

USE OF CD8-TARGETED VIRAL VECTORS

Cross-Reference to Related Applications

[0001] This application claims priority to U.S. provisional application nos. 63/136,202, filed January 11, 2021, entitled “USE OF CD8-TARGETED VIRAL VECTORS,” 63/150,498, filed February 17, 2021, entitled “USE OF CD8-TARGETED VIRAL VECTORS,” 63/168,235, filed March 30, 2021, entitled “USE OF CD8-TARGETED VIRAL VECTORS,” and 63/211,947, filed June 17, 2021, entitled, “USE OF CD8-TARGETED VIRAL VECTORS,” the contents of which are incorporated by reference in their entirety for all purposes.

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 186152004640SeqList.TXT, created January 10, 2022, which is 318,898 bytes in size. The information in the electronic format of the Sequence Listing is incorporated by reference in its entirety.

Field

[0003] The present disclosure relates to methods of transducing resting or non-activated T cells using CD8-targeted viral vectors.

Background

[0004] Viral vectors, including lentiviral vectors, are commonly used for delivery of exogenous agents to cells. However, transduction of the viral vectors to certain target cells can be challenging. Improved viral vectors, including lentiviral vectors, for use in methods for targeting desired cells and improving delivery are needed. The provided disclosure addresses this need.

Summary

[0005] This application is based on, inter alia, the surprising finding that resting or non-activated T cells could be efficiently transduced, both in vitro and in vivo using CD8-targeted viral vectors.

[0006] Provided herein is a method of transducing T cells, the method comprising contacting a non-activated T cell with a lentiviral vector comprising a CD8 binding agent, wherein the lentiviral vector transduces the non-activated T cell. In some examples, the T cell is a CD8+ T cells. In one aspect, the disclosure provides a method of transducing T cells, the method comprising: contacting a non-activated T cell with a lentiviral vector comprising a CD8 binding agent, wherein the lentiviral vector transduces the non-activated T cell, wherein the T cell is a CD8+ T cell. In some examples, the non-activated T cell is surface negative for one or more T cell activation markers selected from the group consisting of CD25, CD44 and CD69.

[0007] In some examples, the non-activated T cell has not been treated with an anti-CD3 antibody (e.g., OKT3). In some examples, the non-activated T cell has not been treated with an anti-

CD28 antibody (e.g., CD28.2). In some examples, the non-activated T cell has not been treated with an anti-CD3 antibody (e.g., OKT3) or with an anti-CD28 antibody (e.g., CD28.2). In some examples, the non-activated T cell has not been treated with a bead coupled to an anti-CD3 antibody (e.g. OKT3) and an anti-CD28 antibody (e.g. CD28.2), optionally wherein the bead is a superparamagnetic bead. In some examples, the bead is a superparamagnetic bead. In some examples, the non-activated T cell has not been treated with a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine. In some examples, the T cell activating cytokine is a human cytokine. In some examples, the non-activated T cell has not been treated with a soluble T cell costimulatory molecule (e.g. anti-CD28 antibody or soluble CD80, soluble CD86, soluble CD137L or soluble ICOS-L).

[0008] In some of any provided examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with a disease or condition (e.g. tumor cells). In some examples, the engineered receptor is an engineered T cell receptor (eTCR). In some examples, the engineered receptor is a chimeric antigen receptor (CAR). In some examples, the CAR comprises an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising intracellular components of a CD3zeta signaling domain and a costimulatory signaling domain. In some examples, the costimulatory signaling domain is a CD28 costimulatory domain. In some examples, the CD28 costimulatory signaling domain comprises the amino acid sequence set forth in SEQ ID NO:98. In some examples, the costimulatory signaling domain is a 4-1BB signaling domain. In some examples, the 4-1BB signaling domain comprises the amino acid sequence set forth in SEQ ID NO:97. In some examples, the CD3zeta signaling domain comprises the sequence set forth in SEQ ID NO:99 or SEQ ID NO:100. In some examples, the transmembrane domain comprises the sequence set forth in any one of SEQ ID NOS:94, 95, and 96. In some examples, the transmembrane domain comprises the sequence set forth in SEQ ID NO:94. In some examples, the transmembrane domain comprises the sequence set forth in SEQ ID NO:95. In some examples, the transmembrane domain comprises the sequence set forth in SEQ ID NO:96. In some examples, the CAR comprises a hinge domain. In some examples, the hinge domain comprises the sequence set forth in any one of SEQ ID NOS:88, 89, 90, 91, 92, 93, and 180. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:88. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:89. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:90. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:91. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:92. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:93. In some examples, the hinge domain comprises the sequence set forth in SEQ ID NO:180.

[0009] In some examples, the antigen binding domain binds to an antigen selected from the group consisting of CD19, CD20, CD22, and BCMA. In some examples, the antigen binding domain binds to CD19. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 108, 109, and 110, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 103, 104, and 105, respectively. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:107, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:102. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:101. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:111. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO:113. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO:115. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO: 117. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO:119. In some examples, the CAR comprises an amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:112. In some examples, the CAR comprises an amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:114. In some examples, the CAR comprises an amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:116. In some examples, the CAR comprises an amino acid sequence encoded by the polynucleotide sequence set forth in SEQ ID NO:118.

[0010] In some examples, the antigen binding domain binds to CD20. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 126, 127, and 182, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 122, 123, and 124, respectively. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:125, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:121. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:120.

[0011] In some examples, the antigen binding domain binds to CD22. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 130, 131, and 132, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 134, 135, and 136, respectively. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 139, 140, and 142, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 143,

144, and 145, respectively. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:129, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:133. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:138, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:142. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:128. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:137.

[0012] In some examples, the antigen binding domain binds to BCMA. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 152, 152, and 154, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 148, 149, and 150, respectively. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 161, 162, and 163, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO: 157, 158, and 159, respectively. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 165, 166, and 167, respectively. In some examples, the antigen binding domain comprises a CDR-H1, a CDRH-2, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO: 174, 175, and 176, respectively, and a CDR-L1, a CDR-L2, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:170, 171, and 172. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:151, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:147. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:160, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:156. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:173, and a VL region comprising the amino acid sequence set forth in SEQ ID NO:169. In some examples, the antigen binding domain comprises a VH region comprising the amino acid sequence set forth in SEQ ID NO:164. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:146. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:155. In some examples, the antigen binding domain comprises the amino acid sequence set forth in SEQ ID NO:168. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO:178. In some examples, the CAR comprises an amino acid sequence set encoded by the polynucleotide sequence set forth in SEQ ID NO:177.

[0013] In some examples, the CAR comprises: (i) an antigen binding domain comprising the VL region set forth in SEQ ID NO:102, a linker comprising the amino acid sequence set forth in SEQ ID

NO:106, and the VH region set forth in SEQ ID NO:107; and/or the scFv set forth in SEQ ID NO:101;(ii) a hinge comprising the amino acid sequence set forth in SEQ ID NO:88; (iii) a transmembrane domain comprising the amino acid sequence set forth in SEQ ID NO:94; (iv) a 4-1BB signaling domain comprises the amino acid sequence set forth in SEQ ID NO:97; and (v) a CD3zeta signaling domain comprising the amino acid sequence set forth in SEQ ID NO:99. In some examples, the CAR comprises the amino acid sequence set forth in SEQ ID NO:113. In some examples, the CAR is encoded by the nucleotide sequence set forth in SEQ ID NO:112.

[0014] In some examples, the non-activated T cell is a human T cell.

[0015] In some examples, the non-activated T cell is in a subject. In some examples, the non-activated T cell is in vitro. In some examples, the non-activated T cell is ex vivo from a subject. In some examples of the provided methods, prior to the contacting, the subject has not been administered a T cell activating treatment.

[0016] In some examples, any of the methods provided herein are carried out in vivo. In some examples, any of the methods provided herein are not ex vivo or are not in vitro.

[0017] In some of any examples of the provided methods, the subject has a disease or condition, such as a cancer. In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells), optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the engineered receptor is a chimeric antigen receptor (CAR). In some examples, the engineered receptor is an engineered T cell receptor (TCR).

[0018] In some of any of the provided methods, the method further comprises editing the T cell to inactivate one or more of *B2M*, *CIITA*, *TRAC*, and *TRB* genes. In some examples, the T cell is edited to inactivate *B2M*, *CIITA*, and *TRAC* genes. In some of any of the provided methods, the method further comprises inserting a gene encoding CD47 at a defined locus. In some examples, the defined locus is selected from the group consisting of a *B2M* locus, a *CIITA* locus, a *TRAC* locus, a *TRB* locus, or a safe harbor locus. In some examples, the safe harbor locus is selected from the group consisting of an *AAVS1* locus, a *CCR5* locus, and a *ROSA26* locus.

[0019] Also provided herein is a transduced T cell produced by the method of any of the provided methods. In some example, the T cell is inactivated at both alleles of the one or more genes. Also provided herein is a composition comprising a provided transduced T cell. In some examples, the composition is a pharmaceutical composition.

[0020] Provided herein is a method of transducing a population of T cells, the method comprising: contacting a population of non-activated T cells with a composition comprising lentiviral vectors

comprising a CD8 binding agent, wherein the population of non-activated T cells is transduced at an efficiency of at least 1%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 5%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35%. In one aspect, the disclosure provides a method of transducing a population of T cells, the method comprising: contacting a population of non-activated T cells with a composition comprising a lentiviral vector comprising a CD8 binding agent, wherein the population of non-activated T cells is transduced at an efficiency of at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 10%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 15%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 20%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 25%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 30%. In some examples, the population of non-activated T cells is transduced at an efficiency of at least 35%.

[0021] In some examples, at least 75% of the T cells in the population of non-activated T cells are surface negative for one or more T cell activation markers selected from the group consisting of CD25, CD44 and CD69 (e.g. at least 80%, at least 85%, at least 90%, at least 95% of the T cells in the population are surface negative for the T cell activation marker). In some examples, the population of non-activated T cells comprises CD8+ T cells (e.g. at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% of the population of non-activated T cells are CD8+ T cells). In some examples, at least 75% of the CD8+ T cells are surface negative for one or more T cell activation markers selected from the group consisting of CD25, CD44 and CD69 (e.g. at least 80%, at least 85%, at least 90%, at least 95% of the CD8+ T cells in the population are surface negative for the T cell activation marker). In some examples, the one or more T cell activation markers is CD25. In some examples, the one or more T cell activation markers is CD44. In some examples, the one or more T cell activation markers is CD69. In some examples, the CD8+ T cells in the population of non-activated T cells are transduced at an efficiency of at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35%.

[0022] In some examples, the population of non-activated T cells has not been treated with an anti-CD3 antibody (e.g., OKT3). In some examples, the population of non-activated T cell has not been treated with an anti-CD28 antibody (e.g., CD28.2). In some examples, the population of non-activated T cells has not been treated with an anti-CD3 antibody (e.g., OKT3) or with an anti-CD28 antibody (e.g., CD28.2). In some examples, the population of non-activated T cells has not been treated with a bead coupled to an anti-CD3 antibody (e.g. OKT3) and an anti-CD28 antibody (e.g. CD28.2), optionally wherein the bead is a superparamagnetic bead. In some examples, the population of non-activated T cells has not been treated with a bead coupled to an anti-CD3 antibody (e.g. OKT3) and an anti-CD28 antibody (e.g. CD28.2). In some examples, the bead is a superparamagnetic bead. In some examples, the population of non-activated T cell has not been

treated with a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine. In some examples, the population of non-activated T cell has not been treated with a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof). In some examples, the T cell activating cytokine is a human cytokine. In some examples, the population of non-activated T cells has not been treated with a soluble T cell costimulatory molecule (e.g. anti-CD28 antibody or soluble CD80, soluble CD86, soluble CD137L or soluble ICOS-L).

[0023] In some examples, the population of non-activated T cells are human cells.

[0024] In some examples, the population of non-activated T cells is in a subject. In some examples, prior to the contacting, the subject had not been administered a T cell activating treatment. In some examples, the population of non-activated T cells is in vitro. In some examples, the population of non-activated T cells is ex vivo from a subject. In some examples, the population of non-activated T cells comprise peripheral blood mononuclear cells (PBMCs) or a subset thereof comprising CD8+ T cells. In some examples, the population of non-activated cells is an enriched population of T cells selected from a biological sample from a subject, optionally wherein the T cells are selected for T cells surface positive for a T cell marker (e.g., CD3 or CD8). In some examples, the population of non-activated cells is an enriched population of T cells selected from a biological sample from a subject. In some examples, the T cells are selected for T cells surface positive for a T cell marker (e.g., CD3 or CD8). In some examples, the T cell marker is CD3. In some examples, the T cell marker is CD8. In some examples, the biological sample is a whole blood sample, apheresis sample or leukapheresis sample. In some examples, the biological sample is a whole blood sample. In some examples, the biological sample is an apheresis sample. In some examples, the biological sample is a leukapheresis sample.

[0025] In some examples, the subject has a disease or condition. In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells), optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells). In some examples, the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the engineered receptor is a chimeric antigen receptor (CAR). In some examples, the engineered receptor is an engineered T cell receptor (TCR).

[0026] In some of any of the provided methods, the method further comprises editing the T cell or population of T cells to inactivate one or more of B2M, CIITA, TRAC, and TRB genes. In some of any

of the provided methods, the population of T cells is edited to inactivate B2M, CIITA, and TRAC genes. In some examples, T cells of the population of T cells are edited to inactivate B2M, CIITA, and TRB genes. In some examples, the method further comprises inserting a gene encoding CD47 at a defined locus. In some examples, the defined locus is selected from the group consisting of a *B2M* locus, a *CIITA* locus, a *TRAC* locus, a *TRB* locus, or a safe harbor locus. In some examples, the safe harbor locus is selected from the group consisting of an *AAVS1* locus, a *CCR5* locus, and a *ROSA26* locus.

[0027] In some of any of the provided methods, the method further comprises expanding the population of transduced T cells. In some examples, the expanding comprises incubation of the transduced cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine. In some examples, the expanding comprises incubation of the transduced cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof). In some examples, the T cell activating cytokine is a human cytokine. In some of any of the provided methods, the method further comprises incubating the transduced T cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof), optionally wherein the T cell activating cytokine is a human cytokine. In some of any of the provided methods, the method further comprises incubating the transduced T cells with one or more T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21, or combinations thereof). In some examples, the T cell activating cytokine is a human cytokine.

[0028] Also provided herein is a population of transduced T cells produced by any of the provided methods. In some examples, at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35% of the cells of the population of non-activated cells are inactivated at the one or more genes. In some examples, at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, or at least 35% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 1% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples about 5% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 10% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 15% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 20% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 25% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, about 30% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some

examples, about 35% of the non-activated CD8+ T cells in the population are transduced and are inactivated at the one or more genes. In some examples, cells of the population are inactivated at both alleles of the one or more genes.

[0029] Also provided herein is a composition comprising the population of transduced T cells, optionally wherein the composition is a pharmaceutical composition. In one aspect, the disclosure provides a composition comprising a population of transduced T cells produced by the method as disclosed herein, optionally wherein the composition is a pharmaceutical composition. Also provided herein is a composition comprising the population of transduced T cells. In some examples, the composition is a pharmaceutical composition. Also provided herein is a pharmaceutical composition comprising the population of transduced T cells. Also provided herein is a method of treating a subject having a disease or condition, the method comprising administering to the subject any of the provided compositions comprising the population of transduced T cells. In some examples, the composition is not administered subcutaneously (SC). In some examples, the composition is not administered intramuscularly (IM). In some examples, the composition is administered intravenously (IV).

[0030] In some of any of the provided compositions, the composition further comprises a cryopreservant. In some examples, the cryopreservant is DMSO.

[0031] Provided herein is a method of in vivo transduction of T cells, the method comprising: administering to a subject a composition comprising lentiviral vectors comprising a CD8 binding agent, wherein the lentiviral vectors transduce T cells within the subject, and wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition. Also provided herein is a method of in vivo transduction of T cells, the method comprising: administering to a subject any of the provided compositions, wherein the lentiviral vectors transduce T cells within the subject, and wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition. In some examples, the subject has a disease or condition.

[0032] Also provided herein is a method of treating a subject having a disease or condition, the method comprising: administering to the subject a composition comprising lentiviral vectors comprising a CD8 binding agent, and wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition. In one aspect, the disclosure provides use of a composition comprising a lentiviral vector comprising a CD8 binding agent in the manufacture of a medicament for treating a subject having a disease or condition, optionally a cancer, wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition, wherein the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells), optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). Also provided herein is a method of treating a subject having a disease or condition, the method comprising administering to the subject any of the provided compositions, wherein the subject is not administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition. In some examples, the disease or condition is a cancer.

[0033] Also provided herein is a method for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof, the method comprising: administering to the subject a composition comprising lentiviral vectors comprising a CD8 binding agent, and wherein the subject is not administered a T cell activating treatment (e.g. before, after, or concurrently) with administration of

the composition. In one aspect, the disclosure provides use of a composition comprising a lentiviral vector comprising a CD8 binding agent in the manufacture of a medicament for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof, wherein the subject is not administered a T cell activating treatment (e.g. before, after, or concurrently) with administration of the composition, wherein the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein expressed on the tumor cells, optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). Also provided herein is a method for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof, the method comprising: administering to the subject a composition provided herein, and wherein the subject is not administered a T cell activating treatment (e.g. before, after, or concurrently) with administration of the composition. Also provided herein is a method for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof, the method comprising administering to the subject a composition provided herein. In some examples, the composition is not administered subcutaneously (SC). In some examples, the composition is not administered intramuscularly (IM). In some examples, the composition is administered intravenously (IV).

[0034] Also provided herein is use of a composition comprising lentiviral vectors comprising a CD8 binding agent for treating a subject having a disease or condition, optionally a cancer. Also provided herein is use of a composition provided herein for formulation of a medicament for treating a subject having a disease or condition, optionally a cancer. Also provided herein is use of a composition comprising lentiviral vectors comprising a CD8 binding agent for treating a subject having a disease or condition. Also provided herein is use of a composition provided herein for formulation of a medicament for treating a subject having a disease or condition. In some examples, the disease or condition is a cancer.

[0035] Also provided herein is a composition comprising lentiviral vectors comprising a CD8 binding agent for use in treating a subject having a disease or condition, optionally a cancer. Also provided herein is a composition provided herein for use in treating a subject having a disease or condition, optionally a cancer. Also provided herein is a composition comprising lentiviral vectors comprising a CD8 binding agent for use in treating a subject having a disease or condition. Also provided herein is a composition provided herein for use in treating a subject having a disease or condition. In some examples, the disease or condition is a cancer.

[0036] Also provided herein is use of a composition comprising lentiviral vectors comprising a CD8 binding agent for formulation of a medicament for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof. Also provided herein is use of a composition provided herein for formulation of a medicament for expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof.

[0037] Provided herein is a composition comprising lentiviral vectors comprising a CD8 binding agent for use in expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof. In one aspect, the disclosure provides a composition comprising a lentiviral vector comprising a CD8 binding agent for use in expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof. Also provided herein is a composition of any provided herein for use in expanding T cells capable of recognizing and killing tumor cells in a subject in need thereof.

[0038] In some of any of the provided examples, the use or the composition for use provided herein is for use in a subject that is not administered or to be administered a T cell activating treatment (e.g. before, after or concurrently) with administration of the composition.

[0039] In some of any of the provided methods, uses or compositions for use provided herein, the disease or condition is a cancer. In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells). In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein expressed on the tumor cells. In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein or antigen expressed by or on cells associated with the disease or condition (e.g. tumor cells), optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the lentiviral vector comprises a transgene encoding an engineered receptor that binds to or recognizes a protein expressed on the tumor cells, optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR)

[0040] In some examples, the T cell activating treatment comprises administration of an anti-CD3 antibody (e.g., OKT3). In some examples, the T cell activating treatment comprises administration of a soluble T cell costimulatory molecule (e.g., anti-CD28 antibody, or a recombinant CD80, CD86, CD137L, ICOS-L). In some examples, the T cell activating treatment comprises administration of a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21). In some examples, the T cell activating cytokine is a human cytokine. In some examples, the T cell activating treatment comprises administration of a T cell activating cytokine (e.g., recombinant IL-2, IL-7, IL-15, IL-21), optionally wherein the T cell activating cytokine is a human cytokine. In some of any examples, the T cell activating treatment comprises administration of recombinant IL-7, optionally human IL-7. In some of any examples, the T cell activating treatment comprises administration of recombinant IL-7. In some examples, the T cell activating treatment comprises administration of recombinant human IL-7. In some of any examples, the T cell activating treatment comprises administration of a lymphodepleting therapy, optionally administration of cyclophosphamide and/or fludarabine. In some of any examples, the T cell activating treatment comprises administration of a lymphodepleting therapy. In some examples, the T cell activating treatment comprises administration of cyclophosphamide and/or fludarabine. In some examples, the T cell activating treatment comprises administration of cyclophosphamide or fludarabine. In some examples, the T cell activating treatment comprises administration of cyclophosphamide. In some examples, the T cell activating treatment

comprises administration of fludarabine. In some examples, the T cell activating treatment comprises administration of cyclophosphamide and fludarabine.

[0041] In some of any of the provided examples, the subject is not administered a T cell activating treatment concurrently with the lentiviral vector. In some of any of the provided examples, the subject is not administered a T cell activating treatment within 1 month before the contacting with the lentiviral vector or before the administration of the composition comprising the lentiviral vectors. In some of any of the provided examples, the subject is not administered a T cell activating treatment within or at or about 1 week, 2 weeks, 3 weeks or 4 weeks, optionally at or about 1, 2, 3, 4, 5, 6 or 7 days, before the contacting with the lentiviral vector or before the administration of the composition comprising the lentiviral vectors. In some of any of the provided examples, the subject is not administered a T cell activating treatment at or about 1, 2, 3, 4, 5, 6 or 7 days, before the contacting with the lentiviral vector or before the administration of the composition comprising the lentiviral vectors. In some of any of the provided examples, the subject is not administered a T cell activating treatment within 1 month after the contacting with the lentiviral vector or after the administration of the composition comprising the lentiviral vectors. In some of any of the provided examples, the subject is not administered a T cell activating treatment within or at or about 1 week, 2 weeks, 3 weeks or 4 weeks, optionally at or about 1, 2, 3, 4, 5, 6 or 7 days, after the contacting with the lentiviral vector or after the administration of the composition comprising the lentiviral vectors. In some of any of the provided examples, the subject is not administered a T cell activating treatment at or about 1, 2, 3, 4, 5, 6 or 7 days, after the contacting with the lentiviral vector or after the administration of the composition comprising the lentiviral vectors.

[0042] In some of any of the provided examples, the lentiviral vector does not comprise or encode a T cell activating agent. In some of any of the provided examples, the lentiviral vector does not comprise or encode a membrane-bound T cell activating agent. In some of any of the provided examples, the lentiviral vector does not comprise or encode a T cell activating agent displayed on the surface. In some of any of the provided examples, the lentiviral vector does not comprise a T cell activating agent displayed on the surface, such as where the T cell activating agent is selected from the group consisting of a CD3 antibody (e.g. anti-CD3 scFv); a T cell activating cytokine (e.g. IL-2, IL-7, IL-15 or IL-21); or a T cell costimulatory molecule (e.g. anti-CD28 antibody, CD80, CD86, CD137L or ICOS-L). In some examples, the T cell activating agent is selected from the group consisting of a CD3 antibody (e.g. anti-CD3 scFv); a T cell activating cytokine (e.g. IL-2, IL-7, IL-15 or IL-21); and a T cell costimulatory molecule (e.g. anti-CD28 antibody, CD80, CD86, CD137L or ICOS-L). In some examples, the T cell activating agent is a polypeptide capable of binding CD3 and/or CD28. In some examples, the T cell activating agent is a polypeptide capable of binding CD3. In some examples, the T cell activating agent is a polypeptide capable of binding CD28. In some examples, the T cell

activating agent is a lymphoproliferative element. In some examples, the T cell activating agent is a cytokine or a cytokine receptor or a signaling domain thereof that activates a STAT3 pathway, a STAT4 pathway, and/or a Jak/STAT5 pathway. In some examples, the T cell activating agent is a T cell survival motif. In some examples, the T cell survival motif is an IL-7 receptor, an IL-15 receptor, or CD28, or a functional portion thereof. In some examples, the T cell activating agent is a microRNA (miRNA) or a short hairpin RNA (shrRNA). In some examples, the miRNA or the shrRNA stimulates the STAT5 pathway. In some examples, the miRNA or the shrRNA inhibits the SOCS pathway. In some examples, the miRNA or the shrRNA stimulates the STAT5 pathway and inhibits the SOCS pathway.

[0043] In some examples, the lentiviral vector does not comprise or encode an inhibitory RNA molecule. In some examples, the inhibitory RNA molecule targets an mRNA transcribed from a gene expressed by T cells. In some examples, the inhibitory RNA molecule targets a gene encoding a component of a T cell receptor (TCR). In some examples, the gene is PD-1, CTLA4, TCR α , TCR β , CD3 ζ , SOCS1, SMAD2, a miR-155 target, IFN γ , TRAIL2, and/or ABCG1.

[0044] In some examples, the lentiviral vector comprises or encodes an inhibitory RNA molecule. In some examples, the inhibitory RNA molecule targets an mRNA transcribed from a gene expressed by T cells. In some examples, the inhibitory RNA molecule targets a gene encoding a component of a T cell receptor (TCR). In some examples, the gene is PD-1, CTLA4, TCR α , TCR β , CD3 ζ , SOCS1, SMAD2, a miR-155 target, IFN γ , TRAIL2, and/or ABCG1.

[0045] In some of any of the provided examples, the CD8 binding agent is an anti-CD8 antibody or an antigen-binding fragment. In some of any of the provided examples, the anti-CD8 antibody or antigen-binding fragment is mouse, rabbit, human, or humanized. In some examples, the antigen-binding fragment is a single chain variable fragment (scFv). In some examples, the anti-CD8 antibody or antigen-binding fragment is a single domain antibody. In some examples, the anti-CD8 antibody or antigen-binding fragment is a camelid (e.g. llama, alpaca, camel) antibody or antigen-binding fragment (e.g. VHH).

[0046] In some of any of the provided examples, the CD8 binding agent binds to a CD8 alpha chain and/or CD8 beta chain. In some of any of the provided examples, the CD8 binding agent binds to a CD8 alpha chain. In some of any of the provided examples, the CD8 binding agent binds to a CD8 beta chain. In some of any of the provided examples, the CD8 binding agent binds to a CD8 alpha chain and a CD8 beta chain.

[0047] In some of any of the provided examples, the CD8 binding agent is exposed on the surface of the lentiviral vector. In some examples, the CD8 binding agent is fused to a transmembrane domain incorporated in the viral envelope.

[0048] In some examples, the lentiviral vector is pseudotyped with a viral fusion protein. In some examples, the viral fusion protein is a VSV-G protein or a functional variant thereof. In some examples, the viral fusion protein is a Cocal virus G protein or a functional variant thereof. In some examples, the viral fusion protein is an Alphavirus fusion protein (e.g. Sindbis virus) or a functional variant thereof. In some examples, the viral fusion protein is a Paramyxoviridae fusion protein (e.g., a Morbillivirus or a Henipavirus) or a functional variant thereof. In some examples, the viral fusion protein is a Morbillivirus fusion protein (e.g., measles virus (MeV), canine distemper virus, Cetacean morbillivirus, Peste-des-petits-ruminants virus, Phocine distemper virus, Rinderpest virus) or a functional variant thereof. In some examples, the viral fusion protein is a Henipavirus fusion protein (e.g., Nipah virus, Hendra virus, Cedar virus, Kumasi virus, Mòjiāng virus) or a functional variant thereof.

[0049] In some of any of the provided examples, the viral fusion protein comprises one or modifications to reduce binding to its native receptor.

[0050] In some of any of the provided examples, the viral fusion protein is fused to the CD8 binding agent. In some examples, the viral fusion protein is or comprises a Nipah virus fusion protein. In some examples, the viral fusion protein is a Nipah virus fusion protein or a functional variant thereof. In some examples, the viral fusion protein comprises a Nipah virus F glycoprotein (NiV-F) or a biologically active portion thereof and a Nipah virus G glycoprotein (NiV-G) or a biologically active portion thereof. In some examples, the CD8 binding agent is fused to the NiV-G or the biologically active portion thereof. In some examples, the viral fusion protein comprises a Nipah virus F glycoprotein (NiV-F) or a biologically active portion thereof and a Nipah virus G glycoprotein (NiV-G) or a biologically active portion thereof, and wherein the CD8 binding agent is fused to the NiV-G or the biologically active portion thereof. In some examples, the CD8 binding agent is fused to the C-terminus of the Nipah virus G glycoprotein or the biologically active portion thereof. In some examples, the CD8 binding protein is fused directly or via a peptide linker.

[0051] In some examples, the NiV-G protein or the biologically active portion thereof is a wild-type NiV-G protein or a functionally active variant or biologically active portion thereof.

[0052] In some examples, the NiV-G protein or the biologically active portion is truncated and lacks up to 40 contiguous amino acid residues at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion has a 5 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:12, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:12. In some examples, the NiV-G protein or the biologically active portion has a 5 amino acid truncation at or near the N-terminus of

the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:12, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:12. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:12. In some examples, the NiV-G protein or the biologically active portion thereof has a 10 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:44, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:44. In some examples, the NiV-G protein or the biologically active portion thereof has a 10 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:44, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:44. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:44. In some examples, the NiV-G protein or the biologically active portion thereof has a 15 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:9, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:45, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:45. In some examples, the NiV-G protein or the biologically active portion thereof has a 15 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:9, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:45, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:45. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:45. In some examples, the NiV-G protein or the biologically active portion thereof has a 20 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:13, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:13. In some examples, the NiV-G protein or the biologically active portion thereof has a 20 amino acid truncation at or near the N-terminus of the wild-type

NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:13, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:13. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:13. In some examples, the NiV-G protein or the biologically active portion has a 25 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:14, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 14. In some examples, the NiV-G protein or the biologically active portion has a 25 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:14, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 14. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:14. In some examples, the NiV-G protein or the biologically active portion has a 30 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:43, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:43. In some examples, the NiV-G protein or the biologically active portion has a 30 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:43, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:43. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:43. In some examples, the NiV-G protein or the biologically active portion has a 34 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:42. In some examples, the NiV-G protein or the biologically active portion has a 34 amino acid truncation at or near the N-terminus of the wild-type

NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:42. In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42. In some examples, the NiV-G protein or the biologically active portion has a 34 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5), optionally wherein the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:42. In some examples, the NiV-G protein or the biologically active portion has a 34 amino acid truncation at or near the N-terminus of the wild-type NiV-G protein (SEQ ID NO:1, SEQ ID NO:4 or SEQ ID NO:5). In some examples, the NiV-G protein or the biologically active portion thereof has the amino acid sequence set forth in SEQ ID NO:42, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO:42.

[0053] In some examples, the NiV-G-protein or the biologically active portion thereof is a mutant NiV-G protein. In some of any of the provided examples, the NiV-G-protein or the biologically active portion thereof is a mutant NiV-G protein that exhibits reduced binding to Ephrin B2 or Ephrin B3. In some of any of the provided examples, the mutant NiV-G protein or the biologically active portion comprises: one or more amino acid substitutions corresponding to amino acid substitutions selected from the group consisting of E501A, W504A, Q530A and E533A with reference to numbering set forth in SEQ ID NO:4. In some examples, the mutant NiV-G protein or the biologically active portion has the amino acid sequence set forth in SEQ ID NO: 17 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 17. In some examples, the mutant NiV-G protein or the biologically active portion has the amino acid sequence set forth in SEQ ID NO: 17. In some examples, the NiV-G protein or the biologically active portion has the amino acid sequence set forth in SEQ ID NO: 18 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 18. In some examples, the NiV-G protein or the biologically active portion has the amino acid sequence set forth in SEQ ID NO: 18.

[0054] In some of any of the provided examples, the NiV-F protein or the biologically active portion thereof is a wild-type NiV-F protein or is a functionally active variant or a biologically active portion thereof. In some of any of the provided examples, the NiV-F protein or

the biologically active portion thereof has a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41, or SEQ ID NO:40 without signal sequence), optionally wherein the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 20 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 20. In some of any of the provided examples, the NiV-F protein or the biologically active portion thereof has a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41). In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 20 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 20. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 20. In some of any of the provided examples, the NiV-F protein or the biologically active portion thereof comprises: i) a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41); and ii) a point mutation on an N-linked glycosylation site, optionally wherein the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 15, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 15. In some of any of the provided examples, the NiV-F protein or the biologically active portion thereof comprises: i) a 20 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41); and ii) a point mutation on an N-linked glycosylation site. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 15, or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 15. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 15. In some of any of the provided embodiments, the NiV-F protein or the biologically active portion thereof has a 22 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41 or SEQ ID NO:40 without signal sequence), optionally wherein the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 16 or 21 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 16 or 21. In some of any of the provided embodiments, the NiV-F protein or the biologically active portion thereof has a 22 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO: 41 or SEQ ID NO:40 without signal sequence). In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 16 or 21 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 16 or 21. In some examples, the NiV-F protein

or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 16 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 16. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 16. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 21 or a sequence of amino acids that exhibits at least at or about 80%, 85%, 90% or 95% sequence identity to the sequence set forth in SEQ ID NO: 21. In some examples, the NiV-F protein or the biologically active portion thereof has the sequence set forth in SEQ ID NO: 21.

[0055] In some examples, the NiV-G protein or the biologically active portion comprises the amino acid sequence set forth in SEQ ID NO: 17, and the NiV-F protein or the biologically active portion thereof comprises the sequence set forth in SEQ ID NO: 21. In some examples, the NiV-G protein or the biologically active portion consists of the amino acid sequence set forth in SEQ ID NO: 17, and the NiV-F protein or the biologically active portion thereof consists of the sequence set forth in SEQ ID NO: 21.

[0056] In some of any of the provided examples, the lentiviral vector comprises a transgene. In some embodiments, the transgene comprises a nucleic acid sequence encoding an RNA sequence capable of RNA interference (e.g. pre-miRNA, siRNA, or shRNA). In some examples, the transgene is selected from the group consisting of a therapeutic gene, a reporter gene, a gene encoding an enzyme, a gene encoding a pro-drug enzyme, a gene encoding an apoptosis inducer, a gene encoding a fluorescent protein, a gene encoding a pro-drug-activating enzyme, a gene encoding an apoptotic protein, a gene encoding an apoptotic enzyme, a gene encoding a suicide protein, a gene encoding a cytokine, a gene encoding an anti-immunosuppressive protein, a gene encoding an epigenetic modulator, a gene encoding a T cell receptor (TCR), a gene encoding a chimeric antigen receptor (CAR), a gene encoding a protein that modifies the cell surface of transduced cells, a gene encoding a protein that modifies the expression of the endogenous TCR, and a gene encoding a switch receptor that converts pro-tumor into anti-tumor signals. In some examples, the transgene encodes an engineered receptor that binds to or recognizes a protein or antigen expressed by cells or a lesion (e.g. tumor) associated with a disease or condition, optionally wherein the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR). In some examples, the transgene encodes an engineered receptor that binds to or recognizes a protein or antigen expressed by cells or a lesion (e.g. tumor) associated with a disease or condition. In some examples, the engineered receptor is a chimeric antigen receptor (CAR) or an engineered T cell receptor (TCR).

[0057] In some examples, the transgene encodes a chimeric antigen receptor (CAR). In some examples, the transgene encodes an engineered T cell receptor (TCR).

[0058] In some examples, the contacting is carried out by ex vivo administration of the lentiviral vector to a subject using a closed fluid circuit. In some examples, the administering is carried out by ex vivo administration of the lentiviral vector to a subject using a closed fluid circuit. In some examples, the ex vivo administration comprises (a) obtaining whole blood from a subject; (b) collecting the fraction of blood containing leukocyte components comprising T cells (e.g. CD8+ T cells); (c) contacting the leukocyte components comprising T cells (e.g. CD8+ T cells) with a composition comprising the lentiviral vector; and (d) reinfusing the contacted leukocyte components comprising T cells (e.g. CD8+ T cells) into the subject, wherein steps (a)-(d) are performed in-line in a closed fluid circuit. In some examples, the contacting in step (c) is for no more than 24 hours, no more than 18 hours, no more than 12 hours, or no more than 6 hours.

[0059] 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.

[0060] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Brief Description of the Drawings

[0061] FIG. 1A depicts Nalm6 tumor growth over time following injection of human peripheral blood mononuclear cells (hPBMC), with prior T cell activation with CD3/CD28 complexes, and then injection a day after of CD8-VHH CD19CAR LV. The results show that CD8-CD19CAR LV and activated hPBMC treatment results in robust control of Nalm6 tumor growth over time.

[0062] FIG. 1B depicts Nalm6 tumor growth over time following injection of non-activated hPBMC (without prior T cell activation), and then injection a day after of CD8-VHH CD19CAR LV. The results show that high dose CD8-VHH CD19CAR LV and non-activated hPBMC treatment results in delayed yet robust control of Nalm6 tumor growth.

[0063] FIG. 1C shows the percent of CD8⁺CD19CAR⁺ cells in total recovered live lymphocytes from spleen, bone marrow or peripheral blood following injection of CD8-VHH CD19CAR LV, as indicated in the top right quadrant of the FACs plots in both PBMC control (top plots) and CD8 fusosome-treated animals (bottom plots).

[0064] FIG. 2A depicts transduction efficiency (%CAR) of human PBMCs, with or without activation by anti-CD3 and anti-CD28 antibodies, following transduction with LV pseudotyped with