence of SEQ ID NOs: 264 or 265.

**[0424]** In some embodiments, the second expression cassette further comprises a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 257. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NO: 257. In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO: 257.

**[0425]** In some embodiments, the FRB comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 252. In some embodiments, the FRB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 252. In some embodiments, the FRB comprises the amino acid sequence of SEQ ID NO: 252.

**[0426]** In some embodiments, the second expression cassette is codon optimized.

**[0427]** In some embodiments, the second expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 266. In some embodiments, the second expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO: 266. In some embodiments, the second expression cassette comprises the nucleotide sequence of SEQ ID NO: 266.

**[0428]** In some embodiments, the second expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 267. In some embodiments, the second expression cassette encodes an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 267. In some embodiments, the second expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO: 267.

**[0429]** In some embodiments, the second expression cassette further comprises a nucleotide sequence encoding FKBP12. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 268 or 269. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 100% identical to the nucleotide sequence of SEQ ID NOs: 268 or 269. In some embodiments, the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NOs: 268 or 269.

**[0430]** In some embodiments, the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 253. In some embodiments, the FKBP12 comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 253. In some embodiments, the FKBP12 comprises the amino acid sequence of SEQ ID NO: 253.

**[0431]** In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NOs: 270 or 271. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide is at least 100% identical to the nucleotide sequence of SEQ ID NOs: 270 or 271. In some embodiments, the nucleotide encoding the synthetic cytokine beta chain polypeptide comprises the nucleotide sequence of SEQ ID NOs: 270 or 271.

**[0432]** In some embodiments, the synthetic cytokine beta chain polypeptide comprises interleukin 2 receptor subunit β (IL2RB).

**[0433]** In some embodiments, the IL2RB comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NOs: 272 or 273. In some embodiments, the IL2RB comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NOs: 272 or 273. In some embodiments, the IL2RB comprises the amino acid sequence of SEQ ID NOs: 272 or 273.

**[0434]** In some embodiments, the third expression cassette further comprises a nucleotide sequence encoding FKBP12.

**[0435]** In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 274. In some embodiments, the nucleotide sequence encoding the FKBP12 is at least 100% identical to the nucleotide sequence of SEQ ID NO: 274. In some embodiments, the nucleotide sequence encoding the FKBP12 comprises the nucleotide sequence of SEQ ID NO: 274.

**[0436]** In some embodiments, the FKBP12 comprises an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 275. In some embodiments, the FKBP12 comprises an amino acid sequence at least 100% identical to the amino acid sequence of SEQ ID NO: 275. In some embodiments, the FKBP12 comprises the amino acid sequence of SEQ ID NO: 275.

**[0437]** In some embodiments, the third expression cassette is codon optimized.

**[0438]** In some embodiments, the third expression cassette comprises a nucleotide sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 276. In some embodiments, the third expression cassette comprises a nucleotide sequence at least 100% identical to the nucleotide sequence of SEQ ID NO: 276. In some embodiments, the third expression cassette comprises the nucleotide sequence of SEQ ID NO: 276.

**[0439]** In some embodiments, the third expression cassette encodes an amino acid sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 277. In some embodiments, the third expression cassette encodes an amino acid sequence at least 100% identical to the amino

acid sequence of SEQ ID NO: 277. In some embodiments, the third expression cassette encodes an amino acid sequence comprising the sequence of SEQ ID NO: 277.

**[0440]** In some embodiments, the third expression cassette further comprises a nucleotide sequence encoding FRB. In some embodiments, the nucleotide sequence encoding the FRB is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 257. In some embodiments, the nucleotide sequence encoding the FRB is at least 100% identical to the nucleotide sequence of SEQ ID NO: 257. In some embodiments, the nucleotide sequence encoding the FRB comprises the nucleotide sequence of SEQ ID NO: 257.

*Intracellular Domain*

**[0441]** In some embodiments, the intracellular signaling domain of the first transmembrane receptor protein comprises an interleukin-2 receptor subunit gamma (IL2Rg) domain.

**[0442]** In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-2RB intracellular domain, and a second dimerization domain.

**[0443]** In some embodiments, the synthetic beta chain comprises an interleukin-2 receptor subunit beta (IL2RB) intracellular domain. IL2RB is also known as IL15RB or CD122. Thus, when referred to herein, IL2RB can also mean IL15RB. That is, the terms are used interchangeably in the present disclosure.

**[0444]** In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-7RB intracellular domain, and a second dimerization domain.

**[0445]** In some embodiments, the synthetic beta chain comprises an interleukin-7 receptor subunit beta (IL7RB) intracellular domain.

**[0446]** In some embodiments, the synthetic cytokine receptor comprises a first transmembrane receptor protein comprising an IL-2RG intracellular domain, a first dimerization domain, a second transmembrane receptor protein comprising an IL-21RB intracellular domain, and a second dimerization domain.

**[0447]** In some embodiments, the synthetic beta chain comprises an interleukin-21 receptor subunit beta (IL21RB) intracellular domain.

*Dimerization Domain*

**[0448]** The dimerization domains may be heterodimerization domains, including but not limited to FK506-Binding Protein of size 12 kD (FKBP) and a FKBP12-rapamycin binding (FRB) domain, which dimerize in the presence of rapamycin or a rapalog.

**[0449]** Alternatively, the first dimerization domain and the second dimerization domain may be a FK506-Binding Protein of size 12 kD (FKBP) and a calcineurin domain, which dimerize in the presence of FK506 or an analogue thereof.

**[0450]** In some embodiments the dimerization domains are homodimerization domains selected from:

i) FK506-Binding Protein of size 12 kD (FKBP);

ii) cyclophiliA (CypA); or

iii) gyrase B (CyrB);

with the corresponding non-physiological ligands being, respectively

i) FK1012, AP1510, AP1903, or AP20187;

ii) cyclosporin-A (CsA); or

iii) coumermycin or analogs thereof.

**[0451]** In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a FKBP domain and a cyclophilin domain.

**[0452]** In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a FKBP domain and a bacterial dihydrofolate reductase (DHFR) domain.

**[0453]** In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are a calcineurin domain and a cyclophilin domain.

**[0454]** In some embodiments, the first and second dimerization domains of the transmembrane receptor proteins are PYR1-like 1 (PYL1) and abscisic acid insensitive 1 (ABI1).

*Transmembrane Domain*

**[0455]** The transmembrane domain is the sequence of the synthetic cytokine receptor that spans the membrane. The transmembrane domain may comprise a hydrophobic alpha helix. In some embodiments, the transmembrane domain is a human protein.

**[0456]** In some embodiments, the TM domain and the intracellular signaling domain are from the same cytokine receptor. In some embodiments, the synthetic gamma chain polypeptide contains an IL-2RG TM domain and an IL-2RG intracellular domain. In some

embodiments, the synthetic beta chain polypeptide contains an IL-2RB TM domain and an IL-2RB intracellular domain. In some embodiments, the synthetic beta chain polypeptide contains an IL-7RB TM domain and an IL-7RB intracellular domain. In some embodiments, the synthetic beta chain polypeptide contains an IL-21RB TM domain and an IL-21RB intracellular domain.

**[0457]** In some embodiments, one or more additional contiguous amino acids of the ectodomain directly adjacent to the TM domain of the cytokine receptor also can be included as part of the polypeptide sequence of a chain of the synthetic cytokine receptor. In some embodiments, 1-20 contiguous amino acids of the ectodomain adjacent to the TM domain of the cytokine receptor is included as part of the polypeptide sequence of a chain of the synthetic cytokine receptor. The portion of the ectodomain may be a contiguous sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids directly adjacent (e.g. N-terminal to) the TM sequence.

**[0458]** In some embodiments, the synthetic cytokine receptor is able to be bound by the non-physiological ligand rapamycin or a rapamycin analog. In some embodiments, the synthetic cytokine receptor is responsive to the non-physiological ligand rapamycin or a rapamycin analog, in which binding of the non-physiological ligand to the dimerization domains of the synthetic cytokine receptor induces cytokine receptor-mediated signaling in the cell, such as via the JAK/STAT pathway.

*Illustrative Polycistronic Constructs*

**[0459]** In some embodiments, the polycistronic construct comprises in 5′ to 3′ order a nucleotide sequence encoding FRB, a nucleotide sequence encoding a synthetic cytokine polypeptide, and a nucleotide sequence encoding a CAR. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises in 5′ to 3′ order a first nucleotide sequence encoding FRB operably linked to IL2RG and a second nucleotide sequence encoding FKBP12 operably linked to IL2RB. In some embodiments, the nucleotide sequence encoding the synthetic cytokine polypeptide comprises in 5′ to 3′ order a first nucleotide sequence encoding FKBP12 operably linked to IL2RG and a second nucleotide sequence encoding sFRB operably linked to IL2RB.

**[0460]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in 5′ to 3′ order on a polycistronic transcript: MND promoter – FRB – [T2A and ER signal sequence] – RACRg – [P2A and ER signal sequence] – RACRb – [P2A and hCSF2R signal sequence] – anti-CD19 CAR.

**[0461]** In some embodiments, the lentiviral particle comprises a T2A nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 278.

**[0462]** GAGGGCCGAGGCAGCCTGCTGACCTGCGGTGATGTGGAAGAAAACCCG GGCCCC (SEQ ID NO: 278).

**[0463]** In some embodiments, the lentiviral particle comprises an ER signal sequence nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 279.

**[0464]** ATGCCCTTGCCCGTGACCGCGTTGCTCCTGCCCTTGGCTCTACTGCTGC ACGCCGCTAGACCC (SEQ ID NO: 279).

**[0465]** In some embodiments, the lentiviral particle comprises a P2A nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 280.

**[0466]** GCCACCAATTTCAGCCTCCTGAAACAAGCCGGTGACGTTGAAGAGAAC CCCGGCCCC (SEQ ID NO: 280).

**[0467]** In some embodiments, the lentiviral particle comprises an ER signal sequence nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 281.

**[0468]** ATGCCCCTGGGGTTGCTGTGGTTGGGACTCGCCCTCCTCGGCGCCCTGC ACGCTCAAGCC (SEQ ID NO: 281).

**[0469]** In some embodiments, the lentiviral particle comprises a P2A nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 282.

**[0470]** GCAACAAACTTTTCTCTGCTGAAGCAGGCCGGCGATGTGGAAGAAAAC CCTGGACCT (SEQ ID NO: 282).

**[0471]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in 5′ to 3′ order on a polycistronic transcript:

(a) a MND promoter;

(b) a CAR;

(c) a cytosolic FRB domain or a portion thereof;

(d) a RACR cell-surface receptor; and

(e) a WPRE sequence

**[0472]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in 5′ to 3′ order:

(a) a CAR;

(b) a cytosolic FRB domain or a portion thereof; and

(c) a RACR cell-surface receptor.

**[0473]** In some embodiments, the lentiviral particle comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 119.

**[0474]** GAACAGAGAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAA
GCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGG
GCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGA
ACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCA
GATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAAC
TAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAGCTC
TATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCTAGC (SEQ ID NO: 119)

**[0475]** In some embodiments, the lentiviral particle comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 120.

**[0476]** MLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRASQDISK
YLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYF
CQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLVAPSQSL
SVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNS
KSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSESKYGPP
CPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMRPVQTT
QEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPEMWHEG
LEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQE
WCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSGATNFSLLKQAGDVEENPGPM
PLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKK
FDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPG
IIPPHATLVFDVELLKLGEGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYFWLER
TMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGA

LGEGPGASPCNQHSPYWAPPCYTLKPETGSGATNFSLLKQAGDVEENPGPMALPV
TALLLPLALLLHAARPILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMM
ERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRI
SKGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFS
QLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPA
SLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSS
PQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQE
RVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPP
GQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV (SEQ ID NO: 120)

**[0477]** In some embodiments, the lentiviral particle comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 121.

**[0478]** In some embodiments, the lentiviral particles of the present disclosure comprises a polynucleotide sequence encoding, in 5′ to 3′ order on a polycistronic transcript:

(a) a MND promoter;

(b) a cytosolic FRB domain or a portion thereof;

(c) a RACR cell-surface receptor;

(d) a CAR; and

(e) a WPRE sequence.

**[0479]** In some embodiments, the lentiviral particles of the present disclosure comprises a polynucleotide sequence encoding, in 5′ to 3′ order:

(a) a cytosolic FRB domain or a portion thereof;

(b) a RACR cell-surface receptor; and

(c) a CAR.

**[0480]** In some embodiments, the lentiviral particle comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 122.

**[0481]** MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ
AYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSGATNFSLLKQ
AGDVEENPGPMPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVV
HYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISP
DYAYGATGHPGIIPPHATLVFDVELLKLGEGSNTSKENPFLFALEAVVISVGSMGLI
ISLLCVYFWLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERL

CLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPETGSGATNFSLLKQAGD
VEENPGPMALPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLYFGERNVKG
MFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLL
QAWDLYYHVFRRISKGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKK
VLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKV
TQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEE
DPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG
SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPD
AGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVGSGATN
FSLLKQAGDVEENPGPMLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRV
TISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTIS
NLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQES
GPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSA
LKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSV
TVSSESKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIF
KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 122)

**[0482]** In some embodiments, the lentiviral particle comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 123.

**[0483]** ATGGAGATGTGGCACGAGGGACTGGAGGAGGCAAGCAGACTGTACTTT
GGCGAGAGGAACGTGAAGGGCATGTTCGAGGTGCTGGAGCCACTGCACGCAA
TGATGGAGAGGGGACCACAGACCCTGAAGGAGACATCTTTCAACCAGGCATA
CGGAAGGGACCTGATGGAGGCACAGGAGTGGTGCCGGAAGTATATGAAGAGC
GGCAATGTGAAGGACCTGCTGCAGGCCTGGGATCTGTACTATCACGTGTTTCG
GAGAATCTCCAAGGGCTCTGGCGCCACCAACTTCTCCCTGCTGAAGCAGGCCG
GCGATGTGGAGGAGAATCCTGGACCAATGCCACTGGGACTGCTGTGGCTGGG
ACTGGCCCTGCTGGGCGCCCTGCACGCCCAGGCCGGCGTGCAGGTGGAGACA
ATCAGCCCTGGCGACGGACGCACCTTTCCAAAGAGGGGACAGACATGCGTGG
TGCACTACACCGGCATGCTGGAGGATGGCAAGAAGTTCGACAGCTCCAGAGA
TAGGAATAAGCCCTTTAAGTTCATGCTGGGCAAGCAGGAAGTGATCAGGGGA
TGGGAGGAGGGAGTGGCACAGATGTCTGTGGGACAGCGGGCCAAGCTGACAA

TCAGCCCAGACTATGCATACGGAGCAACCGGACACCCTGGAATCATCCCACCT
CACGCCACACTGGTGTTTGATGTGGAGCTGCTGAAGCTGGGCGAGGGCAGCA
ACACCTCCAAGGAGAATCCATTTCTGTTCGCCCTGGAGGCCGTGGTCATCTCT
GTGGGCAGCATGGGCCTGATCATCTCCCTGCTGTGCGTGTACTTTTGGCTGGA
GCGCACAATGCCACGGATCCCCACCCTGAAGAACCTGGAGGACCTGGTGACC
GAGTACCACGGCAATTTCTCCGCCTGGTCTGGCGTGAGCAAGGGACTGGCAGA
GTCTCTGCAGCCAGATTATAGCGAGCGGCTGTGCCTGGTGAGCGAGATCCCAC
CCAAGGGAGGCGCCCTGGGAGAGGGACCAGGAGCCTCCCCTTGCAACCAGCA
CTCTCCTTACTGGGCCCCTCCATGTTATACCCTGAAGCCAGAGACAGGCAGCG
GAGCTACTAACTTCTCCCTGCTGAAGCAAGCAGGCGACGTGGAAGAAAATCCT
GGACCAATGGCACTGCCAGTGACCGCCCTGCTGCTGCCTCTGGCCCTGCTGCT
GCACGCAGCCAGACCCATCCTGTGGCACGAAATGTGGCATGAAGGCCTGGAG
GAGGCAAGCAGGCTGTACTTTGGCGAGCGGAATGTGAAAGGAATGTTTGAAG
TGCTGGAGCCTCTGCACGCCATGATGGAGAGGGGCCCTCAGACCCTGAAGGA
GACATCCTTTAACCAGGCCTACGGCAGAGACCTGATGGAGGCCCAGGAGTGG
TGCAGGAAGTATATGAAGTCTGGAAATGTGAAAGACCTGCTGCAGGCCTGGG
ATCTGTATTATCACGTGTTCAGGCGCATCTCTAAGGGCAAGGATACAATCCCT
TGGCTGGGACACCTGCTGGTGGGACTGAGCGGAGCCTTTGGCTTCATCATCCT
GGTGTATCTGCTGATCAACTGCCGCAATACAGGCCCATGGCTGAAGAAGGTGC
TGAAGTGTAACACCCCCGACCCTTCCAAGTTCTTTTCTCAGCTGTCTAGCGAGC
ACGGCGGCGATGTGCAGAAGTGGCTGTCCTCTCCATTTCCCAGCTCCTCTTTCA
GCCCAGGAGGACTGGCACCAGAGATCTCCCCACTGGAGGTGCTGGAGAGGGA
CAAGGTGACCCAGCTGCTGCTGCAGCAGGATAAGGTGCCTGAGCCAGCCTCCC
TGAGCTCCAACCACTCCCTGACCTCTTGCTTTACAAATCAGGGCTACTTCTTTT
TCCACCTGCCAGACGCACTGGAGATCGAGGCATGTCAGGTGTATTTCACATAC
GATCCCTATAGCGAGGAGGACCCTGATGAGGGAGTGGCCGGCGCCCCAACCG
GATCTAGCCCACAGCCTCTGCAGCCACTGAGCGGAGAGGACGATGCATATTGT
ACATTTCCTTCCCGCGACGATCTGCTGCTGTTCTCTCCAAGCCTGCTGGGAGGA
CCAAGCCCACCTTCCACCGCACCAGGCGGCTCCGGGGCAGGGGAGGAGCGGA
TGCCACCCTCTCTGCAGGAGAGAGTGCCAAGGGACTGGGATCCACAGCCACT
GGGACCTCCAACCCCTGGAGTGCCAGACCTGGTGGATTTCCAGCCCCCTCCAG
AGCTGGTGCTGAGAGAGGCAGGAGAGGAGGTGCCTGACGCAGGACCAAGAG
AGGGCGTGAGCTTTCCTTGGTCCAGGCCACCTGGACAGGGAGAGTTCAGAGCC

CTGAACGCCAGGCTGCCCCTGAATACAGACGCCTACCTGTCTCTGCAGGAGCT
GCAGGGCCAGGATCCTACACACCTGGTCGGATCTGGCGCCACCAACTTTAGCC
TGCTGAAGCAGGCAGGCGACGTGGAAGAGAACCCTGGACCAATGCTGCTGCT
GGTGACCAGCCTGCTGCTGTGCGAGCTGCCACACCCTGCCTTCCTGCTGATCC
CAGATATCCAGATGACACAGACCACATCCTCTCTGTCCGCCTCTCTGGGCGAC
AGAGTGACCATCTCTTGTAGGGCCAGCCAGGATATCTCCAAGTACCTGAACTG
GTATCAGCAGAAGCCTGACGGCACAGTGAAGCTGCTGATCTACCACACCTCTA
GGCTGCACAGCGGAGTGCCATCCCGGTTCAGCGGATCCGGATCTGGAACAGA
CTATTCTCTGACCATCAGCAACCTGGAGCAGGAGGATATCGCCACATACTTTT
GCCAGCAGGGCAATACCCTGCCATATACATTCGGCGGAGGAACCAAGCTGGA
GATCACCGGAAGCACATCCGGATCTGGCAAGCCAGGATCCGGAGAGGGATCT
ACAAAGGGAGAGGTGAAGCTGCAGGAGAGCGGACCAGGACTGGTGGCACCC
AGCCAGTCCCTGTCTGTGACCTGTACAGTGTCTGGCGTGAGCCTGCCCGATTA
CGGCGTGTCCTGGATCAGACAGCCACCAAGGAAGGGACTGGAGTGGCTGGGC
GTGATCTGGGGCTCTGAGACCACATACTATAATAGCGCCCTGAAGTCCCGGCT
GACCATCATCAAGGACAACAGCAAGTCCCAGGTGTTTCTGAAGATGAATAGC
CTGCAGACCGACGATACAGCCATCTACTATTGCGCCAAGCACTACTATTACGG
CGGCTCCTACGCCATGGATTATTGGGGCCAGGGCACCTCCGTGACAGTGAGCT
CCGAGTCTAAGTATGGCCCTCCATGCCCCCCTTGTCCTATGTTCTGGGTGCTGG
TGGTGGTGGGAGGCGTGCTGGCCTGTTACTCCCTGCTGGTGACCGTGGCCTTT
ATCATCTTCTGGGTGAAGCGCGGCCGGAAGAAGCTGCTGTATATCTTTAAGCA
GCCCTTCATGAGACCTGTGCAGACCACACAGGAGGAGGACGGCTGCAGCTGT
AGGTTTCCAGAGGAGGAGGAGGGAGGATGCGAGCTGCGCGTGAAGTTCTCTC
GGAGCGCCGATGCCCCTGCCTACCAGCAGGGACAGAACCAGCTGTATAACGA
GCTGAATCTGGGCCGGAGAGAGGAGTACGACGTGCTGGATAAGAGGAGGGGA
AGAGACCCAGAGATGGGAGGCAAGCCTCGGAGAAAGAACCCACAGGAGGGC
CTGTACAATGAGCTGCAGAAGGACAAGATGGCCGAGGCCTATTCCGAGATCG
GCATGAAGGGAGAGAGGCGCCGGGGCAAGGGACACGATGGCCTGTACCAGG
GCCTGAGCACCGCCACAAAGGACACCTATGATGCCCTGCACATGCAGGCCCTG
CCACCCAGGTGA (SEQ ID NO: 123)

**[0484]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in 5′ to 3′ order on a polycistronic transcript:

(a) a MND promoter;

(b) a cytosolic FRB domain or a portion thereof;

(c) a CAR;

(d) TGF-β DN domain or portion thereof; and

(e) a WPRE sequence.

**[0485]** In some embodiments, the lentiviral particles of the present disclosure comprise a polynucleotide sequence encoding, in 5′ to 3′ order:

(a) a cytosolic FRB domain or a portion thereof;

(b) a CAR; and

(c) a TGF-β DN domain or portion thereof.

**[0486]** In some embodiments, the lentiviral particle comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 124.

**[0487]** MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKGSGATNFSLLKQ AGDVEENPGPMLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASLGDRVTISCRA SQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQE DIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVKLQESGPGLV APSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRL TIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSSE SKYGPPCPPCPMFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLLKQAGDVEENPGPM GRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQVTG ISLLPPLGVAISVIIIFYCYRVNRQQKRRR (SEQ ID NO: 124)

**[0488]** In some embodiments, the lentiviral particle comprises a nucleic acid sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 125.

**[0489]** ATGGAGATGTGGCACGAGGGACTGGAGGAGGCATCCAGACTGTACTTC GGCGAGAGGAACGTGAAGGGCATGTTTGAGGTGCTGGAGCCACTGCACGCCA TGATGGAGAGAGGCCCCCAGACCCTGAAGGAGACATCTTTCAACCAGGCCTA

TGGAAGGGACCTGATGGAGGCACAGGAGTGGTGCCGGAAGTACATGAAGAGC
GGCAATGTGAAGGACCTGCTGCAGGCCTGGGATCTGTACTATCACGTGTTCCG
GAGAATCAGCAAGGGCTCCGGCGCCACCAACTTTAGCCTGCTGAAGCAGGCA
GGCGACGTGGAGGAGAATCCAGGACCTATGCTGCTGCTGGTGACATCCCTGCT
GCTGTGCGAGCTGCCACACCCAGCCTTCCTGCTGATCCCCGATATCCAGATGA
CCCAGACCACAAGCTCCCTGAGCGCCTCCCTGGGCGACAGGGTGACAATCTCT
TGTCGGGCCAGCCAGGATATCTCCAAGTATCTGAATTGGTACCAGCAGAAGCC
CGACGGCACCGTGAAGCTGCTGATCTATCACACATCTAGACTGCACAGCGGCG
TGCCTTCCAGGTTTTCTGGCAGCGGCTCCGGCACCGACTACTCTCTGACAATCA
GCAACCTGGAGCAGGAGGATATCGCCACCTATTTCTGCCAGCAGGGCAATACC
CTGCCTTACACATTTGGCGGCGGCACAAAGCTGGAGATCACCGGCTCTACAAG
CGGATCCGGCAAGCCAGGATCCGGAGAGGGATCTACCAAGGGAGAGGTGAAG
CTGCAGGAGAGCGGACCTGGACTGGTGGCACCATCTCAGAGCCTGTCCGTGAC
CTGTACAGTGTCTGGCGTGAGCCTGCCAGATTATGGCGTGAGCTGGATCAGGC
AGCCACCTAGGAAGGGACTGGAGTGGCTGGGCGTGATCTGGGGCTCCGAGAC
CACATACTATAACAGCGCCCTGAAGTCCCGCCTGACCATCATCAAGGACAACT
CTAAGAGCCAGGTGTTCCTGAAGATGAATTCCCTGCAGACCGACGATACAGCC
ATCTACTATTGCGCCAAGCACTACTATTACGGCGGCTCTTATGCCATGGATTAC
TGGGGCCAGGGCACCAGCGTGACAGTGTCTAGCGAGTCCAAGTACGGCCCAC
CCTGCCCTCCATGTCCCATGTTTTGGGTGCTGGTGGTGGTGGGAGGCGTGCTG
GCCTGTTATTCCCTGCTGGTGACCGTGGCCTTCATCATCTTTTGGGTGAAGCGC
GGCCGGAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGAGACCCGTGCA
GACCACACAGGAGGAGGACGGCTGCAGCTGTAGGTTCCCAGAGGAGGAGGAG
GGAGGATGCGAGCTGAGGGTGAAGTTTTCCCGGTCTGCCGATGCCCCTGCCTA
TCAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAATCTGGGCAGGCGCGAG
GAGTACGACGTGCTGGATAAGAGGAGAGGAAGGGACCCTGAGATGGGAGGC
AAGCCAAGGCGCAAGAACCCTCAGGAGGGCCTGTATAATGAGCTGCAGAAGG
ACAAGATGGCCGAGGCCTACTCCGAGATCGGCATGAAGGGAGAGCGGAGAAG
GGGCAAGGGACACGATGGCCTGTATCAGGGCCTGAGCACCGCCACAAAGGAC
ACCTACGATGCACTGCACATGCAGGCCCTGCCACCTAGAGGATCTGGAGCCAC
AAACTTCAGCCTGCTGAAGCAGGCCGGCGATGTGGAGGAGAATCCTGGACCA
ATGGGAAGAGGACTGCTGAGGGGACTGTGGCCACTGCACATCGTGCTGTGGA
CCAGGATCGCCTCTACAATCCCACCCCACGTGCAGAAGAGCGTGAACAATGA

CATGATCGTGACCGATAACAATGGCGCCGTGAAGTTTCCCCAGCTGTGCAAGT
TCTGTGACGTGCGCTTTTCCACCTGTGATAACCAGAAGTCCTGCATGTCTAATT
GTAGCATCACATCCATCTGCGAGAAGCCTCAGGAGGTGTGCGTGGCCGTGTGG
CGGAAGAACGACGAGAATATCACCCTGGAGACAGTGTGCCACGATCCCAAGC
TGCCTTATCACGACTTCATCCTGGAGGATGCCGCCTCTCCTAAGTGTATCATGA
AGGAGAAGAAGAAGCCAGGCGAGACCTTCTTTATGTGCAGCTGTTCCTCTGAC
GAGTGCAACGATAATATCATCTTCTCCGAGGAGTACAACACCTCTAATCCTGA
CCTGCTGCTGGTCATCTTTCAGGTGACAGGCATCTCCCTGCTGCCTCCACTGGG
CGTGGCCATCTCTGTGATCATCATCTTTTATTGTTACAGAGTGAACAGGCAGC
AGAAGCGCCGGCGCTAG (SEQ ID NO: 125)

**[0490]** In some embodiments, the FRB domain comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 251.

**[0491]** MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISK (SEQ ID NO: 251)

**[0492]** In some embodiments, the IL-2 Receptor gamma domain comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 252.

**[0493]** ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETS FNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISK (SEQ ID NO: 252)

**[0494]** In some embodiments, the IL-2 Receptor beta domain comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 253.

**[0495]** GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFML GKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLK L (SEQ ID NO: 253)

**[0496]** In some embodiments, the Rapamycin-Activated Cell-Surface Receptor (RACR) and FRB domain complex comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 254.

**[0497]** MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ
AYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKASRRKRGSGEG
RGSLLTCGDVEENPGPMPLPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLY
FGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK
SGNVKDLLQAWDLYYHVFRRISKGSNTSKENPFLFALEAVVISVGSMGLIISLLCV
YFWLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEI
PPKGGALGEGPGASPCNQHSPYWAPPCYTLKPETGSGATNFSLLKQAGDVEENPG
PMPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDG
KKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGH
PGIIPPHATLVFDVELLKLGEGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGP
WLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLE
RDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYD
PYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPS
TAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAG
EEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV(S
EQ ID NO: 254)

**[0498]** In some embodiments, the Rapamycin-Activated Cell-Surface Receptor (RACR) and FRB domain complex and anti-CD19 CAR comprises a polypeptide sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 99%, or 100% identity to SEQ ID NO: 255.

**[0499]** MEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ
AYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKASRRKRGSGEG
RGSLLTCGDVEENPGPMPLPVTALLLPLALLLHAARPILWHEMWHEGLEEASRLY
FGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMK
SGNVKDLLQAWDLYYHVFRRISKGSNTSKENPFLFALEAVVISVGSMGLIISLLCV
YFWLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEI
PPKGGALGEGPGASPCNQHSPYWAPPCYTLKPETGSGATNFSLLKQAGDVEENPG
PMPLGLLWLGLALLGALHAQAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDG
KKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGH
PGIIPPHATLVFDVELLKLGEGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGP
WLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLE
RDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYD
PYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPS

TAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAG
EEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLVG
SGATNFSLLKQAGDVEENPGPLLLVTSLLLCELPHPAFLLIPDIQMTQTTSSLSASL
GDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDY
SLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITGSTSGSGKPGSGEGSTKGEVK
LQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTY
YNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQ
GTSVTVSSGAVHTRGLDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
EIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 255)

***Pharmaceutical Compositions and Kits***

**[0500]** In some embodiments, the disclosure provides a pharmaceutical composition comprising a particle according to the disclosure and a pharmaceutically acceptable carrier.

**[0501]** In some embodiments, the disclosure provides a kit comprising the particle and instructions for use in transduction of target cells and/or treatment of a subject. The kit may include a pharmaceutically acceptable carrier and/or an injection device. The kit may further include suitable tubing for administering the particles.

***Formulations***

**[0502]** The formulations and compositions of the present disclosure may comprise a combination of any number of viral particles, and optionally one or more additional pharmaceutical agents (polypeptides, polynucleotides, compounds etc.) formulated in pharmaceutically acceptable or physiologically-acceptable compositions for administration to a cell, tissue, organ, or an animal, either alone, or in combination with one or more other modalities of therapy. In some embodiments, the one or more additional pharmaceutical agents further increases transduction efficiency of viral particles.

**[0503]** In some embodiments, the formulations and compositions of the present disclosure may comprise a combination of any number of viral particles.

**[0504]** The present disclosure also provides pharmaceutical compositions comprising an expression cassette or vector (*e.g.*, therapeutic vector) disclosed herein and one or more pharmaceutically acceptable carriers, diluents or excipients. In some embodiments, the pharmaceutical composition comprises a lentiviral vector comprising an expression cassette

disclosed herein, *e.g.*, wherein the expression cassette comprises one or more polynucleotide sequences encoding one or more chimeric antigen receptor (CARs) and variants thereof.

**[0505]** The pharmaceutical compositions that contain the expression cassette or vector genome may be in any form that is suitable for the selected mode of administration, for example, for intraventricular, intramyocardial, intracoronary, intravenous, intra-arterial, intra-renal, intraurethral, epidural, intrathecal, intraperitoneal, or intramuscular administration. The vector genome can be administered, as sole active agent, or in combination with other active agents, in a unit administration form, as a mixture with pharmaceutical supports, to animals and human beings. In some embodiments, the pharmaceutical composition comprises cells transduced *ex vivo* with any of the vector genomes according to the present disclosure.

**[0506]** The pharmaceutical compositions of the present disclosure, formulation of pharmaceutically acceptable excipients and carrier solutions may be useful to those of skill in the art, such as for development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, intraperitoneal, and intramuscular administration and formulation.

**[0507]** In some embodiments, the present disclosure provides formulations or compositions suitable for the delivery of viral vector systems (*i.e.*, viral-mediated transduction) including, but not limited to, retroviral (*e.g.*, lentiviral) vectors.

***Method of Use In vitro* or *Ex Vivo***

**[0508]** The compositions described herein such as fusion proteins or particles described may be used *in vitro* or *ex vivo*. The lentiviral particles described may be used *ex vivo*, in a cell manufacturing process or at a bedside as described, *e.g.*, in Int'l Pat. Pub. No. WO 2022/072885, Int'l Pat. Pub. No. 2019/217954, Int'l Pat. Pub. No. 2020/123649, and Int'l Pat. Pub. No. 2009/072003. In some embodiments, the disclosure provides an *ex vivo* method of transducing target cells, comprising contacting the target cells with the particle according to the present disclosure. In some embodiments, the particles described herein may be used to transduce cells that have not been previously activated. For example, the particles described herein may be useful for transducing cells that have not been previously contacted with cell activation beads or activation reagents (*e.g.* Dynabeads or other reagents comprising anti-CD3 and/or anti-CD28 antibodies or binding fragments thereof). Where a method herein describes use of a lentiviral particle, use of another particle is contemplated

where appropriate and feasible. Where a method herein describes use of a lentiviral particle, use of a composition or fusion molecule is also contemplated where appropriate and feasible. For example, a fusion molecule, contained on the surface of a lentiviral particle, or a pharmaceutical composition may be administered to or contacted with a cell such as an immune cell (e.g. T cell).

**[0509]** Non-limiting examples of cells that can be the target of the lentiviral particle described herein include T lymphocytes, dendritic cells (DC), $T_{reg}$ cells, B cells, Natural Killer cells, and macrophages.

*Ex-Vivo Manufacturing*

**[0510]** In some aspects, the disclosure provides a method of delivering a nucleic acid to a cell *ex vivo*. In some embodiments, the disclosure provides a method of delivering a nucleic acid to an immune cell *ex vivo*. In some embodiments, the lentiviral particles of the disclosure activate and transduce an immune cell *ex vivo*.In some embodiments, the disclosure provides a method of delivering a nucleic acid to a cell in an ex-vivo closed-loop manufacturing process. In some embodiments, an ex-vivo manufacturing process is an extracorporeal process. In exemplary embodiments, the lentiviral vectors disclosed herein permit delivery of a nucleic acid to a target cell during a closed-loop process. Exemplary methods of closed-loop and/or extracorporeal processes are disclosed in US Patent Publication No. 2021/0244871 and WO2022072885, both of which are incorporated herein in their entirety. In some embodiments, the lentiviral vectors as disclosed herein may be used to transduce cells ex vivo. For example, in exemplary closed-loop manufacturing processes, cells are obtained from a subject, washed, incubated and/or contacted with lentiviral particles, optionally washed again, and infused into the subject in a closed-loop system. In such embodiments, the lentiviral particles as disclosed herein are useful even without prior activation of the cells and are capable of binding to the cells in a short incubation and/or contacting step. In some embodiments, the incubation and/or contacting step is approximately or less than one hour. In some embodiments, the incubation and/or contacting step is approximately or less than one hour, approximately or less than two hours, approximately or less than three hours, approximately or less than four hours, or approximately or less than five hours. In some embodiments, the incubation and/or contacting step is less than 12 hours or less than 24 hours. In some embodiments, a nucleic acid is delivered to a cell by transduction with a lentiviral vector such that the nucleic acid enters the cell ex-vivo. In some embodiments, a nucleic acid is delivered to a cell by

contacting the lentiviral vector to the surface of the cell. In such embodiments, the nucleic acid may enter the cell ex-vivo or in vivo after the cells (complexed with the lentiviral vector) are infused back into the subject.

**[0511]** In some embodiments, provided herein are bedside systems and methods for performing cell-based therapies and treatments in a subject-connected, closed-loop continuous-flow manner, including cellular modifications and treatments, e.g., to produce chimeric antigen receptor T (CAR T) cells. In some embodiments of a system described herein, blood is removed from a subject, processed, customized, and returned to the subject in a closed-loop, continuous-flow manner. An arrangement of modules and units are used sequentially for separation and collection of target cells from whole blood, employing for example, leukapheresis and/or other cell enrichment techniques, optionally including cell enrichment, purification and/or washing using an elutriation device, followed by one or more cell customization procedures, e.g., to generate CAR-T cells, optionally followed by cell enrichment, purification, fractionation, and/or washing, after which the processed and modified fraction comprising CAR-T cells are returned to the subject by means of an outlet conduit. One exemplary system is manufactured by Lupagen™ and is a closed-loop, continuous-flow system. Such systems and methods are disclosed in WO2019217964, which is incorporated herein by reference in its entirety.

**[0512]** In some embodiments, the lentiviral vectors as disclosed herein eliminate the need for an ex-vivo activation step. In such embodiments, the isolated cells could be transduced directly after leukapheresis, washing, or selection. It is contemplated that the surface engineering described herein enables the lentiviral particles disclosed herein to activate and transduce cells in a single step. In such embodiments, the lentiviral particles disclosed herein may enable a short or truncated manufacturing process, reducing the time spent in ex-vivo manufacturing by eliminating one or more unit operations (e.g. activation prior to transduction) and/or reducing the amount of time that may be necessary in post-transduction cell culture. Without wishing to be bound by theory, in some embodiments the lentiviral vectors as described herein, in particular those particles comprising a fusion multidomain protein, bind to target cells with a higher avidity than lentiviral particles not comprising a fusion multidomain protein. In such embodiments, the fusion multidomain protein may allow the described lentiviral particles to bind target cells more tightly, reducing the incubation time for transduction and increasing transduction frequency and efficiency. In

some embodiments, the time to effectively bind a lentiviral particle to a target cell may be one hour or less.

**[0513]** It is contemplated that the present disclosure provides an *ex vivo* method of generating an engineered cell comprising contacting a target cell with a particle comprising a fusion molecule comprising an adhesion molecule linked to a costimulatory molecule, a fusion molecule comprising an adhesion molecule linked to an activation molecule, or a fusion molecule comprising an adhesion molecule linked to a costimulatory molecule and an activation molecule wherein the contacting step is performed for approximately one hour, for approximately two hours, approximately three hours, approximately four hours, approximately five hours, approximately six hours, approximately 12 hours, approximately 24 hours, approximately 12-24 hours (inclusive of endpoints), or longer. This method may require the contacting step to be performed in a closed-loop manufacturing or extracorporeal process as described herein. Alternatively, this method may require the contacting step to be performed in a traditional ex-vivo engineered cell manufacturing process. For example, in a perfusion incubator or a centrifuge (such as a Sepax or Rotea machine).

***Method of Use In Vivo***

**[0514]** In some embodiments, the lentiviral particles described herein transduce target cells *in vivo*. In some embodiments, the target cells are immune cells. In some embodiments, the immune cells are T cells. In some embodiments, the lentiviral particles described herein transduce T cells *in vivo*. In some embodiments, the lentiviral particles described herein transduce T cells *in vivo* generating CAR T cells. In some embodiments, the lentiviral particles described herein display a CD58-CD80-anti-CD3 scFv tri-fusion polypeptide and transduce T cells *in vivo* generating CAR T cells. Where a method herein describes use of a lentiviral particle, use of another particle is contemplated where feasible.

**[0515]** In some embodiments, the viral particle is administered via a route selected from the group consisting of extracorporeal, parenteral, intravenous, intramuscular, subcutaneous, intratumoral, intraperitoneal, and intralymphatic. In some embodiments, the viral particle is administered multiple times. In some embodiments, the viral particle is administered by intralymphatic injection of the viral particle. In some embodiments, the viral particle is administered by intraperitoneal injection of the viral particle. In some embodiments, the viral particle is administered by intra-nodal injection – that is, the viral particle may be administered via injection into one or more lymph nodes. In some embodiments, the lymph nodes for administration are the inguinal lymph nodes. In some embodiments, the viral

particle is administered by injection of the viral particle into tumor sites (*i.e.* intratumoral). In some embodiments, the viral particle is administered subcutaneously. In some embodiments, the viral particle is administered systemically. In some embodiments, the viral particle is administered intravenously. In some embodiments, the viral particle is administered intra-arterially. In some embodiments, the viral particle is a lentiviral particle.

**[0516]** In some embodiments, the lentiviral particle is administered by intraperitoneal, subcutaneous, or intranodal injection. In some embodiments, the lentiviral particle is administered by intraperitoneal injection. In some embodiments, the lentiviral particle is administered by subcutaneous injection. In some embodiments, the lentiviral particle is administered by intranodal injection.

**[0517]** The present disclosure provides a method of treatment comprising administering a therapeutically effective dose of lentiviral particles to a subject in need thereof. In some embodiments, a therapeutically effective dose of the lentiviral particles described herein are administered. In some embodiments, a therapeutically effective dose comprises about $0.1\times10^6$ transducing units (TUs), about $0.2\times10^6$ TUs, about $0.3\times10^6$ TUs, about $0.4\times10^6$ TUs, about $0.5\times10^6$ TUs, about $0.6\times10^6$ TUs, about $0.7\times10^6$ TUs, about $0.8\times10^6$ TUs, about $0.9\times10^6$ TUs, about $1\times10^6$ TUs, about $1.2\times10^6$ TUs, about $1.4\times10^6$ TUs, about $1.6\times10^6$ TUs, about $1.8\times10^6$ TUs, about $0.1\times10^6$ TUs, about $0.1\times10^6$ TUs, about $0.1\times10^6$ TUs, about $0.1\times10^6$ TUs, about $2\times10^6$ TUs, about $2.5\times10^6$ TUs, about $3\times10^6$ TUs, about $4\times10^6$ TUs, about $5\times10^6$ TUs, about $6\times10^6$ TUs, about $7\times10^6$ TUs, about $8\times10^6$ TUs, about $9\times10^6$ TUs, about $1\times10^7$ TUs, about $2\times10^7$ TUs, about $3\times10^7$ TUs, about $4\times10^7$ TUs, about $5\times10^7$ TUs, about $6\times10^7$ TUs, about $7\times10^7$ TUs, about $8\times10^7$ TUs, about $9\times10^7$ TUs, about $1\times10^8$ TUs, about $2\times10^8$ TUs, about $3\times10^8$ TUs, about $4\times10^8$ TUs, about $5\times10^8$ TUs, about $6\times10^8$ TUs, about $7\times10^8$ TUs, about $8\times10^8$ TUs, about $9\times10^8$ TUs, about $1\times10^9$ TUs, or about $2\times10^9$ TUs.

**[0518]** In some embodiments, the transduced immune cells comprising the polynucleotide of the present disclosure is administered to the subject.

**[0519]** The disclosure provides a method of treating a malignancy in a subject, comprising administering to the subject the lentiviral particles or pharmaceutical composition of the disclosure. In some embodiments, the malignancy is a B-cell malignancy, a myeloma, or a solid tumor malignancy. The disclosure provides a method of treating diffuse large B-cell lymphoma (DLBCL), Burkitt's type large B-cell lymphoma (B-LBL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), mantle cell

lymphoma (MCL), hematological malignancy, colon cancer, lung cancer, liver cancer, breast cancer, renal cancer, prostate cancer, ovarian cancer, skin cancer, melanoma, bone cancer, brain cancer, squamous cell carcinoma, leukemia, myeloma, B cell lymphoma, kidney cancer, uterine cancer, adenocarcinoma, pancreatic cancer, chronic myelogenous leukemia, glioblastoma, neuroblastoma, medulloblastoma, or sarcoma in a subject, comprising administering to the subject the lentiviral particles or pharmaceutical composition of the disclosure.

***Method of Making***

**[0520]** In some embodiments, the disclosure provides a method of making a particle, comprising introducing a polynucleotide encoding a vector genome into a host cell comprising a polynucleotide encoding a fusion molecule (or fusion protein) as described herein. The fusion molecule (or fusion protein) and the vector genome are expressed by the host cell. The host cell packages the vector genome into a lentiviral particle comprising the fusion molecule (or fusion protein).

**[0521]** In some embodiments, the disclosure provides an *in vivo* method of transducing target cells in a subject in need thereof, comprising administering to the subject a particle or pharmaceutical composition of the disclosure. The particle may be administered by intranodal, intravenous, or subcutaneous injection.

**[0522]** Various disease or disorders may be treated using particles as disclosed herein, or pharmaceutical composition comprising them. The particles may be administered to a subject suffering from or at risk for a B-cell malignancy, relapsed/refractory malignancy, diffuse large B-cell lymphoma (DLBCL), Burkitt's type large B-cell lymphoma (B-LBL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), mantle cell lymphoma (MCL), hematological malignancy, colon cancer, lung cancer, liver cancer, breast cancer, renal cancer, prostate cancer, ovarian cancer, skin cancer, melanoma, bone cancer, brain cancer, squamous cell carcinoma, leukemia, myeloma, B cell lymphoma, kidney cancer, uterine cancer, adenocarcinoma, pancreatic cancer, chronic myelogenous leukemia, glioblastoma, neuroblastoma, medulloblastoma, or sarcoma.

**[0523]** Lentiviral particles of the present disclosure may enhance *in vivo* activity. Lentiviral particles of the present disclosure resist serum inactivation. Lentiviral particles of the present disclosure provide efficient targeting of activated T cells. Lentiviral particles of the present disclosure may require low physical particle per transducing unit compared to two

component glycoproteins. Lentiviral particles of the present disclosure retain potential to transduce a broad range of non-T effector cells. Lentiviral particles of the present disclosure enhance particle to T cell binding. Lentiviral particles of the present disclosure enhance T cell activation. Lentiviral particles of the present disclosure enhance immune cell expansion. Lentiviral particles of the present disclosure enhance immune cell transduction. Lentiviral particles of the present disclosure enhance anti-tumor potency. Lentiviral particles of the present disclosure enhance immune cell persistence.

**[0524]** Some embodiments include a method of making an adhesion molecule, a costimulatory molecule, an activation molecule, or a fusion molecule. The method may include transcribing or translating a nucleic acid (such as a DNA or RNA) that encodes a protein comprising the adhesion molecule, costimulatory molecule, activation molecule, or fusion molecule.

***Kits***

**[0525]** Disclosed herein, in some embodiments, are kits. In some embodiments, the kit includes an adhesion molecule. In some embodiments, the kit includes a costimulatory molecule. In some embodiments, the kit includes an activation molecule. In some embodiments, the kit includes a fusion molecule. In some embodiments, the kit includes a particle. In some embodiments, the kit includes a composition described herein. The kit may include instructions for use, such as instructions for use in a method herein.

## EXAMPLES

**[0526]** The following Examples describe how embodiments of the invention may be made, evaluated, and used. The Examples are intended to be illustrative and non-limiting.

***Example 1***

**[0527]** This Example shows some impacts of incorporating a costimulatory molecule such as CD80, and/or an adhesion protein such as CD58, onto the surface of a lentiviral particle. A schematic of some such lentiviral particles is provided in **FIG. 1**.

*Virus Production*

**[0528]** $1.2 \times 10^6$ 293T cells were seeded into TC-treated 6-well plates in a total volume of 2.5 ml Complete DMEM media per well. 24 hours later, cells were transfected at room temperature.

**[0529]** The following DNA was added to 500 µl serum free OptiMEM™ media: 2µg transfer plasmid, 1µg Gag/pol plasmid, 1µg REV plasmid, and 1µg envelope plasmid. 15 µl (15µg) PEI was then added to the media/DNA mix. The solution was then mixed well

and incubated at room temperature for 20 minutes. The media/DNA/PEI mix was then added to 2.5 ml fresh Complete DMEM media. Seeding media in 293T-containing well was removed and replaced with fresh media containing the transfection reagents and placed in a 37° C humidified incubator. 48 hours later, the supernatant was collected and filtered through a 0.45µm PVDF filter. The virus-containing supernatant was concentrated using an Amicon-Ultra 15 100K column and centrifuged at 3000xg for 30 minutes at 4° C. The virus was then stored at 4° C until use.

*293T Transduction Titers*

**[0530]** $1 \times 10^5$ 293T cells were seeded into TC-treated 12 well plates in 1ml Complete DMEM media. 24 hours later, empty wells were counted 3X to calculate titer. Then virus was added to wells in amounts of: 2ul, 1ul, 0.5ul, 0.2ul, 0.1ul, or 0.05ul virus per well. Virus was diluted 1:100 before adding to 293T cells. 3 days later, 293T cells were harvested for analysis by flow cytometry. Media was removed, and cells were washed in PBS. Cells were then washed in Trypsin and incubate for ~3-5 minutes in a 37° C incubator. Cells were resuspended in 1ml FACS buffer and ~100-200ul were added to a 96 well V bottom plate. Flow cytometry analysis was performed for mCherry expression.

*293T Titer calculation*

**[0531]** TU/ml = (# of cells at time of transduction x %mCherry+ x 100)/(vector volume in ul x 1000)

**[0532]** Engineered particles packaging an anti-CD19 CAR containing either a CD3scFV alone or a CD3scFV+CD80, CD3scFV+CD58, or CD3scFV+CD80+CD58 were added to PBMCs from 2-3 donors.

***Example 2***

**[0533]** This Example shows that incorporation of a costimulatory molecule and/or adhesion molecule on a lentiviral particle enhanced transduction of PBMCs by lentiviral particles as generated in **Example 1**.

*Virus Production*

**[0534]** All solutions used were the same as those described in Example 1. $28 \times 10^6$ 293T cells were seeded into 16x T175 flasks (8x per vector) with 28e6 293T cells each in a total volume of 25ml Complete DMEM media. 24 hours later, cells were transfected. Virus was produced as described in Example 1. All viruses included a Cocal envelope protein.

**[0535]** List of Virus preps made for study:

*1. CD3scfv only*

*2. CD3scfv+CD58*

*3. CD3scfv+CD80*

*PBMC Transduction and staining for flow cytometry*

**[0536]** $50 \times 10^6$ PBMCs were thawed, diluted to $2 \times 10^6$ cells/ml in complete media (*e.g.* RPMI or Optimem). IL-2 was added to a final concentration of 50IU/ml.

**[0537]** 500µl(1e6 cells) were added to the wells of a Non TC-treated 48 well plate. Vector was added to the wells at MOI=10, 5, and 2 based on the SupT1 ddPCR titer and the plates were placed in 37° C incubator.

**[0538]** After 3 days, vector was washed out and replaced with 500µl fresh RPMI media+IL-2 (50IU/ml). Cells were mixed and 100-300µl were added to wells in a 96 well V-bottom plate for activation flow cytometry analysis. Cells were then washed with 200µl FACS buffer. The cell pellets were resuspended in 50-100µl PBS containing LiveDead Stain (1:1000) and incubate at 4° C for 20 min followed by another wash in 200µlFACS buffer. Cells were resuspended in 50µlof FACS buffer + surface stain cocktail, incubated for 30 min at 4° C, washed in 200µlFACS buffer.

*Results and Conclusions*

**[0539]** To assess if lentiviral particles with costimulatory molecules could better activate human T cells, the vector particles were added to human PBMCs at several MOI's. 3 days later, the virus was removed and the cells were given fresh media and analyzed for the activation marker CD25. CD3scfv+CD58 and CD3scfv+CD80 particles potently activated CD8 T cells compared to CD3scfv only (**FIG. 2A** and **FIG. 2B**). Furthermore, CD25 upregulation was dose-dependent (**FIG. 2A** and **FIG. 2B**). CD3scfv only lentiviral particles induced minimal levels of CD25 compared to the particles with CD80 or CD58 (**FIG. 2A** and **FIG. 2B**).

**[0540]** To examine transduction, 6 total days after vector addition, samples were analyzed for anti-CD19 CAR expression. Mirroring the CD25 expression on day 3, CD3scfv+CD58 and CD3scfv+CD80 particles were capable of transducing unstimulated PBMCs while CD3scfv only particles transduced unstimulated PBMCs to a lesser extent (**FIGs. 2C-2F**). Furthermore, transduction occurred in a dose-dependent manner for both CD3 and CD8 T cells (**FIGs. 2C-2F**). The data show that CD3scfv+CD58 and CD3scfv+CD80 particles efficiently activate and transduce unstimulated PBMCs *in vitro* compared to CD3scfv only. Importantly, the enhanced particles results in increased numbers of CAR+ T cells (FIGs. 2D-2F).

**[0541]** To determine if adding costimulatory molecules to particles enhances Rapa-mediated expansion of CAR+ cells *in vitro*, the fold expansion of CD8 T cells was determined using CD3scfv+CD80 particles compared to CD3scfv only particles. PMBCs were cultures in either IL-2 only media or Rapamycin-only media. The addition of the costimulatory molecule did not affect the fold expansion when cultured with IL-2 only (**FIG. 2G**) but the costimulatory molecule induced a dramatic expansion when cultured with Rapamycin-media (**FIG. 2H**). These results demonstrate that adding costimulatory molecules to particles enhanced Rapamycin-mediated expansion of CAR+ cells *in vitro*.

**[0542]** This study demonstrated the ability of the CD3scfv + costimulatory molecules envelope construct to deliver payloads consisting of an anti-CD19 CAR to unstimulated PBMCs *in vitro*. The CD3scfv+CD58 and CD3scfv+CD80 particles induced activation of T cells as measured by CD25 expression and this activation correlated with transduction as measured by % of T cells expressing the anti-CD19 CAR and total CAR+ T cells. Furthermore, activation and transduction occurred in a dose-dependent manner. Costimulatory molecules also enhance Rapamycin-mediated expansion of CAR+ cells *in vitro*. This data further supports the use CD3scfv+CD58 and CD3scfv+CD80 particles to deliver CAR payloads to unstimulated PBMCs *in vitro* and *in vivo*.

***Example 3***

**[0543]** This Example shows that a combination of a costimulatory molecule (CD80 in this case), and an adhesion protein (CD58 in this case) further enhanced T cell activation and transduction. Particles having both molecules were generated. These particles were examined for their ability to activate and transduce unstimulated human PBMCs compared to particles only having anti-CD3scFv.

*Virus Production*

**[0544]** All solutions used were the same as those described in Example 1. $28 \times 10^6$ 293T cells were seeded into 16x T175 flasks (8x per vector) with $28 \times 10^6$ 293T cells each in a total volume of 25ml Complete DMEM media. 24 hours later, cells were transfected. Virus was produced as described in Example 1.

**[0545]** List of Virus preps made for study:

*1. CD3scfv only*

*2. CD3scfv+CD58*

*3. CD3scfv+CD8*

*4. CD3scfv+CD80+CD58*

*PBMC Transduction and Analysis*

**[0546]** PBMCs were transduced and analyzed for expression as described in Example 2.

**[0547]** Supernatant cytokine analysis was measured by Meso Scale Discovery (MSD) 3 days after transduction.

**[0548]** Total K562.CD19, Raji, and Nalm6 tumor cells were tracked over time on an IncuCyte® for up to 15 days.

*Results and Conclusions*

**[0549]** To assess whether lentiviral particles with costimulatory molecules and adhesion molecules enhance T cell activation and transduction, the lentiviral particles were added to human PBMCs at several MOI's. 3 days later, the virus was removed and the cells were given fresh media and analyzed for the activation marker CD25. CD3scfv+CD80+CD58 particles potently activated CD8 T cells compared to CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only (**FIG. 3A** and **FIG. 3B**). Furthermore, CD25 upregulation was dose-dependent and CD3scfv+CD80+CD58 particles activated CD8 T cells at a much lower dose (**FIG. 3A** and **FIG. 3B**). CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only lentiviral particles induced minimal levels of CD25 compared to the CD3scfv+CD80+CD58 particles (**FIG. 3A** and **FIG. 3B**).

**[0550]** To further characterize T cell activation, 3 total days after vector addition, samples were analyzed for cytokine expression. Similar to CD25 expression, CD3scfv+CD80 and CD3scfv+CD80+CD58 particles were capable of inducing IFN-γ production unstimulated PBMCs at lower doses whereas CD3scfv+CD58 and CD3scfv only particles transduced unstimulated PBMCs to a lesser extent (**FIG. 3C**). Furthermore, CD3scfv+CD80+CD58 particles induced robust IL-2 and TNF-α whereas CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only did not (**FIG. 3D** and **FIG. 3E**). The data show that CD3scfv+CD80+CD58 particles efficiently induce cytokine production in unstimulated PBMCs *in vitro* compared to CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only.

**[0551]** To examine the role of CD80 and CD58 on transduction, 3 total days after vector addition, samples were analyzed for anti-CD19 CAR expression with CD3scfv+CD80 and CD3scfv+CD58 mixed particles (**FIG. 3F** and **FIG. 3G)** or CD3scfv+CD80+CD58 on the same particle (**FIG. 3H** and **FIG. 3I** ) compared to CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only. CD3scfv+CD80 and CD3scfv+CD58 mixed particles or CD3scfv+CD80+CD58 on the same particle were both capable of transducing unstimulated PBMCs to a greater extent than CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only (**FIG.**

**3F**, **FIG. 3G, FIG. 3H** and **FIG. 3I**). Furthermore, transduction occurred in a dose-dependent manner for both CD3 and CD8 T cells (**FIG. 3F**, **FIG. 3G, FIG. 3H** and **FIG. 3I**). The data show that both CD58 and CD80 either in mixed particles or on the same particle better activate and transduce unstimulated PBMCs *in vitro* compared to CD3scfv+CD58, CD3scfv+CD80, and CD3scfv only.

**[0552]** To determine whether lentiviral particles with costimulatory and/or adhesion molecules have enhanced particle binding to T cells, the particles were cultured with PBMCs for 6 hours and then were analyzed for particle-associated molecules on T cells (Cocal, CD80, and CD58). Both CD3scfv+CD58 and CD3scfv+CD80+CD58 increased Cocal staining (**FIG. 3J**) and only CD3scfv+CD80+CD58 demonstrated high stating for CD80 (**FIG. 3K**) and CD58 (**FIG. 3L**). The data show that the combination of CD3scfv+CD80+CD58 enhances particle binding to T cells.

**[0553]** To determine whether different T cell subtypes are generated by the lentiviral particles, PBMCs cultured with the lentiviral particles were profiled and gated on viable, CD3+ and CD8+. The cells were further analyzed by flow and principal component analysis was done based on parameters listed CCR7, CD45R, CD45RA, CD27, CD25, CAR+, total cells, CD4, and CD8. The analysis revealed that 3 main clusters of differentiation are produced by the different particles (**FIG. 3M**).

**[0554]** Next, T cell subtypes generated by the particles was profiled. The cells were assessed using CD45RA and CCR7 markers 7 days post transduction at an MOI of 10. Naïve T cells are CD45RA+CCR7+, effector T cells ($T_{eff}$) are CD45RA-CCR7-, central memory T cells ($T_{cm}$) are CD45RA-CCR7+, and terminally differentiated effector memory T cells ($T_{emra}$) are CD45RA+CCR7-. In a first experiment, CD3scfv only particles produced a majority of $T_{eff}$ cells whereas CD3scfv+CD80 particles produced a majority of $T_{cm}$ cells (**FIG. 3N**). In a second experiment, CD3scfv only particles produced both $T_{eff}$ and $T_{cm}$ cells, CD3scfv+CD80 particles produced a majority of $T_{cm}$ cells, CD3scfv+CD58 particles produced a majority of $T_{cm}$ cells, and CD3scfv+CD80+CD58 produced a majority of $T_{cm}$ cells (**FIG. 3O**). The data show that both the addition of CD80 and/or CD58 to the particle consistently produces a $T_{cm}$ cell phenotype. CD45RA-CCR7+ $T_{cm}$ cells are thought to have increased longevity and proliferative capacity and correlate with better antitumor responses *in vivo*.

**[0555]** To assess the ant-tumor efficacy of CAR T cells generated using lentiviral particles expressing costimulatory and/or adhesion molecules, PBMCs were transduced and cultured

with tumor cells. Specifically, particles comprising a nucleotide sequence encoding an anti-CD19 CAR were added to PBMCs at an MOI of 10, along with tumor cells (K562.CD19 or Raji cells) at PBMC:Tumor ratio of 5:1 and put directly on an Incucyte. Tumor cell killing was measured over time. The highest killing was observed with particles composed of at least CD80 in addition to CD3scfv (**FIG. 4A** and **FIG. 4B**). In a subsequent experiment, tumor cell killing was measured 7 days after transduction with an MOI of 10. The total number of CAR+ cells were calculated and incubated with either K562.CD19 or Raji cells at E:T ratios of 0.5 and 1, respectively. CAR T cells generated using a mixture of individual particles with CD80 or CD58. Similarly, the highest killing was observed with particles composed of at least CD80 in addition to CD3scfv, including CD80+CD58 (**FIG. 4C** and **FIG. 4D**). An additional experiment determined the effect for CAR T cells generated with a single lentiviral particle having both CD80 and CD58. Tumor cell killing was measured 7 days after transduction at an MOI 10. The total number of CAR+ cells were calculated and incubated with either K562.CD19 or Nalm6 cells at E:T ratios of 1:1, respectively. The CD80+CD58 dual particle provided the highest cytotoxic function (**FIG. 4E** and **FIG. 4F**).

**[0556]** This study demonstrated the CD3scfv+CD80+CD58 particles induced the highest differentiation of T cells and the highest cytokine production at the lowest MOI. CD3scfv+CD80+CD58 particles further had the highest T cell binding. Furthermore, this study demonstrated that CD3scfv+CD80+CD58 particles provided the highest cytolytic function *in vitro*.

***Example 4***

**[0557]** This Example shows tumor control by *in vivo* transduction of T cells with a lentiviral particle with CD3scfv or CD3scfv+CD80. The lentiviral particle contains a polynucleotide encoding an anti-CD19 CAR. The lentiviral particle was delivered via intravenous injection into NSG MHCI/II KO mice. The mice used in the study were immune-compromised and contain engrafted human T cells and circulating human B cells.

*Study Design*

*Virus preparation, animal strain, cell lines*

**[0558]** 11 female NSG MHCI/II KO mice (Jackson laboratory) were and housed following institutional guidelines (Fred Hutchinson Cancer Research Center).

*Study Protocol*

**[0559]** 11 female NSG MHCI/II KO mice were acclimated for one week after receipt. At day -7, blood from all mice was collected for flow cytometry analysis to quantify degree of

humanization. Mice were randomized according to their total human CD3 levels into the treatment groups described in Table 5.

**Table 5: Study Treatment Groups**

| Group | N | Virus type | Administration route* | Virus Dose (Titre Unit) |
|---|---|---|---|---|
| 1 | 6 | CD3scfv | IV | 50 Million TU Day 0 |
| 2 | 5 | CD3scfv+CD80 | IV | 50 Million TU Day 0 |

*Study Timeline*

**[0560]** At study Day 0 (SD0), $20 \times 10^6$ PBMCs were injected into the intraperitoneal cavity. Mice were then dosed with virus particles according to the table above, followed with a challenge of $5 \times 10^5$ luciferase+ Nalm6 tumor cells intravenously. Tumor burden was measured for the duration of the study. At SD11, blood was collected and CAR T cells were measured.

**[0561]** At SD75, survivor mice were rechallenged with $5 \times 10^6$ Nalm6 cells.

*Results and Conclusions*

**[0562]** On Day 11 of the study, blood was collected from both groups. The level of CAR T cells in the blood was higher in the CD3scfv+CD80 particle-treated group compared to the CD3scfv particle-treated group (**FIG. 5A**). The CD3scfv+CD80 particle-treated group was also able to decrease tumor burden during the initial challenge and subsequent rechallenge compared to the CD3scfv particle-treated group (**FIG. 5B** and **FIG. 5C**).

**[0563]** In summary, when delivered intravenously, CD3scfv and CD3scfv+CD80 engineered lentivirus particles successfully transduced T cells *in vivo.* While both groups decreased tumor burden after initial challenge and subsequent rechallenge, particles with costimulatory molecule CD80 provided greater anti-tumor efficacy and anti-tumor immune response.

***Example 5***

**[0564]** This Example shows transduction with engineered lentiviral particles as described herein to transduce T cells in a short incubation period. Without wishing to be bound by

theory, this study provides proof of concept support that the engineered particles described may be useful in an extracorporeal intravenous system.

**[0565]** PBMCs from 3 healthy donors were thawed and cultured with vector particles containing an anti-CD19 CAR-mCherry payload pseudotyped with either CD3scfv+cocal or CD3scfv+CD80+CD58+Cocal, generally as described in **Example 2**. After the indicated time point, cells were washed in serum-free media containing IL2, human ab serum, HEPES, and glutamine. Cells were then plated in 1 ml serum-free media with IL-2 in a 24 well non-TC-treated plate. 3 days later cells were harvested and CD25 expression was measured by flow cytometry on viable T cells (**FIG. 6A**). The remaining cells were washed and re-plated in 1ml fresh media containing IL-2. 4 days later (Day 7 after transduction) viable T cells were analyzed by flow cytometry for CAR surface expression (**FIG. 6B**). %CAR was measured by staining for anti-CD19 mAb and mCherry expression.

**[0566]** As shown in **FIGs. 6A-6B**, vector particles comprising activation, costimulation, and adhesion molecules (*e.g.* CD3scFv+CD80+CD58 particles) efficiently transduced T cells after short incubation periods to a greater extent than particles comprising a CD3scFv without costimulation and adhesion components. These results indicate that vector particles as described herein may enable T cell transduction during short incubation periods ex vivo, for example in a closed-loop and/or extracorporeal system.

***Example 6***

**[0567]** This Example shows the transduction potential of lentiviral particles comprising a mutated (blinded) envelope protein. Envelope proteins, such as VSV-G or Cocal, can be mutated such that they cannot bind the LDL receptor. These modifications may enhance the specificity of lentiviral particles and reduce or eliminate off-target transduction.

**[0568]** SupT1 cells were cultured with vector particles containing an anti-CD19 CAR-mCherry payload, generated generally as described in Example 2. Specifically, 0.02uL of concentrated particles were added to 3.75 $x10^4$ SupT1 cells and assessed for CAR expression 3 days later. The cells were cultured in the following conditions :

| Condition | Particle Description |
|---|---|
| αCD3/CD58/CD80; no VSVG | CD3scFv+CD80+CD58; no envelope |
| VSVG | VSV-G only |
| VSVG (R354Q) | VSV-G (R354Q) only |
| VSVG (K47Q) | VSV-G (K47Q) only |
| αCD3/CD58/CD80; VSVG | CD3scFv+CD80+CD58; VSV-G |

| | |
|---|---|
| αCD3/CD58/CD80; VSVG (R354Q) | CD3scFv+CD80+CD58; VSV-G (R354Q) |
| αCD3/CD58/CD80; VSVG (K47Q) | CD3scFv+CD80+CD58; VSV-G (K47Q) |

**[0569]** As shown in the top row of **FIG. 7A** lentiviral particles comprising the blinded VSV-G mutant envelopes alone (without CD3scFv+CD80+CD58) exhibited greatly reduced transduction of SupT1 cells compared with a non-blinded VSV-G control. The bottom row depicted in **FIG. 7A** shows that the addition of activation, costimulation, and adhesion molecules in particles comprising blinded VSV-G mutant envelope proteins resulted in increased transduction.

**[0570]** Wishing to confirm the results seen using a T cell line, the experiment was replicated using PBMCs. On day 0, PBMCs from 2 donors were thawed and 2 x 10^6 cells were placed in wells of a 24-well plate. Vector particles containing an anti-CD19 CAR-mCherry payload, generated generally as described in Example 2, were added to each cell-containing well according to the following chart.

| Condition | Particle Description |
|---|---|
| αCD3/CD58/CD80; no VSVG | Anti-CD3scFv+CD80+CD58; no envelope |
| VSVG | VSV-G only |
| VSVG (R354Q) | VSV-G (R354Q) only |
| VSVG (K47Q) | VSV-G (K47Q) only |
| αCD3/CD58/CD80; VSVG | Anti-CD3scFv+CD80+CD58; VSV-G |
| αCD3/CD58/CD80; VSVG (R354Q) | Anti-CD3scFv+CD80+CD58;VSV-G (R354Q) |
| αCD3/CD58/CD80; VSVG (K47Q) | Anti-CD3scFv+CD80+CD58;VSV-G (K47Q) |

**[0571]** On day 3, the media was changed, and cells were re-plated with fresh media and samples were taken for assessment of transduction via flow cytometry. As shown in **FIGs. 7B-7C**, lentiviral particles comprising blinded VSV-G envelopes resulted in reduced transduction compared with the non-blinded VSV-G control in both CD4 (**FIG. 7B**) and CD8 (**FIG. 7C**) T cells. In addition, the addition of CD3scFv+CD80+CD58 to lentiviral particles resulted in increased transduction compared to lentiviral particles without CD3scFv+CD80+CD58. In addition, lentiviral particles comprising CD3scFv+CD80+CD58 without VSV-G also exhibited poor transduction.

**[0572]** On day 5, additional samples were taken for assessment of transduction via flow cytometry. Expression of CAR on day 5 was similar to expression on day 3 (data not shown).

**[0573]** The results of this study support the hypothesis that lentiviral particles comprising a blinded envelope protein and activation, costimulation, and adhesion molecules are capable of transducing primary T cells.

***Example 7***

**[0574]** This Example shows expansion of non-transduced T cells after administration of a lentiviral particle with CD3scfv or CD3scfv+CD80+CD58. The lentiviral particle contains a polynucleotide encoding an anti-CD19 CAR. The lentiviral particle was delivered via intravenous injection into mice.

*Study Design*

**[0575]** Mice were acclimated for one week after receipt. At day -7, blood from all mice was collected for flow cytometry analysis to quantify degree of humanization. Mice were randomized according to their total human CD3 levels into the treatment groups described in the table below.

| **Virus type** | **Administration route** | **Virus Dose (Titre Unit)** |
|---|---|---|
| CD3scfv | IV | 100 Million TU |
| CD3scfv | IV | 50 Million TU |
| CD3scfv+CD58+CD80 | IV | 100 Million TU |
| CD3scfv+CD58+CD80 | IV | 50 Million TU |
| CD3scfv+CD58+CD80 | IV | 25 Million TU |

*Study Timeline*

**[0576]** At study Day 0 (SD0) mice were then dosed with virus particles according to the table above. At SD11, blood was collected, and CAR negative T cells were measured.

*Results and Conclusions*

**[0577]** On Day 11 of the study, blood was collected from both groups. The level of CAR negative T cells in the blood was higher in the CD3scfv+CD58+CD80 particle-treated group and was dose-dependent compared to the CD3scfv particle-treated group (**FIG. 8**). These results indicate, when delivered intravenously, CD3scfv+CD58+CD80 engineered lentivirus particles appears to activate and expand even non-transduced T cells *in vivo.* Without wishing to be bound by theory, this activation of non-transduced T cells may enable

a lower dose of engineered lentiviral particles as non-transduced cells may exhibit anti-tumor activity.

***Example 8***

**[0578]** The Examples shows that combination of a costimulatory molecules CD80, anti-CD3scFv, and an adhesion protein CD58 expressed as a single fusion polypeptide (**FIG. 9A**) further enhances T cell activation and transduction. Particles having the fusion polypeptide were generated. These particles were examined for their ability to activate and transduce unstimulated human PBMCs compared to particles expressing the three proteins separately and compared to particles expressing a CD80/CD58 fusion polypeptide (**FIG. 9B**) and expressing anti-CD3scFv separately.

*Virus Production*

**[0579]** All solutions used were the same as those described in **Example 1**. Virus was produced as described in **Example 1**.

**[0580]** List of Virus preps made for study:

1. α-CD3scfv+CD80+CD58 expressed as a single fusion polypeptide (#498 triple fusion)
2. CD80+CD58 expressed as a single fusion polypeptide and α-CD3scfv expressed separately (#455 dual fusion)
3. α-CD3scfv+CD80+CD58 expressed separately "Separate"

*PBMC Transduction and Analysis*

**[0581]** Three healthy PBMC donors were incubated with the indicated lentiviral particles described above at a multiplicity if infection (MOI) of 10 in 0.9% Sodium Chloride buffer at a concentration of 20e6 cells/ml in a total volume of 100ul in a 96 well U-bottom plate. After 1 hour of incubation, the cells were washed, stained with antibodies, and analyzed by flow cytometry. The percentage of cells with bound Cocal and Cocal geometric mean fluorescence intensity (gMFI) are shown in **FIG. 10**. Cells analyzed by flow cytometry were gated on viable, CD3+, CD14-, CD56-, CD8+, Cocal+ cells. Similar data was seen for CD4+ T cells (data not shown).

*Results and Conclusions*

**[0582]** To assess whether lentiviral particles with costimulatory molecules and adhesion molecules α-CD3scfv+CD80+CD58 expressed as a single fusion polypeptide enhance T cell activation and transduction, the lentiviral particles were added to PBMCs from three healthy PBMC donors at several MOI's and at 2E6 cells/ml in RPMI media. 3 days later, the virus was removed and the cells were washed, given fresh media, and analyzed for the

activation marker CD25 by flow cytometry. Cell were gated on viable, CD3+, CD4+ or CD8+ cells. α-CD3scfv+CD80+CD58 expressed as a single fusion polypeptide "#498" particles potently activated CD4+ (**FIG. 11A** and **FIG. 11C**) and CD8+ (**FIG. 11B** and **FIG. 11D**) T cells. Furthermore, the triple fusion "#498" particles activated CD4+ (**FIG. 11A** and **FIG. 11C**) and CD8+ (**FIG. 11B** and **FIG. 11D**) T cells through displayed CD25 upregulation at a much lower dose as compared to "#455" the dual fusion and "Separate" lentiviral particles.

**[0583]** To further characterize T cell activation, lentiviral particles were added to PBMCs from three healthy PBMC donors at several MOI's and at 2E6 cells/ml in RPMI media. 3 days later, supernatant was harvested and cytokines were measured using V-PLEX™ Proinflammatory Panel 1 Human Kit. Similar to CD25 expression, the triple fusion "#498" particles were capable of inducing more T cell activation-associated cytokines including IFN-γ, IL-2, and TNF-α as compared to "#455" the dual fusion and "Separate" particles. Higher IFN-γ production in unstimulated PBMCs was observed at lower doses (**FIG. 12A**). Furthermore, the triple fusion "#498" particles induced robust IL-2 and TNF-α production as compared to "Fusion-455" and "Separate" particles (**FIG. 12B** and **FIG. 12C**). The data show that the triple fusion "#498" particles efficiently induce cytokine production in unstimulated PBMCs *in vitro* compared to "#455" the dual fusion and "Separate" particles.

**[0584]** T cell subtypes generated by the particles were profiled. The cells were assessed using CCR7, CD27, CD28, and CD57 markers. Lentiviral particles were added to PBMCs from three healthy PBMC donors at several MOI's and at 2E6 cells/ml in RPMI media. 7 days post transduction, cells were washed and CAR surface marker expression was analyzed by flow cytometry. Cells were gated on viable, CD3+, CD4+ or CD8+, CAR+ cells.

**[0585]** Non-terminally differentiated memory T cells are CCR7+CD27+CD28+. Triple fusion-containing particles "#498" produced a greater percentage of CCR7+CD27+CD28+ memory-like CAR+ T cells as compared to dual fusion "#455" and "Separate" particles (**FIG. 13A** and **FIG. 13C**).

**[0586]** CCR7+CD27+CD28+ memory-like CAR+ T cells are thought to have increased longevity and proliferative capacity and correlate with better antitumor responses *in vivo*. Triple fusion particles "#498" produced a smaller percentage of senescence marker CD57 at an MOI of 2 as compared to dual fusion "#455" and "Separate" particles (**FIG. 13B** and **FIG. 13D**).

***Example 9***

**[0587]** This Example shows T cell activation and IFNγ production following *in vivo* transduction of T cells by a lentiviral particle displaying #498 triple fusion polypeptide as compared to #455 dual fusion and "Separate" particles as described above. The lentiviral particle contains a polynucleotide encoding an anti-CD19 CAR.

**[0588]** On study Day –4 NSG MHC I/II dKO mice were injected via tail vein injection with 2.5E5 Nalm6 cells expressing firefly luciferase (ffluc) (**FIG. 14A**). 3 days later (study Day –1), mice were imaged via bioluminescence imaging and randomized to study arms according to tumor burden (total flux), the same day all mice were humanized by injecting 20E6 human PBMCs intraperitoneally in 100 µl of 1X sterile PBS. The mice used in the study were immune-compromised and contain engrafted human T cells and circulating human B cells.

**[0589]** The next day (study Day 0), mice were treated via intraperitoneal injection with different doses of lentiviral particles displaying:

1. α-CD3scfv+CD80+CD58 expressed as a single fusion polypeptide "#498" triple fusion;
2. CD80+CD58 expressed as a single fusion polypeptide and α-CD3scfv expressed separately "#455" dual fusion; or
3. α-CD3scfv+CD80+CD58 expressed separately "Separate".

**[0590]** A control study arm treated mice with 1xPBS (Neg) via intraperitoneal injection. Mice were then weighted two times a week throughout the study to monitor body weight change and imaged weekly to monitor tumor burden. Mice were bleed on study Days 4, 11, 18, 25, and 32 to perform flow cytometry analysis. Study day 4 activation markers CD25 (**FIG. 14B**) and CD71 (**FIG. 14C**) on CD3+ T cells were analyzed. 4 days after lentiviral particle treatment (study Day 4) serum was collected from blood and IFNγ levels in the serum were measured using V-PLEX™ proinflammatory panel 1 human kit (Mesoscale Discovery) (**FIG. 14D**).

**[0591]** Apheresis blood was washed on the Lupagen™ machine and incubated with lentiviral particles at an MOI of 2 for 1 hour in saline. The lentiviral particle contains a polynucleotide encoding an anti-CD19-mCherry transgene.

**[0592]** The particle-bound cells were then washed of unbound particles to generate the "Final" material. Particle-bound cells were assessed by staining for Cocal on various cell populations (CD4+ T cells, CD8+ T cells, NK T cells, NK cells, CD56+ NK cells, monocytes, B cells, and other MFI) and analyzing by flow cytometery. Cocal geometric

mean fluorescence intensity are shown (**FIG. 15A-15C**). The strongest binding was observed with particles displaying the triple fusion "#498" as compared to particles displaying the dual fusion "#455".

***Example 10***

**[0593]** This Examples shows *in vivo* antitumor responses to lentiviral particles displaying a costimulatory and adhesion molecule fusion protein using the Lupagen™ System.

**[0594]** On study Day –4 NSG MHC I/II DKO mice were injected via tail vein injection with 2.5E5 Nalm6 cells expressing GFP/ firefly luciferase (ffluc). 3 days later (study Day –1), mice were imaged via Bioluminescence imaging and randomized to study arms according to tumor burden (total flux). On study Days 0 and 1, mice were injected with PBMCs from 2 different donors either after Lupagen™ wash or after incubation with lentiviral particles comprising "#455" dual fusion or triple fusion "#498" polypeptides in the lentiviral particle surface. On study Day 8 and every week through the study, mice were imaged via Bioluminescence imaging using the IVIS™ spectrum system to analyze tumor burden (total flux) (**FIG. 16C** and **16D**). Serial weekly blood draws were collected to perform flow cytometry analysis and assess CAR T cell expansion and persistence (**FIG. 16A** and **16B**). Disease progression was monitored by Bioluminescence imaging once a week after d-Luciferin subcutaneous injection using the IVIS™ imaging system (**FIG. 16E**).

***Example 11***

**[0595]** This Example shows screening of lentiviral particles displaying variations of CD58 and CD80 dual-fusion polypeptides and screening of lentiviral particles displaying variations of CD58, CD80, and anti-CD3 scFv tri-fusion polypeptides.

**[0596]** Cryopreserved human PBMCs from normal donors were obtained from AllCells™. Human PBMCs were cultured in T cell growth (TCGM) media (RPMI1640 + 5% HuAB serum + 1x GlutaMax + HEPES). For lentiviral transduction, virus was added to the PBMC cells for 3 days. Stimulation and lentiviral infection were then terminated by washing and re-seeding PBMCs in fresh TCGM media.

**[0597]** To analyze T cell activation, about $0.1\times10^6$ cells were pelleted after the 3-day production period following lentiviral transduction described above. Cells were then analyzed by flow cytometry as follows. Cells were resuspended in Fixable Viability Dye eFluor 780 in PBS for 10 minutes, then washed with Cell Staining Buffer. T cell activation was measured by detection of hCD25 marker using an anti-CD25-PE/Cy7 antibody diluted 1:100 in Cell Staining Buffer.

**[0598]** To measure CAR expression levels and transduction efficiencies, about $0.1\times10^6$ cells were pelleted after a 7-day production period following lentiviral transduction. Cells were then analyzed by flow cytometry as follows. Cells were resuspended in Fixable Viability Dye eFluor 780 in PBS for 10 minutes, then washed with Cell Staining Buffer. FMC63 CAR surface expression was detected using an anti-ID-FITC antibody diluted 1:100 in Cell Staining Buffer. Cells were pelleted after a 20 minute incubation in the dark, followed by 2× wash with Cell Staining Buffer. All flow cytometric analysis was done on an Attune™ NxT Flow Cytometer and analyzed with FlowJo™.

**[0599]** Day 7 transduced primary T cells expressing FMC63 CAR were counted, resuspended to a cell density equal to $0.4\times10^6$ CAR+ cells/ml in cell-assay media (RPMI1640 + 10%FBS). 100 µl volume of CAR+ cells (40,000 CAR+ cells) were added to a flat-bottom 96 well plate containing 10,000 Nalm6 cells and incubated at 37° C for effector to target ratio of 4:1. CAR+ cells are serially diluted in cell-assay media prior to plating to achieve lower effector to target ratios. Killing of target cells was analyzed in an IncuCyte™ Live Cell Analysis System. Each well was imaged every 6 hours and the number of Nalm6 cells was quantified to assess the kinetics of T cell cytotoxicity. After 24 hours, supernatant from each well was collected for cytokine measurements according to manufacturer's protocol. Nalm6 target cell lysis was tracked over >4 days. The Nalm6 target cell line was stably labeled with nuclear mKate2 by lentiviral transduction with IncuCyte™ NucLight Red Lentivirus Reagent.

*Results*

**[0600]** Healthy donor PBMCs were transduced with lentiviruses carrying a FMC63 CAR transgene and displaying various surface engineered dual-fusion proteins at MOI 2 and 5. Early activation is determined based on hCD25 staining on Day 3 (**FIG. 17**), and CAR expression level was measured by staining with an anti-FMC63 antibody conjugated to FITC (**FIG. 18**). CAR-T cells were challenged with Nalm6-NIR (**FIG. 19A-19D**) to compare killing kinetics and target-dependent cytokine production levels (**FIG. 20**). Pro-inflammatory cytokine concentrations in co-culture supernatant after 24 hours of assay setup, in combination of killing kinetics indicate that CAR-T cells comprising the #455 dual fusion construct and separately expressed anti-CD3 scFv performed the best in functional assay.

**[0601]** With very low MOI transduction (MOI = 0.5 and 1), lentiviral particles produced using anti-CD3 scFv and dual-fusion plasmids promoted T cell activation on Day 3 (**FIG. 21**), enhanced transduction efficiency and increased CAR expression on Day 7 (**FIG. 22**).

**[0602]** Tri-fusion protein "#498" surface engineered particles were compared against dual-fusion "#455" particles in PBMC transduction using both high and low MOI (MOI=1 and 10). Lentiviral particles produced using tri-fusion versions #479, #496, and #498 enhanced early T cell activation in PBMCs (**FIG. 23**). #496 and #498 outperformed in transduction efficiency and CAR expression on Day 7 (**FIG. 24**). In this experiment, #498 had the most significant effect on CAR+ T cell expansion (**FIG. 25**).

***Example 12***

**[0603]** This Example shows T cell activation and transduction with lentiviral particles displaying a CD58, CD80, and anti-CD3 scFv tri-fusion polypeptide.

**[0604]** Human PBMCs from 3 normal donors were cultured in T cell growth (TCGM) media (RPMI1640 + 5% HuAB serum + 1x GlutaMax + HEPES). For lentiviral transduction, lentiviral particles were added to the PBMC cells.

**[0605]** To analyze T cell activation, cells were pelleted after 3 days and then analyzed by flow cytometry. T cell activation was measured by detection of hCD25 marker using an anti-CD25-PE/Cy7 antibody diluted 1:100 in Cell Staining Buffer. To measure CAR expression levels and transduction efficiencies, cells were pelleted after a 7-day production period following lentiviral transduction. Cells were then analyzed by flow cytometry. Anti-CD19 CAR surface expression was detected and all flow cytometric analysis was done on an Attune™ NxT Flow Cytometer and analyzed with FlowJo™. Day 7 transduced primary T cells expressing anti-CD19 CAR were counted, resuspended, and added to Nalm6 tumor cells. Killing of target Nalm6 cells was analyzed in an IncuCyte™ Live Cell Analysis System. Each well was imaged every 6 hours and the number of Nalm6 cells was quantified to assess the kinetics of T cell cytotoxicity. After 24 hours, supernatant from each well was collected for cytokine measurements according to manufacturer's protocol.

**[0606]** Healthy donor PBMCs (from three donors) were contacted for less than one hour with lentiviruses carrying an anti-CD19 CAR transgene and displaying surface engineered tri-fusion proteins at MOI 2 (**FIG. 28A**). Consistent and efficient binding of T cells to engineered lentiviral particles was observed and measured by percentage of CD3+ T cells positively staining for Cocal (**FIG. 28B**). Selective T cell binding was observed in a Cocal staining peak shift for CD3+ T cells relative to CD3- T cells (**FIG. 28C**). Activation was

determined based on hCD25 staining on Day 3 (**FIG. 28D**), and CAR expression level was measured (**FIG. 28E**). The engineered lentiviral particles demonstrated robust avidity and selectivity for T cell binding following short duration (< 1 hour) culture. Transduced PBMCs were cultured with Nalm6 tumor cells. Specifically, anti-CD19 CAR+ T cells were serial-stimulated with Nalm6 tumor cells every 2-3 days. Total Nalm6 tumor cells were measured over time using an IncuCyte® providing a measurement of tumor cell killing over time (**FIG. 29**). This assay measures the ability of the CAR T cells to expand and kill multiple tumor cells over time and showed that anti-CD19 CAR T cells generated with lentivirus particles displaying a CD58, CD80, and anti-CD3 scFv tri-fusion protein demonstrated serial killing *in vitro*.

**[0607]** In a study of hematologic malignancy in a tumor xenograft model, on Day -4, $2.5 \times 10^5$ Nalm6 cells were intravenously injected into NSG MHCI/II KO mice. At study Day -1, $20 \times 10^6$ PBMCs were injected into the intraperitoneal cavity. At study Day 0, mice were dosed with virus particles displaying a CD58, CD80, and anti-CD3 scFv tri-fusion protein (**FIG. 30A**).

**[0608]** Four days after lentiviral particle administration, cells were harvested and expression of activation markers CD25 (**FIG. 30B**) and CD71 (**FIG. 30C**) and cytokine IFN-γ production (**FIG. 30D**) were measured by flow cytometry on viable CD3+ T cells in the blood. CAR T cell expansion was analyzed at doses of 10 Million and 50 Million transducing units (TU). On Day 11, total anti-CD19 CAR+ T cells found in the blood were analyzed by flow cytometry for CAR surface expression (**FIG. 30E**). Tumor burden was assessed as total flux and measured for the duration of the study using an In vivo Imaging system (IVIS®) (**FIG. 30F**).

***Example 13***

**[0609]** This Example analyzed transduction of T cells by a lentiviral particle displaying either a dual-fusion (#455) construct or a triple fusion (#498) construct. **FIGs. 32H-32I** show total tumor burden (Total flux) over the course of 28 days of the study in the blood of mice injected with PBMCs from Donor 1 (**FIG. 32H**) or Donor 2 (**FIG. 32I**) after Lupagen™ incubation with untreated PBMC control, lentiviral particles displaying either a dual "#455" or triple "#498" fusion construct. The data show that extracorporeal incubation of PBMCs with the lentiviral particles described herein generates potent antitumor responses *in vivo.* Lentiviral particles displaying a CD58, CD80, and anti-CD3 scFv tri-

fusion "#498" polypeptide showed enhanced antitumor activity in Donor 2 with a lower cell dose (Donor 2 - 15e6 cells were injected; Donor 1 - 25e6 cells were injected).

**[0610]** On study Day 49 (rechallange Day 0) the mice were injected via tail vein injection with an additional 2.5E5 Nalm6 cells expressing firefly luciferase (ffluc) to assess clearance of tumor re-challenge (**FIG. 33A**). Tumor burden was assessed as total flux and measured for the duration of the rechallange study for Donor 1 (D1) and Donor 2 (D2) using an In vivo Imaging system (IVIS®) (**FIG. 33C**). **FIG. 33B** shows the tumor burden in NSG MHCI/II KO mice after administration of T cells produced via extracorporeal incubation of PBMCs from Donor 1 (D1) or Donor 2 (D2) incubated with lentiviral particles displaying either a dual fusion "#455" or triple fusion "#498"construct following tumor cell rechallenge at Day 49.Lentiviral particles displaying a CD58, CD80, and anti-CD3 scFv tri-fusion "#498" polypeptide generated anti-CD19 CAR T cells, which showed persistence following primary tumor clearance and protection against tumor rechallenge *in vivo*.

***Example 14***

**[0611]** This Example shows the incorporation of a costimulatory molecule on a lentiviral particle enhances transduction of PBMCs by lentiviral particles as generated in **Example 1**.

*Virus Production*

**[0612]** All solutions used were the same as those described in **Example 1**. 293T cells were seeded into T175 flasks with Complete DMEM media. 24 hours later, cells were transfected. Virus was produced as described in **Example 1**. All viruses included a Cocal envelope protein.

**[0613]** List of Virus preps made for study (viruses contained an anti-CD19 CAR payload):

*1. CD3scfv only*

*2. CD3scfv, CD80, and CD58 proteins separately expressed ("Tri protein")*

*PBMC Transduction and staining for flow cytometry*

**[0614]** $50 \times 10^6$ PBMCs were thawed, diluted to $2 \times 10^6$ cells/ml in complete media (*e.g.* RPMI or Optimem). IL-2 was added to a final concentration of 50IU/ml.

**[0615]** 500µl (1e6 cells) were added to the wells of a Non-TC-treated 48 well plate. Vector was added to the wells at MOI=10, 5, and 2 based on the SupT1 ddPCR titer and the plates were placed in 37° C incubator.

**[0616]** After 3 days, vector was washed out and replaced with 500µl fresh RPMI media+IL-2 (50 IU/ml). Cells were mixed and 100-300µl were added to wells in a 96 well V-bottom plate for activation flow cytometry analysis. Cells were then washed with 200µlFACS

buffer. The cell pellets were resuspended in 50-100µl PBS containing LiveDead Stain (1:1000) and incubate at 4° C for 20 min followed by another wash in 200µlFACS buffer. Cells were resuspended in 50µlof FACS buffer + surface stain cocktail, incubated for 30 min at 4° C, washed in 200µlFACS buffer.

*Results and Conclusions*

**[0617]** To assess if lentiviral particles with separately expressed costimulatory and adhesion molecules can better activate human T cells, the vector particles were added to human PBMCs at several MOI's. 3 days later, the virus was removed and the cells were given fresh media and analyzed for the activation marker CD25. Tri protein particles potently activated CD8 T cells compared to CD3scfv only (**FIG. 36A**). Furthermore, CD25 upregulation was dose-dependent (**FIG. 36A**). CD3scfv only lentiviral particles induced minimal levels of CD25 compared to the Tri protein particles **(FIG. 36A)**. To determine whether lentiviral particles with costimulatory and/or adhesion molecules have enhanced particle binding to T cells, the particles were cultured with PBMCs for 6 hours and then were analyzed for particle-associated molecules on T cells (Cocal). The separate expression of CD58, CD80, and an anti-CD3 scFv increased Cocal staining (**FIG. 36B**)

**[0618]** To examine transduction, 7 total days after transduction, samples were analyzed for anti-CD19 CAR expression. Tri protein particles were capable of transducing unstimulated PBMCs while CD3scfv only particles transduced unstimulated PBMCs to a lesser extent (**FIG. 36C**). Furthermore, transduction occurred in a dose-dependent manner for both CD4+ and CD8+ T cells (**FIG. 36C**). The data show that Tri protein particles efficiently activate and transduce unstimulated PBMCs *in vitro* compared to CD3scfv only. Importantly, the enhanced particles result in increased numbers of CAR+ T cells (**FIG. 36C, right panels: Total CAR+ cells**).

**[0619]** To further characterize T cell activation, 3 total days after vector addition, samples were analyzed for cytokine expression. Tri protein particles were capable of inducing IFN-γ production in unstimulated PBMCs at lower doses whereas CD3scfv only particles transduced unstimulated PBMCs to a lesser extent (**FIG. 36D**). Furthermore, Tri protein particles induced robust IL-2 and TNF-α whereas CD3scfv only did not (**FIG. 36D**). The data show that Tri protein particles efficiently induce cytokine production in unstimulated PBMCs *in vitro* compared to CD3scfv only.

**[0620]** Transduced PBMCs were then cultured with Nalm6 tumor cells. Specifically, anti-CD19 CAR+ T cells were serial-stimulated with Nalm6 tumor cells every 2-3 days. Total

Nalm6 tumor cells were measured over time using an IncuCyte® providing a measurement of tumor cell killing over time (**FIG. 36E**). This assay measures the ability of the CAR T cells to expand and kill multiple tumor cells over time and showed that anti-CD19 CAR T cells generated with lentiviral particles displaying "Tri protein" demonstrated serial killing *in vitro* as compared to particles displaying CD3scfv only.

**[0621]** To determine whether lentiviral particles with costimulatory and/or adhesion molecules have enhanced particle binding to T cells, the particles were cultured with PBMCs for 6 hours and then were analyzed for particle-associated molecules on T cells (Cocal, anti-CD3scFv, CD80, and CD58). Both Tri protein and Fusion particles demonstrated high stating for anti-CD3scFv, CD80, and CD58, and only Fusion particles demonstrated high staining for CD3scfv+CD80+CD58 (data not shown). The data show that the fusion of CD58v+CD3 scFv+CD80 enhances particle binding to T cells. Samples were then analyzed for cytokine expression. Tri protein particles were capable of inducing IFN-γ production in unstimulated PBMCs whereas CD3scfv only particles transduced unstimulated PBMCs to a lesser extent (**FIG. 36F**). Furthermore, Tri protein particles induced robust IL-2 and TNF-α production whereas CD3scfv only did not (**FIG. 36F**). The data show that Tri protein surface engineered particles efficiently induce cytokine production in unstimulated PBMCs as compared to CD3scfv only.

**[0622]** To determine whether different T cell subtypes are generated by the lentiviral particles, PBMCs cultured with the lentiviral particles were profiled and gated on viable, CD4+ and CD8+. The cells were further analyzed by flow cytometry and analysis was done based on the parameters CCR7+ and CD27+ (**FIG. 36G**). Tri protein particles showed an increased population of CCR7+CD27+ T cells as compared to CD3 scFv only. CCR7+CD27+CD28+ memory-like CAR+ T cells are thought to have increased longevity and proliferative capacity and correlate with better antitumor responses *in vivo*.

***Example 15***

**[0623]** This Example shows T cell activation and IFNγ production following *in vivo* transduction of T cells by a lentiviral particle displaying CD58, CD80, and an anti-CD3 scFv separately expressed as compared to CD3scfv only. The lentiviral particles contained a polynucleotide encoding an anti-CD19 CAR.

**[0624]** On study Day –4 NSG MHC I/II dKO mice were injected via tail vein injection with 2.5E5 Nalm6 cells expressing firefly luciferase (ffluc) (**FIG. 37A**). 3 days later (study Day –1), mice were imaged via bioluminescence imaging and randomized to study arms

according to tumor burden (total flux), the same day all mice were humanized by injecting 20E6 human PBMCs intraperitoneally in 100 µl of 1X sterile PBS. The mice used in the study were immune-compromised and contain engrafted human T cells and circulating human B cells.

**[0625]** The next day (study Day 0), mice were treated via intraperitoneal injection with different doses of lentiviral particles displaying:

1. CD58, CD80, and an α-CD3 scFv separately expressed ("Tri protein");
2. α-CD3scfv only.

**[0626]** A control study arm treated mice with 1xPBS (Neg) via intraperitoneal injection. Mice were then weighted two times a week throughout the study to monitor body weight change and imaged weekly to monitor tumor burden. Mice were bleed on study Days 4, 11, 18, 25, and 32 to perform flow cytometry analysis. Study day 4 activation markers CD25 (**FIG. 37B**) and CD71 on T cells were analyzed. A blood draw on Day 11 was collected to perform flow cytometry analysis and assess CAR T cell expansion and persistence (**FIG. 37C, top panel**) and CAR expression level was measured by staining with an anti-FMC63 antibody (**FIG. 37C, bottom panel**). On study Day 6 and every week through the study, mice were imaged via Bioluminescence imaging using the IVIS™ spectrum system to analyze tumor burden (total flux) (**FIG. 37D**).

***Example 16***

**[0627]** This Example shows the incorporation of a costimulatory molecule and an adhesion molecule on a lentiviral particle enhances transduction of PBMCs by lentiviral particles as generated in **Example 1**.

*Virus Production*

**[0628]** All solutions used were the same as those described in **Example 1**. $28 \times 10^6$ 293T cells were seeded into 16x T175 flasks (8x per vector) with 28e6 293T cells each in a total volume of 25ml Complete DMEM media. 24 hours later, cells were transfected. Virus was produced as described in **Example 1**. All viruses included a Cocal envelope protein and an anti-CD19 CAR payload.

**[0629]** List of Virus preps made for study:

*1. CD3scfv, CD80, and CD58 separately expressed ("Tri protein")*

*2. CD58+CD3scFv+CD80 expressed as a fusion protein ("Fusion")*

*Results and Conclusions*

**[0630]** To assess if lentiviral particles with costimulatory molecules can better activate human T cells, the vector particles were added to human PBMCs at several MOI's. CD58+CD3 scFv+CD80 fusion particles potently activated CD4+ and CD8+ T cells compared to the Tri protein particles (**FIG. 38B**). Furthermore, CD25 upregulation was dose-dependent (**FIG. 38B**). To determine whether lentiviral particles with a singular fusion protein comprising costimulatory and/or adhesion molecules have enhanced particle binding to T cells, the particles were cultured with PBMCs and then were analyzed for particle-associated molecules on T cells (Cocal). The fusion particles resulted in increased Cocal staining (**FIG. 38A**).

**[0631]** To examine transduction, 7 total days after transduction, samples were analyzed for anti-CD19 CAR expression. CD58+CD3 scFv+CD80 fusion particles were capable of transducing unstimulated PBMCs at lower doses while Tri protein particles transduced unstimulated PBMCs to a lesser extent (**FIG. 38C**). Furthermore, transduction occurred in a dose-dependent manner for both CD4+ and CD8+ T cells (**FIG. 38C**). The data show that CD58+CD3 scFv+CD80 fusion particles efficiently activate and transduce unstimulated PBMCs *in vitro* compared to Tri protein particles.

**[0632]** To further characterize T cell activation, 3 total days after vector addition, samples were analyzed for cytokine expression. CD58+CD3 scFv+CD80 fusion particles induced robust IL-2 and TNF-α production as compared to Tri protein particles (**FIG. 38D**). The data show that CD58+CD3 scFv+CD80 fusion particles efficiently induce cytokine production in unstimulated PBMCs *in vitro* compared to Tri protein displaying particles.

**[0633]** Transduced PBMCs were then cultured with Nalm6 tumor cells. Specifically, anti-CD19 CAR+ T cells were serial-stimulated with Nalm6 tumor cells every 2-3 days. Total Nalm6 tumor cells were measured over time using an IncuCyte® providing a measurement of tumor cell killing over time. This showed that CAR T cells generated with lentiviral particles displaying a CD58+CD3 scFv+CD80 fusion protein demonstrated improved serial killing *in vitro* as compared to Tri protein displaying particles. In particular, CAR T cells generated with the fusion particles (Fusion) were able to continously control tumor cells for at least 35 days. CAR T cells generated with the separately expressed proteins (Tri protein) exhibited slow tumor outgrowth beginning around day 15.

**[0634]** To determine whether different T cell subtypes are generated by the lentiviral particles, PBMCs cultured with the lentiviral particles were profiled and gated on viable,

CD4+ and CD8+. The cells were further analyzed by flow cytometry based on parameters CCR7+ and CD27+. At most MOIs tested, both the tri protein and fusion particles were capable of generating high levels of CCR7+ and CD27+ CAR+ cells.

***Example 17***

**[0635]** This This Example shows T cell activation and IFNγ production following *in vivo* transduction of T cells by a lentiviral particle displaying the CD58+CD3 scFv+CD80 fusion as compared to particles comprising CD58, CD3 scFv, and CD80 separately expressed. The lentiviral particle contains a polynucleotide encoding an anti-CD19 CAR.

**[0636]** On study Day –4 NSG MHC I/II dKO mice were injected via tail vein injection with 2.5E5 Nalm6 cells expressing firefly luciferase (ffluc). 3 days later (study Day –1), mice were imaged via bioluminescence imaging and randomized to study arms according to tumor burden (total flux), the same day all mice were humanized by injecting 20E6 human PBMCs intraperitoneally in 100 µl of 1X sterile PBS. The mice used in the study were immune-compromised and contain engrafted human T cells and circulating human B cells.

**[0637]** The next day (study Day 0), mice were treated via intraperitoneal injection with different doses of lentiviral particles displaying:

*1. CD3scfv, CD80, and CD58 separately expressed ("Tri protein")*

*2. CD58+CD3scFv+CD80 expressed as a fusion protein ("Fusion")*

**[0638]** A control study arm treated mice with 1xPBS (Vehicle) via intraperitoneal injection. Mice were then weighted two times a week throughout the study to monitor body weight change and imaged weekly to monitor tumor burden. Mice were bleed on study Days 4, 11, 18, 25, and 32 to perform flow cytometry analysis. Study day 4 activation markers CD25 (**FIG. 39A**) and CD71 on T cells were analyzed. A blood draw on Day 11 was collected to perform flow cytometry analysis and assess CAR T cell expansion and persistence (**FIG. 39B, top panel**) and CAR expression level was measured by staining with an anti-FMC63 antibody (**FIG. 39B, bottom panel**). On study Day 6 and every week through the study, mice were imaged via Bioluminescence imaging using the IVIS™ spectrum system to analyze tumor burden (total flux) (**FIG. 39C**). As shown in **FIG. 39C**, tumor growth was better controlled in both of the fusion particle cohorts, with the higher dose exhibiting more robust tumr control. Overall % survival of the mice was analyzed over the course of the study. Lenitviral particles displaying CD58+CD3 scFv+CD80 fusion particles transduced at 50E6 TU showed increased overall survival in mice as compared to lentiviral Tri protein displaying particles.

* * *

**[0639]** The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. Specifically, features described in one section may be combined with features in any other section of the description.

**[0640]** Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

**[0641]** The singular forms “a”, “an”, and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

**[0642]** As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

**[0643]** All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, controls. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

**[0644]** While illustrative embodiments have been described and depicted, it will be appreciated that various changes can be made to these illustrative embodiments without departing from the spirit and scope of the invention.

**CLAIMS**

1. A lentiviral particle, comprising, displayed on the surface of the particle:
   a fusion molecule comprising:
      a) a CD58 extracellular domain, or a functional fragment thereof,
      b) a CD80 extracellular domain, or a functional fragment thereof,
      c) an antigen-binding fragment of an anti-CD3 antibody; and
   a viral glycoprotein (G protein),
      wherein the lentiviral particle comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19.

2. The lentiviral particle of claim 1, wherein the lentiviral particle comprises a polynucleotide encoding a free FKBP12-rapamycin binding (FRB).

3. The lentiviral particle of any one of claims 1-2, wherein the lentiviral particle comprises a polynucleotide encoding a synthetic cytokine gamma chain polypeptide and a synthetic cytokine beta chain polypeptide.

4. The lentiviral particle of any one of claims 1-3, wherein the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv domain, wherein the scFv further comprises a VL comprising a polypeptide sequence at least 80% identical to the polypeptide sequence of SEQ ID NO: 206, and a VH comprising a polypeptide sequence at least 80% identical to the polypeptide sequence of SEQ ID NO: 208.

5. The lentiviral particle of claim 4, wherein the scFv comprises a polypeptide sequence at least 80% identical to the polypeptide sequence of SEQ ID NO: 195.

6. The lentiviral particle of claim 4 or 5, wherein the scFv comprises the polypeptide sequence of SEQ ID NO: 195.

7. The lentiviral particle of any one of claims 1-6, wherein the chimeric antigen receptor comprises a CD8 hinge domain, a CD28 transmembrane domain, a 4-1BB endodomain, and a CD3ζ endodomain.

8. The lentiviral particle of any one of claims 1-7, wherein the chimeric antigen receptor comprises a polypeptide sequence at least 80% identical to the polypeptide sequence of

SEQ ID NO: 205.

9. The lentiviral particle of any one of claims 1-8, wherein the chimeric antigen receptor comprises the polypeptide sequence of SEQ ID NO: 205.

10. The lentiviral particle of any one of claims 1-9, wherein the CD58 extracellular domain, or a functional fragment thereof, comprises a polypeptide sequence that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 10.

11. The lentiviral particle of any one of claims 1-10, wherein the antigen-binding fragment of an anti-CD3 antibody is an scFv domain comprising a polypeptide sequence that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 31.

12. The lentiviral particle of any one of claims 1-11, wherein the CD80 extracellular domain, or a functional fragment thereof, comprises a polypeptide sequence that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 12 or 250.

13. The lentiviral particle of any one of claims 1-12, wherein the fusion molecule comprises a polypeptide sequence that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 33, or wherein the fusion molecule comprises a polypeptide sequence that is at least 80% identical to the polypeptide sequence encoded by SEQ ID NO: 246.

14. The lentiviral particle of any one of claims 1-13, wherein the fusion molecule comprises, in N to C-terminal order, the CD58 extracellular domain, the antigen-binding fragment of an anti-CD3 antibody, and the CD80 extracellular domain.

15. The lentiviral particle of any one of claims 1-14, wherein the viral glycoprotein (G protein) comprises a polypeptide sequence that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 74 or 247.

16. The lentiviral particle of any one of claims 1-15, wherein the lentiviral particle comprises a polynucleotide sequence encoding a free FRB polypeptide that is at least 80% identical to the polypeptide sequence of SEQ ID NOs: 251, 252, or 260.

17. The lentiviral particle of any one of claims 1-16, wherein the lentiviral particle comprises a polynucleotide sequence encoding a synthetic cytokine gamma chain comprising a

polypeptide sequence at least 80% identical to the amino acid sequence of SEQ ID NOs: 264 or 265.

18. The lentiviral particle of any one of claims 1-17, wherein the lentiviral particle comprises polynucleotide sequence encoding a FRB polypeptide that is at least 80% identical to the polypeptide sequence of SEQ ID NO: 252.

19. The lentiviral particle of any one of claims 1-18, wherein the lentiviral particle comprises polynucleotide sequence encoding a synthetic cytokine beta chain polypeptide comprising a polypeptide sequence at least 80% identical to SEQ ID NOs: 272 or 273.

20. The lentiviral particle of any one of claims 1-19, wherein the lentiviral particle comprises polynucleotide sequence encoding a FKBP12 polypeptide comprising a sequence at least 80% identical to SEQ ID NO: 253.

21. A phamaceutical composition for use in treating a CD19+ cancer in a subject in need thereof, wherein the lentiviral particle of any one of claims 1-20 is to be administered to the subject.

22. The phamaceutical composition of claim 21, further comprising rapamycin or a rapamycin analog.

23. A lentiviral particle, comprising, displayed on the surface of the particle: a fusion molecule comprising

a) a CD58 extracellular domain, or a functional fragment thereof,

b) an antigen-binding fragment of an anti-CD3 antibody, and

c) a CD80 extracellular domain, or a functional fragment thereof; and

a viral glycoprotein (G protein);

wherein the lentiviral particle further comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19, a free FRB, a synthetic cytokine gamma chain polypeptide, and a synthetic cytokine beta chain polypeptide,

wherein the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv, a hinge domain, a transmembrane domain, a 41BB endodomain, and a CD3ζ endodomain, and

wherein the scFv comprises a VL comprising SEQ ID NO: 206 and a VH comprising SEQ ID NO: 208, the hinge domain comprises SEQ ID NO: 197, the transmembrane domain

comprises SEQ ID NO: 199, the 41BB endodomain comprises SEQ ID NO: 201, and the CD3ζ endodomain comprises SEQ ID NO: 203.

24. The lentiviral particle of claim 23, further comprising a polycistronic construct comprising in 5′ to 3′ order:
    a. a first expression cassette comprising a nucleotide sequence encoding the free FRB,
    b. a second expression cassette comprising a nucleotide sequence encoding the synthetic cytokine gamma chain polypeptide,
    c. a third expression cassette comprising a nucleotide sequence encoding the synthetic cytokine beta chain polypeptide, and
    d. a fourth expression cassette comprising a nucleotide sequence encoding the chimeric antigen receptor (CAR), wherein each of the expression cassettes are separated by a nucleotide sequence encoding a cleavage site sequence.

25. A lentiviral particle, comprising, displayed on the surface of the particle:
    a fusion molecule comprising
        a) a CD58 extracellular domain, or a functional fragment thereof,
        b) an antigen-binding fragment of an anti-CD3 antibody, and
        c) a CD80 extracellular domain, or a functional fragment thereof; and
    a viral glycoprotein (G protein);
    wherein the lentiviral particle further comprises a polynucleotide encoding a chimeric antigen receptor that specifically binds CD19,
    wherein the chimeric antigen receptor comprises a ligand-binding domain comprising an scFv, a hinge domain, a transmembrane domain, a 41BB endodomain, and a CD3ζ endodomain, and
    wherein the scFv comprises a VL comprising SEQ ID NO: 206 and a VH comprising SEQ ID NO: 208, the hinge domain comprises SEQ ID NO: 197, the transmembrane domain comprises SEQ ID NO: 199, the 41BB endodomain comprises SEQ ID NO: 201, and the CD3ζ endodomain comprises SEQ ID NO: 203.