



(51) International Patent Classification:

C12N 15/867 (2006.01) A61K 31/711 (2006.01)

(21) International Application Number:

PCT/US2024/022527

(22) International Filing Date:

01 April 2024 (01.04.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/455,944 30 March 2023 (30.03.2023) US
63/466,949 16 May 2023 (16.05.2023) US

(71) Applicant: **KELONIA THERAPEUTICS, INC.**
[US/US]; Suite C, 135 William T. Morrissey, Boulevard,
Boston, Massachusetts 02125 (US).

(72) Inventors: **FRIEDMAN, Kevin M.**; 525 East 2nd Street,
Boston, MA 02127 (US). **PERKINS, Molly R.**; 64 Bradlee
Road, Milton, MA 02186 (US). **CONTRASTANO, Shan-**
non G.; 8 Orris Street, #2, Auburndale, MA 02466 (US).

(74) Agent: **BRYAN, Erin E.**; Morse, Barnes-Brown & Pendle-
ton, P.C., Citypoint, 480 Totten Pond Road, 4th Floor,
Waltham, MA 02451 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG,
KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY,
MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA,
NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO,
RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS,
ZA, ZM, ZW.

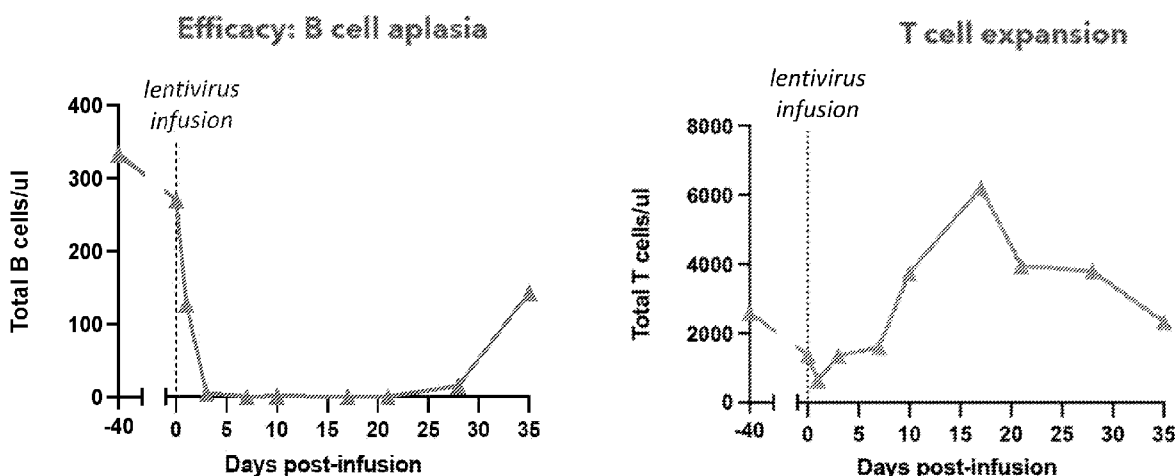
(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, CV,
GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST,
SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ,
RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ,
DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT,
LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,
SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished
upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: *IN VIVO* LENTIVIRUS ANIMAL MODELS

FIGURE 1



(57) Abstract: The present disclosure provides methods for decreasing B cells in non-human primates using recombinant lentiviral vectors.

***IN VIVO* LENTIVIRUS ANIMAL MODELS**

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S. C. § 119(e) of U.S. Provisional Application No. 63/455,944, filed March 30, 2023, and U.S. Provisional Application No. 5 63/466,949, filed May 16, 2023, each of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in xml format in lieu of a paper copy and is hereby incorporated by reference into the specification. The 10 name of the xml file containing the Sequence Listing is KELO-009-WO1_ST26.xml. The xml file is 155 KB, was created on March 28, 2024, and is being submitted electronically via Patent Center, concurrent with the filing of the specification.

Technical Field

The present disclosure relates to methods of using recombinant viruses in animal 15 models. More particularly, the disclosure relates to *in vivo* methods of using recombinant lentiviruses in non-human primate animal models.

Description of the Related Art

Ex vivo manufactured chimeric antigen receptor (CAR) T cell therapies hold a great deal of promise for the treatment of cancer. However, many patients will never receive 20 them because *ex vivo* CAR T cell therapies are too expensive, take too long to manufacture, require toxic lymphodepleting chemotherapy, and are only available at specialized medical centers. In addition, biotechnology companies are geographically restricting access to these medicines because reimbursement does not cover costs associated with manufacturing and treating patients with the medicine.

BRIEF SUMMARY

The present disclosure generally contemplates, in part, to non-human primate models for assessing the safety and efficacy of *in vivo* recombinant lentivirus gene therapies. In various embodiments, the disclosure contemplates, in part, a method to reduce
5 the number of B cells in a non-human primate (NHP) comprising: administering to the NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i) one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and (ii) one or more non-viral membrane-bound tropism polypeptides; and (b) a recombinant
10 HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor; wherein the amount of recombinant lentivirus is sufficient to transduce a population of immune effector cells in the NHP sufficient to reduce the number of B cells in the NHP.

In particular embodiments, at least one of the one or more non-viral membrane-bound tropism polypeptides comprises an extracellular antigen targeting domain, a spacer domain, and a transmembrane domain.
15

In particular embodiments, the extracellular antigen targeting domain binds an antigen expressed on an immune effector cell.

In certain embodiments, the extracellular antigen targeting domain binds an antigen
20 expressed on an immune effector cell selected from the group consisting of: the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3 δ , CD3 ϵ CD3 γ , CD4, CD8 α , and CD8 β .

In further embodiments, the extracellular antigen targeting domain comprises an anti-CD3 ϵ antibody or antigen binding fragment thereof.

25 In various embodiments, the viral envelope further comprises a secondary non-viral membrane-bound tropism polypeptide.

In certain embodiments, the engineered antigen receptor is selected from the group consisting of: a chimeric antigen receptor (CAR), a chimeric costimulatory receptor (CCR), an alpha-beta T cell receptor ($\alpha\beta$ TCR), a gamma delta T cell receptor ($\gamma\delta$ TCR), a dimerizing agent regulated immunoreceptor complex (DARIC), a chimeric TGF- β receptor (CTBR), and a zetamine receptor.
30

In particular embodiments, the immune effector cells comprise one or more of $\alpha\beta$ TCR T cells, $\gamma\delta$ TCR T cells, natural killer (NK) cells, and natural killer T (NKT) cells.

In various embodiments, the disclosure contemplates, in part, a method to reduce the number of B cells in a non-human primate (NHP) comprising: administering to the
5 NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i) one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and (ii) a non-viral membrane-bound tropism polypeptide comprising an anti-CD3 antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain, and
10 optionally a secondary non-viral membrane bound tropism polypeptide; and (b) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor; wherein the amount of recombinant lentivirus is sufficient to transduce a population of T cells in the NHP sufficient to reduce the number of B cells in the NHP.

15 In particular embodiments, the viral envelope further comprises a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof.

In certain embodiments, the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain that binds an antigen expressed on the surface of a B
20 cell, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

In some embodiments, the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain that binds BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D.

25 In various embodiments, the disclosure contemplates, in part, a method to reduce the number of B cells in a non-human primate (NHP) comprising: administering to the NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i) one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and
30 (ii) a non-viral membrane-bound tropism polypeptide comprising an anti-CD3 ϵ antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain, and optionally a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof; and

(b) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an anti-CD20 CAR; wherein the amount of recombinant lentivirus is sufficient to transduce a population of T cells in the NHP sufficient to reduce the number of B cells in the NHP.

5 In particular embodiments, the one or more mutated viral envelope glycoproteins comprise a vesiculovirus envelope glycoprotein, one or more morbillivirus envelope glycoproteins or one or more henipavirus envelope glycoproteins.

In certain embodiments, the vesiculovirus is selected from the group consisting of: vesicular stomatitis Alagoas virus (VSAV; Alagoas vesiculovirus), Carajás virus (CJSV; Carajas vesiculovirus), Chandipura virus (CHPV; Chandipura vesiculovirus), Cocal virus (COCV; Cocal vesiculovirus), vesicular stomatitis Indiana virus (VSIV; Indiana vesiculovirus), Isfahan virus (ISFV; Isfahan vesiculovirus), Maraba virus (MARAV; Maraba vesiculovirus), Morreton virus (MORV; Morreton vesiculovirus), vesicular stomatitis New Jersey virus (VSNJV; New Jersey vesiculovirus), and Piry virus (PIRYV; Piry vesiculovirus).

In some embodiments, the vesiculovirus envelope glycoprotein is a vesiculovirus G protein.

In various embodiments, the vesiculovirus G protein is a COCV G glycoprotein (COCV-G) or a VSIV G glycoprotein (VSIV-G).

20 In some embodiments, the VSIV-G envelope protein comprises one or more of: (a) one or more amino acid substitutions at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, T214, I347, T350, T352, E353, and R354; (b) an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; or (c) amino acid substitutions at K47 and/or R354; or (d) a deletion of residues 1-8.

In various embodiments, the VSIV-G envelope protein comprises one or more amino acid substitutions at H8, K47, Y209, and R354.

30 In particular embodiments, the VSIV-G envelope protein comprises one or more amino acid substitutions selected from the group consisting of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F and R354G; K47F and

R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G; K47Q and R354F; and K47Q and R354Q.

In further embodiments, the VSIV-G envelope protein comprises the amino acid sequence set forth in SEQ ID NO: 2, wherein $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; and $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = Q$.

In particular embodiments, the vesiculovirus G protein is COCV-G.

In some embodiments, the COCV-G envelope protein comprises one or more amino acid substitutions at K47 and/or R354.

In particular embodiments, the COCV-G envelope protein comprises one or more amino acid substitutions selected from the group consisting of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F and R354G; K47F and R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G; K47Q and R354F; and K47Q and R354Q.

In additional embodiments, the COCV-G envelope protein comprises the amino acid sequence set forth in SEQ ID NO: 4, wherein $X_1 = A$ and $X_2 = A$; $X_1 = A$ and $X_2 = G$; $X_1 = A$ and $X_2 = F$; $X_1 = A$ and $X_2 = Q$; $X_1 = G$ and $X_2 = A$; $X_1 = G$ and $X_2 = G$; $X_1 = G$ and $X_2 = F$; $X_1 = G$ and $X_2 = Q$; $X_1 = F$ and $X_2 = A$; $X_1 = F$ and $X_2 = G$; $X_1 = F$ and $X_2 = F$; $X_1 = F$ and $X_2 = Q$; $X_1 = Q$ and $X_2 = A$; $X_1 = Q$ and $X_2 = G$; $X_1 = Q$ and $X_2 = F$; or $X_1 = Q$ and $X_2 = Q$.

In certain embodiments, the one or more morbillivirus envelope glycoproteins are measles virus F (MV-F) and measles virus H (MV-H).

In certain embodiments, the MV-H protein comprises one or more amino acid substitutions at Y481, R533, S548, and F549.

In further embodiments, the MV-H protein comprises one or more amino acid substitutions selected from the group consisting of: Y481A, R533A, S548L, and F549S.

In particular embodiments, the one or more henipavirus envelope glycoproteins are nipah virus F (NiV-F) and nipah virus G (NiV-G).

In some embodiments, the NiV-G protein comprises one or more amino acid substitutions at E501, W504, Q530, and E533.

In various embodiments, the NiV-G protein comprises one or more amino acid substitutions at E501A, W504A, Q530A, and E533A.

In further embodiments, the extracellular antigen targeting domain comprises an antibody or antigen binding fragment selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab₂), a trispecific Fab trimer (Fab₃), an Fv, a single chain Fv protein (scFv), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), a single-domain antibody (sdAb, a camelid VHH, Nanobody), and a centyrin.

In additional embodiments, the spacer domain is hinge domain or stalk obtained or isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β ,

CD28, or Glycophorin A; and/or wherein the transmembrane domain is isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and optionally, wherein the tropism polypeptide comprises a truncated intracellular domain isolated from a polypeptide selected from the group consisting of CD3,
 5 CD4, CD8 α , CD8 β , CD28, or Glycophorin A or a cytoplasmic tail of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

In particular embodiments, the viral envelope further comprises a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL and optionally, comprises a C-terminal truncation
 10 that results in a cytoplasmic domain of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

In various embodiments, the promoter is selected from the group consisting of: an elongation factor 1 α (EF1 α) promoter, a cytomegalovirus (CMV) promoter, a Moloney murine leukemia virus (MoMLV) promoter, a Rous sarcoma virus (RSV) promoter, a 3-
 15 phosphoglycerate kinase (PGK-1) promoter, a herpes simplex virus (HSV) (thymidine kinase) promoter, a chicken β -actin (CAG) promoter, a simian virus 40 (SV40) promoter, an SV40/CD43 promoter, a spleen focus forming virus (SFFV) promoter, and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer binding site substituted (MND) U3 promoter.

20 In some embodiments, the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain selected from the group consisting of: a receptor ectodomain, a ligand, or an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, a
 25 single chain Fv protein (scFv), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), a single-domain antibody (sdAb, a camelid VHH, Nanobody), and a centyrin.

In particular embodiments, the engineered antigen receptor is a CAR comprising a hinge domain isolated or derived from a polypeptide selected from the group consisting of:
 30 CD4, CD8 β , CD8 α , CD28, CD134, CD137, CD152, CD278, IgG1, IgG2, IgG3, and IgG4.

In various embodiments, the engineered antigen receptor is a CAR comprising a transmembrane domain isolated or derived from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3 δ , CD3 ϵ ,

CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

In further embodiments, the engineered antigen receptor is a CAR comprising a
 5 primary signaling domain isolated or derived from a polypeptide selected from the group consisting of: FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d.

In certain embodiments, engineered antigen receptor is a CAR comprising a costimulatory domains isolated or derived from a polypeptide selected from the group
 10 consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD
 15 (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

In some embodiments, the recombinant lentivirus is parenterally administered to the NHP.

20 In various embodiments, the recombinant lentivirus is intravascularly administered to the NHP.

In particular embodiments, the recombinant lentivirus is intravenously or intraarterially administered to the NHP.

In additional embodiments, after administration of the recombinant lentivirus, 1, 2,
 25 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 or more specimens are taken from the NHP, wherein one or more of the specimens is taken from the NHP every day, every other day, every three days, every four days, every five days, every six days, every 7 days, every eight days, every nine days, or every ten or more days for a period of about 10 days to about 60 days or any intervening period of time.

30 In some embodiments, one or more specimens is taken from the NHP and the number of B cells is measured in the specimen.

In particular embodiments, one or more specimens is taken from the NHP and the number of T cells is measured in the specimen.

In some embodiments, one or more specimens is taken from the NHP and the number of CAR⁺ T cells is measured in the specimen.

In certain embodiments, one or more specimens is taken from the NHP and the vector copy number (VCN) of transduced T cells is measured in the one or more
5 specimens.

In particular embodiments, one or more specimens is taken from the NHP and the amount of one or more cytokines and/or chemokines is measured in the one or more specimens.

In further embodiments, one or more specimens is taken from the NHP and the
10 amount of one or more cytokines or chemokines selected from the group consisting of CXCL11, GM-CSF, IFN γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IP-10, MIP-1 α , and TNF α is measured in the one or more specimens.

In various embodiments, the amount of recombinant lentivirus administered to the NHP does not cause a cytokine storm, cytokine release syndrome (CRS), or neurotoxicity
15 in the NHP.

In particular embodiments, the amount of recombinant lentivirus administered to the NHP decreases the number of B cells in a specimen at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more compared to the number of B cells in a specimen from an NHP that was not administered the recombinant lentivirus or compared to the
20 number of B cells in a specimen from the NHP before the NHP was administered the recombinant lentivirus.

In some embodiments, a composition comprising a pharmaceutically acceptable diluent and the recombinant lentivirus is administered to the NHP.

In particular embodiments, the diluent is a pharmaceutically acceptable cell culture
25 medium (*e.g.*, DMEM), Dulbecco's phosphate buffered saline (PBS), Ringer's solution, 5% dextrose in water (D5W), or normal/physiologic saline (0.9% NaCl).

In some embodiments, the amount of recombinant lentivirus administered to the NHP at a dose of about 5 mL/kg to about 15 mL/kg

In various embodiments, the amount of recombinant lentivirus administered to the
30 NHP at a dose of about 5 mL/kg, about 6 mL/kg, about 7 mL/kg, about 8 mL/kg, about 9 mL/kg, about 10 mL/kg, about 11 mL/kg, about 12 mL/kg, about 13 mL/kg, about 14 mL/kg, or about 15 mL/kg.

In particular embodiments, the amount of recombinant lentivirus administered to the NHP is about 1×10^6 TU/mL to about 1×10^{10} TU/mL.

In some embodiments, the amount of recombinant lentivirus administered to the NHP is about 1×10^7 TU/mL to about 1×10^9 TU/mL.

5 In certain embodiments, the amount of recombinant lentivirus administered to the NHP is about 1×10^7 TU/mL, 5×10^7 TU/mL, 1×10^8 TU/mL, 5×10^8 TU/mL, about 1×10^9 TU/mL, about 5×10^9 TU/mL, or about 1×10^{10} TU/mL.

In particular embodiments, the NHP is not administered a lymphodepleting chemotherapy prior to, during, or after administration of the recombinant lentivirus.

10 In various embodiments, the NHP is *Macaca nemestrina*.

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows the effect on lymphocyte populations in a pig-tail macaque administered an *in vivo* HIV-1 derived lentivirus encoding an anti-CD20 CAR. The rightmost panel shows T cell expansion as a function of time after infusion; the leftmost
15 panel shows B cell aplasia as a function of time after infusion.

Figure 2 shows that the *in vivo* HIV-1 derived lentivirus encoding an anti-CD20 CAR did not increase cytokines/chemokines to levels associated with neurotoxicity in *M. nemestrina*. The gray boxes indicate cytokine levels associated with neurotoxicity.

Figure 3 shows the effect on lymphocyte populations in a pig-tail macaque
20 administered an *in vivo* HIV-1 derived lentivirus encoding an anti-CD20 CAR. The rightmost panel shows the vector copy number detected in T cells as a function of time after infusion; the leftmost panel shows B cell aplasia as a function of time after infusion.

BRIEF DESCRIPTION OF THE SEQUENCE IDENTIFIERS

SEQ ID NOs: 1-10 set forth amino acid sequences of fusogens.
25 SEQ ID NO: 11 sets forth the polynucleotide sequence encoding a CAR.
SEQ ID NO 12: sets forth the amino acid sequences encoded by SEQ ID NO: 11.
SEQ ID NOs: 13-92 set forth amino acid sequences of fusogens.
SEQ ID NOs: 93-106 set forth amino acid sequences of linker polypeptides.

SEQ ID NOs: 107-126 set forth amino acid sequences of viral self-cleaving polypeptides.

In the foregoing sequences, X, if present, refers to any amino acid, a specified group of amino acids or the absence of an amino acid.

- 5 Throughout the disclosure, the amino acid position(s) of a fusogen is with reference to the fusogen lacking a signal sequence (*i.e.*, the amino acid sequence after the signal peptide has been cleaved).

DETAILED DESCRIPTION

A. OVERVIEW

- 10 There is a paucity of non-human primate animal models that can be used to assess the safety and efficacy of a lentiviral-based *in vivo* gene therapy designed for human applications. Non-human primate models present unique challenges to lentivirus-based *in vivo* gene therapies for oncology (*e.g.*, chimeric antigen receptor T cell therapy) because they express an array of HIV post-entry mechanisms that restrict HIV-1 replication not
15 present in human cells. In primary human blood cell types (*e.g.*, HSCs, T cells), cyclophilin A (CypA) interacts with the HIV-1 capsid protein (CA) and stimulates replication; tripartite-containing motif 5 α (TRIM5 α) restricts replication but only when the CA-CypA interaction is disrupted.

- In contrast, Old World monkeys such as rhesus macaques (*Macaca mulatta*),
20 cynomolgus macaques (*Macaca fascicularis*), and pig-tail macaques (*Macaca nemestrina*) express different combinations of, and different alleles of, TRIM5 α , CypA, and TRIM5 α -CypA fusion proteins (TRIMCyp) to regulate HIV-1 replication. Rhesus macaques express several variants of TRIM5 α . These TRIM5 α variants and CypA work together to restrict HIV-1 replication with varying efficiencies. TRIMCyp fusion proteins are also seen in
25 some, but not all, populations of rhesus macaques, where it restricts HIV-2 but not HIV-1 replication. Cynomolgus macaques can express TRIM5 α and different TRIM5 α -CypA fusion proteins. Similar to rhesus macaques, TRIM5 α and CypA work together to restrict HIV-1 replication. TRIMCyp fusion protein variants are expressed in cynomolgus macaques but only restrict HIV-2, not HIV-1, infection. In contrast, pig-tail macaques do
30 not express TRIM5 α mRNA. Instead, TRIM5 isoforms TRIM5 θ and TRIM5 η associated

with TRIMCyp are expressed. TRIMCyp restricts HIV-2 but not HIV-1 replication, rendering pig-tail macaques sensitive to HIV-1 infection due to the absence of TRIM5 α and expression of a TRIMCyp that is not able to restrict HIV-1 replication. In preferred embodiments, pig-tail macaques are used as the non-human primate model for the *in vivo* HIV-1 lentivirus-based gene therapies contemplated herein.

The present disclosure provides solutions to the foregoing problems as well as other issues apparent in the field.

The disclosure generally relates to developing *in vivo* lentivirus gene therapy models in non-human primates (NHPs). The disclosure contemplates, in part, a method of reducing B cells in an NHP using recombinant lentivirus.

In various embodiments, a method comprises administering a recombinant lentivirus derived from human immunodeficiency virus (HIV) type 1 (HIV-1) to a NHP, wherein the recombinant lentivirus comprises an envelope that has a component that mediates fusion and one or more components that drive targeting to an immune effector cell, and further comprises a vector that encodes an engineered antigen receptor that targets cells of the B cell lineage in the NHP. In particular embodiments, a method comprises administering a recombinant lentivirus derived from HIV-1 that comprises a viral envelope that comprises one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity, *i.e.*, the ability to bind a cognate receptor on a cell, and one or more non-viral membrane-bound tropism polypeptides engineered to bind an immune effector cell, and a vector comprising encoding an engineered antigen receptor that targets cells of the B cell lineage in the NHP.

The disclosure further contemplates a method to reduce the number of cells of the B cell lineage in an NHP that comprises administering an HIV-1 derived recombinant lentivirus that comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and a non-viral membrane-bound tropism polypeptide comprising an anti-CD3 antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain; optionally, one or more other non-viral membrane bound tropism polypeptides that target an immune effector cell; and a recombinant lentiviral derived from HIV-1 that encodes a promoter operably linked to a polynucleotide encoding an engineered antigen receptor that targets cells of the B cell lineage in the NHP.

The disclosure also contemplates a method to reduce the number of cells of the B cell lineage in an NHP that comprises administering an HIV-1 derived recombinant lentivirus that comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity; a
5 non-viral membrane-bound tropism polypeptide comprising an anti-CD3 antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain; optionally, a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof; and a recombinant HIV-derived lentiviral vector comprising a polynucleotide encoding a
10 promoter operably linked to a polynucleotide encoding a chimeric antigen receptor that binds an antigen expressed on a cell of the B cell lineage.

Techniques for recombinant (*i.e.*, engineered) DNA, peptide and oligonucleotide synthesis, immunoassays, tissue culture, transformation (*e.g.*, electroporation, lipofection), enzymatic reactions, purification and related techniques and procedures may be generally
15 performed as described in various general and more specific references in microbiology, molecular biology, biochemistry, molecular genetics, cell biology, virology and immunology as cited and discussed throughout the present specification. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology*
20 (John Wiley and Sons, updated July 2008); *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (2002); Glover, *DNA Cloning: A Practical Approach*, vol. I & II (IRL Press, Oxford Univ. Press USA, 1985); *Current Protocols in Immunology* (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach,
25 Warren Strober 2001 John Wiley & Sons, NY, NY); *Real-Time PCR: Current Technology and Applications*, Edited by Julie Logan, Kirstin Edwards and Nick Saunders, 2009, Caister Academic Press, Norfolk, UK; Anand, *Techniques for the Analysis of Complex Genomes*, (Academic Press, New York, 1992); Guthrie and Fink, *Guide to Yeast Genetics and Molecular Biology* (Academic Press, New York, 1991); *Oligonucleotide Synthesis* (N. Gait,
30 Ed., 1984); *Nucleic Acid the Hybridization* (B. Hames & S. Higgins, Eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, Eds., 1984); *Animal Cell Culture* (R. Freshney, Ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984); *Next-Generation Genome Sequencing* (Janitz, 2008 Wiley-VCH); *PCR Protocols* (Methods in

Molecular Biology) (Park, Ed., 3rd Edition, 2010 Humana Press); *Immobilized Cells and Enzymes* (IRL Press, 1986); the treatise, *Methods in Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors for Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Harlow and Lane, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998); *Immunochemical Methods in Cell and Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook of Experimental Immunology*, Volumes I-IV (D. M. Weir and CC Blackwell, eds., 1986); Roitt, *Essential Immunology*, 6th Edition, (Blackwell Scientific Publications, Oxford, 1988); *Current Protocols in Immunology* (Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); *Annual Review of Immunology*; as well as monographs in journals such as *Advances in Immunology*.

B. DEFINITIONS

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

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of particular embodiments, preferred embodiments of compositions, methods and materials are described herein. For the purposes of the present disclosure, the following terms are defined below.

The articles “a,” “an,” and “the” are used herein to refer to one or to more than one (*i.e.*, to at least one, or to one or more) of the grammatical object of the article. By way of example, “an element” means one element or one or more elements.

The use of the alternative (*e.g.*, “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

The term “and/or” should be understood to mean either one, or both of the alternatives.

As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In one embodiment, the term “about” or “approximately” refers a range of quantity,

level, value, number, frequency, percentage, dimension, size, amount, weight or length $\pm$ 15%, $\pm$ 10%, $\pm$ 9%, $\pm$ 8%, $\pm$ 7%, $\pm$ 6%, $\pm$ 5%, $\pm$ 4%, $\pm$ 3%, $\pm$ 2%, or $\pm$ 1% about a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

5 In one embodiment, a range, *e.g.*, 1 to 5, about 1 to 5, or about 1 to about 5, refers to each numerical value encompassed by the range. For example, in one non-limiting and merely illustrative embodiment, the range “1 to 5” is equivalent to the expression 1, 2, 3, 4, 5; or 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0; or 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 10 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0.

As used herein, the term “substantially” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher compared to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or 15 length. In one embodiment, “substantially the same” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that produces an effect, *e.g.*, a physiological effect, that is approximately the same as a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

By “enhance” or “promote,” or “increase” or “expand” refers generally to the 20 ability of a recombinant lentivirus comprising a vector encoding an engineered antigen receptor contemplated herein to produce, elicit, or cause a greater effect compared to the effect prior to treatment or the effect caused by either vehicle or a control lentivirus. A measurable effect may include an increase in T cell activation, transduction, expansion, persistence, cytokine secretion, and/or an increase in the ability to reduce or decrease the 25 numbers of target cells in a non-human primate.

By “decrease” or “lower,” or “lessen,” or “reduce,” or “abate” refers generally to the ability of a recombinant lentivirus comprising a vector encoding an engineered antigen receptor contemplated herein to produce, elicit, or cause a lesser effect compared to the effect prior to treatment or the effect caused by either vehicle or a control lentivirus.

30 By “maintain,” or “preserve,” or “maintenance,” or “no change,” or “no substantial change,” refers generally to the ability of a recombinant lentivirus contemplated herein to produce, elicit, or cause a substantially similar or comparable effect compared to the effect caused by either vehicle, a control lentivirus.

Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By “consisting of” is meant including, and
5 limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed elements are required or mandatory, and that no other elements may be present. The phrase “consisting essentially of” means including any elements listed after the phrase, and is limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase
10 “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are present that materially affect the activity or action of the listed elements.

Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional
15 embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be
20 combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

A “recombinant HIV-1 lentivirus” refers to a recombinantly produced or non-naturally occurring lentivirus that contains a viral envelope; HIV-1 lentivirus structural
25 proteins, matrix (MA), capsid (CA), nucleocapsid (NC), and p6 and enzymatic proteins, integrase (IN), protease (PR), and reverse transcriptase (RT); and further comprises a recombinant lentiviral vector that contains HIV-1 genomic elements for packaging, reverse transcription and integration.

The terms “cell of the B cell lineage” or “B cell” refers to cells that show at least
30 one phenotypic, physiological, morphological, functional, or immunological characteristic of a B cell. B cells suitable for use in particular embodiments contemplated herein include but are not limited to B cells that express one or more of the following markers CD1c, CD2, CD5, CD6, CD11a, CD11c, CD14, CD19, CD20, CD21, CD22, CD23, CD24,

CD25, CD27, CD28, CD30, CD35, CD37, CD38, CD39, CD40, CD43, CD45RA, CD45RB, CD45RO, CD49c, CD49d, CD51, CD57, CD62L, CD69, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD83, CD84, CD85, CD86, CD89, CD98, CD95, CD99, CD100, CD106, CD107a, CDw108, CDw121b (IL-1RII), CDw124 (IL-4R), CDw127 (IL-7R), CD184, CD185, CD194, CD196, CD197, CD269, CD270 (TNFRSF14; HVEM), CD272 (BTLA-4), CD273 (PD-L2), CD274 (PD-L1), CD276 (TACI), CD278 (BAFF-R), CD279 (BCMA), CD300, CMRF35, CCR10, κ light chain, λ light chain, IL-10R, IL-11R, IL-13R α 1, LTK, Sca-1, SlamF6, and TNFRSF25; naive B cells, plasma cells, regulatory B cells, marginal zone B cells, follicular B cells, lymphoplasmacytoid cells, plasmablast cells, and memory B cells.

The terms, “binding domain,” “extracellular binding domain,” and “extracellular antigen binding domain” are used interchangeably and refers to a domain that enables an engineered antigen receptor to specifically bind to a target antigen. The binding domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

An “antibody” refers to a polypeptide that comprises at least a light chain immunoglobulin variable region and/or a heavy chain immunoglobulin variable region, which specifically recognizes and binds an epitope on an antigen.

An “isolated antibody or antigen binding fragment thereof” refers to an antibody or antigen binding fragment thereof that has been separated from its natural environment and/or that is derived from a natural, synthetic, semi-synthetic, or recombinant source. An “antigen binding fragment” or “antigen binding portion” refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen.

Illustrative examples of antigen binding fragments suitable for incorporation into engineered antigen receptors contemplated herein include, but are not limited to, a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab₂), a trispecific Fab trimer (Fab₃), an Fv, a single chain Fv protein (“scFv”), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein (“dsFv”), a single-domain antibody (sdAb or Nanobody, *e.g.*, a camelid VHH), a centyrin, other portions of full length antibodies sufficient for antigen binding, and combinations thereof.

A “heavy chain antibody” refers to an antibody that contains two heavy chain variable domains and no light chains; a “camelid antibody” refers to an antibody isolated

from a Camel, Alpaca, or Llama that contains two heavy chain variable domains and no light chains.

An “IgNAR” or “immunoglobulin new antigen receptor” refers to class of antibodies from the shark immune repertoire that consist of homodimers of one variable
 5 new antigen receptor (VNAR) domain and five constant new antigen receptor (CNAR) domains. IgNARs represent some of the smallest known immunoglobulin-based protein scaffolds and are highly stable and possess efficient binding characteristics.

A “single-chain Fv” or “scFv” antibody fragment comprises the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain
 10 and in either orientation (e.g., VL-VH or VH-VL). Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding.

A “single domain antibody,” “sdAb,” or “nanobody” as used herein refers an antibody fragment that contains the smallest known antigen binding unit of the variable
 15 region of a heavy chain antibody, *e.g.*, a camelid VHH or shark VNAR. A “humanized VHH” refers to a single domain non-human VHH that has undergone humanization to reduce potential immunogenicity of the antibody in human recipients. A “humanized VNAR” refers to a single domain non-human VNAR that has undergone humanization to reduce potential immunogenicity of the antibody in human recipients.

20 “Linker,” “peptide linker,” and “polypeptide linker” are used interchangeably and refer to a plurality of amino acid residues between various polypeptide domains added for appropriate spacing, conformation, and function. Linkers include a “variable region linking sequence,” an amino acid sequence that connects the VH and VL domains of an antibody or antigen binding fragment thereof and provides a spacer function compatible with
 25 interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. A linker may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more amino acids long. Illustrative examples of linkers include, but are not limited to glycine polymers; glycine-serine
 30 polymers; glycine-alanine polymers; alanine-serine polymers; GGG; DGGGS (SEQ ID NO: 93); TGEKP (SEQ ID NO: 94); GGRR (SEQ ID NO: 95); GGGGS (SEQ ID NO: 96); GGGGSGGGGS (SEQ ID NO: 97); GGGGSGGGSGGGGS (SEQ ID NO: 98); GGGGSGGGSGGGSGGGGS (SEQ ID NO: 99);

GGGSGGGSGGGSGGGSGGGSGGGGS (SEQ ID NO: 100) ; EGKSSGSGSESKVD (SEQ ID NO: 101); KESGSVSSEQLAQFRSLD (SEQ ID NO: 102); GGRRGGGS (SEQ ID NO: 103); LRQRDGERP (SEQ ID NO: 104); LRQKDGGGSERP (SEQ ID NO: 105); and LRQKDGGSGGGSERP (SEQ ID NO: 106).

5 A “hinge domain,” is a type of domain that may be present in an engineered antigen receptor. A hinge domain plays a role in positioning an antigen binding domain away from the effector cell surface to enable proper cell/cell contact, antigen binding and activation. A hinge domain is placed between a binding domain and a transmembrane domain (TM). A hinge domain may be derived either from a natural, synthetic, semi-synthetic, or
10 recombinant source. A hinge domain may be altered by substituting one or more cysteine and/or proline residues in a naturally occurring immunoglobulin hinge domain with one or more other amino acid residues (*e.g.*, one or more serine residues).

A “transmembrane domain” or “TM domain” refers to a hydrophobic portion of polypeptide that is disposed between an extracellular domain and an intracellular domain
15 and anchors the polypeptide to the plasma membrane of the cell. A TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

An “intracellular signaling domain” refers to a portion of a polypeptide that participates in transducing the message of effective binding of a target antigen by a receptor expressed on an immune effector cell to the immune effector cell’s interior to an elicit
20 effector function (an “effector function” refers to a specialized function of an immune effector cell), *e.g.*, activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors, or other cellular responses elicited with antigen binding to the receptor expressed on the immune effector cell. “Intracellular signaling domains” include the polypeptide domain or functional fragment thereof, which transduces
25 the effector function signal and that directs the cell to perform a specialized function. The term intracellular signaling domain is meant to include any truncated portion of the intracellular signaling domain sufficient to transducing effector function signal.

T cell activation can be said to be mediated by two distinct classes of intracellular signaling domains: primary signaling domains that initiate antigen-dependent primary
30 activation through the TCR (*e.g.*, a TCR/CD3 complex) and costimulatory signaling domains that act in an antigen-independent manner to provide a secondary or costimulatory signal.

A “primary signaling domain” refers to a signaling domain that regulates the primary activation of a TCR complex either in a stimulatory way, or in an inhibitory way. Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

5 A “costimulatory signaling domain” or “costimulatory domain” refers to an intracellular signaling domain of a co-stimulatory molecule. Costimulatory molecules are cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen.

10 A “tropism polypeptide” refers to a polypeptide that binds one or more antigens on a target host cell, *e.g.*, an immune effector cell. A tropism polypeptide comprises an extracellular antigen targeting domain, a spacer domain, and a transmembrane domain.

A “non-viral membrane bound tropism polypeptide” refers to a polypeptide that binds one or more antigens on a target host cell, *e.g.*, an immune effector cell; that is not
15 native to, or derived from, either in whole or in part, a virus; and that is attached to a lipid bilayer, cell membrane, or viral envelope. A non-viral membrane bound tropism polypeptide comprises an extracellular antigen targeting domain, a spacer domain, and a transmembrane domain.

An “extracellular antigen targeting domain” refers to a polypeptide that enables a
20 tropism polypeptide or non-viral membrane-bound tropism polypeptide to specifically bind to a antigen on a target host cell, *e.g.*, an immune effector cell. The extracellular antigen targeting domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source.

A “spacer domain,” refers to a polypeptide disposed between an extracellular
25 antigen targeting domain and a transmembrane domain. A spacer domain positions the extracellular antigen targeting domain away from the viral envelope surface to enable proper virus/target cell contact and binding. A spacer domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. Illustrative examples of spacer domains include but are not limited to hinge or stalk domains from IgG1, IgG2, IgG4, CD2,
30 CD3, CD4, CD8 α , CD8 β , and CD28 and polypeptide linkers of similar amino acid composition and lengths.

Additional definitions are set forth throughout this disclosure.

C. METHODS

Gene therapy development and successful clinical translation is challenging, in part due to difficulty generating clinically relevant pharmacology, toxicology, and safety data from non-human animal models. Moreover, *in vivo* lentivirus-based gene therapies are a new class of gene therapies that do not have well established non-human animal models. Although rodent models are effective surrogates exploring pathways and disease mechanisms and identifying potential treatment targets they are not as useful for evaluating drug safety/toxicity. Non-human primates (NHPs) most closely resemble humans, regarding lifespan, size, telomere length, immune system characteristics, and genetic heterogeneity, conferring high predictive value for the safety/toxicity of cell therapies. Moreover, NHPs can also be used to evaluate the efficacy of a platform *in vivo* lentivirus-based CAR T therapy that targets B cells. The present inventors have established an effective NHP model to evaluate the safety and efficacy of an *in vivo* lentivirus-based gene therapy platform.

In various embodiments, a method to evaluate an *in vivo* lentivirus-based gene therapy platform in an NHP comprises administering an amount of a recombinant lentivirus contemplated herein to the NHP. The term “amount” as used herein, refers to “an amount effective,” “an amount sufficient,” “an effective amount,” or “a sufficient amount” of recombinant lentivirus contemplated herein to achieve a beneficial or desired effect, including a decrease in the number of B cells or B cell aplasia in the NHP. In preferred embodiments, the effective amount of recombinant lentivirus is administered to the NHP and causes a desired effect without causing measurable toxicity.

In particular embodiments, a method to reduce the number of target cells in a NHP comprises administering an amount of a recombinant lentivirus comprising a lentiviral vector encoding an engineered antigen receptor that binds an antigen expressed on a target cell. In preferred embodiments, the recombinant lentivirus and lentiviral vector are derived from HIV-1 and are administered to a pig-tail macaque.

In particular embodiments, a method to reduce the number of B cells in an NHP comprises administering a recombinant lentivirus comprising a viral envelope that comprises one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and one or more non-viral membrane-bound tropism polypeptides, and a recombinant lentiviral vector comprising a polynucleotide

encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor that binds an antigen expressed on the B cell. In certain embodiments, the amount of recombinant lentivirus administered to the NHP is sufficient to transduce a population of immune effector cells sufficient to reduce the number of B cells in the NHP. In particular
5 embodiments, the number of B cells is reduced in the absence of toxicity, *e.g.*, cytokine release syndrome (CRS), or neurotoxicity. In preferred embodiments, the recombinant lentivirus and lentiviral vector are derived from HIV-1 and are administered to a pig-tail macaque.

 In particular embodiments, a method to reduce the number of B cells in an NHP
10 comprises administering a recombinant HIV-1 derived lentivirus comprising (i) a viral envelope that comprises one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity; a non-viral membrane-bound tropism polypeptide comprising an extracellular antigen targeting domain that binds an antigen expressed on an immune effector cell, a spacer domain, and a transmembrane
15 domain; optionally a secondary non-viral membrane bound tropism polypeptide that binds an antigen expressed on an immune effector cell; and (ii) a recombinant lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor that binds an antigen expressed on the B cell. In certain embodiments, the amount of recombinant lentivirus administered to the NHP is
20 sufficient to transduce a population of immune effector cells, *e.g.*, T cells, sufficient to reduce the number of B cells in the NHP. In particular embodiments, the number of B cells is reduced in the absence of toxicity, *e.g.*, cytokine release syndrome (CRS), or neurotoxicity. In preferred embodiments, the recombinant lentivirus and lentiviral vector are derived from HIV-1 and are administered to a pig-tail macaque.

25 In particular embodiments, a method to reduce the number of B cells in an NHP, *e.g.*, *Macaca nemestrina*, comprises administering a recombinant HIV-1 derived lentivirus comprising (i) a viral envelope that comprises one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity; a non-viral membrane-bound tropism polypeptide comprising an extracellular antigen
30 targeting domain comprising an antibody or antigen binding fragment thereof that binds an antigen expressed on a T cell, *e.g.*, CD3, a spacer domain, and a transmembrane domain; a secondary non-viral membrane bound tropism polypeptide that binds an antigen expressed on a T cell cell, *e.g.*, CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof; and

(ii) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding a chimeric antigen receptor that binds an antigen expressed on the B cell, *e.g.*, CD19, CD20, CD22, CD38, BCMA, GPCR5D. In certain embodiments, the amount of recombinant lentivirus administered to the NHP is sufficient to transduce a population of T cells, sufficient to reduce the number of B cells in the NHP. In particular embodiments, the number of B cells is reduced in the absence of toxicity, *e.g.*, cytokine release syndrome (CRS), or neurotoxicity.

In particular embodiments, a composition comprising a recombinant lentivirus contemplated herein is parenterally administered to an NHP. Illustrative examples of parenteral methods of administration include but are not limited to intravascular (intravenous or intraarterial), intraosseous, intraperitoneal, intraventricular, intracerebral, intracranial, intraspinal, intrathecal, intramuscular, and intramedullary. In particular embodiments, a composition comprising a recombinant lentivirus contemplated herein is intravascularly administered to an NHP. In particular embodiments, a composition comprising a recombinant lentivirus contemplated herein is intraarterially administered to an NHP. In preferred embodiments, a composition comprising a recombinant lentivirus contemplated herein is intravenously administered to an NHP.

In particular embodiments a composition comprising a recombinant lentivirus contemplated herein is parenterally administered to an NHP at an amount of about 1×10^6 TU/mL to about 1×10^{10} TU/mL, about 1×10^7 TU/mL to about 1×10^{10} TU/mL, about 1×10^8 TU/mL to about 1×10^{10} TU/mL, about 1×10^6 TU/mL to about 1×10^9 TU/mL, about 1×10^6 TU/mL to about 1×10^8 TU/mL, or about 1×10^7 TU/mL to about 1×10^9 TU/mL. In particular embodiments a composition comprising a recombinant lentivirus contemplated herein is parenterally administered to an NHP at an amount of about 1×10^6 TU/mL, about 5×10^6 TU/mL, about 1×10^7 TU/mL, 5×10^7 TU/mL, 1×10^8 TU/mL, 5×10^8 TU/mL, about 1×10^9 TU/mL, about 5×10^9 TU/mL, or 1×10^{10} TU/mL about 1×10^7 TU/mL, 5×10^7 TU/mL, 1×10^8 TU/mL, 5×10^8 TU/mL, about 1×10^9 TU/mL, about 5×10^9 TU/mL, or about 1×10^{10} TU/mL.

In particular embodiments a composition comprising a recombinant lentivirus contemplated herein is parenterally administered to an NHP at a rate of dose of about 5 mL/kg to about 15 mL/kg, about 6 mL/kg to about 14 mL/kg, about 7 mL/kg to about 13 mL/kg, about 8 mL/kg to about 12 mL/kg, or about 9 mL/kg to about 11 mL/kg. In particular embodiments a composition comprising a recombinant lentivirus contemplated

herein is parenterally administered to an NHP at a rate of dose of about 5 mL/kg, about 6 mL/kg, about 7 mL/kg, about 8 mL/kg, about 9 mL/kg, about 10 mL/kg, about 11 mL/kg, about 12 mL/kg, about 13 mL/kg, about 14 mL/kg, or about 15 mL/kg.

In particular embodiments, after a recombinant lentivirus contemplated herein is
5 administered to an NHP, one or more specimens are taken from the NHP and analyzed. Specimens include but are not limited to saliva, blood, plasma, blood cells, lymph, lymph nodes, cerebral spinal fluid, urine, stool, bone, bone marrow, brain, liver, spleen, thymus, stomach, colon, kidneys, heart, lungs, muscle, and other organ tissues. A specimen may be analyzed by any method known in the art including but not limited to PCR, qPCR, RT-
10 qPCR, northern blot, southern blot, immunohistochemistry (IHC), flow cytometry, and the like. A specimen may be analyzed to measure the amounts and types of cells present including but not limited to the amount of target cells and the amount of genetically modified cells, the amount of cytokines produced, the vector copy number and expansion of the genetically modified cells.

15 Specimens may also be used to assess the specificity of the gene therapy by measuring the biodistribution of the recombinant lentivirus in off-target and on-target cells and tissues. In particular embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 more specimens are taken from the NHP. In particular embodiments, one or more specimens is taken every day, every other day, every three days, every four days, every five
20 days, every six days, every 7 days, every eight days, every nine days, or every ten or more days for a period of about 10 days, about 15 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, about 30 days, about 31 days, about 32 days, about 33 days, about 34 days, about 35 days, about 36 days, about 37 days, about 38 days, about 39 days, about 40
25 days, about 41 days, about 42 days, about 43 days, about 44 days, about 45 days, about 46 days, about 47 days, about 48 days, about 49 days, about 50 days, about 51 days, about 52 days, about 53 days, about 54 days, about 55 days, about 56 days, about 57 days, about 58 days, about 59 days, or about 60 days.

In particular embodiments, the number of B cells or the extent of B cell aplasia in
30 an NHP administered a recombinant lentivirus contemplated herein is measured in one or more specimens taken from the NHP.

In particular embodiments, the number and/or phenotype of T cells in an NHP administered a recombinant lentivirus contemplated herein is measured in one or more specimens taken from the NHP.

5 In particular embodiments, the number of CAR⁺ T cells in an NHP administered a recombinant lentivirus contemplated herein is measured in one or more specimens taken from the NHP.

In particular embodiments, the VCN of CAR⁺ T cells (transduced T cells) in an NHP administered a recombinant lentivirus contemplated herein is measured in one or more specimens taken from the NHP.

10 In particular embodiments, one or more cytokines in an NHP administered a recombinant lentivirus contemplated herein is measured in one or more specimens taken from the NHP. Illustrative examples of cytokines measured in the one or more specimens include but are not limited to CXCL11, GM-CSF, IFN γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IP-10, MIP-1 α , and TNF α .

15 In particular embodiments, an NHP is administered a composition comprising a recombinant lentivirus contemplated herein and the number of B cells is decreased in the NHP compared to the number of B cells in the NHP before administration of the composition comprising the recombinant lentivirus. In particular embodiments, the decrease in the number of B cells in the NHP is measurable one, two, three, four, or five
20 days after the administration of the recombinant lentivirus to the NHP. In particular embodiments, the number of B cells in a blood specimen decreases by at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more compared to the number of B cells in a blood specimen before administration of the recombinant lentivirus to the NHP. In particular embodiments, the number of B cells in a blood specimen decreases by at least
25 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more within one, two, three, four, or five days after administration compared to the number of B cells in a blood specimen before administration of the recombinant lentivirus to the NHP.

In preferred embodiments, an NHP administered a recombinant lentivirus contemplated herein is not administered a lymphodepleting chemotherapy prior to, during,
30 or after administration of the recombinant lentivirus.

In particular embodiments, a composition comprises a recombinant lentivirus and a pharmaceutically acceptable carrier suitable for enteral or parenteral, *e.g.*, intravascular (intravenous or intraarterial), intraosseous, intraperitoneal, intraventricular, intracerebral,

intracranial, intraspinal, intrathecal, intramuscular, and intramedullary, administration and formulation.

D. RECOMBINANT LENTIVIRUSES

Recombinant lentiviruses have been used as a gene delivery platform for treatments
5 of severe genetic diseases and cancer. A “lentivirus” refers to a complex retrovirus. Among retroviruses, lentiviruses are the most efficient at transducing resting or growth-arrested cells.

Illustrative lentiviruses suitable for deriving or engineering recombinant lentiviruses contemplated in particular embodiments herein include, but are not limited to human
10 immunodeficiency virus (HIV) including HIV type 1 (HIV-1) and HIV type 2 (HIV-2); visna-maedi virus (VMV); caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

In preferred embodiments, a recombinant lentivirus is derived or engineered from
15 an HIV-1 lentivirus.

In particular embodiments, a recombinant lentivirus comprises an envelope engineered to target and fuse with an immune effector cell.

E. VIRAL ENVELOPE

Lentiviruses comprise a lentiviral vector and an outer surface, a lipid bilayer, cell
20 membrane, or viral envelope that mediates virus – cell attachment and fusion. The outer surface can be pseudotyped or engineered to express one or more non-viral membrane-bound tropism polypeptides that enable selective and/or specific binding of the virus to the desired cell type. The outer surface can also be engineered to express a viral polypeptide that mediates virus – cell fusion but that lacks the ability to bind its cognate receptor on the
25 cell surface of both target and non-target cells. Accordingly, the lentivirus comprises a viral envelope that specifically binds and fuses to a desired target cell but not a non-target cell.

In particular embodiments, a recombinant lentivirus comprises an outer surface, a lipid bilayer, cell membrane, or viral envelope comprising one or more mutated viral
30 envelope glycoproteins that mediate fusion of the viral particle with the target host cell but that do not bind its cognate receptor expressed on the target host cell or other non-target

cells, and one or more non-viral membrane-bound tropism polypeptides that bind an antigen expressed on the target host cell, *e.g.*, an immune effector cell.

In particular embodiments, one or more mutated viral envelope glycoproteins comprise a vesiculovirus envelope glycoprotein or one or more morbillivirus envelope glycoproteins or henipavirus envelope glycoproteins. In particular embodiments, a mutated viral envelope glycoprotein comprises a heterologous signal peptide that is eventually cleaved after translocation to the ER membrane.

Illustrative examples of vesiculoviruses suitable for use in particular embodiments from which G glycoproteins can be isolated include, but are not limited to vesicular stomatitis Alagoas virus (VSAV; Alagoas vesiculovirus), Carajás virus (CJSV; Carajas vesiculovirus), Chandipura virus (CHPV; Chandipura vesiculovirus), Cocal virus (COCV; Cocal vesiculovirus), vesicular stomatitis Indiana virus (VSIV, f.k.a. VSV; Indiana vesiculovirus), Isfahan virus (ISFV; Isfahan vesiculovirus), Maraba virus (MARAV; Maraba vesiculovirus), Morreton virus (MORV; Morreton vesiculovirus), vesicular stomatitis New Jersey virus (VSNJV; New Jersey vesiculovirus), and Piry virus (PIRYV; Piry vesiculovirus).

In particular embodiments, a vesiculovirus G envelope protein comprises one or more amino acid substitutions that enable the polypeptide to mediate fusion of the viral particle and the target host cell but that ablate the polypeptide's ability to bind its cognate receptor expressed on the target host cell, *e.g.*, LDL-R. In particular embodiments, a vesiculovirus G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 15-322 disclosed in U.S. Patent Application No. 20200216502, each said sequence incorporated by reference herein in its entirety, or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that enables the polypeptide to mediate fusion of the viral particle and the target host cell but that ablate the polypeptide's ability to bind its cognate receptor expressed on the target host cell, *e.g.*, LDL-R. In particular embodiments, a vesiculovirus G envelope protein comprises amino acid substitution at positions 47 and/or 354 (or 358 in some strains, *e.g.*, CJSV, VSNJV) with reference to the vesiculovirus G envelope protein lacking a signal peptide.

In particular embodiments, a vesiculovirus is vesicular stomatitis Indiana virus (VSIV). In particular embodiments, a mutated viral envelope glycoprotein is derived from a VSIV envelope glycoprotein (*e.g.*, SEQ ID NO: 1:
KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPKSHKAIQA

DGWMCHASKWVTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPG
 FPPQSCGYATVTDAEAVIVQVTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHN
 STTWHSDYKVKGLCDSNLISMDITFFSEDGELSSLGKEGTGFRS NYFAYETGGKA
 CKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQTSVDVSLI
 5 QDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETR
 YIRVDIAAPILSRMVGMISGTTTERELWDDWAPYEDVEIGPNGVLRTSSGYKFPLY
 MIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEG
 WFSSWKSSIASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK) or
 an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto comprising
 10 one or more modifications that enable the polypeptide to mediate fusion of the viral particle
 and a cell but that substantially ablate or ablate the polypeptide's ability to bind its cognate
 receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a mutated viral
 envelope glycoprotein is derived from a VSIV-G polypeptide set forth in SEQ ID NO: 1
 comprising L47I and/or H80Q amino acid substitutions, such substitutions being present in
 15 naturally occurring variants of VSIV.

In particular embodiments, a mutated VSIV-G envelope protein comprises one or
 more of: one or more amino acid substitutions at H8, N9, Q10, K47, K50, A51, S183,
 S179, N180, I182, M184, Y209, T214, I347, T350, T352, E353, and R354 (substitution
 with any amino acid; a conservation substitution; a disruptive substitution; substitution with
 20 D, E, A, G, F, or Q; or substitution with A, G, F, or Q); an insertion of TT between N9 and
 Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10,
 an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47,
 and an insertion of GGS between N208 and Y209; or a deletion of residues 1-8. In
 particular embodiments, a VSIV-G envelope protein comprises one or more amino acid
 25 substitutions at H8, K47, Y209, and R354. In particular embodiments, a VSIV-G envelope
 protein comprises one or more amino acid substitutions at H8, K47, Y209, and R354,
 wherein each substitution can be with A, G, F, or Q. Amino acid positions are with
 reference to a VSIV-G envelope protein that lacks a signal peptide, *e.g.*, SEQ ID NO: 1. In
 particular embodiments, a mutated viral envelope glycoprotein is derived from a VSIV-G
 30 polypeptide set forth in SEQ ID NO: 1 comprising L47I and/or H80Q amino acid
 substitutions, such substitutions present in naturally occurring variants of VSIV.

In particular embodiments, a mutated VSIV-G polypeptide comprises one or more
 amino acid substitutions at K47, I182, and/or R354 (substitution with any amino acid; a

conservation substitution; a disruptive substitution; substitution with D, E, A, G, F, or Q; or substitution with A, G, F, or Q). In particular embodiments, a mutated VSIV-G polypeptide comprises amino acid substitutions at K47, I182, or R354; K47 and I182; K47 and R354; I182 and R354; or at K47, I182, and R354 of SEQ ID NO: 1.

- 5 In particular embodiments, a mutated VSIV-G polypeptide comprises one or more of the following amino acid substitutions: K47A, K47Q, I182E, I182D, R354A, and/or R354Q. In particular embodiments, a mutated VSIV-G polypeptide comprises the following amino acid substitutions: K47A, K47Q, I182E, I182D, R354A, or R354Q; K47A and I182E; K47A and I182D; K47Q and I182E; K47Q and I182D; I182E and R354A; 10 I182E and R354Q; I182D and R354A; I182D and R354Q; K47A and R354A; K47A and R354Q; K47Q and R354A; K47Q and R354Q; K47A, I182E, and R354A; K47A, I182D, and R354A; K47Q, I182E, and R354A; K47Q, I182D, and R354A; K47A, I182E, and R354Q; K47A, I182D, and R354Q; K47Q, I182E, and R354Q; or K47Q, I182D, and R354Q of SEQ ID NO: 1.
- 15 In particular embodiments, a VSIV-G envelope protein comprises one or more amino acid substitutions at H8, K47, Y209, and R354. In particular embodiments, a VSIV-G envelope protein comprises one or more amino acid substitutions at H8, K47, Y209, and R354, wherein each substitution can be with A, G, F, or Q. In particular embodiments, a VSIV-G envelope protein comprises one or more amino acid substitutions at K47 and R354 20 (with reference to a VSIV-G envelope protein that lacks a signal peptide, *e.g.*, SEQ ID NO: 1). In particular embodiments, a VSIV-G envelope protein comprises one or more amino acid substitutions selected from the group consisting of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F and R354G; K47F and R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G; K47Q and R354F; and K47Q and R354Q. In preferred embodiments, a VSIV-G envelope comprises the amino acid substitutions K47Q or K47A and R354A or R354Q. Amino acid positions are with reference to a VSIV-G envelope protein that lacks a signal peptide.

- In particular embodiments, a VSIV-G envelope protein comprises an amino acid 30 sequence set forth in SEQ ID NO: 2 (wherein $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = A$, $X_3 = H$,

- and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; and $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = Q$) or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and the target host cell but that does not bind its cognate receptor expressed on the target host cell, *e.g.*, LDL-R.

Table 1

SEQ ID NO	Amino Acid Sequence
2	KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWNHNDLIGTAX ₁ QVKMPX ₂ SHKAIQADGWMCHASKW VTTCDFRWYGPKYITX ₃ SIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDG ELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSS ISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVLDLSYLAPKNPGTGPFTIINGTL

	KYFETRYIRVDIAAPILSRMVGMISSGTTTEX ₄ ELWDDWAPYEDVEIGPNGVLRRTSSGYKFPLYMI GHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES ₄ LFFGDTGLSKNPIELVEGW ₄ FSSWKSSIASF FFIIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEMNRLGK
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In particular embodiments, a mutated VSIV-G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 13-76 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a mutated VSIV-G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 61-76 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a mutated VSIV-G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 61, 65, 69, and 73 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R.

Table 2

SEQ ID NO:	AMINO ACID SEQUENCE
13	KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMISSGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES ₄ LFFGDTGLSKNPIELVEGW ₄ FSSWKSS IASFFFFIIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEMNRLGK
14	KFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMISSGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES ₄ LFFGDTGLSKNPIELVEGW ₄ FSSWKSS

	IASFFFIIGLIIGLFLVLRVGIHLCKIKLHHTKKRQIYTDIEMNRLGK
15	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHHTKKRQIYTDIEMNRLGK
16	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHHTKKRQIYTDIEMNRLGK
17	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHHTKKRQIYTDIEMNRLGK
18	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHHTKKRQIYTDIEMNRLGK
19	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN

	GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
20	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
21	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
22	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
23	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
24	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED

	GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLICIKLKHTKKRQIYTDIEMNRLGK
25	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLICIKLKHTKKRQIYTDIEMNRLGK
26	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLICIKLKHTKKRQIYTDIEMNRLGK
27	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLICIKLKHTKKRQIYTDIEMNRLGK
28	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPASHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLICIKLKHTKKRQIYTDIEMNRLGK
29	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW

	VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHL CIKHKHTKKRQIYTDIEMNRLGK
30	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHL CIKHKHTKKRQIYTDIEMNRLGK
31	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHL CIKHKHTKKRQIYTDIEMNRLGK
32	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHL CIKHKHTKKRQIYTDIEMNRLGK
33	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS

	IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
34	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
35	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
36	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
37	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
38	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN

	GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
39	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
40	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
41	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
42	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
43	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED

	GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
44	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPGSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
45	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
46	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
47	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
48	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPF SHKAIQADGWMCHASKW

	VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
49	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
50	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
51	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
52	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS

	IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
53	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
54	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
55	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
56	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
57	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPFSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN

	GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
58	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
59	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
60	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPF SHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
61	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
62	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHS DYKVKGLCD SNLISMDITFFSED

	GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
63	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
64	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
65	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
66	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTPVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
67	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW

	VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
68	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITQSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
69	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEAELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
70	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEGELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLHTKKRQIYTDIEMNRLGK
71	KFTIVFPHNQGNWKNVP SNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTP SVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTD AEAIVIQ VTPHHVLVDEYTG EWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEFELWDDWAPYEDVEIGPNGVLR TSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGW FSSWKSS

	IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
72	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEQELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
73	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEAELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
74	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEGELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
75	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRVDIAAPILSRMVGMIISGTTTEFELWDDWAPYEDVEIGPNGVLRRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESFFGDTGLSKNPIELVEGWFSWKSS IASFFFIIGLIIGLFLVLRVGIHLCKIKLKHTKKRQIYTDIEMNRLGK
76	KFTIVFPHNQGNWKNVPSNYHYCPSSSDLNWHNDLIGTALQVKMPQSHKAIQADGWMCHASKW VTTCDFRWYGPKYITHSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQ VTPHHVLVDEYTGWVDSQFINGKCSNYICPTVHNSTTWHSYKVKGLCDSNLISMDITFFSED GELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEG SSISAPSQTSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIIN

	GTLKYFETRYIRVDIAAPILSRMVGMI SGTTTEQELWDDWAPYEDVEIGPNGVLRTSSGYKFPL YMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNP IELVEGWFS SWKSS IASFFFFIIGLIIGLFLVLRVGIHLCKIKLKH TKKRQIYTDIEMNRLGK
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In particular embodiments, a vesiculovirus is coccal virus (COCV). In particular
embodiments, a mutated viral envelope glycoprotein is derived from a COCV envelope
glycoprotein (COCV-G; *e.g.*, SEQ ID NO: 3:

KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHNDLLGITMKVKMPKTHKAIQA
5 DGWMCHAAKWITTCDFRWYGP KYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGF
PPQNCGYATVTDSVAVVVQATPHHVLVDEYTG EWIDSQFPNGKCETEECETVHN
STVWYS DYKVTGLCDATLVDTEITFFSEDGKKESIGKPNTGYRSNYFAYEKGDKV
CKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPVGATISAPTQTSVDVSLI
LDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIINGTLKYFETR
10 YIRIDIDNPIISKMVGKISGSQTERELWTEWFPYEGVEIGPNGILKTPTGYKFPLFMI
GHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFS
SWKSTVVTTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK) or an
amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto comprising
one or more modifications that enable the polypeptide to mediate fusion of the viral particle
15 and a cell but that substantially ablate or ablate the polypeptide's ability to bind its cognate
receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a COCV-G envelope
protein comprises one or more amino acid substitutions at K47 and/or R354. In particular
embodiments, a COCV-G envelope protein comprises one or more amino acid substitutions
at K47 and/or R354, wherein each amino acid can be substituted with A, G, F, or Q. In
20 particular embodiments, a COCV-G envelope protein comprises one or more amino acid
substitutions at K47 and/or R354 (with reference to a COCV-G envelope protein that lacks
a signal peptide, *e.g.*, SEQ ID NO: 3). In particular embodiments, a COCV-G envelope
protein comprises one or more amino acid substitutions selected from the group consisting
of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and
25 R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F
and R354G; K47F and R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G;
K47Q and R354F; and K47Q and R354Q. In particular embodiments, a COCV-G
envelope protein comprises an amino acid sequence set forth in SEQ ID NO: 4 (wherein
X₁= A and X₂ = A; X₁= A and X₂ = G; X₁= A and X₂ = F; X₁= A and X₂ = Q; X₁= G and
30 X₂ = A; X₁= G and X₂ = G; X₁= G and X₂ = F; X₁= G and X₂ = Q; X₁= F and X₂ = A; X₁=

F and $X_2 = G$; $X_1 = F$ and $X_2 = F$; $X_1 = F$ and $X_2 = Q$; $X_1 = Q$ and $X_2 = A$; $X_1 = Q$ and $X_2 = G$; $X_1 = Q$ and $X_2 = F$; or $X_1 = A$ and $X_2 = Q$) or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor

5 expressed on a cell, *e.g.*, LDL-R.

Table 3

SEQ ID NO	Amino Acid Sequence
4	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPX ₁ THKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTD ₁ SVAVVQA TPHHVLVDEYTGWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTETFFSEDGK KESIGKPN ₁ TGYRSNYFAYEKGD ₁ KVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPVGATI SAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIINGTLK YFETRYIRIDIDNPIISKMVGKISGSQTEX ₂ ELWTEWFFPYEGVEIGPNGILKPTGYKFPLFMIG HGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSW ₁ KSTVVTFF FAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK

In particular embodiments, a mutated COCV-G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 77-92 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a mutated COCV-G envelope protein comprises an amino acid sequence set forth in any one of SEQ ID NOs: 89-92 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R. In particular embodiments, a mutated VSIV-G envelope protein comprises an amino acid sequence set forth in SEQ ID NOs: 89 or 92 or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto that mediates fusion of the viral particle and a cell but that substantially ablates or ablates the polypeptide's ability to bind its cognate receptor expressed on a cell, *e.g.*, LDL-R.

Table 4

SEQ ID NO:	AMINO ACID SEQUENCE
77	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPATHKAIQADGWMCHAAKW

	ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTG EWIDSQFPNGKCETE ECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEAELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSFRK
78	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPATHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTG EWIDSQFPNGKCETE ECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEGELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSFRK
79	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPATHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTG EWIDSQFPNGKCETE ECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEFELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSFRK
80	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPATHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTG EWIDSQFPNGKCETE ECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEQELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSFRK
81	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPGTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTG EWIDSQFPNGKCETE ECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEAELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST

	VVTTTTFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
82	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPGTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEGELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTTTTFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
83	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPGTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEFELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTTTTFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
84	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPGTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEQELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTTTTFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
85	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPFTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEAELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSSWKST VVTTTTFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
86	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPFTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDKVCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN

	GTLKYFETRYIRIDIDNPIISKMGKISGSQTEGELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
87	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPFTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEFELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
88	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPFTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEQELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
89	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPQTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEAELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
90	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPQTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECPCVG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMGKISGSQTEGELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTFFFAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
91	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHDLLGITMKVKMPQTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDSDVAVVVQ ATPHHVLVDEYTGEWIDSQFPNGKCETEECETVHNSTVWYSDYKVTGLCDATLVDTEITFFSED

	GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECVPG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEFELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTTTTAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK
92	KFSIVFPQSQKGNWKNVPSSYHYCPSSSDQNWHNDLLGITMKVKMPQTHKAIQADGWMCHAAKW ITTCDFRWYGPKYITHSIHSIQPTSEQCKESIKQTKQGTWMSPGFPPQNCGYATVTDVAVVVQ ATPHHVLVDEYTGWIDSQFPNGKCEETECETVHNSTVWYSYKVTGLCDATLVDTEITFFSED GKKESIGKPNTGYRSNYFAYEKGDVKCKMNYCKHAGVRLPSGVWFEFVDQDVYAAAKLPECVPG ATISAPTQTSVDVSLILDVERILDYSLCQETWSKIRSKQPVSPVDLSYLAPKNPGTGPAFTIIN GTLKYFETRYIRIDIDNPIISKMVGKISGSQTEQELWTEWFPYEGVEIGPNGILKTPTGYKFPL FMIGHGMLDSDLHKTSQAEVFEHPHLAEAPKQLPEEETLFFGDTGISKNPVELIEGWFSWKST VVTTTTAIGVFILLYVVARIVIAVRYRYQGSNNKRIYNDIEMSRFRK

In particular embodiments, one or more mutated morbillivirus envelope glycoproteins are derived from measles virus F (MV-F) and measles virus H (MV-H). In particular embodiments, a recombinant particle comprises one or more measles virus viral envelope glycoproteins modified to lack cell binding activity and retain fusogenic activity.

- 5 In some embodiments, a recombinant particle comprises a modified MV-F glycoprotein and an MV-H glycoprotein modified to lack cell binding activity and retain fusogenic activity.

In particular embodiments, one or more mutated morbillivirus envelope glycoproteins are derived from measles virus F (MV-F) polypeptide (*e.g.*, SEQ ID NO: 5:

- 10 QIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLPNITLLNNCTRVEIAEYRRL
 LRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITA
 GIALHQSMNLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPS
 MNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSIALGGDINKVLE
 KLGYSGGDLLGILESIRGIKARITHVDTESYFIVLSIAYPTLSEIKGVIVHRLEGVSYN
 15 IGSQEWYTTVPKYVATQGYLISNFEDESSCTFMPEGTVCSQNALYPMSPLLQECLR
 GSTKSCARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAAD
 HCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPISLERLDVGTNLGNIAIAKLEDA
 KELLESSDQILRSMKGLSSTSIVYILIAVCLGGLIGIPALICCCRGR) or an amino acid
 sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto and a measles virus H
 20 (MV-H) polypeptide (*e.g.*, SEQ ID NO: 6:
 MGSRIVINREHLMIDRPYVLLAVLFVFMFLSLIGLLAIAGIRLHRAAIYTAIEIHKSLST

NLDVTNSIEHQVKDVLTPLFKIIGDEVGLRTPQRFTDLVKFISDKIKFLNPDREYDF
 RDLTWCINPPERIKLDYDQYCADVAAEELMNALVNSTLLETTRTTNQFLAVSKGN
 CSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLSSK
 RSELSQLSMYRVFEVGVIRNPGLGAPVFHMTNYLEQPVSNDLSCNMVALGELKL
 5 AALCHGEDSITIPYQSGSGKGVSFQLVKLG VWKSPPTDMQSWVPLSTDDPVIDRLYL
 SSHRGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKIQALCENPEWAPLKD
 NRIPSYGVLSVDLSLTVELKIKIASGFGPLITHGSGMDLYKSNHNNVYWLTIPPMK
 NLALGVINTLEWIPRFKVSPYLFTVPIKEAGGDCHAPTYPAEVDGDVKLSSNLVI
 LPGQDLQYVLATYDTSRVEHAVVYYYVYSPSRFSYFYPFRLPIKGVPIELQVECFT
 10 WDQKLWCRHFCVLADSESGGHITHSGMVGMGVSCTVTREDGTN) or an amino
 acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto, wherein the MV-H
 protein lacks cell binding activity and retains fusogenic activity. In particular
 embodiments, the MV-H polypeptide comprises one or more amino acid substitutions at
 positions Y463, R515, S530, and F531 of a MV-H polypeptide (*e.g.*, SEQ ID NO 6). In
 15 particular embodiments, the MV-H polypeptide comprises one or more of the amino acid
 substitutions Y463A, R515A, S530L, and F531S in an MV-H polypeptide (*e.g.*, SEQ ID
 NO: 7:

MGSRIVINREHLMIDRPYVLLAVLFVMFLSLIGLLAIAGIRLHRAAIYTAIEHKSLSLST
 NLDVTNSIEHQVKDVLTPLFKIIGDEVGLRTPQRFTDLVKFISDKIKFLNPDREYDF
 20 RDLTWCINPPERIKLDYDQYCADVAAEELMNALVNSTLLETTRTTNQFLAVSKGN
 CSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLSSK
 RSELSQLSMYRVFEVGVIRNPGLGAPVFHMTNYLEQPVSNDLSCNMVALGELKL
 AALCHGEDSITIPYQSGSGKGVSFQLVKLG VWKSPPTDMQSWVPLSTDDPVIDRLYL
 SSHRGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKIQALCENPEWAPLKD
 25 NRIPSYGVLSVDLSLTVELKIKIASGFGPLITHGSGMDLYKSNHNNVYWLTIPPMK
 NLALGVINTLEWIPRFKVSPALFNVPIKEAGGDCHAPTYPAEVDGDVKLSSNLVI
 LPGQDLQYVLATYDTSAVEHAVVYYYVYSPSRSSYFYPFRLPIKGVPIELQVECFT
 WDQKLWCRHFCVLADSESGGHITHSGMVGMGVSCTVTREDGTN) or an amino
 acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto.

30 In particular embodiments, one or more mutated henipaviruses envelope
 glycoproteins are derived from nipah virus F (NiV-F) and nipah virus G (NiV-G). In
 particular embodiments, a recombinant particle comprises one or more nipah virus viral
 envelope glycoproteins modified to lack cell binding activity and retain fusogenic activity.

In some embodiments, a recombinant particle comprises a modified NiV-F glycoprotein and a NiV-G glycoprotein modified to lack cell binding activity and retain fusogenic activity.

- In particular embodiments, one or more mutated henipavirus envelope glycoproteins are derived from a nipah virus F (NiV-F) polypeptide (*e.g.*, SEQ ID NO: 8: LHYEKLKSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNSMSQCTGSVMENYKTR LNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEA MKNADNINKLKSSIESTNEAVVKLQETAECTVYVLTALQDYINTNLVPTIDKISCK QTELSLDLALSKYLSDLLFVFGPNLQDPVNSMTIQAISQAFGGNYETLLRTLGYA
- 5 TEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQELLPVSFNNDNSEW ISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPREL VVSSHVPRFALSNGVLFANCISVTCQCQTTGRAISQSGETLLMIDNTTCPTAVLG NVIISLGKYLGSVNYNSEGIAIGPPVFTDKVDISSQISSMNQSLQQSKDYIKEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFISFIIVEKKRNT or an amino acid
- 10 sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto and a nipah virus G (NiV-G) polypeptide (*e.g.*, SEQ ID NO: 9: MKKINEGLLDKILSAFNTVIALLGSIIVMNIMIIQNYTRSTDNQAIVIKDALQGIQ QQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQSTASINENVNEKCKFTL PPLKIHENISCPNPLPFREYRPQTEGVSNLVGLPNNICLQKTSNQILKPKLISYTLP
- 20 VVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVLDRGDEVPS LFMTNVWTPPNPNTVYHCSAVYNNEFYVLCVSTVGDPILNSTYWSGSLMMT RLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDTLYFPAVGFLV RTEFKYNDNSNCPITKCQYSKPENCRLSMGIRPNSHYILRSGLLKYNLSDGENPKVV FIEISDQRLSIGSPSKIYDSLQGPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNT
- 25 VISRPGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTV FKDNEILYRAQLASEDTNAQKTITNCFLKNKIWCISLVEIYDTGDNVIRPKLFAV KIPEQCT) or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical thereto, wherein the NiV-G polypeptide lacks cell binding activity and retains fusogenic activity. In particular embodiments, the NiV-G polypeptide comprises one or more amino
- 30 acid substitutions at positions E468, W471, Q497, and E500 of an NiV-G polypeptide (*e.g.*, SEQ ID NO: 9). In particular embodiments, the NiV-G polypeptide comprises one or more of the amino acid substitutions E468A, W471A, Q497A, and E500A in an NiV-G polypeptide (*e.g.*, SEQ ID NO: 10:

MKKINEGLLLDSKILSAFNTVIALLGSIIVMNIMIIQNYTRSTDNQAVIKDALQGIQ
 QQIKGLADKIGTEIGPKVSLIDTSSTITIPANIGLLGSKISQSTASINENVNEKCKFTL
 PPLKIHECNISCPNPLPFREYRPQTEGVSNLVGLPNNICLQKTSNQILKPKLISYTLF
 VVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGVSKQRIIGVGEVLDRGDEVPS
 5 LFMTNVWTPPNPNTVYHCSAVYNNEFYVLCVSTVGDPIINSTYWSGSLMMT
 RLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDLYFPAVGFLV
 RTEFKYNDSPITKCKQYKPCNRLSMGIRPNHYILRSGLLKYNLSDGENPKVV
 FIEISDQRLSIGSPSKIYDSLQGPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNT
 VISRPGQSQCPRFNTCPAICAEGVYNDAFLIDRINWISAGVFLDSNATAANPVFTV
 10 FKDNEILYRAQLASEDTNAQKTITNCFLKNKIWCISLVEIYDTGDNVIRPKLFAV
 KIQEQCT) or an amino acid sequence at least 95%, 96%, 97%, 98%, or 99% identical
 thereto, wherein the NiV-G polypeptide lacks cell binding activity and retains fusogenic
 activity.

In particular embodiments, a recombinant lentivirus contemplated herein comprises
 15 an outer surface, a lipid bilayer, cell membrane, or viral envelope comprising or expressing
 one or more non-viral membrane bound tropism polypeptides that are engineered to
 specifically bind a target host cell. In particular embodiments, a recombinant lentivirus
 contemplated herein comprises a viral envelope comprising or expressing a primary non-
 viral membrane bound tropism polypeptide and a secondary non-viral membrane bound
 20 tropism polypeptide that are both engineered to specifically bind a target host cell.

In particular embodiments, a non-viral membrane bound tropism polypeptide
 comprises an extracellular antigen targeting domain, a spacer domain, and a transmembrane
 domain. In particular embodiments, a non-viral membrane bound tropism polypeptide
 comprises from N-terminus to C-terminus, an extracellular antigen targeting domain, a
 25 spacer domain, and a transmembrane domain. In particular embodiments, a non-viral
 membrane bound tropism polypeptide comprises an extracellular antigen targeting domain,
 a spacer domain, a transmembrane domain, and a cytoplasmic tail (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8,
 9, or 10 amino acids or more).

In particular embodiments, a non-viral membrane-bound tropism polypeptide
 30 comprises an extracellular antigen targeting domain that binds an antigen expressed on a
 target host cell. In particular embodiments, a non-viral membrane-bound tropism
 polypeptide comprises an extracellular antigen targeting domain that binds an antigen
 expressed on an immune effector cell. In particular embodiments, a non-viral membrane-

bound tropism polypeptide comprises an extracellular antigen targeting domain that comprises an antibody or antigen binding fragment thereof that binds an antigen expressed on the surface of an immune effector cell.

In particular embodiments, a non-viral membrane-bound tropism polypeptide
 5 comprises an extracellular antigen targeting domain that comprises an antibody or antigen binding fragment selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab₂), a trispecific Fab trimer (Fab₃), an Fv, a single chain Fv protein (scFv), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), a single-
 10 domain antibody (sdAb, a camelid VHH, Nanobody), and a centyrin.

In particular embodiments, a non-viral membrane-bound tropism polypeptide comprises an extracellular antigen targeting domain that comprises an scFv or one or more VHHs that bind an antigen expressed on an immune effector cell.

In particular embodiments, a non-viral membrane-bound tropism polypeptide
 15 comprises an extracellular antigen targeting domain that binds the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3δ, CD3ε CD3γ, CD4, CD8α, and CD8β. In particular embodiments, the extracellular antigen targeting domain comprises an scFv, a murine scFv, a humanized scFv, or a human scFv or one or more VHHs that binds the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3δ, CD3ε CD3γ, CD4,
 20 CD8α, and CD8β.

In particular embodiments, a non-viral membrane-bound tropism polypeptide comprises an anti-CD3ε antibody or antigen binding fragment thereof. Illustrative examples of anti-CD3ε antibodies or antigen binding fragments thereof suitable for using in particular embodiments include scFvs or other antigen binding fragments isolated from
 25 clone 10D12 (Miltenyi), clone REA994 (Miltenyi), CD3-1 (Mabtech), and SP34-2 (BD Biosciences), and variants thereof that bind NHP CD3ε having at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto.

In particular embodiments, a non-viral membrane-bound tropism polypeptide comprises an anti-CD3ε scFv, a spacer domain, a transmembrane domain, and a
 30 cytoplasmic tail (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more).

In particular embodiments, a non-viral membrane-bound tropism polypeptide comprises an extracellular antigen targeting domain; spacer domain comprising a hinge domain or stalk obtained or isolated from a polypeptide selected from the group consisting

of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and/or a transmembrane domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and optionally, a truncated intracellular domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A or a cytoplasmic tail of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

In particular embodiments, a non-viral membrane-bound tropism polypeptide comprises an extracellular antigen targeting domain that binds the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3 δ , CD3 ϵ , CD3 γ , CD4, CD8 α , and CD8 β ; spacer domain comprising a hinge domain or stalk obtained or isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and/or a transmembrane domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and optionally, a truncated intracellular domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A or a cytoplasmic tail of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

In particular embodiments, a recombinant lentivirus comprises an envelope that comprises (a) one or more mutated viral envelope glycoproteins that mediate fusion of the viral particle with the target host cell but that do not bind its cognate receptor expressed on the target host cell or other non-target cells; (b) a non-viral membrane-bound tropism polypeptide comprises an extracellular antigen targeting domain that binds the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3 δ , CD3 ϵ , CD3 γ , CD4, CD8 α , and CD8 β ; spacer domain comprising a hinge domain or stalk obtained or isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and/or a transmembrane domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and optionally, a truncated intracellular domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A or a cytoplasmic tail of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more; and (c) a secondary non-viral membrane bound tropism polypeptide.

In particular embodiments, a secondary non-viral membrane bound tropism polypeptide comprises an extracellular antigen targeting domain that binds a T cell surface antigen including but not limited to the alpha or beta chains of a TCR, CD28, CD134 (OX40), CD137 (4-1BB), and CD278 (ICOS).

In particular embodiments, a secondary non-viral membrane bound tropism polypeptide comprises an extracellular antigen targeting domain comprising an antibody or antigen binding fragment thereof that specifically binds a T cell surface antigen including but not limited to the alpha or beta chains of a TCR, CD28, CD134 (OX40), CD137 (4-
5 1BB), and CD278 (ICOS).

In particular embodiments, a secondary non-viral membrane bound tropism polypeptide comprises all or part of a co-stimulatory molecule including but not limited to CD80, CD86, OX40L, 4-1BBL, and ICOSL or a functional fragment thereof. In particular
10 embodiments, the functional fragment is a CD80, CD86, OX40L, 4-1BBL, and ICOSL comprising a C-terminal truncation that results in a cytoplasmic domain of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

In particular embodiments, a recombinant lentivirus comprises an envelope that comprises (a) one or more mutated viral envelope glycoproteins that mediate fusion of the viral particle with the target host cell but that do not bind its cognate receptor expressed on
15 the target host cell or other non-target cells; (b) a non-viral membrane-bound tropism polypeptide comprises an extracellular antigen targeting domain that binds the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3δ, CD3ε CD3γ, CD4, CD8α, and CD8β, a spacer domain, and a transmembrane domain; and further comprises a secondary non-viral membrane bound tropism polypeptide comprising all or part of a co-stimulatory
20 molecule including but not limited to CD80, CD86, OX40L, 4-1BBL, and ICOSL or a functional fragment thereof, *e.g.*, a CD80, CD86, OX40L, 4-1BBL, and ICOSL, comprising a C-terminal truncation that results in a cytoplasmic domain of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

F. LENTIVIRAL VECTORS

25 Lentiviral vectors are useful tools to deliver genetic material to cells. A “lentiviral vector” is a nucleic acid molecule derived from a lentiviral genome that is used to transfer or deliver another nucleic acid from a recombinant lentivirus into a cell and/or into the cell’s genome. A recombinant lentiviral vector is based on or derived from a lentivirus genome that has been engineered to remove lentiviral accessory proteins but leave elements
30 intact for packaging, reverse transcription and integration. Recombinant lentiviruses contemplated herein comprise genomes that lack one or more lentiviral accessory genes, *e.g.*, the genes *env*, *vif*, *vpr*, *vpu* and *nef*, thereby increasing the safety of the lentiviruses.

In particular embodiments, a recombinant lentivirus contains two copies of a lentiviral vector, a genomic RNA comprising backbone sequences derived from a lentivirus genome. It is understood that many different sources of lentiviral vector sequences can be used, or combined and numerous substitutions and alterations in certain of the lentiviral

5 vector sequences may be accommodated without impairing the ability of a vector to package polynucleotides into a recombinant lentivirus or lentiviral particle and to transfer the polynucleotide into a host cell. Illustrative examples of lentiviral vectors suitable for use in particular embodiments contemplated herein include but are not limited to those described in Naldini *et al.*, (1996a, 1996b, and 1998); Zufferey *et al.*, (1997); Dull *et al.*,

10 1998, U.S. Pat. Nos. 6,013,516; and U.S. Pat. No. 5,994,136, each of which are incorporated herein by reference in their entireties.

In various embodiments, a lentiviral vector contemplated herein comprises one or more LTRs, and one or more, or all, of the following accessory elements: a Psi (Ψ) packaging signal, a cPPT/FLAP, an export element, poly (A) sequences, and may

15 optionally comprise a WPRE or HPRE, an insulator element, a selectable marker, and/or a cell suicide gene.

In particular embodiments, a lentiviral vector integrates into the host cell genome. In certain embodiments, a lentiviral vector is integration defective, episomal, and does not integrate in the host cell genome. As used herein, the term “integration defective

20 lentivirus” or “IDLV” refers to a lentivirus having an integrase that lacks the capacity to integrate the viral vector into the host cell genome. Illustrative mutations in HIV-1 integrase suitable to reduce integrase activity include, but are not limited to: H12N, H12C, H16C, H16V, S81R, D41A, K42A, H51A, Q53C, D55V, D64E, D64V, E69A, K71A, E85A, E87A, D116N, D116I, D116A, N120G, N120I, N120E, E152G, E152A, K156E,

25 K156A, E157A, K159E, K159A, K160A, R166A, D167A, E170A, H171A, K173A, K186Q, K186T, K188T, E198A, R199C, R199T, R199A, D202A, K211A, Q214L, Q216L, Q221L, W235F, W235E, K236S, K236A, K246A, G247W, D253A, R262A, R263A and K264H. In particular embodiments, an HIV-1 integrase deficient integrase comprises a D64V, D161I, D116A, E152G, or E152A mutation; D64V, D116A, and

30 E152G mutations; D64V, D116A, and E152A mutations; or a D64V mutation.

A “long terminal repeat” or “LTR” in its natural unmodified sequence context is a direct repeat and contains U3, R and U5 regions domains located at the ends of the lentiviral genome. LTRs generally provide functions fundamental to lentiviral gene

expression and replication. The U3 region contains the enhancer and promoter elements to initiate transcription of the viral genome; the R region contains a trans-activation responsive (TAR) element that mediates activation of transcription through its binding to the Tat viral protein and a polyadenylation sequence; and the U5 region and adjacent primer binding site (PBS) play important roles in initiating reverse transcription.

A “FLAP element” or “cPPT/FLAP” refers to a nucleic acid whose sequence includes the central polypurine tract and central termination sequences (cPPT and CTS) of a lentivirus, *e.g.*, HIV-1 or HIV-2. Suitable FLAP elements are described in U.S. Pat No. 6,682,907 and in Zennou, *et al.*, 2000, *Cell*, 101:173.

A “packaging signal” or “packaging sequence” refers to Psi [Ψ] sequences located within the lentiviral genome which are required for insertion of the lentiviral genomic RNA into the viral capsid or particle, see *e.g.*, Clever *et al.*, 1995. *J. of Virology*, Vol. 69, No. 4; pp. 2101-2109.

An “export element” refers to a cis-acting post-transcriptional regulatory element which regulates the transport of an RNA transcript from the nucleus to the cytoplasm of a cell. Examples of RNA export elements include, but are not limited to, the human immunodeficiency virus (HIV-1) rev response element (RRE) (see *e.g.*, Cullen *et al.*, 1991. *J. Virol.* 65: 1053; and Cullen *et al.*, 1991. *Cell* 58: 423).

Expression of heterologous sequences in viral vectors may be increased by incorporating posttranscriptional regulatory elements, efficient polyadenylation sites, and optionally, transcription termination signals into the vectors. A variety of posttranscriptional regulatory elements can increase expression of a heterologous nucleic acid, *e.g.*, woodchuck hepatitis virus posttranscriptional regulatory element (WPRE; Zufferey *et al.*, *J. Virol.* 73: 2886, 1999); the posttranscriptional regulatory element present in hepatitis B virus (HPRE) (Huang *et al.*, *Mol. Cell. Biol.* 13:7476-7486, 1993); and the like (Liu *et al.*, *Genes Dev.* 9:1766, 1995).

Lentiviral vectors preferably contain several safety enhancements as a result of modifying the LTRs. “Self-inactivating” (SIN) vectors refer to lentiviral vectors in which a deletion in the U3 region of the 3' LTR to prevent viral transcription beyond the first round of viral replication. HIV-1 based lentivectors can tolerate significant U3 deletions, including the removal of the LTR TATA box (*e.g.*, deletions from -418 to -18), without significant reductions in vector titers.

An additional safety enhancement is provided by replacing the U3 region of the 5' LTR with a heterologous promoter to drive transcription of the viral genome during production of viral particles. Examples of heterologous promoters which can be used include, for example, viral simian virus 40 (SV40) (*e.g.*, early or late), cytomegalovirus (CMV) (*e.g.*, immediate early), Moloney murine leukemia virus (MoMLV), Rous sarcoma virus (RSV), and herpes simplex virus (HSV) (thymidine kinase) promoters.

In preferred embodiments, an HIV-1 based lentiviral vector (*i.e.*, a lentiviral vector derived from HIV-1) comprises a polynucleotide comprising or encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor.

10 G. ENGINEERED ANTIGEN RECEPTORS

In various embodiments, a recombinant lentivirus contemplated herein comprises a lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding one or more engineered antigen receptors. Engineered antigen receptors include but are not limited to a chimeric antigen receptor (CAR), a chimeric costimulatory receptor (CCR), an alpha-beta T cell receptor ($\alpha\beta$ TCR), a gamma delta T cell receptor ($\gamma\delta$ TCR), a dimerizing agent regulated immunoreceptor complex (DARIC), a chimeric TGF- β receptor (CTBR), and a zetakine receptor.

In particular embodiments, the engineered antigen receptor is designed redirect an immune effector cell to bind or target an antigen on a target cell, *e.g.*, a B cell. In particular embodiments, the engineered antigen receptor comprises an extracellular antigen binding domain that binds to alpha folate receptor (FR α), $\alpha v\beta 6$ integrin, B cell maturation antigen (BCMA), B7-H3 (CD276), B7-H6, carbonic anhydrase IX (CAIX), CD16, CD 19, CD20, CD22, CD30, CD33, CD37, CD38, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD133, CD138, CD171, carcinoembryonic antigen (CEA), C- type lectin-like molecule-1 (CLL-1), CD2 subset 1 (CS-1), chondroitin sulfate proteoglycan 4 (CSPG4), cutaneous T cell lymphoma-associated antigen 1 (CTAGE1), epidermal growth factor receptor (EGFR), epidermal growth factor receptor variant IP (EGFRvIII), epithelial glycoprotein 2 (EGP2), epithelial glycoprotein 40 (EGP40), epithelial cell adhesion molecule (EPCAM), ephrin type-A receptor 2 (EPHA2), fibroblast activation protein (FAP), Fc Receptor Like 5 (FCRL5), fetal acetylcholinesterase receptor (AChR), ganglioside G2 (GD2), ganglioside G3 (GD3), Glypican-3 (GPC3), G Protein-Coupled Receptor Class C Group 5 Member D (GPCR5D), EGFR family including ErbB2 (HER2),

IL-10R α , IL-13R α 2, Kappa, cancer/testis antigen 2 (LAGE-1A), Lambda, Lewis-Y (LeY), LI cell adhesion molecule (LI- CAM), melanoma antigen gene (MAGE)-A1, MAGE- A3, MAGE-A4, MAGE-A6, MAGE A10, melanoma antigen recognized by T cells 1 (MelanA or MART1), Mesothelin (MSLN), MUC1, MUC16, MHC class I chain related proteins A
 5 (MICA), MHC class I chain related proteins B (MICB), neural cell adhesion molecule (NCAM), cancer/testis antigen 1 (NY-ESO-1), polysialic acid; placenta- specific 1 (PLAC1), preferentially expressed antigen in melanoma (PRAME), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), receptor tyrosine kinase-like orphan receptor 1 (ROR1), synovial sarcoma, X breakpoint 2 (SSX2), Survivin, tumor
 10 associated glycoprotein 72 (TAG72), tumor endothelial marker 1 (TEM1/CD248), tumor endothelial marker 7-related (TEM7R), trophoblast glycoprotein (TPBG), UL 16-binding protein (ULBP) 1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, vascular endothelial growth factor receptor 2 (VEGFR2), or Wilms tumor 1 (WT-1).

In particular embodiments, the engineered antigen receptor comprises an
 15 extracellular antigen binding domain that binds to a B cell antigen selected from the group consisting of CD1c, CD2, CD5, CD6, CD11a, CD11c, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD35, CD37, CD38, CD39, CD40, CD43, CD45RA, CD45RB, CD45RO, CD49c, CD49d, CD51, CD57, CD62L, CD69, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD83,
 20 CD84, CD85, CD86, CD89, CD98, CD95, CD99, CD100, CD106, CD107a, CDw108, CDw121b (IL-1RII), CDw124 (IL-4R), CDw127 (IL-7R), CD184, CD185, CD194, CD196, CD197, CD269, CD270 (TNFRSF14; HVEM), CD272 (BTLA-4), CD273 (PD-L2), CD274 (PD-L1), CD276 (TACI), CD278 (BAFF-R), CD279 (BCMA), CD300, CMRF35, CCR10, κ light chain, λ light chain, IL-10R, IL-11R, IL-13R α 1, LTK, Sca-1,
 25 SlamF6, and TNFRS25.

In certain embodiments, the engineered antigen receptor comprises an extracellular antigen binding domain that binds to BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D.

I. T CELL RECEPTORS

30 In particular embodiments, a lentiviral vector comprises a polynucleotide encoding a promoter operably linked to a polynucleotide encoding a TCR. Naturally occurring TCRs comprise two subunits, an alpha chain and a beta chain subunit ($\alpha\beta$ TCR), or a gamma chain

and a delta chain subunit ($\gamma\delta$ TCR), each of which is a unique protein produced by a recombination event in each T cell's genome. In one embodiment, the TCR is an $\alpha\beta$ TCR. In one embodiment, the TCR is a $\gamma\delta$ TCR.

In particular embodiments, polynucleotides encoding TCRs are preferably isolated
5 from their natural context in a (naturally-occurring) chromosome of a T cell and incorporated into a lentiviral vector contemplated herein. In particular embodiments, the TCR is an exogenous TCR because it is introduced into immune effector cells that do not normally express the particular TCR. The essential aspect of the TCRs is that it has high avidity for a tumor antigen presented by a major histocompatibility complex (MHC) or
10 similar immunological component. In contrast to TCRs, CARs are engineered to bind target antigens in an MHC independent manner.

A TCR can be expressed with additional polypeptides attached to the amino-terminal or carboxyl-terminal portion of the alpha chain or beta chain of a TCR, or of the gamma chain or delta chain of a TCR so long as the attached additional polypeptide does
15 not interfere with the ability of the chains to form a functional T cell receptor and recognize antigen.

Antigens that are recognized by the engineered TCRs contemplated in particular embodiments include, but are not limited to B cell antigens contemplated herein.

2. *CHIMERIC ANTIGEN RECEPTORS*

In particular embodiments, a lentiviral vector comprises a polynucleotide encoding
20 a promoter operably linked to a polynucleotide encoding a chimeric antigen receptor (CAR). CARs combine antibody-based specificity to a desired antigen with a T cell receptor-activating intracellular domain to generate a chimeric to redirect immune effector cell specificity in a major histocompatibility (MHC) independent manner. CARs exploit
25 cell-specific targeting abilities of monoclonal antibodies, soluble ligands or cell specific co-receptors and thereby triggering proliferation of immune effector cells, cytokine production, phagocytosis or production of molecules that can mediate cell death of the target antigen expressing cell.

In various embodiments, a CAR comprises an extracellular antigen binding domain
30 that binds to a specific target antigen, a hinge domain, a transmembrane domain and one or more intracellular signaling domains.

The present disclosure contemplates all permutations of CAR architectures, from first generation CARs to third generation CARs. A first-generation CAR comprises an extracellular antigen binding domain, a hinge domain, a transmembrane domain, and a primary signaling domain; a second-generation CAR comprises an extracellular antigen binding domain, a hinge domain, a transmembrane domain, a costimulatory domain, and a primary signaling domain; and a third-generation CAR comprises an extracellular antigen binding domain, a hinge domain, a transmembrane domain, two costimulatory signaling domains and a primary signaling domain.

An antigen binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule of interest. In particular embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof. In particular embodiments, the extracellular binding domain comprises an antibody or antigen binding fragment thereof is selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab₂), a trispecific Fab trimer (Fab₃), an Fv, an single chain Fv protein ("scFv"), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody). In one embodiment, the binding domain comprises an scFv. In one embodiment, the binding domain comprises one or more VHH antibodies.

In particular embodiments, the CAR comprises an extracellular domain that binds an antigen selected from the group consisting of: CD1c, CD2, CD5, CD6, CD11a, CD11c, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD35, CD37, CD38, CD39, CD40, CD43, CD45RA, CD45RB, CD45RO, CD49c, CD49d, CD51, CD57, CD62L, CD69, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD83, CD84, CD85, CD86, CD89, CD98, CD95, CD99, CD100, CD106, CD107a, CDw108, CDw121b (IL-1RII), CDw124 (IL-4R), CDw127 (IL-7R), CD184, CD185, CD194, CD196, CD197, CD269, CD270 (TNFRSF14; HVEM), CD272 (BTLA-4), CD273 (PD-L2), CD274 (PD-L1), CD276 (TACI), CD278 (BAFF-R), CD279 (BCMA), CD300, CMRF35, CCR10, κ light chain, λ light chain, IL-10R, IL-11R, IL-13R α 1, LTK, Sca-1, SlamF6, and TNFRSF25.

In certain embodiments, the CAR comprises an extracellular antigen binding domain that binds to BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D.

In a preferred embodiment, the CAR comprises a LEU16 scFv directed against CD20.

In particular embodiments, a CAR comprises a hinge domain. In particular embodiments, the hinge domain comprises the CH2 and CH3 domains of IgG1, IgG4, or IgD. Illustrative hinge domains suitable for use in the CARs described herein include the hinge region derived from the extracellular regions of type 1 membrane proteins such as CD28, CD8 α , and CD4, which may be wild-type hinge regions from these molecules or may be altered.

The transmembrane (TM) domain of the CAR fuses the extracellular binding portion and intracellular signaling domain and anchors the CAR to the plasma membrane of the immune effector cell. The TM domain may be derived either from a natural, synthetic, semi-synthetic, or recombinant source. Illustrative TM domains may be derived from (*i.e.*, comprise) at least the transmembrane region(s) of the alpha, beta, gamma, or delta chain of the T-cell receptor, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154, AMN, and PD-1. In particular embodiments, a CAR contemplated herein comprises a TM domain and a short oligo- or polypeptide linker, preferably between 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length that links the TM domain and the adjacent intracellular signaling domain of the CAR. A glycine-serine linker provides a particularly suitable linker.

In particular embodiments, a CAR comprises an intracellular signaling domain that comprises one or more “co-stimulatory signaling domains” and a “primary signaling domain.”

Primary signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Illustrative examples of ITAM containing primary signaling domains suitable for use in CARs contemplated in particular embodiments include those derived from FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d. In particular preferred embodiments, a CAR comprises a CD3 ζ primary signaling domain and one or more co-stimulatory signaling domains.

Illustrative examples of co-stimulatory domains suitable for use in CARs contemplated in particular embodiments include those isolated from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28,

CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, NKD2C, SLP76, TRIM, and ZAP70. In particular embodiments, a CAR comprises one or more co-stimulatory signaling domains selected from the group consisting of CD28, CD137, and CD134, and a CD3 ζ primary signaling domain.

- 5 In particular embodiments, a CAR comprises: an extracellular domain that binds to CD20; a CD8 α hinge and transmembrane domain; a CD137 costimulatory domain and a CD3 ζ primary signaling domain. In preferred embodiments, the CAR comprises a polynucleotide sequence set forth in SEQ ID NO: 11 and an amino acid sequence set forth in SEQ ID NO: 12. The skilled person would understand that a lentiviral vector comprising
- 10 a polynucleotide encoding s CAR comprises a polynucleotide sequence encoding a signal peptide but that when the CAR is processed and translocated to the ER membrane the signal peptide is cleaved such that the CAR is expressed on a transduced immune effector cell without the signal peptide.

SEQ ID NO	Sequence
11	GACTGCCACCACCATGGAGACAGATACCCTGCTCCTGTGGGTCCTCCTGTTGTGGGTGCCAGGAT CTACAGGAGAGGTGCAGCTGCAACAGTCCGGCGCTGAGTTGGTGAAGCCGGGCGCATCCGTGAAA ATGTCCTGTAAGGCATCTGGTTACACATTCACATCCTACAACATGCATTGGGTAAAGCAGACGCC AGGCCAAGGGCTGGAGTGGATCGGAGCCATCTATCCTGGCAATGGGGACACCTCCTATAATCAGA AGTTTAAGGGCAAGGCAACACTGACAGCCGACAAAAGCTCGTCCACCGCTTATATGCAGCTTTCC AGCCTGACCTCCGAGGATTCCGCAGACTACTATTGCGCCCGATCAAATTACTATGGAAGTTCCCTA CTGGTTTTTTCGATGTCTGGGGCGCCGGTACCACTGTACGGTGTCAAGCGGCTCCACAAGCGGGG GTGGCTCCGGTGGCGGATCCGGAGGCGGTGGGAGCTCCGACATCGTGTGACCCAGTCTCCTGCA ATCCTGTCCGCTTCTCCAGGTGAGAAAGTGACCATGACCTGTCTGTCTTCTTCGAGCGTCAATTA CATGGACTGGTACCAGAAAAAGCCCGGCGAGCTCTCCCAAGCCATGGATATATGCCACTAGCAACC TGGCCAGCGGCGTGCCCGCACGCTTTTCCGGGTCTGGCAGCGGCACCTCTTACTCTCTTACCATA TCGCGCGTGGAGGCTGAGGACGCGGCTACCTATTACTGTCAACAGTGGTCCTTTAACCCCTCCAAC CTTTGGAGGGGGCACCAAGCTCGAGATCAAAACACGACGCCAGCGCCGCGACCACCAACACCGG CGCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGC GCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGCCCGGAC TTGTGGGTCCTTCTCCTGTCACTGGTTATCACCCCTTTACTGCAAACGGGGCAGAAAGAACTCC TGTATATATTCAAACAACCATTTATGAGACCAGTACAACTACTCAAGAGGAAGATGGCTGTAGC TGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGA CGCCCCCGGTACAAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGG AGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGAAAGCCGAGAAGGAAG AACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGAT

	TGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAG CCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTGAAAGTTGTCTCCT CCTGCACTGACTGACTGATACAATCGATTCTGGCAGGATCCGCAGGCCCTCTGCTAGCTTGACTG ACTGAGTCGA
12	METDTLLLWVLLLWVPGSTGEVQLQQSGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGQGL EWIGAIYPNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSADYYCARSNYYGSSYWFDF VWGAGTTTVTVSSGSTSGGSGGGSGGGSSDIVLTQSPAILSASPGEKVTMTCRASSSVNYMDWY QKKPGSSPKPWYATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPTFGGG TKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVL LLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKG ERRRGKGHGGLYQGLSTATKDTYDALHMQALPPR

3. *CHIMERIC COSTIMULATORY RECEPTORS*

Chimeric costimulatory receptors (CCRs) are molecules that combine antibody-based specificity to a desired antigen with a T cell receptor-costimulatory domain but that lack a primary signaling domain. CCRs redirect immune effector cell specificity in an MHC independent manner and enhance the immune effector cell response in the presence of a CAR.

In particular embodiments, a lentiviral vector comprises a polynucleotide encoding a promoter operably linked to a polynucleotide encoding a CAR that comprises a first costimulatory domain and a primary signaling domain; a ribosomal skip or self-cleaving viral peptide; and a CCR comprises a second costimulatory domain, wherein the first and second costimulatory domains are different from each other and synergize to immune effector cell proliferation, persistence, cytokine production, and/or phagocytosis or production of molecules that can mediate cell death of the target antigen expressing cell.

In particular embodiments, a CCR that comprises an extracellular antigen binding domain comprising an antibody or antigen binding fragment thereof; a hinge domain; a transmembrane domain; and a costimulatory signaling domains.

In particular embodiments, a CCR that comprises an extracellular antigen binding domain comprising an antibody or antigen binding fragment thereof that binds a B cell antigen that is different from the B cell antigen bound by a CAR.

In particular embodiments, a CCR comprises an extracellular antigen binding domain that binds BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D; a hinge domain selected from the group consisting of a CD4 hinge, a CD8 β hinge, a CD8 α

hinge, a CD28 hinge, a CD134 hinge, a CD137 hinge, a CD152 hinge, an IgG1 hinge, an IgG2 hinge, an IgG3 hinge, an IgG4 hinge; a transmembrane domain isolated or derived from a polypeptide selected from the group consisting of an alpha, beta, gamma, or delta chain of the T-cell receptor, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, 5 CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1); and a costimulatory signaling domain isolated or derived from a polypeptide selected from the group consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD7, CD27, CD28, CD30, CD40, ICAM, CD83, CD94, CD134 10 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, SLP76, TRAT1, TNFR2, TNFRS14, TNFRS18, TNFRS25, and ZAP70.

4. DIMERIZATION ACTIVATED RECEPTOR INITIATION COMPLEXES

In particular embodiments, a lentiviral vector comprises a polynucleotide encoding a promoter operably linked to a polynucleotide encoding a DARIC. A DARIC provides a 15 binding component and a signaling component that are each expressed as separate fusion proteins comprising an extracellular multimerization domain. The multimerization domains of the components associate by binding to a bridging factor (see U.S. Pat. Appl. No. 2016/0311901, which is incorporated herein by reference in its entirety). Although the bridging factor leads to formation of the DARIC system components, formation of the 20 DARIC complex does not produce significant signaling on its own. The described DARIC complexes contemplated herein only initiate physiologically relevant signals when the DARIC binding component binds to a target antigen expressed or displayed on a target cell. In particular embodiments, a DARIC binding component comprises an antigen binding domain that binds to an antigen selected from the group consisting of: CD1c, CD2, CD5, 25 CD6, CD11a, CD11c, CD14, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD35, CD37, CD38, CD39, CD40, CD43, CD45RA, CD45RB, CD45RO, CD49c, CD49d, CD51, CD57, CD62L, CD69, CD70, CD72, CD73, CD74, CDw75, CDw76, CD77, CDw78, CD79a, CD79b, CD80, CD83, CD84, CD85, CD86, CD89, CD98, CD95, CD99, CD100, CD106, CD107a, CDw108, CDw121b (IL-1RII), CDw124 30 (IL-4R), CDw127 (IL-7R), CD184, CD185, CD194, CD196, CD197, CD269, CD270 (TNFRSF14; HVEM), CD272 (BTLA-4), CD273 (PD-L2), CD274 (PD-L1), CD276

(TACI), CD278 (BAFF-R), CD279 (BCMA), CD300, CMRF35, CCR10, κ light chain, λ light chain, IL-10R, IL-11R, IL-13R α 1, LTK, Sca-1, SlamF6, and TNFRS25.

In certain embodiments, the DARIC comprises an extracellular antigen binding domain that binds to BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D.

5 In a preferred embodiment, the DARIC binding component comprises a LEU16 scFv directed against CD20.

H. POLYPEPTIDES

Polypeptides, fusion polypeptides, and polypeptide variants are contemplated herein. Exemplary polypeptides include, but not limited to, mutated viral envelope
10 glycoproteins, non-viral membrane-bound tropism polypeptides, CAR polypeptides, CCR polypeptides, DARIC binding and signaling components, CTBRs, zetakines, fusion polypeptides and fragments thereof, *e.g.*, SEQ ID NOs: 1-10, and 12-126. “Polypeptide,” “peptide,” and “protein” are used interchangeably, unless specified to the contrary, and according to conventional meaning, *i.e.*, as a sequence of amino acids. Polypeptides are not
15 limited to a specific length, *e.g.*, they may comprise a full-length protein sequence, a fragment of a full-length protein, or a fusion protein, and may include post-translational modifications, *e.g.*, glycosylations, acetylations, phosphorylations and the like.

An “isolated peptide” or an “isolated polypeptide” is a synthetic polypeptide, a semi-synthetic polypeptide, or a polypeptide obtained or derived from a recombinant
20 source.

Polypeptides include “polypeptide variants.” Polypeptide variants may differ from a naturally occurring polypeptide in one or more amino acid substitutions, deletions, additions and/or insertions. Such variants may be naturally occurring or may be synthetically generated. In particular embodiments, polypeptides include polypeptide
25 variants having at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 86%, 97%, 98%, or 99% amino acid identity to any reference sequence contemplated herein, typically where the variant maintains at least one biological activity of the reference sequence. In particular embodiments, a polypeptide
30 variant is a viral envelope glycoprotein that has been modified to preserve its fusogenic activity and disable its binding activity to its cognate receptor expressed on a cell. In particular embodiments, a polypeptide variant is a viral envelope glycoprotein that has at

least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity to a reference sequence and that has been modified to preserve its fusogenic activity and disable its binding activity to
 5 its cognate receptor expressed on a cell.

Polypeptides variants include biologically active “polypeptide fragments.”

Illustrative examples of biologically active polypeptide fragments include but are not limited to signal peptides, binding domains, hinges, transmembrane domains, intracellular domains, and the like. As used herein, the term “biologically active fragment”
 10 or “minimal biologically active fragment” refers to a polypeptide fragment that retains at least 100%, at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, at least 10%, or at least 5% of the naturally occurring polypeptide activity. In particular embodiments, a biologically active fragment is a polypeptide comprising an N-terminal and/or C-terminal truncation of 1, 2, 3, 4, 5, 6, 7, 8,
 15 9, 10 or more amino acids. In particular embodiments, a mutated viral envelope glycoprotein or non-viral membrane-bound tropism polypeptide comprises a truncation of the cytoplasmic domain that results in a cytoplasmic tail or stub of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

Polypeptides contemplated in particular embodiments comprise a signal peptide.

Signal peptides are often present at the N-terminus of polypeptides and assist in the trafficking, processing and maturation of the expressed polypeptide. The signal peptide is usually cleaved in the ER and the mature polypeptide comprises an amino acid sequence lacking the signal peptide. Illustrative examples of signal peptides suitable for use in particular DARIC signaling components include but are not limited to an IgG1 heavy chain
 20 signal polypeptide, an IgK light chain signal polypeptide, a CD8 α signal polypeptide, or a human GM-CSF receptor alpha signal polypeptide. In various preferred embodiments, a polypeptide comprises GM-CSF α signal polypeptide.

In particular embodiments, polypeptides contemplated herein may comprise one or more amino acids denoted as “X” or “X_n” wherein n is an integer that denotes the particular
 30 X amino acid. “X” if present in an amino acid SEQ ID NO, refers to any one or more amino acids or particular amino acids if disclosed.

As noted above, polypeptides may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are

generally known in the art. For example, amino acid sequence variants of a reference polypeptide can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (Proc. Natl. Acad. Sci. USA. 82: 488-492. (1985)), Kunkel *et al.*, (Methods in Enzymol, 154: 367-382. (1987)), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.*, (Molecular Biology of the Gene, Fourth Edition, Benjamin/Cummings, Menlo Park, Calif. (1987)) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.*, Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C. (1978)).

10 In certain embodiments, a polypeptide variant comprises one or more conservative substitutions. A “conservative substitution” is one in which an amino acid is substituted for another amino acid that has similar properties. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, 15 glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids.

In one embodiment, where expression of two or more polypeptides is desired, the 20 polynucleotide sequences encoding them can be separated by an IRES sequence as disclosed elsewhere herein or a polypeptide cleavage signal.

In another embodiment, two or more polypeptides, can be expressed as a fusion protein that comprises one or more polypeptide cleavage signals between the polypeptides.

Exemplary polypeptide cleavage signals include, but are not limited to, protease 25 cleavage sites, nuclease cleavage sites and ribosomal skipping polypeptides or self-cleaving viral polypeptides (see, *e.g.*, in Ryan *et al.*, 1997. J. Gener. Virol. 78, 699-722; deFelipe and Ryan, 2004. Traffic, 5(8); 616-26; and Scymczak *et al.* (2004) Nature Biotech. 5, 589-594).

Exemplary protease cleavage sites include, but are not limited to the cleavage sites of potyvirus NIa proteases (*e.g.*, tobacco etch virus protease), poty virus HC proteases, 30 potyvirus PI (P35) proteases, byovirus NIa proteases, byovirus RNA-2-encoded proteases, aphthovirus L proteases, enterovirus 2A proteases, rhinovirus 2 A proteases, picoma 3C proteases, comovirus 24K proteases, nepovirus 24K proteases, RTSV (rice tungro spherical

virus) 3C-like protease, PYVF (parsnip yellow fleck virus) 3C-like protease, heparin, thrombin, factor Xa and enterokinase.

Illustrative examples of ribosomal skipping polypeptides include but are not limited to: a viral 2A peptide or sequence (Donnelly et al, 2001. J. Gen. Virol. 82: 1027- 1041). In a particular embodiment, the viral 2A peptide is an aphthovirus 2A peptide, a potyvirus 2A peptide, or a cardiovirus 2A peptide.

In one embodiment, the viral 2A peptide is selected from the group consisting of: a foot-and-mouth disease virus (FMDV) 2A peptide, an equine rhinitis A virus (ERAV) 2A peptide, a Theilovirus 2A peptide, and an encephalomyocarditis virus 2A peptide.

Illustrative examples of viral 2A sequences include, but are not limited to:
 GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 107); ATNFSLLKQAGDVEENPGP (SEQ ID NO: 108); LLKQAGDVEENPGP (SEQ ID NO: 109);
 GSGEGRGSLTTCGDVEENPGP (SEQ ID NO: 110); EGRGSLTTCGDVEENPGP (SEQ ID NO: 111); LLTTCGDVEENPGP (SEQ ID NO: 112);
 GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 113);
 QCTNYALLKLAGDVESNPGP (SEQ ID NO: 114); LLKLAGDVESNPGP (SEQ ID NO: 115); GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 116);
 VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 117); LLNFDLLKLAGDVESNPGP (SEQ ID NO: 118); TLNFDLLKLAGDVESNPGP (SEQ ID NO: 119);
 NFDLLKLAGDVESNPGP (SEQ ID NO: 120); QLLNFDLLKLAGDVESNPGP (SEQ ID NO: 121); APVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 122);
 VTELLYRMKRAETCYCPRLLAHPTEARHKQKIVAPVKQT (SEQ ID NO: 123);
 LNFDLLKLAGDVESNPGP (SEQ ID NO: 124);
 LLAIHPTEARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 125); and
 EARHKQKIVAPVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 126).

In particular embodiments, a lentiviral vector comprises or encodes a promoter operably linked to a polynucleotide encoding a fusion protein.

I. POLYNUCLEOTIDES

Polynucleotides encoding mutated viral envelope glycoproteins, non-viral membrane-bound tropism polypeptides, CAR polypeptides, CCR polypeptides, DARIC binding and signaling components, CTBRs, zetakines, fusion polypeptides and variants

thereof are contemplated herein. As used herein, the terms “polynucleotide” or “nucleic acid” refer to deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and DNA/RNA hybrids. Polynucleotides may be single-stranded or double-stranded and either recombinant, synthetic, or isolated. Polynucleotides include, but are not limited to: pre-messenger RNA (pre-mRNA), messenger RNA (mRNA), RNA, circular RNA (circRNA), synthetic RNA, genomic RNA (viral genomic RNA), genomic DNA (gDNA), PCR amplified DNA, complementary DNA (cDNA), synthetic DNA, or recombinant DNA. Polynucleotides refer to a polymeric form of nucleotides of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 1000, at least 5000, at least 10000, or at least 15000 or more nucleotides in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide, as well as all intermediate lengths. It will be readily understood that “intermediate lengths,” in this context, means any length between the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*, 151, 152, 153, *etc.*, 201, 202, 203, *etc.* In particular embodiments, polynucleotides or variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence.

As used herein, “isolated polynucleotide” refers to a polynucleotide that has been purified from the sequences which flank it in a naturally-occurring state. In particular embodiments, an isolated polynucleotide is a synthetic polynucleotide, a semi-synthetic polynucleotide, or a polynucleotide obtained or derived from a recombinant source, or other polynucleotide that does not exist in nature and that has been made by the hand of man.

In various embodiments, a polynucleotide comprises or encodes a lentiviral vector.

In various embodiments, a polynucleotide comprises a genomic RNA encoding a lentiviral vector.

Illustrative examples of polynucleotides include, but are not limited to, polynucleotides encoding polypeptides set forth in SEQ ID NOs: 1-10 and 12-126, and SEQ ID NO: 11.

In particular embodiments, polynucleotides may be codon-optimized. As used herein, the term “codon-optimized” refers to substituting codons in a polynucleotide encoding a polypeptide in order to increase the expression, stability and/or activity of the polypeptide. Factors that influence codon optimization include, but are not limited to one or

more of: (i) variation of codon biases between two or more organisms or genes or synthetically constructed bias tables, (ii) variation in the degree of codon bias within an organism, gene, or set of genes, (iii) systematic variation of codons including context, (iv) variation of codons according to their decoding tRNAs, (v) variation of codons according to GC %, either overall or in one position of the triplet, (vi) variation in degree of similarity to a reference sequence for example a naturally occurring sequence, (vii) variation in the codon frequency cutoff, (viii) structural properties of mRNAs transcribed from the DNA sequence, (ix) prior knowledge about the function of the DNA sequences upon which design of the codon substitution set is to be based, (x) systematic variation of codon sets for each amino acid, and/or (xi) isolated removal of spurious translation initiation sites.

The recitations “sequence identity” or, for example, comprising a “sequence 50% identical to,” as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Included are polynucleotides and polypeptides having at least about 50%, 55%, 60%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 86%, 97%, 98%, or 99% sequence identity to any of the reference sequences described herein.

A vector may comprise a polynucleotide comprising or encoding one or more exogenous, endogenous, or heterologous expression control sequences operably linked to a polynucleotide encoding one or more polypeptides contemplated herein. “Expression control sequences,” “control elements,” or “regulatory sequences” present in a vector are those non-translated regions of the vector including but not limited to an origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5′ and 3′ untranslated regions, all of which interact with host cellular proteins to carry out transcription and translation.

The term “promoter” as used herein refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. An RNA polymerase initiates and transcribes polynucleotides operably linked to the promoter. In particular embodiments, promoters operative in mammalian cells comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another

sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

The term “operably linked”, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one
 5 embodiment, the term refers to a functional linkage between an expression control sequence (such as a promoter, and/or enhancer) and a second polynucleotide sequence encoding a polypeptide, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

Illustrative expression control sequences suitable for use in particular embodiments
 10 include, but are not limited to, a cytomegalovirus (CMV) immediate early promoter, a viral simian virus 40 (SV40) (*e.g.*, early or late), a Moloney murine leukemia virus (MoMLV) LTR promoter, a Rous sarcoma virus (RSV) LTR, a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and Pl 1 promoters from vaccinia virus, an elongation factor 1 - alpha (EFl α) promoter, early growth response 1 (EGR1), ferritin H
 15 (FerH), ferritin L (FerL), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), eukaryotic translation initiation factor 4A1 (EIF4A1), heat shock 70kDa protein 5 (HSPA5), heat shock protein 90kDa beta, member 1 (HSP90B1), heat shock protein 70kDa (HSP70), P-kinesin (P-KIN), the human ROSA 26 locus (Irions *et al.*, Nature Biotechnology 25, 1477 - 1482 (2007)), a spleen focus forming virus (SFFV) promoter, a
 20 Ubiquitin C promoter (UBC), a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken P-actin (CAG) promoter, a P-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) U3 promoter (Haas *et al.* Journal of Virology. 2003;77(17): 9439-9450).

25 Elements directing the efficient termination and polyadenylation of heterologous nucleic acid transcripts increases heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In particular embodiments, vectors comprise a polyadenylation sequence 3' to a sequence to be transcribed and/or expressed. The terms “polyadenylation (or polyA) site,”
 30 “polyadenylation (or polyA) signal” or “polyadenylation (or polyA) sequence” as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation signals can promote mRNA stability by addition of a polyA tail to the 3' end of the coding sequence and thus,

contribute to increased translational efficiency. Cleavage and polyadenylation are directed by a poly(A) signal in the RNA. The core poly(A) signal for mammalian pre-mRNAs has two recognition elements flanking a cleavage-polyadenylation site. Typically, an almost invariant AAUAAA hexamer lies 20-50 nucleotides upstream of a more variable element
5 rich in U or GU residues. Cleavage of the nascent transcript occurs between these two elements and is coupled to the addition of up to 250 adenosines to the 5' cleavage product. In particular embodiments, the core poly(A) signal is an ideal polyA signal (e.g., AATAAA, ATTAAA, AGTAAA). In particular embodiments, the poly(A) sequence is an SV40 polyA sequence, a bovine growth hormone polyA sequence (BGHpA), a rabbit β -globin
10 polyA sequence (r β gpA), variants thereof, or another suitable heterologous or endogenous polyA sequence known in the art. In particular embodiments, the poly(A) sequence is synthetic.

J. CELLS

Recombinant lentiviruses contemplated herein are engineered to bind and transduce
15 a cell. In particular embodiments, a recombinant lentivirus contemplated herein is engineered to bind and transduce immune effector cells. Cells may be non-genetically modified to express one or more of the polypeptides contemplated herein, or in particular preferred embodiments, cells may be genetically modified to express one or more of the polypeptides contemplated herein. As used herein, the term “genetically engineered” or
20 “genetically modified” refers to the addition of extra genetic material in the form of DNA or RNA into the total genetic material in a cell. The terms, “genetically modified cells” or “modified cells” are used interchangeably in particular embodiments.

In particular embodiments, a recombinant lentivirus comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that mediate virus-target cell
25 fusion and one or more non-viral membrane-bound tropism polypeptides that bind an antigen expressed on a target cell, *e.g.*, an immune effector cell.

An “immune effector cell” is any cell of the immune system that has one or more effector functions (*e.g.*, cytotoxic cell killing activity, secretion of cytokines, induction of ADCC and/or CDC). Illustrative types of immune effector cells contemplated in particular
30 embodiments include, without limitation, T lymphocytes, dendritic cells (DC), Treg cells, natural killer (NK) cells, natural killer T (NKT) cells, and macrophages. The terms “T cell” or “T lymphocyte” are art-recognized and are intended, in particular embodiments, to

include thymocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, and/or activated T lymphocytes. Illustrative examples of T lymphocytes suitable for use in particular embodiments, include but not limited to cytotoxic T cells (CTLs; CD8⁺ T cells), TILs, helper T cells (HTLs; CD4⁺ T cells), CD4⁺CD8⁺ T cells, CD4⁺CD8⁻ T cells, or any other subset of T cells that has an effector function. In a particular embodiment, the cells comprise $\alpha\beta$ T cells. In a particular embodiment, the cells comprise $\gamma\delta$ T cells.

In particular embodiments, a recombinant lentivirus comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that mediate virus-target cell fusion and one or more non-viral membrane-bound tropism polypeptides that bind one or more antigens expressed on an immune effector cell, wherein the antigen is selected from the group consisting of: CD3 δ , CD3 ϵ , CD3 γ , CD4, CD8 α , CD8 β , the alpha or beta chains of a TCR, CD28, CD134 (OX40), CD137 (4-1BB), and CD278 (ICOS).

In particular embodiments, immune effector cells include natural killer (NK) cells. NK cells do not express T cell antigen receptors (TCR), CD3 or surface immunoglobulins (Ig) B cell receptor, but usually express the surface markers CD 16 (Fc γ RIII) and CD56 in humans. In particular embodiments, a recombinant lentivirus comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that mediate virus-target cell fusion and one or more non-viral membrane-bound tropism polypeptides that bind an antigen on an immune effector cell, wherein the antigen is CD 16 (Fc γ RIII) and/or CD56.

In particular embodiments, immune effector cells include natural killer T (NKT) cells.

In particular embodiments, a progenitor of an immune effector cell is transduced with a recombinant lentivirus contemplated herein and is subsequently induced to differentiate, or differentiates, into one or more immune effector cells. In particular embodiments, progenitors of immune effectors cells include hematopoietic stem cells (HSCs) contained within the CD34⁺ population of cells derived from cord blood, bone marrow or mobilized peripheral blood which naturally differentiate into mature immune effector cells, or which can be induced to differentiate into mature immune effector cells. In particular embodiments, a recombinant lentivirus comprises a viral envelope comprising one or more mutated viral envelope glycoproteins that mediate virus-target cell fusion and one or more non-viral membrane-bound tropism polypeptides that bind an antigen on a hematopoietic stem cell, wherein the antigen is selected from the group consisting of: CD7,

CD33, CD34, CD45, CD49f, CD90, CD98, CD110, CD117, CD123, CD133, CD184, CD201, FMS-like tyrosine kinase 3 (FLT3), and thrombopoietin receptor.

K. COMPOSITIONS AND FORMULATIONS

Formulations and compositions contemplated herein comprise a recombinant lentivirus and/or immune effector cells modified *ex vivo* 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 particular embodiments, a composition comprises a recombinant lentivirus comprising one or more mutated viral envelope glycoproteins that mediate virus-target cell fusion, one or more non-viral membrane-bound tropism polypeptides, and a lentiviral vector comprising a polynucleotide comprising or encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor, *e.g.*, a CAR.

In particular embodiments, the composition is a pharmaceutical composition. A “pharmaceutical composition” refers to a composition formulated in pharmaceutically-acceptable or physiologically - acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

“Pharmaceutically acceptable” refers to molecular entities and compositions that do not produce excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio when administered to a human.

In particular embodiments, a composition comprises a pharmaceutically acceptable carrier and a recombinant lentivirus contemplated herein. The term “pharmaceutically acceptable carrier” refers to a diluent, adjuvant, excipient, or vehicle and the like with which a recombinant lentivirus is physiologically compatible with administration to a human, including but not limited to pharmaceutically acceptable cell culture media, Dulbecco's phosphate buffered saline (PBS), Ringer's solution, 5% dextrose in water (D5W), and normal/physiologic saline (0.9% NaCl).

In particular embodiments, a composition is substantially free of mycoplasma, endotoxin, and microbial contamination. By “substantially free” with respect to endotoxin is meant that there is less endotoxin per dose of cells than is allowed by the FDA for a

biologic, which is a total endotoxin of 5 EU/kg body weight per day, which for an average 70 kg person is 350 EU per total dose of cells. In particular embodiments, compositions contemplated herein contain about 0.5 EU/mL to about 5.0 EU/mL, or about 0.5 EU/mL, 1.0 EU/mL, 1.5 EU/mL, 2.0 EU/mL, 2.5 EU/mL, 3.0 EU/mL, 3.5 EU/mL, 4.0 EU/mL, 4.5
5 EU/mL, or 5.0 EU/mL.

It would be understood by the skilled artisan that particular embodiments contemplated herein may comprise other formulations, such as those that are well known in the pharmaceutical art, and are described, for example, in *Remington: The Science and Practice of Pharmacy*, Volume I and Volume II. 23rd Edition. Edited by
10 Adeboye Adejare. Academic Press, 2020, which is incorporated by reference herein, in its entirety.

All publications, patent applications, and issued patents cited in this specification
15 are herein incorporated by reference as if each individual publication, patent application, or issued patent were specifically and individually indicated to be incorporated by reference.

Although the foregoing embodiments have been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings contemplated herein that certain
20 changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

EXAMPLE 1NHP MODEL FOR *IN VIVO* LENTIVIRAL GENE THERAPY

To evaluate the safety and efficacy of an *in vivo* HIV-1 based lentiviral gene
5 therapy platform, a non-human primate (NHP) model was established in the pig-tail
macaque, *Macaca nemestrina*. Pig-tail macaques do not express TRIM5 α and are
permissible to HIV-1 infection.

The model was interrogated using a recombinant HIV-1 based lentivirus
comprising a lentiviral vector encoding an MNDU3 promoter operably linked to an anti-
10 CD20 CAR, *e.g.*, SEQ ID NOs: 11, 12. Briefly, HEK 293 cells were transiently transfected
with plasmids encoding HIV-1 gag and pol; HIV-1 REV; a mutated VSIV-G protein
comprising K47Q and R354A mutations; a primary non-viral membrane bound tropism
polypeptide comprising an anti-CD3 ϵ antibody that bind *M. nemestrina* CD3 ϵ , *e.g.*, SP34-
2, and a CD8 α hinge and transmembrane domain; a secondary non-viral membrane bound
15 tropism polypeptide comprising a CD80 polypeptide comprising a C-terminal truncation;
and a transfer vector encoding an HIV-1 based lentiviral vector encoding an MNDU3
promoter operably linked to an anti-CD20 CAR. Transfected cells were cultured and
lentivirus containing supernatant was harvested, and lentivirus was purified, titered,
formulated in Dulbecco's modified eagle's medium (DMEM) without phenol red and
20 stored in frozen aliquots.

Blood samples were drawn from a pig-tail macaque prior to intravenous infusion of
recombinant lentivirus to establish baseline blood cell counts and to establish baseline
cytokine levels.

The pig-tail macaque was administered 5.0×10^8 TU/kg of the lentivirus at about 10
25 mL/kg for about 30 minutes.

After administration, blood specimens were collected on days 1, 3, 7, 10, 17, 21, 28,
and 35 to analyze blood cell counts and cytokine and chemokine levels in serum. Urine
and feces were collected once prior to administration and again on days 1, 3, and 7 post-
administration. Necropsy was performed at day 35: bone marrow, blood and CSF
30 collections were supplemented after euthanasia with a thorough harvest of tissues for IHC,

gDNA extraction and RNA extraction; portions of the spleen and lymph node were collected for flow cytometry analysis.

- Characterization of lymphoid cell populations were monitored by flow cytometry. Briefly, red blood cells were lysed using ammonium-chloride-potassium (ACK) buffer (ThermoFisher A1049201), stained with Ghost Dye™ Red, followed by staining with cell surface antibodies (see table below), fixed and signal acquired.

mAb specificity	Clone	Fluorescent Dye	Company catalog #
mAb dilution buffer	NA	NA	BD#554657
Brilliant buffer	NA	NA	BD# 742055
CD45	D058-1283	BUV395	BD# 564099
CD22	S-HCL-1	PE-Cy7	ab233586
CD20	2H7	BV510	BL# 302340
CD14	26ic	PerCP-Cy5.5	CP# 103466
CD8	3B5	Pacific blue	Inv# MHCD0828
CD159a (NKG2a)	REA110	APC	MB# 130-113-563
CD4	L200	PE-Cy7	BD#560644
CD3	SP34.2	BV786	BD# 563918
	Ghost Dye™ Red 710	L/D	To# 13-0871-T100
Abbreviation: mAb = Monoclonal Antibody. Inv = Invitrogen To = Tonbo (Cytex) BD = BD-Biosciences, BL = Biolegend CP = Caprico Biotech Ab = Abcam			

Flow cytometric analysis was performed on the Cytex Aurora and data was analyzed using FlowJo software. Final counts were determined by CBC lymphocyte counts in combination with population analysis by flow cytometry.

- Figure 1 shows data indicating that the *in vivo* HIV-1 derived lentivirus was functional and efficacious in *M. nemestrina*. Figure 1, right panel, shows that the recombinant HIV-1 derived lentivirus encoding the anti-CD20 CAR transduced T cells and led to T cell expansion. Figure 1, left panel, shows that T cells transduced with the HIV-1 derived lentivirus encoding the anti-CD20 CAR potently reduced B cells and mediated durable B cell aplasia.

Characterization of cytokine and chemokine levels in serum were analyzed using the Meso Scale Discovery (MSD) UPLEX electrochemiluminescent sandwich immunoassay. Two panels of cytokines and chemokines were measured for each sample and each sample was analyzed in technical duplicates. Multispot assay plates were coated with human biotinylated capture antibodies (antibodies were validated as cross-reactive with pig-tail macaques) prior to addition of serum samples, followed by conjugated detection antibodies, per manufacturer's instructions. Levels of cytokines and chemokines were measured using MESO QuickPlex SQ 120MM machine and analyzed via MSD Discovery Workbench software. Standard curves were generated using lyophilized proteins of known concentrations provided by MSD and prepared following manufacturer's instructions.

Figure 2 shows that the *in vivo* HIV-1 derived lentivirus was safe at 5×10^8 TU/kg and did not increase cytokine/chemokines to levels associated with neurotoxicity in *M. nemestrina*.

EXAMPLE 2

FURTHER EVALUATION OF *IN VIVO* LENTIVIRAL GENE THERAPY IN NHP MODEL

To further evaluate the therapeutic window, a lower dose of lentivirus was used in the *Macaca nemestrina* model established in Example 1. Blood samples were drawn from a pig-tail macaque prior to intravenous infusion of recombinant lentivirus to establish baseline blood cell counts and to establish baseline cytokine levels. The pig-tail macaque was administered 5.0×10^7 TU/kg of the lentivirus manufactured in Example 1 at about 10 mL/kg for about 30 minutes.

After administration, blood specimens were collected on days 1, 3, 7, 10, 14, and 21 to analyze blood cell counts, vector copy number (VCN), and cytokine and chemokine levels in serum. Urine and feces were collected once prior to administration and again on days 1, 3, and 7 post-administration. Necropsy will be performed at day 35: bone marrow, blood and CSF collections will be supplemented after euthanasia with a thorough harvest of tissues for IHC, gDNA extraction and RNA extraction; portions of the spleen and lymph node will also be collected for flow cytometry analysis.

Characterization of lymphoid cell populations were monitored by flow cytometry. Briefly, red blood cells were lysed using ammonium-chloride-potassium (ACK) buffer

(ThermoFisher A1049201), stained with Ghost Dye™ Red, followed by staining with cell surface antibodies (see table below), fixed and signal acquired.

mAb specificity	Clone	Fluorescent Dye	Company catalog #
mAb dilution buffer	NA	NA	BD#554657
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CD22	S-HCL-1	PE-Cy7	ab233586
CD20	2H7	BV510	BL# 302340
CD14	26ic	PerCP-Cy5.5	CP# 103466
CD8	3B5	Pacific blue	Inv# MHCD0828
CD159a (NKG2a)	REA110	APC	MB# 130-113-563
CD4	L200	PE-Cy7	BD#560644
CD3	SP34.2	BV786	BD# 563918
	Ghost Dye™ Red 710	L/D	To# 13-0871-T100
Abbreviation: mAb = Monoclonal Antibody. Inv = Invitrogen To = Tonbo (Cytex) BD = BD-Biosciences, BL = Biolegend CP = Caprico Biotech Ab = Abcam			

Flow cytometric analysis was performed on the Cytex Aurora and data was analyzed using FlowJo software. Final counts were determined by CBC lymphocyte counts in combination with population analysis by flow cytometry.

Figure 3 shows data indicating that the *in vivo* HIV-1 derived lentivirus was functional and efficacious in *M. nemestrina*. Figure 3, right panel, shows the vector copy number (VCN) in T cells transduced with the HIV-1 derived lentivirus encoding the anti-CD20 CAR. Figure 3, left panel, shows T cells transduced with the HIV-1 derived lentivirus encoding the anti-CD20 CAR potentially reduced B cells and mediated durable B cell aplasia.

Characterization of cytokine and chemokine levels in serum will be analyzed using the Meso Scale Discovery (MSD) UPLEX electrochemiluminescent sandwich immunoassay. Two panels of cytokines and chemokines will be measured for each sample and each sample is analyzed in technical duplicates. Multispot assay plates will be coated with human biotinylated capture antibodies (antibodies were validated as cross-reactive

with pig-tail macaques) prior to addition of serum samples, followed by conjugated detection antibodies, per manufacturer's instructions. Levels of cytokines and chemokines will be measured using MESO QuickPlex SQ 120MM machine and analyzed via MSD Discovery Workbench software. Standard curves will be generated using lyophilized

5 proteins of known concentrations provided by MSD and prepared following manufacturer's instructions.

In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but

10 should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. A method to reduce the number of B cells in a non-human primate (NHP) comprising: administering to the NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i) one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and (ii) one or more non-viral membrane-bound tropism polypeptides; and (b) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor;

wherein the amount of recombinant lentivirus is sufficient to transduce a population of immune effector cells in the NHP sufficient to reduce the number of B cells in the NHP.

2. The method of claim 1, wherein at least one of the one or more non-viral membrane-bound tropism polypeptides comprises an extracellular antigen targeting domain, a spacer domain, and a transmembrane domain.

3. The method of claim 2, wherein the extracellular antigen targeting domain binds an antigen expressed on an immune effector cell.

4. The method of claim 2 or claim 3, wherein the extracellular antigen targeting domain binds an antigen expressed on an immune effector cell selected from the group consisting of: the alpha, beta, gamma, or delta chain of the T cell receptor, CD2, CD3 δ , CD3 ϵ , CD3 γ , CD4, CD8 α , and CD8 β .

5. The method of any one of claims 2 to 4, wherein the extracellular antigen targeting domain comprises an anti-CD3 ϵ antibody or antigen binding fragment thereof.

6. The method of any one of claims 2 to 5, wherein the viral envelope further comprises a secondary non-viral membrane-bound tropism polypeptide.

7. The method of any one of claims 1 to 6, wherein the engineered antigen receptor is selected from the group consisting of: a chimeric antigen receptor (CAR), a chimeric costimulatory receptor (CCR), an alpha-beta T cell receptor ($\alpha\beta$ TCR), a gamma

delta T cell receptor ($\gamma\delta$ TCR), a dimerizing agent regulated immunoreceptor complex (DARIC), a chimeric TGF- β receptor (CTBR), and a zetakine receptor.

8. The method of any one of claims 1 to 7, wherein the immune effector cells comprise one or more of $\alpha\beta$ TCR T cells, $\gamma\delta$ TCR T cells, natural killer (NK) cells, and natural killer T (NKT) cells.

9. A method to reduce the number of B cells in a non-human primate (NHP) comprising: administering to the NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i) one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and (ii) a non-viral membrane-bound tropism polypeptide comprising an anti-CD3 antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain, and optionally a secondary non-viral membrane bound tropism polypeptide; and (b) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an engineered antigen receptor;

wherein the amount of recombinant lentivirus is sufficient to transduce a population of T cells in the NHP sufficient to reduce the number of B cells in the NHP.

10. The method of and one of claims 1 to 9, wherein the viral envelope further comprises a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof.

11. The method of any one of claims 1 to 10, wherein the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain that binds an antigen expressed on the surface of a B cell, a hinge domain, a transmembrane domain, and one or more intracellular signaling domains.

12. The method of and one of claims 1 to 11, wherein the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain that binds BCMA, CD19, CD20, CD22, CD38, CD79A, CD79B, and GPCR5D.

13. A method to reduce the number of B cells in a non-human primate (NHP) comprising: administering to the NHP, an amount of a recombinant HIV-1 derived lentivirus, wherein the recombinant lentivirus comprises (a) a viral envelope comprising (i)

one or more mutated viral envelope glycoproteins that retain fusogenic activity and lack cognate receptor binding activity and (ii) a non-viral membrane-bound tropism polypeptide comprising an anti-CD3 ϵ antibody or antigen binding fragment thereof, a spacer domain, and a transmembrane domain, and optionally a secondary non-viral membrane bound tropism polypeptide selected from the group consisting of CD80, CD86, CD137L, OX40L, and ICOSL or variants thereof; and (b) a recombinant HIV-1 derived lentiviral vector comprising a polynucleotide encoding a promoter operably linked to a polynucleotide encoding an anti-CD20 CAR;

wherein the amount of recombinant lentivirus is sufficient to transduce a population of T cells in the NHP sufficient to reduce the number of B cells in the NHP.

14. The method of any one of claims 1 to 13, wherein the one or more mutated viral envelope glycoproteins comprise a vesiculovirus envelope glycoprotein, one or more morbillivirus envelope glycoproteins or one or more henipavirus envelope glycoproteins.

15. The method of claim 14, wherein the vesiculovirus is selected from the group consisting of: vesicular stomatitis Alagoas virus (VSAV; Alagoas vesiculovirus), Carajás virus (CJSV; Carajas vesiculovirus), Chandipura virus (CHPV; Chandipura vesiculovirus), Cocal virus (COCV; Cocal vesiculovirus), vesicular stomatitis Indiana virus (VSIV; Indiana vesiculovirus), Isfahan virus (ISFV; Isfahan vesiculovirus), Maraba virus (MARAV; Maraba vesiculovirus), Morreton virus (MORV; Morreton vesiculovirus), vesicular stomatitis New Jersey virus (VSNJV; New Jersey vesiculovirus), and Piry virus (PIRYV; Piry vesiculovirus).

16. The method of claim 14 or claim 15, wherein the vesiculovirus envelope glycoprotein is a vesiculovirus G protein.

17. The method of any one of claims 14 to 16, wherein the vesiculovirus G protein is a COCV G glycoprotein (COCV-G) or a VSIV G glycoprotein (VSIV-G).

18. The method of claim 17, wherein the VSIV-G envelope protein comprises one or more of:

(a) one or more amino acid substitutions at H8, N9, Q10, K47, K50, A51, S183, S179, N180, I182, M184, Y209, T214, I347, T350, T352, E353, and R354;

(b) an insertion of TT between N9 and Q10, an insertion of GGS between H8 and N9, an insertion of GGS between N9 and Q10, an insertion of TT between N208 and Y209, an insertion of GGS between P46 and K47, and an insertion of GGS between N208 and Y209; or

(c) amino acid substitutions at K47 and/or R354; or

(d) a deletion of residues 1-8.

19. The method of claim 17 or claim 18, wherein the VSIV-G envelope protein comprises one or more amino acid substitutions at H8, K47, Y209, and R354.

20. The method of any one of claims 17 to 19, wherein the VSIV-G envelope protein comprises one or more amino acid substitutions selected from the group consisting of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F and R354G; K47F and R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G; K47Q and R354F; and K47Q and R354Q.

21. The method of any one of claims 17 to 20, wherein the VSIV-G envelope protein comprises the amino acid sequence set forth in SEQ ID NO: 2, wherein $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = A$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = G$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$,

$X_2 = F$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = F$; $X_1 = L$, $X_2 = F$, $X_3 = H$, and $X_4 = Q$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = F$; $X_1 = I$, $X_2 = Q$, $X_3 = Q$, and $X_4 = Q$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = A$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = G$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = F$; $X_1 = L$, $X_2 = Q$, $X_3 = Q$, and $X_4 = Q$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; $X_1 = I$, $X_2 = Q$, $X_3 = H$, and $X_4 = Q$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = A$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = G$; $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = F$; and $X_1 = L$, $X_2 = Q$, $X_3 = H$, and $X_4 = Q$.

22. The method of any one of claims 14 to 17, wherein the vesiculovirus G protein is COCV-G.

23. The method of claim 22, wherein the COCV-G envelope protein comprises one or more amino acid substitutions at K47 and/or R354.

24. The method of claim 22 or claim 23, wherein the COCV-G envelope protein comprises one or more amino acid substitutions selected from the group consisting of: K47A and R354A; K47A and R354G; K47A and R354F; K47A and R354Q; K47G and R354A; K47G and R354G; K47G and R354F; K47G and R354Q; K47F and R354A; K47F and R354G; K47F and R354F; K47F and R354Q; K47Q and R354A; K47Q and R354G; K47Q and R354F; and K47Q and R354Q.

25. The method of any one of claims 22 to 24, wherein the COCV-G envelope protein comprises the amino acid sequence set forth in SEQ ID NO: 4, wherein $X_1 = A$ and $X_2 = A$; $X_1 = A$ and $X_2 = G$; $X_1 = A$ and $X_2 = F$; $X_1 = A$ and $X_2 = Q$; $X_1 = G$ and $X_2 = A$; $X_1 = G$ and $X_2 = G$; $X_1 = G$ and $X_2 = F$; $X_1 = G$ and $X_2 = Q$; $X_1 = F$ and $X_2 = A$; $X_1 = F$ and $X_2 = G$; $X_1 = F$ and $X_2 = F$; $X_1 = F$ and $X_2 = Q$; $X_1 = Q$ and $X_2 = A$; $X_1 = Q$ and $X_2 = G$; $X_1 = Q$ and $X_2 = F$; or $X_1 = Q$ and $X_2 = Q$.

26. The method of claim 14, wherein the one or more morbillivirus envelope glycoproteins are measles virus F (MV-F) and measles virus H (MV-H).

27. The method of claim 26, wherein the MV-H protein comprises one or more amino acid substitutions at Y481, R533, S548, and F549.
28. The method of claim 26 or claim 27, wherein the MV-H protein comprises one or more amino acid substitutions selected from the group consisting of: Y481A, R533A, S548L, and F549S.
29. The method of claim 14, wherein the one or more henipavirus envelope glycoproteins are nipah virus F (NiV-F) and nipah virus G (NiV-G).
30. The method of claim 29, wherein the NiV-G protein comprises one or more amino acid substitutions at E501, W504, Q530, and E533.
31. The method of claim 29 or claim 30, wherein the NiV-G protein comprises one or more amino acid substitutions at E501A, W504A, Q530A, and E533A.
32. The method of any one of claims 2 to 31, wherein the extracellular antigen targeting domain comprises an antibody or antigen binding fragment selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab₂), a trispecific Fab trimer (Fab₃), an Fv, a single chain Fv protein (scFv), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).
33. The method of any one of claims 1 to 32, wherein the spacer domain is hinge domain or stalk obtained or isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and/or wherein the transmembrane domain is isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A; and optionally, wherein the tropism polypeptide comprises a truncated intracellular domain isolated from a polypeptide selected from the group consisting of CD3, CD4, CD8 α , CD8 β , CD28, or Glycophorin A or a cytoplasmic tail of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.
34. The method of any one of claims 1 to 33, wherein the viral envelope further comprises a secondary non-viral membrane bound tropism polypeptide selected from the

group consisting of CD80, CD86, CD137L, OX40L, and ICOSL and optionally, comprises a C-terminal truncation that results in a cytoplasmic domain of about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids or more.

35. The method of any one of claims 1 to 34, wherein the promoter is selected from the group consisting of: an elongation factor 1 α (EF1 α) promoter, a cytomegalovirus (CMV) promoter, a Moloney murine leukemia virus (MoMLV) promoter, a Rous sarcoma virus (RSV) promoter, a 3-phosphoglycerate kinase (PGK-1) promoter, a herpes simplex virus (HSV) (thymidine kinase) promoter, a chicken β -actin (CAG) promoter, a simian virus 40 (SV40) promoter, an SV40/CD43 promoter, a spleen focus forming virus (SFFV) promoter, and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer binding site substituted (MND) U3 promoter.

36. The method of any one of claims 1 to 35, wherein the engineered antigen receptor is a CAR comprising an extracellular antigen binding domain selected from the group consisting of: a receptor ectodomain, a ligand, or an antibody or antigen binding fragment thereof selected from the group consisting of: a Camel Ig, a Llama Ig, an Alpaca Ig, Ig NAR, a Fab' fragment, a F(ab')₂ fragment, a bispecific Fab dimer (Fab2), a trispecific Fab trimer (Fab3), an Fv, a single chain Fv protein (scFv), a bis-scFv, (scFv)₂, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein ("dsFv"), and a single-domain antibody (sdAb, a camelid VHH, Nanobody).

37. The method of any one of claims 1 to 36, wherein the engineered antigen receptor is a CAR comprising a hinge domain isolated or derived from a polypeptide selected from the group consisting of: CD4, CD8 β , CD8 α , CD28, CD134, CD137, CD152, CD278, IgG1, IgG2, IgG3, and IgG4.

38. The method of any one of claims 1 to 37, wherein the engineered antigen receptor is a CAR comprising a transmembrane domain isolated or derived from a polypeptide selected from the group consisting of: alpha, beta, gamma, or delta chain of the T-cell receptor, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154, CD278, amnionless (AMN), and programmed cell death 1 (PDCD1).

39. The method of any one of claims 1 to 38, wherein the engineered antigen receptor is a CAR comprising a primary signaling domain isolated or derived from a polypeptide selected from the group consisting of: FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD3 ζ , CD22, CD79a, CD79b, and CD66d.

40. The method of any one of claims 1 to 39, wherein the engineered antigen receptor is a CAR comprising a costimulatory domains isolated or derived from a polypeptide selected from the group consisting of: Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, caspase recruitment domain family member 11 (CARD11), CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD94, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DNAX-Activation Protein 10 (DAP10), Linker for activation of T-cells family member 1 (LAT), SH2 Domain-Containing Leukocyte Protein Of 76 kD (SLP76), T cell receptor associated transmembrane adaptor 1 (TRAT1), TNFR2, TNFRS14, TNFRS18, TNFRS25, and zeta chain of T cell receptor associated protein kinase 70 (ZAP70).

41. The method of any one of claims 1 to 40, wherein the recombinant lentivirus is parenterally administered to the NHP.

42. The method of any one of claims 1 to 41, wherein the recombinant lentivirus is intravascularly administered to the NHP.

43. The method of any one of claims 1 to 42, wherein the recombinant lentivirus is intravenously or intraarterially administered to the NHP.

44. The method of any one of claims 1 to 43, wherein after administration of the recombinant lentivirus, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 or more specimens are taken from the NHP, wherein one or more of the specimens is taken from the NHP every day, every other day, every three days, every four days, every five days, every six days, every 7 days, every eight days, every nine days, or every ten or more days for a period of about 10 days to about 60 days or any intervening period of time.

45. The method of any one of claims 1 to 44, wherein one or more specimens is taken from the NHP and the number of B cells is measured in the specimen.

46. The method of any one of claims 1 to 45, wherein one or more specimens is taken from the NHP and the number of T cells is measured in the specimen.

47. The method of any one of claims 1 to 46, wherein one or more specimens is taken from the NHP and the number of CAR⁺ T cells is measured in the specimen.

48. The method of any one of claims 1 to 47, wherein one or more specimens is taken from the NHP and the vector copy number (VCN) of transduced T cells is measured in the one or more specimens.

49. The method of any one of claims 1 to 48, wherein one or more specimens is taken from the NHP and the amount of one or more cytokines and/or chemokines is measured in the one or more specimens.

50. The method of any one of claims 1 to 49, wherein one or more specimens is taken from the NHP and the amount of one or more cytokines or chemokines selected from the group consisting of CXCL11, GM-CSF, IFN γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IP-10, MIP-1 α , and TNF α is measured in the one or more specimens.

51. The method of any one of claims 1 to 50, wherein the amount of recombinant lentivirus administered to the NHP does not cause a cytokine storm, cytokine release syndrome (CRS), or neurotoxicity in the NHP.

52. The method of any one of claims 1 to 51, wherein the amount of recombinant lentivirus administered to the NHP decreases the number of B cells in a specimen at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or more compared to the number of B cells in a specimen from an NHP that was not administered the recombinant lentivirus or compared to the number of B cells in a specimen from the NHP before the NHP was administered the recombinant lentivirus.

53. The method of any one of claims 1 to 52, wherein a composition comprising a pharmaceutically acceptable diluent and the recombinant lentivirus is administered to the NHP.

54. The method of claim 53, wherein the diluent is a pharmaceutically acceptable cell culture medium (*e.g.*, DMEM), Dulbecco's phosphate buffered saline (PBS), Ringer's solution, 5% dextrose in water (D5W), or normal/physiologic saline (0.9% NaCl).

55. The method of any one of claims 1 to 54, wherein the amount of recombinant lentivirus administered to the NHP at a dose of about 5 mL/kg to about 15 mL/kg

56. The method of any one of claims 1 to 55, wherein the amount of recombinant lentivirus administered to the NHP at a dose of about 5 mL/kg, about 6 mL/kg, about 7 mL/kg, about 8 mL/kg, about 9 mL/kg, about 10 mL/kg, about 11 mL/kg, about 12 mL/kg, about 13 mL/kg, about 14 mL/kg, or about 15 mL/kg.

57. The method of any one of claims 1 to 56, wherein the amount of recombinant lentivirus administered to the NHP is about 1×10^6 TU/mL to about 1×10^{10} TU/mL.

58. The method of any one of claims 1 to 57, wherein the amount of recombinant lentivirus administered to the NHP is about 1×10^7 TU/mL to about 1×10^9 TU/mL.

59. The method of any one of claims 1 to 58, wherein the amount of recombinant lentivirus administered to the NHP is about 1×10^7 TU/mL, 5×10^7 TU/mL, 1×10^8 TU/mL, 5×10^8 TU/mL, about 1×10^9 TU/mL, about 5×10^9 TU/mL, or about 1×10^{10} TU/mL.

60. The method of any one of the preceding claims, wherein the NHP is not administered a lymphodepleting chemotherapy prior to, during, or after administration of the recombinant lentivirus.

61. The method of any one of the preceding claims, wherein the NHP is *Macaca nemestrina*.

FIGURE 1

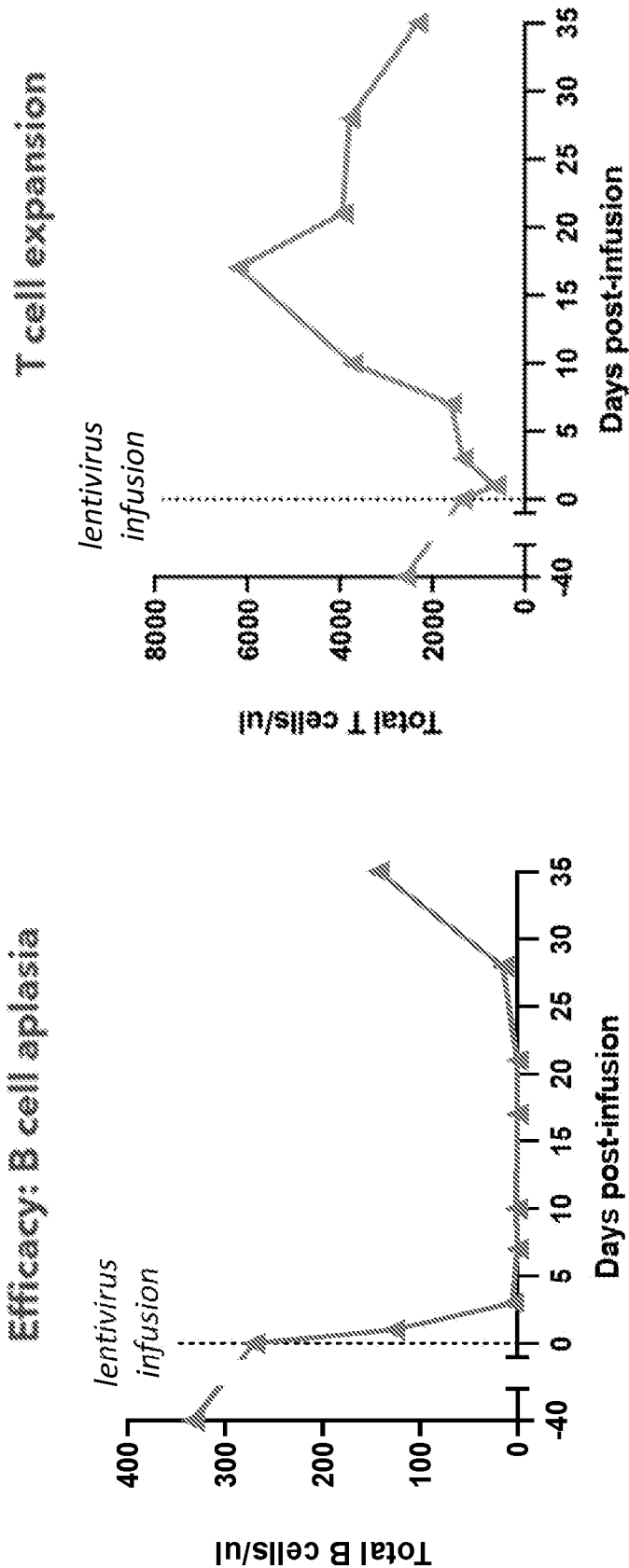
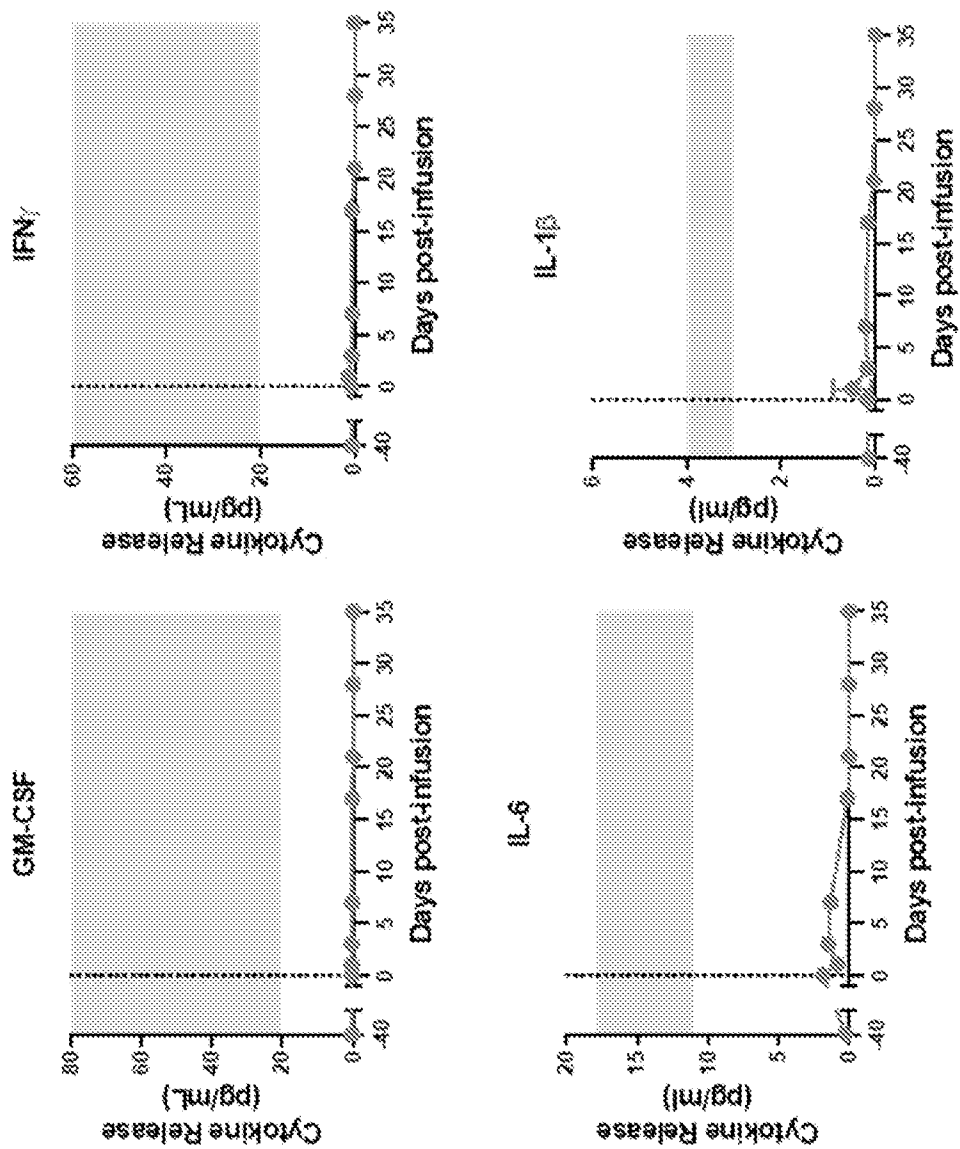


FIGURE 2



Shaded areas indicate peak biomarker levels associated with neurotoxicity after CAR T in NHP

FIGURE 3

