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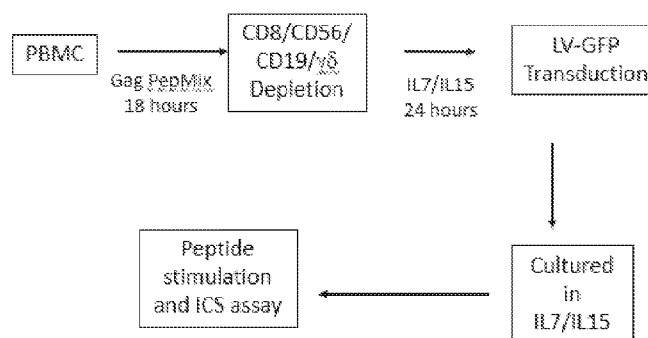


Figure 14

(57) Abstract: The present disclosure relates generally to immunization and immunotherapy for the treatment or prevention of HIV. In particular, the methods include purifying peripheral blood mononuclear cells (PBMC) from a source, stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8+ T cells, CD4+ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the depleted PBMC with a viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC.

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METHODS OF MANUFACTURING GENETICALLY-MODIFIED LYMPHOCYTES**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This application claims priority to: U.S. Provisional Patent Application No. 62/648,804, filed on March 27, 2018 entitled "METHODS OF MANUFACTURING GENETICALLY-MODIFIED LYMPHOCYTES," the disclosure of which is incorporated herein by reference.

FIELD

[0002] The present disclosure relates generally to the field of immunization and immunotherapy for the treatment and prevention of HIV. In particular, the disclosed methods relate to obtaining, processing, and proliferating leukocytes, including depletion of non-target cells, from HIV+ individuals seeking a cure in order to prepare a cell product suitable for infusion to such HIV+ individuals.

BACKGROUND

[0003] Combination antiretroviral therapy (cART) (also known as Highly Active Antiretroviral Therapy or HAART) limits HIV-1 replication and slows disease progression, but drug toxicities and the emergence of drug-resistant viruses are challenges for long-term control in HIV-infected persons. Additionally, traditional antiretroviral therapy, while successful at delaying the onset of AIDS or death, has yet to provide a cure. Alternative treatment strategies are needed.

[0004] Intense interest in immunotherapy for HIV infection has been precipitated by emerging data indicating that the immune system has a major, albeit usually insufficient, role in limiting HIV replication. Some studies have tested vaccines against HIV, but success has been limited to date. Additionally, there has been interest in augmenting HIV immunotherapy by utilizing gene therapy techniques, but as with other immunotherapy approaches, success has been limited.

[0005] Gene therapy is one of the ripest areas of biomedical research with the potential to create new therapeutics that may involve the use of viral vectors. In view of the wide variety of potential genes available for therapy, an efficient means of delivering these genes is needed to fulfill the promise of gene therapy as a means of treating infectious and non-infectious diseases. Several viral systems including murine retrovirus, adenovirus, parvovirus (adeno-associated virus), coxsackie virus, measles virus, picornavirus, flavivirus,

vaccinia virus, and herpes virus have been proposed as therapeutic gene transfer vectors. However, *in vivo* application of viral vectors is often limited by host immune responses against viral structural proteins and/or transduced gene products.

[0006] Although lentiviral vectors do not generally induce cytotoxicity and do not elicit strong host immune responses, some lentiviruses such as HIV-1, which encode several immunostimulatory gene products, have the potential to cause cytotoxicity and induce strong immune responses *in vivo*. Another important issue related to the use of lentiviral vectors is that of possible cytopathogenicity or functional unresponsiveness of T cells upon exposure to certain cytotoxic viral proteins. Likewise, the possibility of generating replication-competent, virulent lentivirus by recombination is often a concern. For HIV immunotherapy using gene therapy techniques, the failure to obtain sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications typically results in a rapid reemergence of HIV upon termination of antiretroviral therapy.

[0007] Previous efforts to achieve a cure for HIV have fallen short for these reasons, among others. Accordingly, there remains a need for improved treatments of HIV.

SUMMARY

[0008] In one aspect, a method is provided. The method includes purifying peripheral blood mononuclear cells (PBMC) from a source, stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the depleted PBMC with a viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC. In embodiments, the at least one subset of cells comprises any one or more of CD8⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, neutrophils, basophils, eosinophils, mast cells, dendritic cells, and NKT cells.

[0009] In embodiments, the culturing occurs in a static culture system or a semi-static culture system. In embodiments, the at least one HIV-specific peptide comprises a pool of synthetic, overlapping peptides. In embodiments, the pool of synthetic, overlapping peptides represents the HIV Gag polyprotein. In embodiments, the depleting comprises separating the at least one subset of cells from the depleted PBMC. In embodiments, the separating comprises magnetic bead separation. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the HIV

RNA sequence comprises a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. In embodiments, the at least one genetic element comprises a microRNA or a shRNA. In embodiments, the at least one genetic element comprises a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 30.

[0010] In another aspect, a genetically-modified lymphocyte for treatment of a subject infected with HIV is provided. The genetically-modified lymphocyte is prepared by a process including the steps of purifying peripheral blood mononuclear cells (PBMC) comprising at least one lymphocyte from the subject, stimulating the at least one lymphocyte with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element, culturing the PBMC for at least one day, and harvesting the cultured PBMC.

[0011] In another aspect, a genetically-modified lymphocyte for treatment of a subject immunized with a preventive or therapeutic HIV vaccine is provided. The genetically-modified lymphocyte is prepared by a process including the steps of purifying peripheral blood mononuclear cells (PBMC) comprising at least one lymphocyte from the subject, stimulating the at least one lymphocyte with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element, culturing the PBMC for at least one day, and harvesting the cultured PBMC.

[0012] In another aspect, a genetically-modified lymphocyte for treatment of a subject exposed to, but not infected with HIV is provided. The genetically-modified lymphocyte is prepared by a process including the steps of purifying peripheral blood mononuclear cells (PBMC) comprising at least one lymphocyte from the subject, stimulating the at least one lymphocyte with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element, culturing the PBMC for at least one day, and harvesting the cultured

PBMC.

[0013] In another aspect, a method is provided that comprises stimulating PBMC with at least one HIV-specific peptide; depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells; and transducing the depleted PBMC with a viral delivery system encoding at least one genetic element. In embodiments, the at least one HIV-specific peptide comprises a HIV gag peptide. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the HIV RNA sequences comprise an HIV Vif sequence, an HIV Tat sequence, or a variant thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **Figure 1** depicts a flow diagram of an *ex vivo* treatment method of the present disclosure.

[0015] **Figure 2** depicts CD4⁺ T cell alteration and prevention of new infection in accordance with the present disclosure.

[0016] **Figure 3** depicts an exemplary lentiviral vector system comprised of a therapeutic vector, a helper plasmid, and an envelope plasmid. The therapeutic vector shown here is a preferred therapeutic vector, which is also referred to herein as AGT103, and contains miR30CCR5-miR21Vif-miR185-Tat.

[0017] **Figure 4** depicts an exemplary 3-vector lentiviral vector system in a circularized form.

[0018] **Figure 5** depicts an exemplary 4-vector lentiviral vector system in a circularized form.

[0019] **Figure 6** depicts a further exemplary 3-vector lentiviral vector system in a circularized form.

[0020] **Figure 7** depicts exemplary vector sequences. Positive (i.e., genomic) strand sequences of the promoter and miR cluster were developed for inhibiting the spread of CCR5-tropic HIV strains. The upper sequence (SEQ ID NO: 86) is the EF-1alpha promoter, which contains a restriction recognition site on its 3' end. The bottom three sequences are the miR30 CCR5 (SEQ ID NO: 87), miR21 Vif (SEQ ID NO: 88), and miR185 Tat (SEQ ID NO: 89) sequences that contain restriction recognition sites. The portions of the sequences not underlined represent the restriction recognition sites.

[0021] **Figure 8** depicts a flow diagram for an exemplary method of producing CD4⁺ T cells, including a step of depleting CD8⁺ T cells.

[0022] **Figure 9** depicts data demonstrating a substantial increase of CD4⁺ T cells when Gag peptide-stimulated cells were depleted of CD8⁺ T cells.

5 [0023] **Figure 10** depicts data demonstrating an increase in CD4⁺ cells and an outgrowth of V δ 1 cells when CD8⁺ T cells were depleted.

[0024] **Figure 11** depicts data demonstrating that CD8⁺ T cell depletion increased the yield of target cells by approximately 6-fold.

10 [0025] **Figure 12** depicts data demonstrating a 3-fold increase of CD4⁺ T cells when Gag peptide-stimulated cells were depleted of CD8⁺ T cells.

[0026] **Figure 13** depicts data demonstrating that CD8⁺ T cell depletion resulted in expansion of CD56⁺ NK cells.

[0027] **Figure 14** depicts a flow diagram for an exemplary method of producing CD4⁺ T cells, including a step of depleting CD8⁺ T cells, CD56⁺ cells, CD19⁺ B cells and $\gamma\delta$ cells.

15 [0028] **Figure 15** depicts data demonstrating the effect on CD4⁺ T cell yield using various cell depletion strategies including (i) no depletion; (ii) CD8⁺ T cell depletion; (iii) CD8⁺ T cell depletion and $\gamma\delta$ cell depletion; and (iv) CD8⁺ T cell depletion, $\gamma\delta$ cell depletion, and B cell depletion.

20 [0029] **Figure 16** depicts data demonstrating the effect on CD4⁺ T cell yield using various cell depletion strategies including (i) no depletion; (ii) CD8⁺ T cell depletion; and (iii) CD8⁺ T cell depletion, CD56⁺ cell depletion, $\gamma\delta$ cell depletion, and B cell depletion,

[0030] **Figure 17** depicts a flow diagram for an exemplary method of producing CD4⁺ T cells, including a 4-way cell depletion step to deplete CD8⁺ T cells, CD56⁺ cells, CD19⁺ cells, and $\gamma\delta$ cells.

25 [0031] **Figure 18** depicts data demonstrating results of CD4⁺ T cell expression using a 4-way cell depletion process.

DETAILED DESCRIPTION

Overview

30 [0032] Disclosed herein are methods and compositions for treating and/or preventing human immunodeficiency virus (HIV) disease to achieve a functional cure. The methods and compositions include integrating lentivirus, non-integrating lentivirus, and related viral vector technology as described below.

[0033] Disclosed herein are therapeutic viral vectors (*e.g.*, lentiviral vectors),

immunotherapies, and methods for their use for treating HIV infection. In embodiments, methods and compositions for achieving a functional cure for HIV infection are provided. As depicted in Figure 1 herein, various aspects and embodiments of the disclosure may include a first stimulation event, for example a first therapeutic immunization with vaccines intended to produce strong immune responses against HIV in HIV-infected patients, for example with stable suppression of viremia due to daily administration of HAART. In embodiments, the leukocyte fraction is purified from an apheresis product. This is followed by (1) stimulation of purified leukocytes with at least one synthetic peptide representing an immunogenic HIV protein, (2) depleting unnecessary cells by a physical separation method, (3) transducing the stimulated and depleted leukocyte population with a therapeutic lentivirus vector, (4) transferring transduced cells to a static culture vessel for at least one day of growth, (5) harvesting cells from the static culture vessel, (6) substituting the growth medium with cryopreservation solution and freezing cells ready for infusion, and (7) re-infusion back into the original patient.

[0034] The various methods and compositions can be used to prevent infused cells, such as CD4⁺ T cells, from becoming infected with HIV once they are again present in the body of a person with HIV infection. For example, as illustrated in Figure 2, to prevent infused cells from becoming infected, CCR5 expression can be modulated to prevent virus penetration. Further, destruction of any residual infecting viral RNA can also be targeted. In respect of the foregoing, and in reference to Figure 2 herein, compositions and methods are provided to stop the HIV viral cycle in cells that become infected with HIV even in the absence of CCR5, or cells that were already infected before transduction and the need is to prevent the release of infectious virus. To stop the HIV viral cycle, viral RNA produced by latently-infected cells, such as latently-infected CD4⁺ T cells, is targeted.

[0035] Previous efforts to achieve a cure for HIV have fallen short due to, among others, the failure to obtain sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications. When this number is below a critical threshold, a cure as described herein is not achieved. For example, upon termination of antiretroviral therapy HIV re-emergence generally follows. Thereafter, patients often experience rapid destruction of HIV-specific CD4 T cells, followed by return to progression of disease despite prior genetic therapy. By employing selective enrichment for HIV-specific T cells in accordance with the compositions and methods described herein, a new HIV treatment regimen has been developed including, in various embodiments, a cure.

Definitions and Interpretation

[0036] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.*: Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane, *Using Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan *et al.*, *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

[0037] As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, “about” will mean up to plus or minus 10% of the particular term.

[0038] As used herein, the terms “administration of” or “administering” an active agent means providing an active agent of the disclosure to the subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

[0039] As used herein, the term “AGT103” refers to a particular embodiment of a

lentiviral vector that contains a miR30-CCR5/miR21-Vif/miR185-Tat microRNA cluster sequence, as detailed herein.

[0040] As used herein, the term “AGT103-T” refers to a cell that has been transduced with a lentivirus that contains the AGT103 lentiviral vector.

5 [0041] As used herein, the term “CCR5” refers to C-C chemokine receptor 5. Reference herein to “CCR5delta32” is reference to a mutant genotype in the CCR5 gene.

[0042] As used herein, the term “CD” refers to a particular cluster of differentiation protein. A non-limiting example of this terminology as used herein is CD4 protein expression. Examples of such proteins include but are not limited to CD4.

10 [0043] As used herein, the term “cART” refers to combination antiretroviral therapy. The term “cART” may be used synonymously with HAART (Highly Active Antiretroviral Therapy).

[0044] As used herein, the phrase “coding sequence” describes any viral vector sequence capable of being transcribed or reverse transcribed. A “coding sequence” includes, without
15 limitation, exogenous sequences (*e.g.*, sequences on vectors that have been transduced or transfected into cells) capable of being transcribed or reverse transcribed.

[0045] As used herein, the transitional term “comprising,” when used to define compositions and methods, means that the compositions and methods include the recited elements, but does not exclude others. As used herein, “consisting of,” when used to define
20 compositions and methods, means that the compositions and methods exclude more than trace elements of other ingredients for compositions and substantial method steps. Embodiments defined by each of these transitional terms are within the scope of this disclosure. For example, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and
25 compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of). Further, as used herein, the term “includes” means includes without limitation.

[0046] As used herein, the term “engraftment” refers to the ability for one skilled in the art to determine a quantitative level of sustained engraftment in a subject following infusion
30 of a cellular source (see for *e.g.*: Rosenberg et al., *N. Engl. J. Med.* 323:570-578 (1990); Dudley et al., *J. Immunother.* 24:363-373 (2001); Yee et al., *Curr. Opin. Immunol.* 13:141-146 (2001); Rooney et al., *Blood* 92:1549-1555 (1998)).

[0047] As used herein, the terms, “expression,” “expressed,” or “encodes” refer to the

process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

5 [0048] As used herein, the term “functional cure”, as referenced above and herein, and as further defined herein, refers to a state or condition wherein HIV+ individuals who previously required ongoing HIV therapies such as cART or HAART, may survive with low or undetectable virus replication using lower doses, intermittent doses, alternate drug combinations or single agents, or discontinued dosing of such HIV therapies. An individual
10 may be said to have been “functionally cured” while still requiring adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of all or virtually all HIV such that no recurrence is detected within a specified time frame, for example, 1 month, 3 months, 6 months, 1 year, 3 years, and 5 years, and all other time frames as may be defined.

15 [0049] As used herein, the phrase “HIV-specific peptide” refers to any peptide that is capable of generating an anti-HIV response in a T cell, including a CD4+ T cell, or is otherwise related to, or expressed or encoded by, HIV, including without limitation any GAG peptide.

[0050] As used herein, the term “HIV vaccine” encompasses immunogens plus vehicle
20 plus adjuvant intended to elicit HIV-specific immune responses. The term “HIV vaccine” is within the meaning of the term “stimulatory agent” as described herein. A “HIV vaccine” may include purified or whole inactivated virus particles that may be HIV or recombinant virus vectors capable of expressing HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides, in addition to recombinant bacterial vectors,
25 plasmid DNA or RNA capable of directing cells to producing HIV proteins, glycoproteins or protein fragments able to elicit specific immunity. Alternately, specific methods for immune stimulation including anti-CD3/CD28 beads, T cell receptor-specific antibodies, mitogens, superantigens, cytokines and other chemical or biological stimuli may be used to activate dendritic, T or B cells for the purposes of enriching HIV-specific CD4 T cells prior to
30 transduction or for *in vitro* assay of lentivirus-transduced CD4 T cells. Activating substances may be soluble, polymeric assemblies, liposome or endosome-based or linked to beads. Cytokines including interleukin-2, 6, 7, 12, 15, 23 or others may be added to improve cellular responses to stimuli and/or improve the survival of CD4 T cells throughout the

culture and transduction intervals. Alternately, and without limiting any of the foregoing, the term “HIV vaccine” encompasses the MVA/HIV62B vaccine, and variants and analogs thereof. The MVA/HIV62B vaccine is a known highly attenuated double recombinant MVA vaccine. The MVA/HIV62B vaccine was constructed through the insertion of HIV-1 gag-pol and env sequences into the known MVA vector (*see, e.g.: Goepfert et al. (2014) J. Infect. Dis.* 210(1): 99-110, and see WO2006026667, both of which are incorporated herein by reference). The term “HIV vaccine” also includes any one or more vaccines provided in Table 1, below and in any similar tables contained in the priority documents (all of which are incorporated herein in their entirety).

Table 1

IAVI Clinical Trial ID*	Prime**
HVTN 704 AMP	VRC-HIVMAB060-00-AB
VAC89220HPX2004	Ad26.Mos.HIV Trivalent
01-I-0079	VRC4302
04/400-003-04	APL 400-003 GENEVAX-HIV
10-1074	10-1074
87 I-114	gp160 Vaccine (Immuno-AG)
ACTG 326; PACTG 326	ALVAC vCP1452
Ad26.ENVA.01	Ad26.EnvA-01
Ad5HVR48.ENVA.01	Ad5HVR48.ENVA.01
ANRS VAC 02	rgp 160 + peptide V3 ANRS VAC 02
ANRS VAC 04	LIPO-6
ANRS VAC 05	ALVAC vCP125
ANRS VAC 07	ALVAC vCP300
ANRS VAC 08	ALVAC-HIV MN120TMG strain (vCP205)
ANRS VAC 09 bis	LIPO-6
ANRS VAC 12	LPHIV1
ANRS VAC 14	gp160 MN/LAI
ANRS VAC 16	LPHIV1
ANRS VAC 18	LIPO-5
APL 400-003RX101	APL 400-003 GENEVAX-HIV

AVEG 002	HIVAC-1e
AVEG 003	VaxSyn gp160 Vaccine (MicroGeneSys)
AVEG 004	gp160 Vaccine (Immuno-AG)
AVEG 005A/B	Env 2-3
AVEG 006X; VEU 006	MN rgp120
AVEG 007A/B	rgp120/HIV-1 SF-2
AVEG 011	UBI HIV-1 Peptide Immunogen, Multivalent
AVEG 013A	gp160 Vaccine (Immuno-AG)
AVEG 014A/B	TBC-3B
AVEG 017	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
AVEG 019	p17/p24:Ty- VLP
AVEG 020	gp120 C4-V3
AVEG 021	P3C541b Lipopeptide
AVEG 022	ALVAC-HIV MN120TMG strain (vCP205)
AVEG 028	Salmonella typhi CVD 908-HIV-1 LAI gp 120
AVEG 031	APL 400-047
AVEG 034/034A	ALVAC vCP1433
C060301	GTU-MultiHIV
C86P1	HIV gp140 ZM96
Cervico-vaginal CN54gp140-hsp70 Conjugate Vaccine (TL01)	CN54gp140
CM235 and SF2gp120	CM235 (ThaiE) gp120 plus SF2(B) gp120
CombiHIVvac (KombiVICHvak)	CombiHIVvac
CRC282	P2G12
CUTHIVAC002	DNA-C CN54ENV
DCVax-001	DCVax-001
DNA-4	DNA-4
DP6?001	DP6?001 DNA
DVP-1	EnvDNA

EN41-UGR7C	EN41-UGR7C
EnvPro	EnvPro
EuroNeut41	EN41-FPA2
EV01	NYVAC-C
EV02 (EuroVacc 02)	DNA-C
Extention HVTN 073E/SAAVI 102	Sub C gp140
F4/AS01	F4/AS01
FIT Biotech	GTU-Nef
Guangxi CDC DNA vaccine	Chinese DNA
HGP-30 memory responses	HGP-30
HIV-CORE002	ChAdV63.HIVconsv
HIV-POL-001	MVA-mBN32
HIVIS 01	HIVIS-DNA
HIVIS 02	MVA-CMDR
HVRF-380-131004	Vichrepol
HVTN 040	AVX101
HVTN 041	rgp120w61d
HVTN 044	VRC-HIVDNA009-00-VP
HVTN 045	pGA2/JS7 DNA
HVTN 048	EP HIV-1090
HVTN 049	Gag and Env DNA/PLG microparticles
HVTN 050/Merck 018	MRKAd5 HIV-1 gag
HVTN 052	VRC-HIVDNA009-00-VP
HVTN 054	VRC-HIVADV014-00-VP
HVTN 055	TBC-M335
HVTN 056	MEP
HVTN 059	AVX101
HVTN 060	HIV-1 gag DNA
HVTN 064	EP HIV-1043
HVTN 065	pGA2/JS7 DNA
HVTN 067	EP-1233

HVTN 070	PENNVAX-B
HVTN 072	VRC-HIVDNA044-00-VP
HVTN 073	SAAVI DNA-C2
HVTN 076	VRC-HIVDNA016-00-VP
HVTN 077	VRC-HIVADV027-00-VP
HVTN 078	NYVAC-B
HVTN 082	VRC-HIVDNA016-00-VP
HVTN 084	VRC-HIVADV054-00-VP
HVTN 086, SAAVI 103	SAAVI MVA-C
HVTN 087	HIV-MAG
HVTN 088	Oligomeric gp140/MF59
HVTN 090	VSV-Indiana HIV gag vaccine
HVTN 092	DNA-HIV-PT123
HVTN 094	GEO-D03
HVTN 096	DNA-HIV-PT123
HVTN 097	ALVAC-HIV vCP1521
HVTN 100	ALVAC-HIV-C (vCP2438)
HVTN 101	DNA-HIV-PT123
HVTN 104	VRC-HIVMAB060-00-AB
HVTN 105	AIDSVAX B/E
HVTN 106	DNA Nat-B env
HVTN 110	Ad4-mgag
HVTN 112	HIV-1 nef/tat/vif, env pDNA vaccine
HVTN 116	VRC-HIVMAB060-00-AB
HVTN 205	pGA2/JS7 DNA
HVTN 702	ALVAC-HIV-C (vCP2438)
HVTN 703 AMP	VRC-HIVMAB060-00-AB
HVTN 908	pGA2/JS7 DNA
IAVI 001	DNA.HIVA
IAVI 016	MVA.HIVA
IAVI A001	tgAAC09

IAVI A003	AAV1-PG9
IAVI B001	Ad35-GRIN/ENV
IAVI B002	Adjuvanted GSK investigational HIV vaccine formulation 1
IAVI B003	Ad26.EnvA-01
IAVI B004	HIV-MAG
IAVI C001	ADVAX
IAVI C002	ADMVA
IAVI D001	TBC-M4
IAVI N004 HIV-CORE 004	Ad35-GRIN
IAVI R001	rcAd26.MOS1.HIVEnv
IAVI S001	SeV-G
IDEA EV06	DNA-HIV-PT123
IHV01	Full-Length Single Chain (FLSC)
IMPAACT P1112	VRC-HIVMAB060-00-AB
IPCAVD006	MVA mosaic
IPCAVD008	Trimeric gp140
IPCAVD009	Ad26.Mos.HIV Trivalent
ISS P-001	Tat vaccine
LFn-p24 vaccine	LFn-p24
MCA-0835	3BNC117
Mucovac2	CN54gp140
MV1-F4	Measles Vector - GSK
MYM-V101	Virosome-Gp41
NCHECR-AE1	pHIS-HIV-AE
PEACHI-04	ChAdV63.HIVconsv
PedVacc001 & PedVacc002	MVA.HIVA
PolyEnv1	PolyEnv1
PXVX-HIV-100-001	Ad4-mgag
RISVAC02	MVA-B
RV 151 / WRAIR 984	LFn-p24
RV 158	MVA-CMDR

SG06RS02	HIV gp140 ZM96
TAB9	TAB9
TaMoVac II	HIVIS-DNA
UBI V106	UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent
UCLA MIG-001	TBC-3B
UKHVCSpoke003	DNA - CN54ENV and ZM96GPN
V3-MAPS	V3-MAPS
VAX 002	AIDSVAX B/B
VAX 003	AIDSVAX B/E
VRC 602	VRC-HIVMAB060-00-AB
VRC 607	VRCHIVMAB080-00-AB

*IAVI is the International AIDS Vaccine Initiative, whose clinical trials database is publicly available at <http://www.iavi.org/trials-database/trials>.

** As used herein, the term “Prime” refers to the composition initially used as an immunological inoculant in a given clinical trial as referenced in Table 1 herein.

[0051] As used herein, the term “*in vivo*” refers to processes that occur in a living organism. The term “*ex vivo*” refers to processes that occur outside of a living organism. For example, *in vivo* treatment refers to treatment that occurs within a patient’s body, while *ex vivo* treatment is one that occurs outside of a patient’s body, but still uses or accesses or interacts with tissues from that patient. Thereafter, an *ex vivo* treatment step may include a subsequent *in vivo* treatment step.

[0052] As used herein, the term “miRNA” refers to a microRNA, and also may be referred to herein as “miR”. The term “microRNA cluster” refers to at least two microRNAs that are situated on a vector in close proximity to each other and are co-expressed.

[0053] As used herein, the term “packaging cell line” refers to any cell line that can be used to express a lentiviral particle.

[0054] As used herein, the term “PBMC” refers to peripheral blood mononuclear cells.

[0055] As used herein, the term “percent identity,” in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms

available to persons of ordinary skill in the art) or by visual inspection. Depending on the application, the “percent identity” can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0056] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

[0057] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

[0058] The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (*CABIOS*, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0059] The nucleic acid and protein sequences of the present disclosure can further be used as a “query sequence” to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, word length = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the disclosure. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

[0060] As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0061] As used herein, the term “pharmaceutically acceptable carrier” refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, *e.g.*, an acid addition salt or a base addition salt (*see, e.g.*, Berge *et al.* (1977) *J Pharm Sci* 66:1-19).

[0062] As used herein, the term “physical method of selection” refers to any physical method that can be used to positively or negatively select for a cell type within a larger mixture of cells (*e.g.*, PBMC). A non-limiting example of a physical method of selection is magnetic bead sorting.

[0063] As used herein, the term “SEQ ID NO” is synonymous with the term “Sequence ID No.”

[0064] As used herein, the term “small RNA” refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or

less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). “Small RNA” of the disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, for example through pathways that result in the destruction of the target gene mRNA.

[0065] As used herein, the term “static culture” refers to a cell culture environment which is not physically agitated, rocked, or otherwise subjected to intentional movement for the duration of the cell culture interval.

[0066] As used herein, the term “semi-static culture” refers to a cell culture environment which is subjected to minimal intentional movement, rocking, or physical agitation for the duration of the cell culture interval.

[0067] As used herein, the term “stimulatory agent” refers to any exogenous agent that can stimulate an immune response, and includes, without limitation, a vaccine, a HIV vaccine, and HIV or HIV-related peptides. A stimulatory agent can preferably stimulate a T cell response.

[0068] As used herein, the term “subject” includes a human patient but also includes other mammals. The terms “subject,” “individual,” “host,” and “patient” may be used interchangeably herein.

[0069] As used herein, the term “T regulatory cells” refers to a subpopulation of immunosuppressive T cells that derive from progenitor cells in the bone marrow and mature in the thymus. T regulatory cells can be identified by the expression of the markers CD4, CD25, and/or FOXP3.

[0070] As used herein, the phrase “target sequence” includes any nucleotide sequence capable of being targeted by a regulatory factor. An example of a target sequence includes, but is not limited to, mRNA. Examples of regulatory factors include any regulatory RNA such as, but not limited to, microRNA and shRNA.

[0071] As used herein, the term “Tat” refers to the HIV *tat* gene and its gene product, and variants thereof.

[0072] As used herein, the term “therapeutically effective amount” refers to a sufficient quantity of the active agents of the present disclosure, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient’s

condition or its severity, and the age, weight, *etc.*, of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, *e.g.*, the route of administration, the condition of the subject, as well as other factors understood by those in the art.

5 [0073] As used herein, the term “therapeutic vector” is synonymous with a lentiviral vector such as the AGT103 vector.

[0074] As used herein, the terms “treatment” or “treating” generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable
10 effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

[0075] As used herein, the term “vaccine”, which is used interchangeably with the term
15 “therapeutic vaccine” refers to an exogenous agent that can elicit an immune response in an individual and includes, without limitation, purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, or virus-like particles (VLPs).

[0076] As used herein, the term “variant” refers to a nucleotide sequence that, when
20 compared to a reference sequence, contains at least one of a single nucleotide polymorphism, a single nucleotide variation, a conversion, an inversion, a duplication, a deletion, or a substitution. A “variant” includes amino acid sequences that derive from “variant” nucleotide sequences, as well as post-transcriptional and post translational modifications related thereto.

25 [0077] The term “Vif” refers to the HIV *vif* gene and its gene product, and variants thereof.

Description of Aspects of the Disclosure

[0078] As detailed herein, in one aspect, a method of treating cells infected with HIV is provided. The method generally includes contacting peripheral blood mononuclear cells
30 (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting step is carried out *ex vivo*; depleting non-target cell populations; transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a period of time

sufficient to achieve such transduction. In embodiments, the transduced PBMC are cultured from about 1 to about 35 days. In embodiments, the method further includes infusing the transduced PBMC into a subject. In embodiments, the subject is a human. In embodiments, the stimulatory agent is a peptide or mixture of peptides, and in embodiments, includes a gag peptide. In further embodiments, the stimulatory agent includes a vaccine. In embodiments, the vaccine is a HIV vaccine, and in further embodiments, the HIV vaccine is a MVA/HIV62B vaccine, or a variant or analog thereof. In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In
5 embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In other embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the HIV RNA sequence includes a HIV Vif sequence, a HIV Tat sequence, variants thereof, or RNA
10 sequences from other HIV genes that prevent HIV infection or reduce virus expression in already-infected cells. In embodiments, the at least one genetic element includes at least one of a microRNA or a shRNA. In further embodiments, the at least one genetic element comprises a microRNA cluster.

[0079] In embodiments, the at least one genetic element includes a microRNA having at
20 least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 1. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 1.
25 In embodiments, the at least one genetic element includes SEQ ID NO: 1.

[0080] In embodiments, the at least one genetic element includes a microRNA having at
least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 2. In
30 embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 2. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 2. In embodiments, the at least one genetic element includes SEQ ID NO: 2.

[0081] In embodiments, the at least one genetic element includes a microRNA having at

least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 3. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 3. In
5 embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 3. In embodiments, the at least one genetic element includes SEQ ID NO: 3.

[0082] In embodiments, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at
10 least 94%, at least 95% or more percent identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has less than 80% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has greater than 95% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster includes SEQ ID NO: 30.

[0083] In another aspect, a method is disclosed which includes obtaining peripheral blood
15 from HIV+ individuals; fractionating the blood to obtain a PBMC population; contacting the PBMC population with purified antigen-presenting cells or peptides or proteins representing components of HIV; culturing the contacted PBMC population for about 1 to about 12 days to expand an antigen-specific population; depleting one or more non-target cell populations to produce a fraction enriched with cells that respond to peptide stimulation; transducing the
20 enriched cell fraction *ex vivo* with a viral delivery system as detailed herein; culturing the transduced cell fraction for a period of time sufficient to ensure adequate cell proliferation; and harvesting the transduced cells.

[0084] In embodiments, the PBMC population is further purified to produce a purified fraction of PBMC. In embodiments, further purified fractions of PBMC are contacted with
25 peptides or proteins representing components of HIV.

[0085] In another aspect, a method is disclosed that includes contacting lymphocytes with at least one HIV peptide and transducing the lymphocytes with a viral delivery system encoding at least one genetic element.

[0086] In embodiments, the lymphocytes that are contacted comprise any one or more of
30 T cells, B cells, and NK cells. In embodiments, the lymphocytes that are contacted comprise only T cells. In embodiments, the T cells comprise any one or more of CD4+ T cells, CD8+ T cells, and $\gamma\delta$ cells. In embodiments, the T cells comprise only CD4+ T cells.

[0087] In embodiments, the lymphocytes that are contacted are derived from PBMC. In

embodiments, the method further includes depleting at least one subset of cells in the PBMC. In embodiments, the at least one subset of cells that are depleted includes any one or more of B cells, NK cells, CD8+ T cells, and $\gamma\delta$ cells.

5 [0088] In embodiments, the lymphocytes that are contacted are derived from a population of leukocytes. In embodiments, the method further comprises depleting at least one subset of cells in the leukocytes. In embodiments, the at least one subset of cells that are depleted includes any one or more of neutrophils, eosinophils, basophils, monocytes, and certain lymphocytes including any one or more of NK cells, B cells, CD8+ T cells, and $\gamma\delta$ cells.

10 [0089] In embodiments, the lymphocytes that are contacted are derived from a population of cells. In embodiments, the method further comprises depleting at least one subset of cells from the population of cells. In embodiments, the at least one subset of cells that are depleted includes any one or more of CD8+T cells, $\gamma\delta$ cells, NK cells, B cells, neutrophils, basophils, mast cells, monocytes, dendritic cells, T regulatory cells, NKT cells, and erythrocytes. In embodiments, the at least one subset of cells that are depleted includes any
15 one or more of any cell type that is not a CD4+ T cell.

[0090] In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the lentiviral particle is any lentiviral particle described herein.

[0091] In another aspect, a method of manufacturing a cell product for treating HIV infection in a subject is disclosed. The method generally includes obtaining blood
20 leukocytes; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) or defined fractions of PBMC. In embodiments, the method further includes contacting the PBMC or purified fraction of PBMC *ex vivo* with a therapeutically effective amount of a stimulatory agent; depleting one or more non-target cell populations in order to increase the proportion of antigen-specific T cells; transducing
25 the PBMC or purified fraction of PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; culturing the transduced PBMC or a purified fraction of PBMC for a period of time sufficient to achieve transduction and growth of the modified cell population; and harvesting the transduced PBMC or a purified fraction of PBMC. In embodiments, the transduced PBMC or purified fraction of PBMC are cultured from about 1
30 to about 35 days. In embodiments, the method further involves infusing the transduced PBMC or purified fraction of PBMC into a subject. In embodiments, the subject is a human. In embodiments, the at least one of the first stimulatory agents includes a peptide or mixture of peptides, represents one, two, three or more of proteins encoded by the HIV genome, and

includes multiple examples of individual peptides to represent sequence variation among known HIV isolates from the country or region where treatment is intended or to accommodate unusual variation among histocompatibility genes for specific patient populations. In embodiments, at least one of the first stimulatory agents includes a gag peptide. In embodiments, the at least one of the first stimulatory agents includes a vaccine. In embodiments, the vaccine is a HIV vaccine, and in further embodiments, the HIV vaccine is a MVA/HIV62B vaccine, or a variant or analog thereof. In embodiments, the first stimulatory agent is a mixture of gag peptides.

[0092] In embodiments, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5. In embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the HIV RNA sequence includes a HIV Vif sequence, a HIV Tat sequence, or variants thereof. In embodiments, the at least one genetic element includes a microRNA or a shRNA, or a cluster thereof. In embodiments, the at least one genetic element comprises a synthetic microRNA cluster.

[0093] In embodiments, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 1. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 1. In embodiments, the at least one genetic element includes SEQ ID NO: 1.

[0094] In embodiments, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 2. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 2. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 2. In embodiments, the at least one genetic element includes SEQ ID NO: 2.

[0095] In embodiments, the at least one genetic element includes a microRNA having at

least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 3. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 3. In
5 embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 3. In embodiments, the at least one genetic element includes SEQ ID NO: 3.

[0096] In embodiments, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at
10 least 94%, at least 95% or more percent identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has less than 80% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has greater than 95% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster includes SEQ ID NO: 30.

[0097] In another aspect, a method of manufacturing a cell product for treating HIV in a
15 subject is disclosed. In embodiments, the method includes contacting PBMC or a purified fraction of PBMC with at least one antigen and depleting one or more non-target cell populations in the PBMC. In embodiments, the one or more non-target cell populations includes any one or more of B cells, NK cells, CD8⁺ T cells, and $\gamma\delta$ cells. In embodiments, the at least one antigen is a peptide or mixture of peptides encoded by the HIV genome. In
20 embodiments, the peptide is a gag peptide or the mixture of peptides comprises a gag peptide.

[0098] In another aspect, a method of manufacturing a cell product for treating HIV in a subject is disclosed. In embodiments, the method includes contacting leukocytes or a purified fraction of leukocytes with at least one antigen and depleting one or more non-target
25 cell populations of leukocytes. In embodiments, the one or more non-target cell populations includes any one or more of neutrophils, basophils, eosinophils, and monocytes. In embodiments, the one or more non-target cell populations further includes specific lymphocytes including any one or more of B cells, NK cells and CD8⁺ T cells. In embodiments, the at least one antigen is a peptide or a mixture of peptides encoded by the
30 HIV genome. In embodiments, the peptide is a gag peptide or the mixture of peptides comprises a gag peptide.

[0099] In another aspect, a lentiviral vector is disclosed. The lentiviral vector includes at least one encoded genetic element, wherein the at least one encoded genetic element

comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence.

[0100] In another aspect a lentiviral vector is disclosed in the at least one encoded genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, a sequence from another portion of the HIV genome, or a variant thereof. The at least one encoded genetic element may include a microRNA or a shRNA. The at least one encoded genetic element may include a microRNA cluster.

[0101] In embodiments, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 1. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 1. In embodiments, the at least one genetic element includes SEQ ID NO: 1.

[0102] In embodiments, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 2. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 2. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 2. In embodiments, the at least one genetic element includes SEQ ID NO: 2.

[0103] In embodiments, the at least one genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 3. In embodiments, the microRNA has less than 80% sequence identity with SEQ ID NO: 3. In embodiments, the microRNA has greater than 95% sequence identity with SEQ ID NO: 3. In embodiments, the at least one genetic element includes SEQ ID NO: 3.

[0104] In embodiments, the microRNA cluster includes a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at

least 94%, at least 95% or more percent identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has less than 80% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster has greater than 95% sequence identity with SEQ ID NO: 30. In embodiments, the microRNA cluster includes SEQ ID NO: 30.

5 [0105] In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector as described herein; at least one envelope plasmid for expressing an envelope protein preferably optimized for infecting a cell; and at least one helper plasmid for expressing a gene of interest, for example any of gag, pol, and rev genes, wherein when the lentiviral vector, the at least one envelope plasmid, and the at
10 least one helper plasmid are transfected into a packaging cell, wherein a lentiviral particle is produced by the packaging cell, wherein the lentiviral particle is capable of modulating a target sequence of interest, for example inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

[0106] In embodiments, the envelope protein is optimized for infecting an endocytic
15 compartment. In embodiments, the envelope protein is any of the envelope proteins described herein.

[0107] In another aspect, a lentiviral particle capable of infecting a cell is disclosed. The lentiviral particle includes at least one envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. The envelope protein may be optimized for
20 infecting a T cell. In embodiments, the envelope protein is optimized for infecting a CD4+ T cell.

[0108] In embodiments, the particle is a pseudotyped particle. In embodiments, the pseudotyped particle is any pseudotyped particle described herein. In embodiments, the pseudotyped particle comprises an envelope protein from a virus that is not a lentivirus. In
25 embodiments, the virus from which envelope protein derives is any virus described herein.

[0109] In another aspect, a modified cell is disclosed. In embodiments, the modified cell is a CD4+ T cell. In embodiments, the CD4+ T cell is infected with a lentiviral particle as described herein. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen based on the prior immunization with a stimulatory agent. In a further
30 embodiment, the HIV antigen that is recognized by the CD4+ T cell includes a gag antigen. In a further embodiment, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

[0110] In another aspect, a modified cell is disclosed. In embodiments, the modified cell

is a CD4⁺ T cell that has been infected with a lentiviral particle and an exogenous antigen. In embodiments, the lentiviral particle is any lentiviral particle described herein. In embodiments, the exogenous antigen is an antigen derived from HIV. In embodiments, the antigen derived from HIV is any HIV antigen described herein.

5 [0111] In another aspect, a method of selecting a subject for a therapeutic treatment regimen is disclosed. The method generally includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC) and determining a first quantifiable measurement associated with at least one factor associated with the PBMC; contacting the
10 PBMC *ex vivo* with a therapeutically effective amount of a second stimulatory agent, and determining a second measurement associated with the at least one factor associated with the PBMC, whereby when the second quantifiable measurement is different (*e.g.*, higher) than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may be T cell proliferation or IFN gamma production.

15 [0112] In another aspect, a method is disclosed. The method includes purifying peripheral blood mononuclear cells (PBMC) from a source, stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells; transducing the depleted PBMC with a
20 viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC.

[0113] In embodiments, the purifying the PBMC comprises removing any one or more of residual red blood cells, residual platelets, and residual inflammatory cells. In embodiments, the purifying comprises separating any one or more of residual red blood cells, residual
25 platelets, and residual inflammatory cells on the basis of cell density. In embodiments, the separating on the basis of cell density comprises differential centrifugation. However, any suitable method of purifying and/or separating the PBMC is contemplated.

[0114] In embodiments, the purifying comprises separating any one or more of residual red blood cells, residual platelets, and residual inflammatory cells using magnetic bead
30 separation. In embodiments, the purifying comprises separating other contaminants from the PBMC using magnetic bead separation. In embodiments, the magnetic bead separation comprises using antibodies to aid in positive selection of leukocytes. In embodiments, the magnetic bead separation comprises using antibodies to aid in negative selection of residual

red blood cells, residual platelets residual inflammatory cells, and/or other contaminants. In embodiments, the antibodies are monoclonal antibodies. In embodiments, the purifying the PBMC comprises using an automated system.

5 [0115] In embodiments, the transduced PBMC are cultured for more than one day, for example, more than 24 hours, more than 30 hours, more than 36 hours, more than 42 hours, more than 48 hours, more than 48 hours, more than 54 hours, more than 60 hours, more than 66 hours, more than 72 hours, more than 78 hours, more than 84 hours, more than 90 hours, or more than 96 hours.

10 [0116] In embodiments, the culturing occurs in a static culture system. In embodiments, the culturing occurs in a semi-static culture system. In embodiments, the cultured cells adhere to the culture plate or flask. In embodiments, the cultured cells are in a suspension. In embodiments, some of the cultured cells adhere to the culture plate or flask and some cells are in a suspension.

15 [0117] In embodiments, the at least one HIV-specific peptide comprises a pool of synthetic peptides. In embodiments, synthetic peptides of the pool of synthetic peptides overlap. In embodiments, synthetic peptides of the pool of synthetic peptides do not overlap. In embodiments, the pool of synthetic peptides comprises approximately 150 individual peptides. In embodiments, the pool of peptides may include sequence variants of individual peptides that are included to match the prevailing HIV sequences found in a particular region or sub-population, or to better match the requirements for peptide binding and T cell receptor recognition dictated by particular haplotypes in the major histocompatibility gene clusters. In embodiments, the pool of synthetic peptides comprises less than 150 individual peptides, for example, less than 140 individual peptides, less than 130 individual peptides, less than 120 individual peptides, less than 110 individual peptides, less than 100 individual peptides, less than 90 individual peptides, less than 80 individual peptides, less than 70 individual peptides, less than 60 individual peptides, or less than 50 individual peptides. In
25 embodiments, the pool of synthetic peptides comprises more than 150 individual peptides, for example, more than 160 individual peptides, more than 170 individual peptides, more than 180 individual peptides, more than 190 individual peptides, more than 200 individual peptides, more than 210 individual peptides, more than 220 individual peptides, more than 230 individual peptides, more than 240 individual peptides, or more than 250 individual peptides.
30

[0118] In embodiments, the pool of synthetic peptides represents the HIV Gag

polyprotein. However, the pool of synthetic peptides may represent any HIV-specific peptide.

[0119] In embodiments, stimulating the PBMC with at least one HIV-specific peptide comprises infecting PBMC with recombinant vaccinia. In embodiments, the vaccinia expresses Gag proteins. In embodiments, the vaccinia comprises any one or more of Gag proteins, Pol proteins, and Env proteins. In embodiments, the vaccinia targets white blood cells. In embodiments, the vaccinia targets any one or more of neutrophils, eosinophils, basophils, lymphocytes and monocytes. In embodiments, the vaccinia targets macrophages. In embodiments, the vaccinia targets any antigen presenting cell. In embodiments, the antigen presenting cell is a B cell. In embodiments, the antigen presenting cell is a dendritic cell.

[0120] In embodiments, stimulating the PBMC with at least one HIV-specific peptide comprises adding a purified Gag protein directly to PBMC culture. In embodiments, the purified Gag protein comprises a vaccine. In embodiments, the purified Gag protein comprises Gag p24 protein vaccine. In embodiments, stimulating the PBMC with at least one HIV-specific peptide comprises adding a purified Env protein directly to PBMC culture. In embodiments, stimulating the PBMC with at least one HIV-specific peptide comprises adding a purified Pol protein directly to PBMC culture.

[0121] In embodiments, stimulating the PBMC with at least one HIV-specific peptide comprises adding customized peptides that comprise various sequence variations of known HIV sequences. In embodiments the customized peptides comprise an array of peptides.

[0122] In embodiments, the depleting comprises separating the at least one subset of cells from the depleted PBMC. In embodiments, the at least one subset of cells comprises CD8⁺ T cells. In embodiments, the at least one subset of cells comprises CD8⁺ T cells, CD56⁺ NK cells, B cells, and $\gamma\delta$ cells. In embodiments, the at least one subset of cells comprises CD8⁺ T cells and $\gamma\delta$ cells. In embodiments, the at least one subset of cells comprises CD8⁺ T cells, $\gamma\delta$ cells, and B cells. However, the at least one subset of cells may comprise any subset of cells present in the PBMC including, without limitation, any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells.

[0123] In embodiments, the separating comprises magnetic bead separation. In embodiments, depleting comprises a method of using iron-labeled antibodies against cell surface markers to identify any unwanted subset of cells. In embodiments, the method further comprises removing the unwanted subset of cells by retaining them on a magnetic

column.

[0124] In embodiments, the depleting comprises positive selection for Gag-responsive cells that are secreting interferon-gamma. In embodiments, the Gag-responsive cells are macrophages, B lymphocytes, and/or T lymphocytes. In embodiments, the Gag-responsive
5 cells secrete other types of interferons such as interferon-alpha, interferon-beta, or interferon-kappa.

[0125] In embodiments, the depleting comprises utilizing unlabeled antibodies that identify unwanted cell types. In embodiments, the method further comprises retaining antibody-bound cells on a column. In embodiments, the column is coated with Protein A.
10 In embodiments, the column is coated with Protein G. In embodiments, the column is coated with anti-immunoglobulin or another immunoglobulin binding compound that removes effectively the cells bound to the targeting antibodies.

[0126] In embodiments, the depleting comprises purifying target cells based on density. In embodiments, the depleting comprises using density gradient centrifugation.

15 [0127] In embodiments, the depleting comprises labeling unwanted leukocytes with primary antibodies and killing the cell with secondary antibodies that bind to constant regions of the monoclonal antibodies. In embodiments, the secondary antibody is chemically linked to a toxin. In embodiments, the toxin is Diphtheria toxin. In embodiments, the antibodies are monoclonal. In embodiments, the antibodies are polyclonal.

20 [0128] In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the HIV RNA sequence comprises a HIV Vif sequence, a HIV Tat sequence, or a variant thereof.

[0129] In embodiments, the at least one genetic element comprises a non-coding RNA. In
25 embodiments, the non-coding RNA is a microRNA. In embodiments, the non-coding RNA is a siRNA. In embodiments, the non-coding RNA is a piRNA. In embodiments, the non-coding RNA is a snoRNA. In embodiments, the non-coding RNA is a snRNA. In embodiments, the non-coding RNA is a exRNA. In embodiments, the at least one genetic element comprises a microRNA or a shRNA.

30 [0130] In embodiments, the at least one genetic element comprises a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 30. In embodiments, the at least one genetic element comprises a microRNA having less than 80% identity with at least

one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 30, for example, 75% identity, 70% identity, 65% identity, 60% identity, 55% identity, or 50% identity.

[0131] In another aspect, a genetically-modified lymphocyte for treatment of a subject infected with HIV is disclosed. The genetically-modified lymphocyte is prepared by a process comprising the steps of purifying peripheral blood mononuclear cells (PBMC) from the subject, stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, T regulatory cells, and NKT cells, transducing the depleted PBMC with a viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC.

[0132] In embodiments, the subject infected with HIV is in the latent period of HIV infection. In embodiments, the subject infected with HIV is experiencing few or no clinical symptoms of the HIV infection. In embodiments, the subject infected with HIV is in the acute phase of HIV infection. In embodiments, the subject infected with HIV is in the chronic phase of the HIV infection. In embodiments, the subject infected with HIV has experienced viremia in which HIV has entered the bloodstream. In embodiments, the subject infected with HIV has a depleted CD4⁺ T cells as a result of the HIV infection. In embodiments, the subject infected with HIV has a depleted CD4⁺ T cells in any one or more of the peripheral blood, the gastrointestinal tract, the lymph nodes, and the lymphatic tissue. In embodiments, the CD4⁺ T cells are preferentially depleted in the gastrointestinal tract. In embodiments, the subject infected with HIV has depleted CCR5⁺ CD4⁺ T cells in any one or more the peripheral blood, the gastrointestinal tract, the lymph nodes, and the lymphatic tissue.

[0133] In another aspect, a genetically-modified lymphocyte for treatment of a subject immunized with a preventive or therapeutic HIV vaccine is disclosed. The genetically-modified lymphocyte is prepared by a process comprising the steps of purifying peripheral blood mononuclear cells (PBMC) from the subject, stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, neutrophils, T regulatory cells, and NKT cells, transducing the depleted PBMC with a viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC.

[0134] In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that comprises a whole organism. In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that comprises a live-attenuated organism. In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that comprises an antiretroviral. In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that comprises an antibody. In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that causes stimulation of T cell immunity. In embodiments, the subject immunized with a preventive or therapeutic vaccine is immunized with a vaccine that stimulates endogenous antibodies. In embodiments, the subject immunized with a preventive or therapeutic HIV vaccine is immunized with RV 144 prime and boost vaccines.

[0135] In another aspect, a genetically-modified lymphocyte for treatment of a subject exposed to, but not infected with HIV, or made immune to HIV through preventive vaccination, is disclosed. These individuals may have been exposed to infectious HIV one or multiple times particularly through contact with genital fluids and subsequently developed an immune response against HIV but are not-infected by diagnostic criteria including assay for plasma viral RNA or isolation of infectious virus from PBMC. These individuals may have received one or more HIV preventive vaccines in a single or multiple doses. Because they have detectable HIV immunity it is possible to manufacture the AGT103-T product and by infusing genetically-modified lymphocytes, increase the level of HIV immunity and make it sufficiently durable to withstand subsequent exposures to infectious HIV. In embodiments, the genetically-modified lymphocyte is prepared by a process comprising the steps of purifying peripheral blood mononuclear cells (PBMC) from the subject; stimulating the PBMC with at least one HIV-specific peptide, depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells, transducing the depleted PBMC with a viral delivery system encoding at least one genetic element, culturing the transduced PBMC for at least one day, and harvesting the cultured PBMC.

Human Immunodeficiency Virus (HIV)

[0136] Human Immunodeficiency Virus, which is also commonly referred to as “HIV”, is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS is a

condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending upon the HIV subtype and genetics of the host population. Infection with HIV occurs by the transfer of bodily fluids, including but not limited to blood, semen, vaginal fluid, pre-ejaculate, saliva, tears, lymph or cerebro-spinal fluid, or breast milk, or use of contaminated blood or tissue products. HIV may be present in an infected individual as both free virus particles and within infected immune cells.

[0137] HIV infects vital cells in the human immune system such as helper T cells, although tropism can vary among HIV subtypes. Immune cells that may be specifically susceptible to HIV infection include but are not limited to CD4⁺ T cells, macrophages, and dendritic cells. HIV infection leads to low levels of CD4⁺ T cells through a number of mechanisms, including but not limited to apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected CD4⁺ T cells by cytotoxic lymphocytes that recognize infected cells. When CD4⁺ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections and cancer.

[0138] Structurally, HIV is distinct from many other retroviruses. The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and at least nine genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu, and sometimes a tenth tev, which is a fusion of tat, env and rev), encoding 19 proteins. Three of these genes, gag, pol, and env, contain information needed to make the structural proteins for new virus particles.

[0139] HIV replicates primarily in CD4 T cells and causes cellular destruction or dysregulation to reduce host immunity. Because HIV establishes infection as an integrated provirus and may enter a state of latency wherein virus expression in a particular cell decreases below the level required for cytopathology affecting that cell or detection by the host immune system, HIV is difficult to treat and has not been eradicated even after prolonged intervals of combination antiretroviral therapy (cART). In most cases, HIV infection causes fatal disease although survival may be prolonged by cART.

[0140] A major goal in the fight against HIV is to develop strategies for curing disease. Prolonged cART has not accomplished this goal, so investigators have turned to alternative procedures. Early efforts to improve host immunity by therapeutic immunization (using a vaccine after infection has occurred) had marginal or no impact. Likewise, treatment

intensification had moderate or no impact.

[0141] Some progress has been made using genetic therapy, but positive results are sporadic and found only among rare human beings carrying defects in one or both alleles of the gene encoding CCR5, which plays a critical role in viral penetration of host cells.

5 However, many investigators are optimistic that genetic therapy holds the best promise for eventually achieving an HIV cure.

[0142] The methods and compositions of the disclosure are able to achieve a cure that may or may not include complete eradication of all HIV from the body. As mentioned above, a cure is defined as a state or condition wherein HIV+ individuals who previously
10 required cART, may survive with low or undetectable virus replication and using lower or intermittent doses of cART, or are potentially able to discontinue cART altogether. As used herein, a cure may still possibly require adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a cure is the eventual eradication of HIV to prevent all possibility of recurrence.

15 [0143] The primary obstacles to achieving a cure lie in the basic biology of HIV itself. Virus infection deletes CD4 T cells that are critical for nearly all immune functions. Most importantly, HIV infection and depletion of CD4 T cells requires activation of individual cells. Activation is a specific mechanism for individual CD4 T cell clones that recognize pathogens or other molecules, using a rearranged T cell receptor.

20 [0144] In the case of HIV, infection activates a population of HIV-specific T cells that become infected and are consequently depleted before other T cells that are less specific for the virus, which effectively cripples the immune system's defense against the virus. The capacity for HIV-specific T cell responses is rebuilt during prolonged cART; however, when cART is interrupted the rebounding virus infection repeats the process and again deletes the
25 virus-specific cells, resetting the clock on disease progression.

[0145] A cure is possible if enough HIV-specific CD4 T cells are protected to allow for a host's native immunity to confront and control HIV once cART is interrupted. In one embodiment, the present disclosure provides methods and compositions for improving the effectiveness of genetic therapy to provide a cure of HIV disease. In another embodiment,
30 the present disclosure provides methods and compositions for enhancing host immunity against HIV to provide a cure. In yet another embodiment, the present disclosure provides methods and compositions for enriching HIV-specific CD4 T cells in a patient to achieve a cure.

[0146] In embodiments, treatment results in enriching a subject's HIV-specific CD4 T cells by about 100%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1000%.

Gene Therapy

5 [0147] Viral vectors are used to deliver genetic constructs to host cells for the purposes of disease therapy or prevention.

[0148] Genetic constructs can include, but are not limited to, functional genes or portions of genes to correct or complement existing defects, DNA sequences encoding regulatory proteins, DNA sequences encoding regulatory RNA molecules including antisense, short
10 homology RNA, long non-coding RNA, small interfering RNA or others, and decoy sequences encoding either RNA or proteins designed to compete for critical cellular factors to alter a disease state. Gene therapy involves delivering these therapeutic genetic constructs to target cells to provide treatment or alleviation of a particular disease.

[0149] There are multiple ongoing efforts to utilize genetic therapy in the treatment of
15 HIV disease, but thus far, the results have been poor. A small number of treatment successes were obtained in rare HIV patients carrying a spontaneous deletion of the CCR5 gene (an allele known as CCR5delta32).

[0150] Lentivirus-delivered nucleases or other mechanisms for gene
20 deletion/modification may be used to lower the overall expression of CCR5 and/or help to lower HIV replication. At least one study has reported having success in treating the disease when lentivirus was administered in patients with a genetic background of CCR5delta32. However, this was only one example of success, and many other patients without the CCR5delta32 genotype have not been treated as successfully. Consequently, there is a substantial need to improve the performance of viral genetic therapy against HIV, both in
25 terms of performance for the individual viral vector construct and for improved use of the vector through a strategy for achieving functional HIV cure.

[0151] For example, some existing therapies rely on zinc finger nucleases to delete a portion of CCR5 in an attempt to render cells resistant to HIV infection. However, even after optimal treatment, only 30% of T cells had been modified by the nuclease at all, and of
30 those that were modified, only 10% of the total CD4 T cell population had been modified in a way that would prevent HIV infection. In contrast, the disclosed methods result in virtually every cell carrying a lentivirus transgene having a reduction in CCR5 expression below the level needed to allow HIV infection.

[0152] For the purposes of the disclosed methods, gene therapy can include, but is not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIM5-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

Immunotherapy

[0153] Historically, vaccines have been a go-to weapon against deadly infectious diseases, including smallpox, polio, measles, and yellow fever. Unfortunately, there is no currently approved vaccine for HIV. The HIV virus has unique ways of evading the immune system, and the human body seems incapable of mounting an effective immune response against it. As a result, scientists do not have a clear picture of what is needed to provide protection against HIV.

[0154] However, immunotherapy may provide a solution that was previously unaddressed by conventional vaccine approaches. Immunotherapy, also called biologic therapy, is a type of treatment designed to boost the body's natural defenses to fight infections or cancer. It uses materials either made by the body or in a laboratory to improve, target, or restore immune system function.

[0155] In embodiments, immunotherapeutic approaches may be used to enrich a population of HIV-specific CD4 T cells for the purpose of increasing the host's anti-HIV immunity. In embodiments, integrating or non-integrating lentivirus vectors may be used to transduce a host's immune cells for the purposes of increasing the host's anti-HIV immunity. In embodiments, a vaccine comprising HIV proteins including but not limited to a killed particle, a virus-like particle, HIV peptides or peptide fragments, a recombinant viral vector, a recombinant bacterial vector, a purified subunit or plasmid DNA combined with a suitable vehicle and/or biological or chemical adjuvants to increase a host's immune responses may be used to enrich the population of virus-specific T cells or antibodies, and these methods may be further enhanced through the use of HIV-targeted genetic therapy using lentivirus or other viral vector.

Methods

[0156] In one aspect, the disclosure provides methods for using viral vectors to achieve a cure for HIV disease. The methods generally include immunotherapy to enrich the

proportion of HIV-specific CD4 T cells, followed by lentivirus transduction to deliver inhibitors of HIV and CCR5 and CXCR4 as required.

[0157] In one embodiment, the methods include a first stimulation event to enrich a proportion of HIV-specific CD4 T cells. The first stimulation can include administration of one or more of any agent suitable for enriching a patient's HIV-specific CD4+ T cells including but not limited to a vaccine.

[0158] Therapeutic vaccines can include one or more HIV protein with protein sequences representing the predominant viral types of the geographic region where treatment is occurring. Therapeutic vaccines will include purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for immunization. Vaccinations may be administered according to standard methods known in the art and HIV patients may continue antiretroviral therapy during the interval of immunization and subsequent *ex vivo* lymphocyte culture including lentivirus transduction.

[0159] In some embodiments, HIV+ patients are immunized with an HIV vaccine, increasing the frequency of HIV-specific CD4 T cells by about 2, about 25, about 250, about 500, about 750, about 1000, about 1250, or about 1500-fold (or any amount in between these values). The vaccine may be any clinically utilized or experimental HIV vaccine, including the disclosed lentiviral, other viral vectors or other bacterial vectors used as vaccine delivery systems. In another embodiment, the vectors encode virus-like particles (VLPs) to induce higher titers of neutralizing antibodies. In another embodiment, the vectors encode peptides or peptide fragments associated with HIV including but not limited to gag, pol, and env, tat, rev, nef, vif, vpr, vpu, and tev, as well as LTR, TAR, RRE, PE, SLIP, CRS, and INS. Alternatively, the HIV vaccine used in the disclosed methods may comprise purified proteins, inactivated viruses, virally vectored proteins including HIV-specific antibodies or antibody-like molecules or CD4-like molecules, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), or biological or chemical adjuvants including cytokines and/or chemokines.

[0160] In one embodiment, the methods include *ex vivo* re-stimulation of CD4 T cells from persons or patients previously immunized by therapeutic vaccination, using purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and

methods for re-stimulation. *Ex vivo* re-stimulation may be performed using the same vaccine or immune stimulating compound used for *in vivo* immunization, or it may be performed using a different vaccine or immune stimulating compound than those used for *in vivo* immunization. Moreover, in some embodiments, the patient does not require prior therapeutic vaccination or re-stimulation of CD4 T cells if the individual has sufficiently high antigen-specific CD4 T cell responses to HIV proteins. In these embodiments, such a patient may only require *ex vivo* stimulation of CD4 T cells with viral antigens, vaccines or peptides followed by selection for HIV-specific T cells based on the response to stimulation. Enriched cell preparations may include 1%, 5%, 10%, 20%, 30%, 40%, 50% or more of the HIV-specific CD4⁺ T cells and are used for lentivirus transduction of genes able to protection from HIV-mediated depletion. Stimulation with polyclonal mitogen plus cytokines increases the number of enriched and transduced T cells until appropriate levels are reached for infusion back into the original patient.

[0161] In embodiments, peripheral blood mononuclear cells (PBMCs) are obtained by leukapheresis and treated *ex vivo* to obtain about 1×10^9 CD4 T cells of which about 0.1%, about 1%, about 5% or about 10% or about 30% or about 40% or about 50% are both HIV-specific in terms of antigen responses, and HIV-resistant by virtue of carrying the therapeutic transgene delivered by the disclosed lentivirus vector. Alternatively, about 1×10^7 , about 1×10^8 , about 1×10^9 , about 1×10^{10} , about 1×10^{11} , or about 1×10^{12} CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells are isolated for *ex vivo* re-stimulation.

[0162] The isolated CD4 T cells can be cultured in appropriate medium throughout re-stimulation with HIV vaccine antigens, which may include antigens present in the prior therapeutic vaccination. Antiretroviral therapeutic drugs including inhibitors of reverse transcriptase, protease or integrase may be added to prevent virus re-emergence during prolonged *ex vivo* culture. CD4 T cell re-stimulation may be used to enrich the proportion of HIV-specific CD4 T cells in culture. The same procedure may also be used for analytical objectives wherein smaller blood volumes with peripheral blood mononuclear cells obtained by purification, are used to identify HIV-specific T cells and measure the frequency of this sub-population. The proportion of HIV-specific CD4 T cells in culture may be enriched or increased through depletion of one or more other cell subsets.

[0163] The PBMC fraction may be enriched for HIV-specific CD4 T cells by contacting the cells with HIV proteins matching or complementary to the components of the vaccine

previously used for *in vivo* immunization. *Ex vivo* re-stimulation can increase the relative frequency of HIV-specific CD4 T cells by about 2, about 5, about 10, about 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold. The methods may additionally include combining *in vivo* therapeutic immunization and *ex vivo* re-stimulation of CD4 T cells with *ex vivo* lentiviral transduction and culturing.

[0164] Thus, in one embodiment, the re-stimulated PBMC or fraction of PBMC that has been enriched for HIV-specific CD4 T cells can be cultured for 1, 2, 3, 4, 5 or up to 12, 20 or 30 days before activating again with a polyclonal mitogen or other plant- or fungal based agglutinins or other reagents capable of recognizing cell surface CD3 and CD28 to cross link these molecules and cause polyclonal T cell activation. After polyclonal stimulation, cells may be transduced with therapeutic anti-HIV lentivirus or other vectors and maintained in culture for a sufficient period of time for such transduction, for example from about 1 to about 21 days, including up to about 35 days. Alternatively, the cells may be cultured for about 1- about 18 days, about 1- about 15 days, about 1- about 12 days, about 1- about 9 days, or about 3- about 7 days. Thus, the transduced cells may be cultured for about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days. Activation with a polyclonal mitogen may or may not be included in the cell product manufacturing process.

[0165] In further embodiments, once the transduced cells have been cultured for a sufficient period of time, transduced CD4 T cells are infused back into the original patient. Infusion can be performed using various devices and methods known in the art. In some embodiments, infusion may be accompanied by pre-treatment with cyclophosphamide or similar compounds to increase the efficiency of re-engraftment.

[0166] In some embodiments, a CCR5-targeted therapy may be added to a subject's antiretroviral therapy regimen, which was continued throughout the treatment process. Examples of CCR5-targeted therapies include but are not limited to Maraviroc (a CCR5 antagonist) or Rapamycin (immunosuppressive agent that lowers CCR5). In some embodiments, the antiretroviral therapy may be ceased and the subject can be tested for virus rebound. If no rebound occurs, adjuvant therapy can also be removed and the subject can be tested again for virus rebound.

[0167] In various embodiments, continued virus suppression with reduced or no

antiretroviral therapy including cART or HAART, and reduced or no adjuvant therapy for about 26 weeks can be considered a functional cure for HIV. Other definitions of a cure are described herein.

[0168] The lentiviral and other vectors used in the disclosed methods may encode at least one, at least two, at least three, at least four, or at least five genes, or at least six genes, or at least seven genes, or at least eight genes, or at least nine genes, or at least ten genes, or at least eleven genes, or at least twelve genes of interest. Given the versatility and therapeutic potential of HIV-targeted gene therapy, a viral vector of the disclosure may encode genes or nucleic acid sequences that include but are not limited to (i) an antibody directed to an antigen associated with an infectious disease or a toxin produced by the infectious pathogen, (ii) cytokines including interleukins that are required for immune cell growth or function and may be therapeutic for immune dysregulation encountered in HIV and other chronic or acute human viral or bacterial pathogens, (iii) factors that suppress the growth of HIV *in vivo* including CD8 suppressor factors, (iv) mutations or deletions of chemokine receptor CCR5, mutations or deletions of chemokine receptor CXCR4, or mutations or deletions of chemokine receptor CXCR5, (v) antisense DNA or RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, (vi) small interfering RNA against specific receptors or peptides associated with HIV or host protein associated with HIV, or (vii) a variety of other therapeutically useful sequences that may be used to treat HIV or AIDS.

[0169] Additional examples of HIV-targeted gene therapy that can be used in the disclosed methods include, but are not limited to, affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatively on CD8 T cells), modification of signal transduction pathways to avoid cell death cause by viral proteins, increased expression of HIV restriction elements including TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIM5-alpha complexes, tetherin (BST2), and similar proteins identified as being capable of reducing HIV replication in mammalian cells.

[0170] In some embodiments, a patient may be undergoing cART or HAART concurrently while being treated according to the methods of the disclosure. In other embodiments, a patient may undergo cART or HAART before or after being treated according to the methods of the disclosure. In some embodiments, cART or HAART is maintained throughout treatment according to the methods of the disclosure and the patient may be monitored for HIV viral burden in blood and frequency of lentivirus-transduced CD4

T cells in blood. Preferably, a patient receiving cART or HAART prior to being treated according to the methods of the disclosure is able to discontinue or reduce cART or HAART following treatment according to the methods of the disclosure.

5 [0171] For efficacy purposes, the frequency of transduced, HIV-specific CD4 T cells, which is a novel surrogate marker for gene therapy effects, may be determined, as discussed in more detail herein.

Compositions

10 [0172] In various aspects, the disclosure provides lentiviral vectors capable of delivering genetic constructs to inhibit HIV penetration of susceptible cells. For instance, one mechanism of action in accordance herein is to reduce mRNA levels for CCR5 and/or CXCR4 chemokine receptors for reducing the rates for viral entry into susceptible cells.

[0173] Alternatively, the disclosed lentiviral vectors are capable of inhibiting the formation of HIV-infected cells by reducing the stability of incoming HIV genomic RNA. And in yet another embodiment, the disclosed lentivirus vectors are capable of preventing 15 HIV production from a latently infected cell, wherein the mechanism of action is to cause instability of viral RNA sequences through the action of inhibitory RNA including short-homology, small-interfering or other regulatory RNA species.

[0174] The therapeutic lentiviruses disclosed generally comprise at least one of two types of genetic cargo. First, the lentiviruses may encode genetic elements that direct expression of 20 small RNA capable of inhibiting the production of chemokine receptors CCR5 and/or CXCR4 that are important for HIV penetration of susceptible cells. The second type of genetic cargo includes constructs capable of expressing small RNA molecules targeting HIV RNA sequences for the purpose of preventing reverse transcription, RNA splicing, RNA translation to produce proteins, or packaging of viral genomic RNA for particle production 25 and spreading infection. An exemplary structure is diagrammed in Figure 3.

[0175] As shown in Figure 3 (top panel), an exemplary construct may comprise numerous sections or components. For example, in one embodiment, an exemplary LV construct may comprise the following sections or components:

- RSV - a Rous Sarcoma virus long terminal repeat;
- 30 • 5'LTR - a portion of an HIV long terminal repeat that can be truncated to prevent replication of the vector after chromosomal integration;
- Psi - a packaging signal that allows for incorporation of the vector RNA genome into viral particles during packaging;

- RRE - a Rev Responsive element can be added to improve expression from the transgene by mobilizing RNA out of the nucleus and into the cytoplasm of cells;
- cPPT - a Poly purine tract that facilitates second strand DNA synthesis prior to integration of the transgene into the host cell chromosome;
- Promoter - a promoter initiates RNA transcription from the integrated transgene to express micro-RNA clusters (or other genetic elements of the construct), and in some embodiments, the vectors may use an EF-1 promoter;
- Anti-CCR5 - a micro RNA targeting messenger RNA for the host cell factor CCR5 to reduce its expression on the cell surface;
- Anti-Rev/Tat - a micro RNA targeting HIV genomic or messenger RNA at the junction between HIV Rev and Tat coding regions, which is sometimes designated miRNA Tat or given a similar description in this application;
- Anti-Vif - a micro RNA targeting HIV genomic or messenger RNA within the Vif coding region;
- WPRE - a woodchuck hepatitis virus post-transcriptional regulatory element is an additional vector component that can be used to facilitate RNA transport of the nucleus; and
- deltaU3 3'LTR - a modified version of a HIV 3' long terminal repeat where a portion of the U3 region has been deleted to improve safety of the vector.

[0176] One of ordinary skill in the art will recognize that the above components are merely examples, and that such components may be reorganized, substituted with other elements, or otherwise changed, so long as the construct is able to prevent expression of HIV genes and decrease the spread of infection.

[0177] Vectors of the disclosure may include either or both of the types of genetic cargo discussed above (i.e., genetic elements that direct expression of a gene or small RNAs, such as siRNA, shRNA, or miRNA that can prevent translation or transcription), and the vectors of the disclosure may also encode additionally useful products for the purpose of treatment or diagnosis of HIV. For instance, in some embodiments, these vectors may also encode green fluorescent protein (GFP) or truncated and biologically inactive cell surface molecules for the purpose of tracking the vectors or antibiotic resistance genes for the purposes of selectively maintaining genetically-modified cells *in vivo*.

[0178] The combination of genetic elements incorporated into the disclosed vectors is not

particularly limited. For example, a vector herein may encode a single small RNA, two small RNAs, three small RNA, four small RNAs, five small RNAs, six small RNAs, seven small RNAs, eight small RNAs, nine small RNAs, or ten small RNAs, or eleven small RNAs, or twelve small RNAs. Such vectors may additionally encode other genetic elements to function in concert with the small RNAs to prevent expression and infection of HIV.

[0179] Those of ordinary skill in the art will understand that the therapeutic lentivirus may substitute alternate sequences for the promoter region, targeting of regulatory RNA, and types of regulatory RNA. Further, the therapeutic lentivirus of the disclosure may comprise changes in the plasmids used for packaging the lentivirus particles; these changes are required to increase levels of production in vitro.

Lentiviral Vector System

[0180] A lentiviral virion (particle) in accordance with various aspects and embodiments herein is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). In various embodiments, one vector containing a nucleic acid sequence encoding the lentiviral pol proteins is provided for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol proteins are expressed by multiple vectors. In other embodiments, vectors containing a nucleic acid sequence encoding the lentiviral Gag proteins for forming a viral capsid, operably linked to a promoter, are provided. In embodiments, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence. In other embodiments, the gag nucleic acid is on a separate vector from all the pol nucleic acid sequences that encode pol proteins.

[0181] Numerous modifications can be made to the vectors herein, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions. In embodiments, the gag, pol and env vector(s) do not contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

[0182] The vector(s) forming the particle preferably do not contain a nucleic acid sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope

protein.

[0183] In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelopes one can “infect” virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment. Examples of viruses from which such env genes and envelope proteins can derive include the influenza virus (*e.g.*, the Influenza A virus, Influenza B virus, Influenza C virus, Influenza D virus, Isavirus, Quarantavirus, and Thogotovirus), the Vesiculovirus (*e.g.*, Indiana vesiculovirus), alpha viruses (*e.g.*, the Semliki forest virus, Sindbis virus, Aura virus, Barmah Forest virus, Bebaru virus, Cabassou virus, Getah virus, Highlands J virus, Trocara virus, Una Virus, Ndumu virus, and Middleburg virus, among others), arenaviruses (*e.g.*, the lymphocytic choriomeningitis virus, Machupo virus, Junin virus and Lassa Fever virus), flaviviruses (*e.g.*, the tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus, Apoi virus, Bagaza virus, Edge Hill virus, Jugra virus, Kadam virus, Dakar bat virus, Modoc virus, Powassan virus, Usutu virus, and Sal Vieja virus, among others), rhabdoviruses (*e.g.*, vesicular stomatitis virus, rabies virus), paramyxoviruses (*e.g.*, mumps or measles) and orthomyxoviruses (*e.g.*, influenza virus).

[0184] Other env gene envelope proteins that can preferably be used include those derived from endogenous retroviruses (*e.g.*, feline endogenous retroviruses and baboon endogenous retroviruses) and closely related gammaretroviruses (*e.g.*, the Moloney Leukemia Virus, MLV-E, MLV- A, Gibbon Ape Leukemia Virus, GALV, Feline leukemia virus, Koala retrovirus, Trager duck spleen necrosis virus, Viper retrovirus, Chick syncytial virus, Gardner-Arnstein feline sarcoma virus, and Porcine type-C oncovirus, among others). These gammaretroviruses can be used as sources of env genes and envelope proteins for targeting primary cells. The gammaretroviruses are particularly preferred where the host cell is a primary cell.

[0185] Envelope proteins can be selected to target a specific desired host cell. For example, targeting specific receptors such as a dopamine receptor can be used for brain delivery. Another target can be vascular endothelium. These cells can be targeted using an envelope protein derived from any virus in the Filoviridae family (*e.g.*, Cuevaviruses, Dianloviruses, Ebolaviruses, and Marburgviruses). Species of Ebolaviruses include Tai Forest ebolavirus, Zaire ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus, and Reston ebolavirus.

[0186] In addition, in embodiments, glycoproteins can undergo post-transcriptional modifications. For example, in an embodiment, the GP of Ebola, can be modified after translation to become the GP1 and GP2 glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (*e.g.*, FIV or SHIV [U.S. Patent No. 5,654,195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

[0187] Lentiviral vector systems as provided herein typically include at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (*e.g.*, Figure 4). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (*e.g.*, Figure 5). Accordingly, both 3-vector (*e.g.*, Figures 4 and 6) and 4-vector (*e.g.*, Figure 5) systems can be used to produce a lentivirus as described herein. In embodiments, the therapeutic vector, at least one envelope plasmid and at least one helper plasmid are transfected into a packaging cell, for example a packaging cell line. A non-limiting example of a packaging cell line is the 293T/17 HEK cell line. When the therapeutic vector, the envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, a lentiviral particle is ultimately produced.

[0188] In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line, a lentiviral particle is produced by the packaging cell line, wherein the lentiviral particle is capable of inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

[0189] In another aspect, the lentiviral vector, which is also referred to herein as a therapeutic vector, includes the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 33-34), Psi sequence (RNA packaging site) (SEQ ID NO: 35), RRE (Rev-response element) (SEQ ID NO: 36), cPPT (polypurine tract) (SEQ ID NO: 37), EF-1 α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPRE)

(SEQ ID NOS: 31 or 67), and Δ U3 3' LTR (SEQ ID NO: 38). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[0190] In another aspect, a helper plasmid includes the following elements: CAG promoter (SEQ ID NO: 40); HIV component gag (SEQ ID NO: 42); HIV component pol (SEQ ID NO: 43); HIV Int (SEQ ID NO: 44); HIV RRE (SEQ ID NO: 45); and HIV Rev (SEQ ID NO: 46). In another aspect, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[0191] In another aspect, an envelope plasmid includes the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 49) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 51). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[0192] In various aspects, the plasmids used for lentiviral packaging are modified by substitution, addition, subtraction or mutation of various elements without loss of vector function. For example, and without limitation, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A.

[0193] In another aspect, the HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. For example, the VSV-G glycoprotein can be substituted with membrane glycoproteins derived from gammaretroviruses (*e.g.*, gibbon ape leukemia virus, GALV, murine leukemia virus 10A1, MLV, Koala retrovirus, Trager duck spleen necrosis virus, Viper retrovirus, Chick syncytial virus, Gardner-Arnstein feline sarcoma virus, and Porcine type-C oncovirus, among others), endogenous retroviruses (*e.g.*, feline endogenous virus (RD114), human endogenous retrovirus such as HERV-W, and baboon endogenous retrovirus, BaEV, among others), Lyssavirus (*e.g.*, Rabies virus, FUG), mammarenavirus (*e.g.*, lymphocytic choriomeningitis virus, LCMV, Influenza viruses such as the Influenza A virus, Influenza A fowl plague virus, FPV, Influenza B virus, Influenza C virus, Influenza D virus, Isavirus, Quaranjavirus, and Thogotovirus), Alphavirus (*e.g.*, Ross River alphavirus, RRV, or Ebola viruses, EboV, such as Sudan ebolavirus, Tai Forest

ebolavirus, Zaire ebolavirus, Bundibugyo ebolavirus, and Reston ebolavirus).

[0194] Various lentiviral packaging systems can be acquired commercially (*e.g.*, Lenti-
vpak packaging kit from OriGene Technologies, Inc., Rockville, MD), and can also be
designed as described herein. Moreover, it is within the skill of a person ordinarily skilled in
the art to substitute or modify aspects of a lentiviral packaging system to improve any
number of relevant factors, including the production efficiency of a lentiviral particle.

Bioassays

[0195] In various aspects, the present disclosure includes bioassays for determining the
success of HIV treatment for achieving a functional cure. These assays provide a method for
measuring the efficacy of the disclosed methods of immunization and treatment by
measuring the frequency of transduced, HIV specific CD4 T cells in a patient. HIV-specific
CD4 T cells are recognizable because, among others, they proliferate, change the
composition of cell surface markers, induce signaling pathways including phosphorylation,
and/or express specific marker proteins that may be cytokines, chemokines, caspases,
phosphorylated signaling molecules or other cytoplasmic and/or nuclear components.
Specific responding CD4 T cells are recognized for example, using labeled monoclonal
antibodies or specific in situ amplification of mRNA sequences, that allow sorting of HIV-
specific cells using flow cytometry sorting, magnetic bead separation or other recognized
methods for antigen-specific CD4 T cell isolation. The isolated CD4 T cells are tested to
determine the frequency of cells carrying integrated therapeutic lentivirus. Single cell testing
methods may also be used including microfluidic separation of individual cells that are
coupled with mass spectrometry, PCR, ELISA or antibody staining to confirm
responsiveness to HIV and presence of integrated therapeutic lentivirus.

[0196] Thus, in various embodiments, following application of a treatment according to
the disclosure (*e.g.*, (a) immunization, (b) *ex vivo* leukocyte/lymphocyte culture and/or
depletion of non-target cells; (c) re-stimulation with purified proteins, inactivated viruses,
virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants
including cytokines and/or chemokines, vehicles; (d) transduction of the enriched T cells
with a viral delivery system; and (e) infusion of the enriched, transduced T cells), a patient
may be subsequently assayed to determine the efficacy of the treatment. A threshold value of
target T cells in the body may be established to measure a functional cure at a determined
value, for example, at about 1×10^8 HIV-specific CD4 T cells bearing genetic modification
from therapeutic lentivirus. Alternatively, the threshold value may be about 1×10^5 , about

1x10⁶, about 1x10⁷, about 1x10⁸, about 1x10⁹, or about 1x10¹⁰ CD4 T cells in the body of the patient.

[0197] HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus can be determined using any suitable method, such as but not limited to flow cytometry, cell
5 sorting, FACS analysis, DNA cloning, PCR, RT-PCR or Q-PCR, ELISA, FISH, western blotting, southern blotting, high throughput sequencing, RNA sequencing, oligonucleotide primer extension, or other methods known in the art.

[0198] While methods for defining antigen specific T cells with genetic modifications are known in the art, utilizing such methods to combine identifying HIV-specific T cells with
10 integrated or non-integrated gene therapy constructs as a standard measure for efficacy is a novel concept in the field of HIV treatment, as described variously herein.

Doses and Dosage Forms

[0199] The disclosed methods and compositions can be used for treating HIV+ patients during various stages of their disease. Accordingly, dosing regimens may vary based upon
15 the condition of the patient and the method of administration.

[0200] In various embodiments, HIV-specific vaccines for the initial *in vivo* immunization are administered to a subject in need in varying doses. In general, vaccines delivered by intramuscular injection include about 10 µg to about 300 µg, about 25 µg to about 275 µg, about 50 µg to about 250 µg, about 75 µg to about 225 µg, or about 100 µg to
20 about 200 µg of HIV protein, either total virus protein prepared from inactivated virus particles, virus-like particles or purified virus protein from recombinant systems or purified from virus preparations. Recombinant viral or bacterial vectors may be administered by any and all of the routes described. Intramuscular vaccines will include about 1 µg to about 100 µg, about 10 µg to about 90 µg, about 20 µg to about 80 µg, about 30 µg to about 70 µg,
25 about 40 µg to about 60 µg, or about 50 µg of suitable adjuvant molecules and be suspended in oil, saline, buffer or water in volumes of 0.1 to 5 ml per injection dose, and may be soluble or emulsion preparations. Vaccines delivered orally, rectally, buccally, at genital mucosal or intranasally, including some virally-vectored or bacterially-vectored vaccines, fusion proteins, liposome formulations or similar preparations, may contain higher amounts
30 of virus protein and adjuvant. Dermal, sub-dermal or subcutaneous vaccines utilize protein and adjuvant amounts more similar to oral, rectal or intranasal-delivered vaccines. Depending on responses to the initial immunization, vaccination may be repeated 1-5 times using the same or alternate routes for delivery. Intervals may be of 2-24 weeks between

immunizations. Immune responses to vaccination are measured by testing HIV-specific antibodies in serum, plasma, vaginal secretions, rectal secretions, saliva or bronchoalveolar lavage fluids, using ELISA or similar methodology. Cellular immune responses are tested by in vitro stimulation with vaccine antigens followed by staining for intracellular cytokine accumulation followed by flow cytometry or similar methods including lymphoproliferation, expression of phosphorylated signaling proteins or changes in cell surface activation markers. Upper limits of dosing may be determined based on the individual patient and will depend on toxicity/safety profiles for each individual product or product lot.

[0201] Immunization may occur once, twice, three times, or repeatedly. For instance, an agent for HIV immunization may be administered to a subject in need once a week, once every other week, once every three weeks, once a month, every other month, every three months, every six months, every nine months, once a year, every eighteen months, every two years, every 36 months, or every three years.

[0202] Immunization will generally occur at least once before *ex vivo* expansion and enrichment of CD4 T cells, and immunization may occur once, twice, three times, or more after *ex vivo* leukocyte/lymphocyte culture/re-stimulation and infusion.

[0203] In one embodiment, HIV vaccines for immunization are administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a wide variety of nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition comprising an HIV vaccine can also be formulated for injection.

[0204] HIV vaccine compositions for the purpose of immunization can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intracisternally, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, or via a buccal or nasal spray formulation.

[0205] Further, the HIV vaccine compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, and a suspension. Further, the composition may be a controlled

release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the composition may be a transdermal delivery system.

[0206] In another embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In yet another embodiment, the solid dosage form includes one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form is in immediate release or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

[0207] In a further embodiments, the pharmaceutical composition comprising a HIV vaccine is formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

[0208] In yet a further embodiment, the pharmaceutical composition comprising an HIV vaccine is formulated as a nasal dosage form. Such dosage forms of the present disclosure comprise solution, suspension, and gel compositions for nasal delivery.

[0209] In one embodiment, the pharmaceutical composition is formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In other embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In particular embodiments, the composition comprising HIV vaccine or a pharmaceutically acceptable salt thereof is formulated to be suitable for administration to a pediatric patient.

[0210] In one embodiment, the pharmaceutical composition is formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emulsions, non-aqueous solutions or suppositories. In other embodiments, the non-aqueous solutions or suspensions includes propylene glycol, polyethylene glycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate. As a base for suppositories, witepsol, macrogol, tween 61, cacao oil, laurin oil or glycerinated gelatin can be used.

[0211] The dosage of the pharmaceutical composition can vary depending on the patient's

weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

[0212] For the purposes of re-stimulation, lymphocytes, PBMCs, and/or CD4 T cells are generally removed from a patient and isolated for re-stimulation and culturing. The isolated cells may be contacted with the same HIV vaccine or activating agent used for immunization or a different HIV vaccine or activating agent. In one embodiment, the isolated cells are contacted with about 10 ng to 5 µg of an HIV vaccine or activating agent per about 10⁶ cells in culture (or any other suitable amount). More specifically, the isolated cells may be contacted with about 50 ng, about 100 ng, about 200 ng, about 300 ng, about 400 ng, about 500 ng, about 600 ng, about 700 ng, about 800 ng, about 900 ng, about 1 µg, about 1.5 µg, about 2 µg, about 2.5 µg, about 3 µg, about 3.5 µg, about 4 µg, about 4.5 µg, or about 5 µg of an HIV vaccine or activating agent per about 10⁶ cells in culture.

[0213] Activating agents or vaccines are generally used once for each in vitro cell culture but may be repeated after intervals of about 15 to about 35 days. For example, a repeat dosing could occur at about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

[0214] For transduction of the enriched, re-stimulated cells, the cells may be transduced with lentiviral vectors or with other known vector systems as disclosed, for example, in Figure 4 or Figure 6. The cells being transduced may be contacted with about 1-1,000 viral genomes (measured by RT-PCR assay of culture fluids containing lentivirus vector) per target cell in culture (or any other suitable amount). Lentivirus transduction may be repeated 1-5 times using the same range of 1-1,000 viral genomes per target cell in culture.

Cellular Enrichment

[0215] In various embodiments, cells such as T cells are obtained from an HIV infected patient and cultured. Culturing can occur in multiwell plates in a culture medium comprising conditioned media ("CM"). The levels of supernatant p24gag ("p24") and viral RNA levels may be assessed by standard means. Those patients whose CM-cultured cells have peak p24 supernatant levels of less than 1 ng/ml may be suitable patients for large-scale T-cell expansion in CM with or without the use of additional anti-viral agents. Additionally, different drugs or drug combinations of interest may be added to different wells and the impact on virus levels in the sample may be assessed by standard means. Those drug combinations providing adequate viral suppression are therapeutically useful combinations.

It is within the capacity of a competent technician to determine what constitutes adequate viral suppression in relation to a particular subject. In order to test the effectiveness of drugs of interest in limiting viral expansion, additional factors such as anti-CD3 antibodies may be added to the culture to stimulate viral production. Unlike culture methods for HIV infected cell samples known in the art, CM allows the culture of T cells for periods of over two months, thereby providing an effective system in which to assay long term drug effectiveness.

[0216] In a preferred embodiment, the HIV infected cells are obtained from a subject with susceptible transcription mediating protein sequences and susceptible HIV regulatory element sequences. In a more preferred embodiment, the HIV infected cells are obtained from a subject having wild-type transcription-mediating protein sequences and wild-type HIV regulatory sequences.

[0217] Stable transduction of primary cells of the hematopoietic system and/or hematopoietic stem cells may be obtained by contacting, *in vitro* or *ex vivo*, the surface of the cells with both a lentiviral vector and at least one molecule which binds the cell surface. The cells may be cultured in a ventilated vessel comprising two or more layers under conditions conducive to growth and/or proliferation. In some embodiments, this approach may be used in conjunction with non-CD4⁺ T cell depletion and/or broad polyclonal expansion.

[0218] In another approach to T cell enrichment, PBMCs are stimulated with a peptide and enriched for cells secreting a cytokine, such as interferon-gamma. This approach generally involves stimulating a mixture of cells containing T cells with antigen, and effecting a separation of antigen-stimulated cells according to the degree to which they are labeled with the product. Antigen stimulation is achieved by exposing the cells to at least one antigen under conditions effective to elicit antigen-specific stimulation of at least one T cell. Labeling with the product is achieved by modifying the surface of the cells to contain at least one capture moiety, culturing the cells under conditions in which the product is secreted, released and specifically bound (“captured” or “entrapped”) to said capture moiety; and labeling the captured product with a label moiety, where the labeled cells are not lysed as part of the labeling procedure or as part of the separation procedure. The capture moiety may incorporate detection of cell surface glycoproteins CD3 or CD4 to refine the enrichment step and increase the proportion of antigen-specific T cells in general, of CD4⁺ T cells in specific.

[0219] The following examples are given to illustrate aspects of the present disclosure. It should be understood, however, that the disclosure is not to be limited to the specific conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

5 **Examples**

Example 1: Development of a Lentiviral Vector System

[0220] A lentiviral vector system was developed as summarized in Figure 3 (linear form) and Figure 4 (circularized form). Referring first to the top portion of Figure 3, a representative therapeutic vector has been designed and produced with the following
10 elements being from left to right: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 33-34), Psi sequence (RNA packaging site) (SEQ ID NO: 35), RRE (Rev-response element) (SEQ ID NO: 36), cPPT (polypurine tract) (SEQ ID NO: 37), EF-1 α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID
15 NOS: 31 or 67), and Δ U3 3' LTR (SEQ ID NO: 38). The therapeutic vector detailed in Figure 3 is also referred to herein as AGT103.

[0221] Referring next to the middle portion of Figure 3, a helper plasmid has been designed and produced with the following elements being from left to right: CAG promoter (SEQ ID NO: 40); HIV component gag (SEQ ID NO: 42); HIV component pol (SEQ ID
20 NO: 43); HIV Int (SEQ ID NO: 44); HIV RRE (SEQ ID NO: 45); and HIV Rev (SEQ ID NO: 46).

[0222] Referring next to the lower portion of Figure 3, an envelope plasmid has been designed and produced with the following elements being from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 49) and vesicular stomatitis virus G
25 glycoprotein (VSV-G) (SEQ ID NO: 51).

[0223] Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, VA) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid (as shown in Figure 3). The transfection of 293T/17 HEK cells, which produced functional viral particles, employed
30 the reagent Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by

anion-exchange chromatography. The concentration of lentiviral particles can be expressed in terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per cell by quantitative PCR, or by
5 infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

[0224] As mentioned above, a 3-vector system (i.e., a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the 3-vector system is shown in Figure 4. The schematic of Figure 4 is a circularized version of the linear
10 system previously described in Figure 3. Briefly, and with reference to Figure 4, the top-most vector is a helper plasmid, which, in this case, includes Rev. The vector appearing in the middle of Figure 4 is the envelope plasmid. The bottom-most vector is the previously described therapeutic vector.

[0225] Referring more specifically to Figure 4, the Helper plus Rev plasmid includes a
15 CAG enhancer (SEQ ID NO: 39); a CAG promoter (SEQ ID NO: 40); a chicken beta actin intron (SEQ ID NO: 41); a HIV gag (SEQ ID NO: 42); a HIV Pol (SEQ ID NO: 43); a HIV Int (SEQ ID NO: 44); a HIV RRE (SEQ ID NO: 45); a HIV Rev (SEQ ID NO: 46); and a rabbit beta globin poly A (SEQ ID NO: 47).

[0226] The Envelope plasmid includes a CMV promoter (SEQ ID NO: 49); a beta globin
20 intron (SEQ ID NO: 50); a VSV-G (SEQ ID NO: 51); and a rabbit beta globin poly A (SEQ ID NO: 56).

[0227] In an alternate vector system, and with respect to Figure 6, the vector sequences are provided herein as SEQ ID NOs: 81-83.

[0228] Synthesis of a 2-vector lentiviral packaging system including Helper (plus Rev)
25 and Envelope plasmids.

[0229] Materials and Methods:

[0230] Construction of the helper plasmid: The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the
30 fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTCATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 68) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID

NO: 69). The sequence for the Gag, Pol, Integrase fragment was as follows:

[0231] GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGG
 AATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTG
 CGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAAT
 5 TGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCT
 ATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCCAAAAGTTAA
 ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAG
 AAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT
 ACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGT
 10 AGATTTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGG
 AATACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGT
 GGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGC
 ATTTACCATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAA
 TGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGAC
 15 AAAAATCTTAGAGCCTTTTAGAAAAACAAAATCCAGACATAGTCATCTATCAATA
 CATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA
 AATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTACCACACCAGACA
 AAAACATCAGAAAGAACCTCCATTCCCTTTGGATGGGTTATGAACTCCATCCTG
 ATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTC
 20 AATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCA
 GGGATTAAAGTAAGGCAATTATGTAACTTCTTAGGGGAACCAAAGCACTAACA
 GAAGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGA
 GATTCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTTAAT
 AGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAG
 25 AGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCCAC
 ACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAG
 CATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAAC
 ATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGG
 AGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAAC
 30 CCATAATAGGAGCAGAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACT
 AAATTAGGAAAAGCAGGATATGTAAGTACAGAGGAAGACAAAAAGTTGTCCC
 CCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTT
 GCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATATGCATTGGG

AATCATTCAAGCACAAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAAT
 AGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACA
 AAGGAATTGGAGGAAATGAACAAGTAGATAAATTGGTCAGTGCTGGAATCAGG
 AAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATA
 5 TCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCACCTGTAGTAGC
 AAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGC
 ATGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTTAG
 AAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAG
 AAGTAATTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAG
 10 CAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTCCACC
 AGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGC
 ATTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAATTA
 AAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGT
 ACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGT
 15 ACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAA
 GAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGAC
 AGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGG
 GGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAG
 CAAAGATCATCAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCA
 20 AGTAGACAGGATGAGGATTAA (SEQ ID NO: 70)

[0232] Next, a DNA fragment containing the Rev, RRE, and rabbit beta globin poly A
 sequence with XbaI and XmaI flanking restriction sites was synthesized by MWG Operon.
 The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites
 The DNA sequence was as follows:

25 [0233] TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAG
 AACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGA
 GGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAG
 AGACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCT
 GCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTA
 30 ACGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTG
 GTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTT
 CCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCT
 GACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTT

GCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCAT
 CAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAAC
 AGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCT
 TGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGT
 5 TGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTA
 AAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGG
 CTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCC
 TGCTGTCCATTCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTA
 TATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTT
 10 TACTAGCCAGATTTTTCTCCTCTCCTGACTACTCCAGTCATAGCTGTCCCTCTT
 CTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATA
 GCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC
 GGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTA
 ATTGCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGAAACCTGTCGTGCCAGCGG
 15 ATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCC
 GCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTT
 TTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAG
 TGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGC
 AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGC
 20 ATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATC
 AGCGGCCGCCCCGGG (SEQ ID NO: 71)

[0234] Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG
 enhancer/promoter plus a chicken beta actin intron sequence. A DNA fragment containing
 the CAG enhancer/promoter/intron sequence with MluI and EcoRI flanking restriction sites
 25 was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at
 the MluI and EcoRI restriction sites. The DNA sequence was as follows:

[0235] ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGC
 CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA
 CCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTA
 30 ACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACT
 GCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC
 GTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGG
 GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTC

GAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCC
 CAATTTTGTATTTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGG
 GGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGC
 GAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTC
 5 CTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGG
 CGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCG
 CGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGG
 GACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTT
 CTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCG
 10 GGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGC
 GTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTT
 TGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGG
 TCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGG
 GGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCTGCACCC
 15 CCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGG
 GCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGC
 CGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGC
 GGCCCCGGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGCAGCCATTGCCTTT
 TATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGA
 20 GCCGAAATCTGGGAGGCGCCGCCGCACCCCTCTAGCGGGCGCGGGCGAAGCG
 GTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGC
 GCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTG
 CCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCG
 GGAATTC (SEQ ID NO: 72)

25 [0236] Construction of the VSV-G Envelope plasmid:

[0237] The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was
 synthesized by MWG Operon with flanking EcoRI restriction sites. The DNA fragment was
 then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and the
 correct orientation was determined by sequencing using a CMV specific primer. The DNA
 30 sequence was as follows:

[0238] GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTATTCATTGGGGTGA
 ATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATG
 TTCCTTCTAATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGA

CTTAATAGGCACAGCCTTACAAGTCAAATGCCCAAGAGTCACAAGGCTATTCA
 AGCAGACGGTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGC
 TGGTATGGACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAG
 AACAATGCAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCA
 5 GGCTTCCCTCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTG
 ATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGG
 GTTGATTCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTC
 CATAACTCTACAACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCT
 AACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCC
 10 TGGGAAAGGAGGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAACTGGA
 GGCAAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCA
 GGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTG
 AATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAA
 GTCTAATTCAGGACGTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCT
 15 GGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTG
 CTCCTAAAAACCCAGGAACCGGTCTTGCTTTCACCATAATCAATGGTACCCTAA
 AATACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAA
 GAATGGTCGGAATGATCAGTGGAACCTACCACAGAAAGGGAACCTGTGGGATGAC
 TGGGCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGT
 20 TCAGGATATAAGTTTCTTTATACATGATTGGACATGGTATGTTGGACTCCGATC
 TTCATCTTAGCTCAAAGGCTCAGGTGTTTGAACATCCTCACATTCAAGACGCTGC
 TTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAA
 AATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCC
 TCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGG
 25 TATCCATCTTTGCATTAAATTAAGCACACCAAGAAAAGACAGATTTATACAGA
 CATAGAGATGAGAATTC (SEQ ID NO: 73)

[0239] A 4-vector system (i.e., a 3-vector lentiviral packaging system) has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in Figure 5. Briefly, and with reference to Figure 5, the top-most vector is a helper plasmid, which, in this case, does not include Rev. The vector second from the top is a separate Rev plasmid. The vector second from the bottom is the envelope plasmid. The bottom-most vector is the previously described therapeutic vector.

[0240] Referring, in part, to Figure 5, the Helper plasmid includes a CAG enhancer (SEQ

ID NO: 39); a CAG promoter (SEQ ID NO: 40); a chicken beta actin intron (SEQ ID NO: 41); a HIV gag (SEQ ID NO: 42); a HIV Pol (SEQ ID NO: 43); a HIV Int (SEQ ID NO: 44); a HIV RRE (SEQ ID NO: 45); and a rabbit beta globin poly A (SEQ ID NO: 47).

[0241] The Rev plasmid includes a RSV promoter and a HIV Rev (SEQ ID NO: 75); and a rabbit beta globin poly A (SEQ ID NO: 60).

[0242] The Envelope plasmid includes a CMV promoter (SEQ ID NO: 49); a beta globin intron (SEQ ID NO: 50); a VSV-G (SEQ ID NO: 51); and a rabbit beta globin poly A (SEQ ID NO: 56).

[0243] *Synthesis of a 3-vector lentiviral packaging system including Helper, Rev, and Envelope plasmids.*

[0244] *Materials and Methods:*

[0245] *Construction of the Helper plasmid without Rev:*

[0246] The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by MWG Operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

[0247] TCTAGAAGGAGCTTTGTTCCCTTGGGTTCTTGGGAGCAGCAGGAAGCAC
TATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGG
TATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCT
GTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGT
GGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAA
TTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAA
ATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGG
ACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTATAGAGT
TTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGG
TCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCC
TTGACTTGAGGTTAGATTTTTTTTATATTTTGTGTTATTTTTTTCTTTAACA
TCCCTAAAATTTTCTTACATGTTTTACTAGCCAGATTTTTCTCTCTCTGACT
ACTCCCAGTCATAGCTGTCCCTCTTCTTATGAAGATCCCTCGACCTGCAGCCC
AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTC
ACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGC
CTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTTCCAG

TCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAG
 TCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTC
 TCCGCCCCATGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCG
 GCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTTGGAGGCCCTAGGCTTTT
 5 GCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG
 CATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTG
 TCCAAACTCATCAATGTATCTTATCACCCGGG (SEQ ID NO: 74)

[0248] *Construction of the Rev plasmid:*

[0249] The RSV promoter and HIV Rev sequence was synthesized as a single DNA
 10 fragment by MWG Operon with flanking MfeI and XbaI restriction sites. The DNA
 fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI
 restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA
 sequence was as follows:

[0250] CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGG
 15 TGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAG
 GATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATA
 CACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGA
 GAGAAAAAGCACCGTGATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGC
 CTTATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATT
 20 CCGCATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATAACAATAAACGCCA
 TTTGACCATTACACCATTTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAAC
 CGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACAC
 CGGGACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAG
 GAAGAAGCGGAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAG
 25 TTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGA
 AGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTTCGATTAG
 TGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAG
 CTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACCTCTG
 GGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAATCTCCTACAATATTGG
 30 AGTCAGGAGCTAAAGAATAGTCTAGA (SEQ ID NO: 75)

[0251] The plasmids for the 2-vector and 3-vector packaging systems could be modified
 with similar elements and the intron sequences could potentially be removed without loss of
 vector function. For example, the following elements could replace similar elements in the

2-vector and 3-vector packaging system:

[0252] *Promoters:* Elongation Factor-1 (EF-1) (SEQ ID NO: 4), phosphoglycerate kinase (PGK) (SEQ ID NO: 52), and ubiquitin C (UbC) (SEQ ID NO: 53) can replace the CMV (SEQ ID NO: 49) or CAG promoter (SEQ ID NO: 80). These sequences can also be further varied by addition, substitution, deletion or mutation.

[0253] *Poly A sequences:* SV40 poly A (SEQ ID NO: 54) and bGH poly A (SEQ ID NO: 55) can replace the rabbit beta globin poly A (SEQ ID NO: 47). These sequences can also be further varied by addition, substitution, deletion or mutation.

[0254] *HIV Gag, Pol, and Integrase sequences:* The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 56); HIV Pol (SEQ ID NO: 57); and HIV Int (SEQ ID NO: 58) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

[0255] *Envelope:* The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 59), gibbon ape leukemia virus (GALV) (SEQ ID NO: 60), Rabies (FUG) (SEQ ID NO: 61), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 62), influenza A fowl plague virus (FPV) (SEQ ID NO: 63), Ross River alphavirus (RRV) (SEQ ID NO: 64), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 65), or Ebola virus (EboV) (SEQ ID NO: 66). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

[0256] In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'delta LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'delta LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

Example 2: Development of an Anti-HIV Lentivirus Vector

[0257] The purpose of this example was to develop an anti-HIV lentivirus vector.

[0258] Inhibitory RNA Designs. The sequence of Homo sapiens chemokine C-C motif receptor 5 (CCR5) (GC03P046377) mRNA was used to search for potential siRNA or shRNA candidates to knockdown CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT RNAi Designer from Thermo Scientific. Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

[0259] The genomic sequence of Bal strain of human immunodeficiency virus type 1 (HIV-1 85US_BaL, accession number AY713409) was used to search for potential siRNA or shRNA candidates to knockdown HIV replication levels in human cells. Based on sequence homology and experience, the search focused on regions of the Tat and Vif genes of HIV although an individual of skill in the art will understand that use of these regions is non-limiting and other potential targets might be selected. Importantly, highly conserved regions of gag or pol genes could not be targeted by shRNA because these same sequences were present in the packaging system complementation plasmids needed for vector manufacturing. As with the CCR5 (NM 000579.3, NM 001100168.1-specific) RNAs, potential HIV-specific RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Gene-E Software Suite hosted by the Broad Institute (broadinstitute.org/mai/public) or the BLOCK-iT RNAi Designer from Thermo Scientific (madesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=shrna&pid=6712627360706061801). Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters.

[0260] *Vector Constructions.* For CCR5, Tat or Vif shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered, purified and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Exemplary vector sequences that were determined to restrict HIV replication can be found in Figure 7. The shRNA sequences with the highest activity against CCR5, Tat, and Vif gene expression were assembled into the miR30, miR21, and miR185 backbones. This resulted in miR30 CCR5 (SEQ ID NO: 87), miR21 Vif (SEQ ID NO: 88), and miR185 Tat (SEQ ID NO: 89) sequences. The underlined sequences of SEQ ID NOS: 87, 88, and 89 represent the backbones with the shRNA sequences. The sequences that are not underlined of SEQ ID NOS: 87, 88, 89 represent the restriction recognition sequences.

[0261] *Development of Vectors*

[0262] It should be noted that not all vectors developed for these experiments necessarily worked as might be predicted. More specifically, a lentivirus vector against HIV might include three main components: 1) inhibitory RNA to reduce the level of HIV binding proteins (receptors) on the target cell surface to block initial virus attachment and penetration; 2) overexpression of the HIV TAR sequence that will sequester viral Tat protein and decrease its ability to transactivate viral gene expression; and 3) inhibitory RNA that attack important and conserved sequences within the HIV genome.

[0263] With respect to the first point above, a key cell surface HIV binding protein is the

chemokine receptor CCR5. HIV particles attach to susceptible T cells by binding to the CD4 and CCR5 cell surface proteins. Because CD4 is an essential glycoprotein on the cell surface that is important for the immunological function of T cells, this was not chosen as a target to manipulate its expression levels. However, people born homozygous for null mutations in the CCR5 gene and completely lacking receptor expression, live normal lives save for enhanced susceptibility to a few infectious diseases and the possibility of developing rare autoimmunity. Thus, modulating CCR5 was determined to be a relatively safe approach and was a primary target in the development of anti-HIV lentivirus vectors.

[0264] With respect to the second point above, the viral TAR sequence is a highly structured region of HIV genomic RNA that binds tightly to viral Tat protein. The Tat:TAR complex is important for efficient generation of viral RNA. Over-expression of the TAR region was envisioned as a decoy molecule that would sequester Tat protein and decrease the levels of viral RNA. However, TAR proved toxic to most mammalian cells including cells used for manufacturing lentivirus particles. Further, TAR was inefficient for inhibiting viral gene expression in other laboratories and has been discarded as a viable component in HIV gene therapy.

[0265] In various embodiments, viral gene sequences have been identified that meet 3 criteria: i) Sequences that are reasonably conserved across a range of HIV isolates representative of the epidemic in a geographic region of interest; ii) reduction in RNA levels due to the activity of an inhibitory RNA in a viral vector will reduce the corresponding protein levels by an amount sufficient to meaningfully reduce HIV replication; and iii) the viral gene sequence(s) targeted by inhibitory RNA are not present in the genes required for packaging and assembling viral vector particles during manufacturing. In various embodiments, a sequence at the junction of HIV Tat and Rev genes and a second sequence within the HIV Vif gene have been targeted by inhibitory RNA. The Tat/Rev targeting has an additional benefit of reducing HIV envelope glycoprotein expression because this region overlaps with the envelope gene in the HIV genome.

[0266] Various methods for vector development and testing relies first on identifying suitable targets (as described herein) followed by constructing plasmid DNAs expressing individual or multiple inhibitory RNA species for testing in cell models, and finally constructing lentivirus vectors containing inhibitory RNA with proven anti-HIV function. The lentivirus vectors are tested for toxicity, yield during in vitro production, and effectiveness against HIV in terms of reducing CCR5 expression levels or lowering viral

gene products to inhibit virus replication.

[0267] Table 2 below demonstrates progression through multiple versions of inhibitory constructs until arriving at a clinical candidate. Initially, shRNA (short homology RNA) molecules were designed and expressed from plasmid DNA constructs.

5 [0268] Plasmids 1-4, as detailed in Table 2 below, tested shRNA sequences against Gag, Pol and RT genes of HIV. While each shRNA was active for suppressing viral protein expression in a cell model, there were two important problems that prevented further development. First, the sequences were targeted to a laboratory isolate of HIV that was not representative of Clade B HIV strains currently circulating in North America and Europe.

10 Second, these shRNA targeted critical components in the lentivirus vector packaging system and would severely reduce vector yield during manufacturing. Plasmid 5, as detailed in Table 2, was selected to target CCR5 and provided a lead candidate sequence. Plasmids 6, 7, 8, 9, 10, and 11, as detailed in Table 2, incorporated the TAR sequence and it was found they produced unacceptable toxicity for mammalian cells including cells used for lentivirus

15 vector manufacturing. Plasmid 2, as detailed in Table 2, identified a lead shRNA sequence capable of reducing Tat RNA expression. Plasmid 12, as detailed in Table 2, demonstrated the effectiveness of shCCR5 expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 13, as detailed in Table 2, demonstrated the effectiveness of a shVif expressed as a microRNA (miR) in a lentiviral vector and

20 confirmed it should be in the final product. Plasmid 14, as detailed in Table 2, demonstrated the effectiveness of shTat expressed as a microRNA (miR) in a lentiviral vector and confirmed it should be in the final product. Plasmid 15, as detailed in Table 2, contained the miR CCR5, miR Tat and miR Vif in the form of a miR cluster expressed from a single promoter. These miR do not target critical components in the lentivirus vector packaging

25 system and proved to have negligible toxicity for mammalian cells. The miRs within the cluster were equally effective to individual miR that were tested previously, and the overall impact was a substantial reduction in replication of a CCR5-tropic HIV BaL strain.

Table 2: Development of HIV Vectors

Plasmid	Sequence	Material	Description	Remarks	Decision
1	SEQ ID NO: 9	Lentiviral vector	shRNA construct for RT of LAI strain	Wrong target, lab virus, no virus test	Abandon

2	SEQ ID NO: 76	Lentiviral vector	H1 promoter shRNA Tat/Rev overlap	Tat protein knock- down >90%	Lead
<i>Vector Construction:</i> For Rev/Tat shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. The two Rev/Tat shRNA sequences that were synthesized were the Rev/Tat shRNA coding sequence #1 (5'-GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTT T-3') (SEQ ID NO: 10) and the Rev/Tat shRNA coding sequence #2 (5'-ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCTTCTTCCTGCCATTTTTT-3') (SEQ ID NO: 77). Two different Rev/Tat target sequences were tested for their ability to decrease Tat mRNA expression: The Rev/Tat shRNA target sequence #1 (5'-GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and the Rev/Tat shRNA target sequence #2 (5'-ATGGCAGGAAGAAGCGGAG-3') (SEQ ID NO: 76). <i>Functional test for shRNA against Rev/Tat:</i> Reduction in Tat expression was tested using a luciferase reporter plasmid which contained a Rev/Tat target sequence inserted into the 3'-UTR (untranslated region of the mRNA). Separate luciferase reporter plasmids containing the Rev/Tat shRNA target sequence #1 (SEQ ID NO: 9) and the Rev/Tat shRNA target sequence #2 (SEQ ID NO: 76) were tested. The Rev/Tat shRNA coding sequence #1 (SEQ ID NO: 10) was co-transfected with a luciferase reporter plasmid containing the Rev/Tat shRNA target sequence #1 (SEQ ID NO: 9). The Rev/Tat shRNA coding sequence #2 (SEQ ID NO: 77) was co-transfected with a luciferase reporter plasmid containing the Rev/Tat shRNA target sequence #2 (SEQ ID NO: 76). There was a 90% reduction in light emission in the luciferase reporter plasmid containing the Rev/Tat shRNA target sequence #1 (SEQ ID NO: 9). This is in contrast to the less than 10% reduction in light emission in the luciferase reporter plasmid containing the Rev/Tat target sequence #2 (SEQ ID NO: 76). <i>Conclusion:</i> The Rev/Tat shRNA coding sequence #1 (SEQ ID NO: 10) was superior to the RevTat shRNA coding sequence #2 (SEQ ID NO: 77) in terms of reducing mRNA levels in the luciferase assay system. This indicates potent inhibitory activity of the Rev/Tat shRNA coding sequence #1 (SEQ ID NO: 10) and it was selected as a lead candidate for further development.					

3	SEQ ID NO: 12	Lentiviral vector	shRNA construct for LAI Gag	Inhibits Gag expression but will inhibit packaging	Abandon
<i>Vector Construction:</i> For Gag shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon, and tested for its ability to decrease Gag mRNA expression. The Gag shRNA sequence that was synthesized was the Gag shRNA coding sequence (5'-GAAGAAATGATGACAGCATTTCAAGAGAATGCTGTCATCATTTCTTCTTTTT-3') (SEQ ID NO: 12). The Gag shRNA target sequence (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) was tested for its ability to reduce Gag mRNA expression. Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences). <i>Functional test for shRNA against Gag:</i> Reduction in Gag expression was tested using a luciferase reporter plasmid which contained the Gag shRNA target sequence (SEQ ID NO: 11) inserted into the 3'-UTR (untranslated region of the mRNA). The Gag shRNA coding sequence (SEQ ID NO: 12) was co-transfected with the luciferase reporter plasmid containing luciferase and the Gag shRNA target sequence (SEQ ID NO: 11). Co-transfection of these sequences resulted in nearly a 90% reduction in light emission from the luciferase reporter plasmid compared to control treatments. <i>Conclusion:</i> This Gag shRNA coding sequence (SEQ ID NO: 12) is potent against HIV Gag expression, but was abandoned. The lentivirus packaging system requires production of Gag from the helper plasmid and shRNA inhibition of Gag will reduce lentivirus vector yield. This shRNA coding sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.					
4	SEQ ID NO: 14	Lentiviral vector	shRNA construct for Pol	Inhibits Pol expression but will inhibit packaging	Abandon
<i>Vector Construction:</i> A Pol shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon, and tested for its ability to decrease Pol mRNA expression. The Pol shRNA sequence that was synthesized was the Pol shRNA coding sequence (5'-CAGGAGATGATACAGTTCAAGAGACTGTATCATCTGCTCCTGTTTTT-3') (SEQ ID					

NO: 14). The Pol shRNA target sequence (5'- CAGGAGCAGATGATACAG -3') (SEQ ID NO: 13) was tested for its ability to reduce Pol mRNA expression. Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

Functional tests for shRNA against HIV Pol: Reduction in Pol expression was tested using a luciferase reporter plasmid which contained the Pol shRNA target sequence (SEQ ID NO: 13) inserted into the 3'-UTR (untranslated region of the mRNA). The Pol shRNA coding sequence (SEQ ID NO: 14) was co-transfected with the luciferase reporter plasmid containing luciferase and the Pol shRNA target sequence (SEQ ID NO: 13). Co-transfection of these sequences resulted in a 60% reduction in light emission from the luciferase reporter plasmid compared to control treatments.

Conclusion: This Pol shRNA coding sequence (SEQ ID NO: 14) is potent against HIV Pol expression, but was abandoned. The lentivirus packaging system requires production of Pol from the helper plasmid and shRNA inhibition of Pol will reduce lentivirus vector yield. This shRNA sequence could be used as an oligonucleotide inhibitor of HIV or incorporated into an alternate viral vector packaging system that uses a different vector genome or is modified to resist inhibition by this shRNA.

5	SEQ ID NO: 16	Lentiviral vector	shRNA construct for CCR5	Best of 5 candidates, Extracellular CCR5 protein reduction >90%	Lead
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Vector Construction: Various CCR5 shRNA coding sequences were constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

CCR5 shRNA target sequence #1 is (5'-GTGTCAAGTCCAATCTATG-3') (SEQ ID NO: 15), which corresponds to CCR5 shRNA coding sequence #1 (5'-GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 16). CCR5 target sequence #1 (SEQ ID NO: 15) is located within the Homo Sapiens CCR5 mRNA target sequence #1 (SEQ ID NO: 25).

CCR5 shRNA target sequence #2 is (5'-GAGCATGACTGACATCTAC-3') (SEQ ID NO:

17), which corresponds to CCR5 shRNA coding sequence #2 (5'-GAGCATGACTGACATCTACTTCAAGAGAGTAGATGTCAGTCATGCTCTTTTT-3') (SEQ ID NO: 18). CCR5 target sequence #2 (SEQ ID NO: 17) is located within the Homo Sapiens CCR5 mRNA target sequence #2 (SEQ ID NO: 26).

CCR5 shRNA target sequence #3 is (5'-GTAGCTCTAACAGGTTGGA-3') (SEQ ID NO: 19), which corresponds to CCR5 shRNA coding sequence #3 (5'-GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCTGTTAGAGCTACTTTTT-3') (SEQ ID NO: 20). CCR5 target sequence #3 (SEQ ID NO: 19) is located within the Homo Sapiens CCR5 mRNA target sequence #3 (SEQ ID NO: 27).

CCR5 shRNA target sequence #4 is (5'-GTTTCAGAACTACCTCTTA-3') (SEQ ID NO: 21), which corresponds to CCR5 shRNA coding sequence #4 (5'-GTTTCAGAACTACCTCTTATTCAAGAGATAAGAGGTAGTTTCTGAACTTTTT-3') (SEQ ID NO: 22). CCR5 target sequence #4 (SEQ ID NO: 21) is located within the Homo Sapiens CCR5 mRNA target sequence #4 (SEQ ID NO: 28).

CCR5 shRNA target sequence #5 is (5'-GAGCAAGCTCAGTTTACACC-3') (SEQ ID NO: 23), which corresponds to CCR5 shRNA coding sequence #5 (5'-GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAACTGAGCTTGCTCTTTTT-3') (SEQ ID NO: 24). CCR5 target sequence #5 (SEQ ID NO: 23) is located within the Homo Sapiens CCR5 mRNA target sequence #5 (SEQ ID NO: 29).

Functional test for shRNA against CCR5: The ability of the CCR5 shRNA sequences to knock-down CCR5 RNA expression was initially tested by separately co-transfecting each of the CCR5 shRNA target sequences (SEQ ID NOS: 15, 17, 19, 21, and 23) with its corresponding CCR5 shRNA coding sequence (SEQ ID NOS: 16, 18, 20, 22, 24, and 26). CCR5 mRNA expression was then assessed by qPCR analysis using CCR5-specific primers.

Conclusion: Based on the reduction in CCR5 mRNA levels CCR5 shRNA coding sequence #1 (SEQ ID NO: 16) was the most potent in reducing CCR5 gene expression. This shRNA was selected as a lead candidate.

6	SIH-U6-TAR	Lentiviral vector	U6 promoter-TAR	Toxic to cells	Abandon
7	SIH-U6-TAR-H1-	Lentiviral vector	U6 promoter-TAR-H1-	Toxic to cells	Abandon

	shCCR5		shCCR5		
8	U6-TAR-H1-shRT	Lentiviral vector	U6 promoter-TAR-H1-RT	Suppress HIV, toxic to cells, poor packaging	Abandon
9	U6-TAR-7SK-shRT	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon
10	U6-TAR-H1-shRT-H1-shCCR5	Lentiviral vector	U6 promoter-TAR-H1-RT-H1-shCCR5	Toxic, poor packaging, H1 repeats	Abandon
11	U6-TAR-7SK-shRT-H1-CCR5	Lentiviral vector	Change shRNA promoter to 7SK	Toxic, poor packaging	Abandon

Vector Construction: A TAR decoy sequence containing flanking KpnI restriction sites was synthesized by MWG operon and inserted into the pSIH lentiviral vector (System Biosciences) at the KpnI site. In this vector, TAR expression is regulated by the U6 promoter. The TAR decoy sequence is (5'-

CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTCTCGACAGCGACCAGATCTG
AGCCTGGGAGCTCTCTGGCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAATT
CTTACTGAGCAAGAAA-3') (SEQ ID NO: 8). Expression of the TAR decoy sequence was determined by qPCR analysis using specific primers for the TAR sequence. Additional vectors were constructed also containing the TAR sequence. The H1 promoter and shRT sequence was inserted in this vector in the XhoI site. The H1 promoter and shRT sequence is (5'-

GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCCTAGG
CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAG
GGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCAT
AAACGTGAAATGTCTTTGGATTGGAATCTTATAAGTTCTGTATGAGACCACTT
GGATCCGCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTC
CGCTTTTT-3') (SEQ ID NO: 78). This vector could express TAR and knockdown RT. The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression. Another vector was constructed containing U6 TAR, H1 shRT, and H1 shCCR5. The H1 shCCR5 sequence was inserted into the SpeI site of the plasmid containing U6 TAR and H1 shRT. The H1 CCR5 sequence is (5'-

GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCCTAGG

CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAG
GGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCAT
AAACGTGAAATGTCTTTGGATTGGGAATCTTATAAGTTCTGTATGAGACCACTT
GGATCCGTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTT
TTT-3') (SEQ ID NO: 79). The 7SK promoter was also substituted for the H1 promoter to regulate shRT expression.

Functional test for TAR decoy activity: We tested the effect of SIH-U6-TAR on packaging efficiency. When TAR sequence was included, the yield of vector in the SIH packaging system was reduced substantially.

Conclusion: Lentivirus vectors expressing the TAR decoy sequence are unsuitable for commercial development due to low vector yields. These constructs were abandoned.

12	SEQ ID NO: 1	Lentiviral vector	microRNA sequence	Extracellular CCR5 protein reduction >90%	Lead
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Vector Construction: A CCR5 microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences). The EF-1 promoter was substituted for a CMV promoter that was used in the plasmid construct Test Material 5. The EF-1 promoter was synthesized by MWG Operon containing flanking ClaI and BsrGI restriction sites and inserted into the pCDH vector containing shCCR5-1. The EF-1 promoter sequence is (5'-
CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACT
GGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC
CGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTG
TGTGGTTCCCGCGGGCCTGGCCTCTTACGGGTTATGGCCCTTGCGTGCCTTGAAT
TACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGG
AAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT
TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCAC
CTTCGCGCCTGTCTCGCTGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTGTATG
ACCTGCTGCGACGCTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGAT
CTGCACACTGGTATTTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGT
CCCAGCGCACATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCG

GACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCC
GTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTG
AGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGAC
GCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT
TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG
GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG
GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGC
CAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCT
TGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTACAGGT
GTCGTGA-3') (SEQ ID NO: 4).

Functional test for lentivirus CDH-shCCR5-1: The ability of CCR5 miR and CCR5 shRNA sequences to knock-down CCR5 expression was determined by transducing CEM-CCR5 T cells with CCR5 miR or shRNA sequences. Cell surface CCR5 expression was measured by staining with a fluorescently-labeled monoclonal antibody against CCR5 and measuring the intensity of the staining by analytical flow cytometry. The most effective shRNA sequence for targeting CCR5 was CCR5 shRNA coding sequence #1 (SEQ ID NO: 16). However, the most effective CCR5 target sequence for constructing the synthetic microRNA sequence was overlapping with CCR5 shRNA coding sequence #5 (SEQ ID NO: 24); this conclusion was based on sequence alignments and experience with miRNA construction. Finally, the miR30 hairpin sequence was used to construct the synthetic miR30 CCR5 coding sequence which is (5'-

AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTTGCTCTACTGTGAA
GCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCA
AGGGGCTT-3') (SEQ ID NO: 1). The CCR5 miRNA target sequence is (5'-
GAGCAAGCTCAGTTTACA-3') (SEQ ID NO: 5).

At multiplicity of infection equal to 5, generating on average 1.25 genome copies of integrated lentivirus per cell, CCR5 expression levels were reduced by $\geq 90\%$ indicating potent inhibition of CCR5 mRNA by the miR30 CCR5 coding sequence (SEQ ID NO: 1) when it was transduced into cells as part of a lentivirus vector.

Conclusion: The miR30 CCR5 coding sequence (SEQ ID NO: 1) is potent for reducing CCR5 cell surface expression and is a lead candidate for a therapeutic lentivirus for HIV.

13	SEQ ID NO: 2	Lentiviral vector	microRNA sequence	Vif protein reduction>80%	Lead
<i>Vector Construction:</i> A Vif microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. Oligonucleotide sequences were inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience with constructing synthetic miRNA, the miR21 hairpin sequence was used to construct the synthetic miR21 Vif coding sequence which is (5'- CATCTCCATGGCTGTACCACCTTGTCTGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA-3') (SEQ ID NO: 2). The Vif miRNA coding sequence is (5'- GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6). The Vif miRNA target sequence is (5'-AAGTTCAGAAGTACACATCCC) (SEQ ID NO: 84). <i>Functional test for potency of miR21Vif:</i> After transfection of the miR21 Vif coding sequence (SEQ ID NO: 2), Vif expression was determined by measuring Vif protein expression by immunoblot analysis using an anti-Vif monoclonal antibody to identify the Vif protein. <i>Conclusion:</i> the miR21 Vif coding sequence (SEQ ID NO: 2) reduced Vif protein expression by ≥ 10-fold compared to control treatments as determined by quantitative image analysis of immunoblot data. This was sufficient to justify the miR21 Vif coding sequence (SEQ ID NO: 2) as a lead candidate for our therapeutic lentivirus.					
14	SEQ ID NO: 3	Lentiviral vector	microRNA sequence	Tat RNA reduction>80%	Lead
<i>Vector Construction:</i> A Tat microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. The microRNA cluster (SEQ ID NO: 30-miR30CCR5 miR21Vif miR185 Tat cluster coding sequence) was inserted into the pCDH lentiviral vector (System Biosciences) containing an EF-1 promoter. Based on sequence alignments and experience in the construction of synthetic miRNA, the miR185 hairpin sequence was selected for constructing a synthetic miR185 Tat coding sequence which is (5'- GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG-3') (SEQ ID NO: 3). The Tat miRNA coding sequence is (5'-					

TCCGCTTCTTCCTGCCATAG-3') (SEQ ID NO: 7). The Tat miRNA target sequence is (5'-CTATGGCAGGAAGAAGCGGA-3') (SEQ ID NO: 85).

Functional test for potency of miR185 Tat: After transfection of the pCDH lentiviral vector containing the miR30CCR5 miR21Vif miR185Tat cluster coding sequence (SEQ ID NO: 30), Tat expression was determined by measuring Tat mRNA expression with RT-PCR analysis using Tat specific primers. The ability of the miR185 Tat coding sequence (SEQ ID NO: 3) to knock down Tat mRNA expression was analyzed against a similar microRNA in which the Tat coding sequence was inserted into the miR155 backbone.

Conclusion: The miR185 Tat coding sequence (SEQ ID NO: 3) was approximately twice as potent for reducing Tat mRNA compared to using the miR155 backbone containing the Tat miRNA coding sequence. Accordingly, the miR185 Tat coding sequence (SEQ ID NO: 3) was selected as the lead candidate for our therapeutic lentivirus.

15	SEQ ID NO: 30	Lentiviral vector	microRNA cluster sequence	CCR5 reduction>90%, Vif protein reduction>80%, Tat RNA reduction>80%, >95% inhibition of HIV replication	Candidate
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Vector Construction: A miR30CCR5 miR21Vif miR185Tat microRNA cluster coding sequence (SEQ ID NO: 30) was constructed with a synthetic DNA fragment containing BsrGI and NotI restriction sites that was synthesized by MWG Operon. The DNA fragment was inserted into the pCDH lentiviral vector (System Biosciences) containing the EF-1 promoter. The miR cluster sequence is (5'-

AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTTGCTCTACTGTGAA
GCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCA
AGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTT
CTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTT
TGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGA
TTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGC
ACCTTCCCTCCCAATGACCGCGTCTTCGTC-3') (SEQ ID NO: 30) and incorporates Test Material 12, Test Material 13 and Test Material 14 into a single cluster that can be expressed under control of the EF-1 promoter.

Functional test for potency of the Lentivirus Vector AGT103 containing the miR30CCR5 miR21Vif miR185Tat microRNA cluster coding sequence (SEQ ID NO: 30): The AGT103 vector was tested for potency against CCR5 using the assay for reduction in cell surface CCR5 expression (Test Material 12). The AGT103 vector was tested for potency against Vif using the assay for reduction in cell surface Vif expression (Test Material 13). The AGT103 vector was tested for potency against Tat using the assay for reduction in cell surface Tat expression (Test Material 14).

Conclusion: Potency for reducing CCR5 expression by the miRNA cluster was similar to potency observed for the miR30CCR5 alone. Potency for reducing Vif expression by the miRNA cluster was similar to potency observed for the miR21Vif alone. Potency for reducing Tat expression by the miRNA cluster was similar to potency observed for the miR185Tat alone. The miR30CCR5 miR21Vif miR Vif miR185 Tat microRNA cluster coding sequence (SEQ ID NO: 30) is potent for reducing cell surface CCR5 levels and for inhibiting two HIV genes. Thus, AGT103 containing this miRNA cluster was selected as the therapeutic vector construct for our HIV functional cure program.

[0269] *Functional Assays.* Individual lentivirus vectors containing CCR5, Tat or Vif shRNA coding sequences (e.g., SEQ ID NOS: 1, 2, and 3) and, for experimental purposes, expressing green fluorescent protein (GFP) under control of the CMV Immediate Early Promoter, and designated AGT103/CMV-GFP were tested for their ability to knockdown CCR5, Tat or Vif expression. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days; protein and RNA were analyzed for CCR5, Tat or Vif expression. Protein levels were tested by Western blot assay or by labeling cells with specific fluorescent antibodies (CCR5 assay), followed by analytical flow cytometry comparing modified and unmodified cell fluorescence using either the CCR5-specific or isotype control antibodies.

[0270] *Starting Testing of Lentivirus.* T cell culture medium was made using RPMI 1640 supplemented with 10% FBS and 1% penicillin–streptomycin. Cytokine stocks of IL-2 10,000 units/ml, IL-12 1µg/ml, IL-7 1µg/ml, IL-15 1µg/ml were also prepared in advance.

[0271] Prior to transduction with the lentivirus, an infectious viral titer was determined and used to calculate the amount of virus to add for the proper multiplicity of infection (MOI).

[0272] *Day 0-12: Antigen-specific enrichment.* On day 0, cryopreserved PBMC were thawed, washed with 10 ml 37°C medium at 1200 rpm for 10 minutes and resuspended at a concentration of 2×10^6 /ml in 37°C medium. The cells were cultured at 0.5 ml/well in a 24-well plate at 37°C in 5% CO₂. To define the optimal stimulation conditions, cells were stimulated with combinations of reagents as listed in Table 3 below:

Table 3

1	2	3	4	5	6
IL-2+IL-12	IL-7+IL-15	Peptides+ IL-2+IL-12	Peptides+ IL-7+IL-15	MVA+ IL- 2+IL-12	MVA+ IL- 7+IL-15

[0273] Final concentrations: IL-2=20 units/ml, IL-12=10 ng/ml, IL-7=10 ng/ml, IL-15=10 ng/ml, peptides=5 µg/ml individual peptide, MVA MOI=1.

[0274] On days 4 and 8, 0.5 ml fresh medium and cytokine at listed concentrations (all concentrations indicate the final concentration in the culture) were added to the stimulated cells.

[0275] *Day 12-24: non-specific expansion and lentivirus transduction.* On day 12, the stimulated cells were removed from the plate by pipetting and resuspended in fresh T cell culture medium at a concentration of 1×10^6 /ml. The resuspended cells were transferred to T25 culture flasks and stimulated with DYNABEADS® Human T-Activator CD3/CD28 following the manufacturer's instruction plus cytokine as listed above; flasks were incubated in the vertical position.

[0276] On day 14, AGT103/CMV-GFP was added at MOI 20 and cultures were returned to the incubator for 2 days. At this time, cells were recovered by pipetting, collected by centrifugation at 1300 rpm for 10 minutes, resuspended in the same volume of fresh medium, and centrifuged again to form a loose cell pellet. That cell pellet was resuspended in fresh medium with the same cytokines used in previous steps, with cells at 0.5×10^6 viable cells per ml.

[0277] From days 14 to 23, the number of the cells was evaluated every 2 days and the cells were diluted to 0.5×10^6 /ml with fresh media. Cytokines were added every time.

[0278] On day 24, the cells were collected and the beads were removed from the cells. To remove the beads, cells were transferred to a suitable tube that was placed in the sorting magnet for 2 minutes. Supernatant containing the cells was transferred to a new tube. Cells were then cultured for 1 day in fresh medium at 1×10^6 /ml. Assays were performed to determine the frequencies of antigen-specific T cells and lentivirus transduced cells.

[0279] To prevent possible viral outgrowth, amprenavir (0.5 ng/ml) was added to the cultures on the first day of stimulation and every other day during the culture.

[0280] *Examine antigen-specific T cells by intracellular cytokine staining for IFN-gamma.* Cultured cells after peptide stimulation or after lentivirus transduction at 1×10^6 cells/ml were stimulated with medium alone (negative control), Gag peptides (5 µg/ml individual peptide), or PHA (5 µg/ml, positive control). After 4 hours, BD GolgiPlug™ (1:1000, BD Biosciences) was added to block Golgi transport. After 8 hours, cells were washed and stained with extracellular (CD3, CD4 or CD8; BD Biosciences) and intracellular (IFN- gamma; BD Biosciences) antibodies with BD Cytfix/Cytoperm™ kit following the manufacturer's instruction. Samples were analyzed on a BD FACSCalibur™ Flow Cytometer. Control samples labeled with appropriate isotype-matched antibodies were included in each experiment. Data were analyzed using Flowjo software.

[0281] Lentivirus transduction rate was determined by the frequency of GFP+ cells. The transduced antigen-specific T cells are determined by the frequency of CD3+CD4+GFP+IFN gamma + cells; tests for CD3+CD8+GFP+IFN gamma + cells are included as a control.

[0282] These results indicate that CD4 T cells, the target T cell population, can be transduced with lentiviruses that are designed to specifically knock down the expression of HIV-specific proteins, thus producing an expandable population of T cells that are immune to the virus. This example serves as a proof of concept indicating that the disclosed lentiviral constructs can be used in combination with vaccination to produce a functional cure in HIV patients.

Example 3: Clinical Study for Treatment of HIV

[0283] AGT103-T is a genetically modified autologous PBMC containing $> 1 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector.

[0284] A Phase I clinical trial will test the safety and feasibility of infusing *ex vivo* modified autologous CD4 T cells (AGT103-T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts > 500 cells per mm^3 of blood and stable virus suppression below 200 copies per ml of plasma while on cART. All study participants will continue receiving their standard antiretroviral medications through the Phase I clinical trial. Up to 40 study participants receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro

testing to measure the frequency of CD4⁺ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polyprotein. Subjects in the upper half of vaccine responders, based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not
5 continue in the study. We anticipate that the cut-off for higher responders is a HIV-specific CD4⁺ T cell frequency $\geq 0.065\%$ of total CD4 T cells. Subjects enrolled into the gene therapy arm of our trial undergo leukapheresis followed by purification of PBMC (using Ficoll density gradient centrifugation or negative selection with antibodies) that are cultured *ex vivo* and stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12
10 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during *ex vivo* culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The
15 culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1 \times 10^{10}$ autologous PBMC. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include the product must include $\geq 0.5 \times 10^7$ HIV-specific CD4 T cells that
20 are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103-T subjects receive one dose of busulfuram (or Cytosan) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ PBMC containing genetically modified CD4 T cells.

[0285] A Phase II study will evaluate efficacy of AGT103-T cell therapy. Phase II study
25 participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that
30 will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws.

Persistently high viremia requires participants to return to their original antiretroviral drug regimen with or without Maraviroc according to recommendations of their HIV care physician.

[0286] If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

[0287] Patient Selection

[0288] Inclusion Criteria:

- Aged between 18 and 60 years.
- Documented HIV infection prior to study entry.
- Must be willing to comply with study-mandated evaluations; including not changing their antiretroviral regimen (unless medically indicated) during the study period.
- CD4+ T-cell count >500 cell per millimeter cubed (cells/mm³)
- CD4+ T-cell nadir of >400 cells/mm³
- HIV viral load <1,000 copies per milliliter (mL)

Exclusion Criteria:

- Any viral hepatitis
- Acute HIV infection
- HIV viral load >1,000 copies/mL
- Active or recent (prior 6 months) AIDS defining complication
- Any change in HIV medications within 12 weeks of entering the study
- Cancer or malignancy that has not been in remission for at least 5 years with the exception of successfully treated basal cell carcinoma of the skin
- Current diagnosis of NYHA grade 3 or 4 congestive heart failure or uncontrolled angina or arrhythmias
- History of bleeding problems
- Use of chronic steroids in past 30 days
- Pregnant or breast feeding

- Active drug or alcohol abuse
- Serious illness in past 30 days
- Currently participating in another clinical trial or any prior gene therapy

[0289] Safety assessments

- 5
- Acute infusion reaction
 - Post-infusion safety follow-up

[0290] Efficacy assessments – Phase I

- 10
- Number and frequency of modified CD4 T cells.
 - Durability of modified CD4 T cells.
 - In vitro response to Gag peptide re-stimulation (ICS assay) as a measure of memory T cell function.
 - Polyfunctional anti-HIV CD8 T cell responses compare to pre- and post-vaccination time points.
 - Frequency of CD4 T cells making doubly spliced HIV mRNA after in vitro
- 15 stimulation.

[0291] Efficacy assessments – Phase II

- 20
- Number and frequency of genetically modified CD4 T cells.
 - Maintenance of viral suppression ($< 2,000$ vRNA copies per ml but 2 consecutive weekly draws not exceeding 5×10^4 vRNA copies per ml are permitted) with Maraviroc monotherapy.
 - Continued virus suppression during and after Maraviroc withdrawal.
 - Stable CD4 T cell count.

[0292] AGT103-T consists of up to 1×10^{10} genetically modified, autologous CD4⁺ T cells containing $\geq 1 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103

25 lentivirus vector. A Phase I clinical trial will test the safety and feasibility of infusing *ex vivo* modified autologous CD4 T cells (AGT103-T) in adult research participants with confirmed HIV infection, CD4⁺ T-cell counts > 500 cells per mm^3 of blood and stable virus suppression below 200 copies per ml of plasma while on cART. Up to 40 study participants receive two doses by intramuscular injection 8 weeks apart, of recombinant modified vaccinia Ankara

30 (rMVA) expressing HIV Gag, Pol and Env proteins. Seven to 10 days after the second immunization a blood sample is collected for in vitro testing to measure the frequency of CD4⁺ T-cells that respond to stimulation with a pool of overlapping, synthetic peptides representing the HIV-1 Gag polyprotein. Subjects in the upper half of vaccine responders,

based on measuring the frequency of Gag-specific CD4 T cells are enrolled in the gene therapy arm and subjects in the lower half of responders do not continue in the study. We anticipate that the cut-off for higher responders is a HIV-specific CD4⁺ T cell frequency $\geq 0.065\%$ of total CD4 T cells. Subjects enrolled into the gene therapy arm of our trial undergo leukapheresis and the CD4⁺ T cells are enriched by negative selection. The enriched CD4 subset is admixed with 10% the number of cells from the CD4-negative subset to provide a source and antigen-presenting cells. The enriched CD4 T cells are stimulated with HIV Gag peptides plus interleukin-2 and interleukin-12 for 12 days, then stimulated again with beads decorated with CD3/CD28 bispecific antibody. The antiretroviral drug Saquinavir is included at 100 nM to prevent emergence of autologous HIV during *ex vivo* culture. One day after CD3/CD28 stimulation cells are transduced with AGT103 at multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which time they expand by polyclonal proliferation. The culture period is ended by harvesting and washing cells, setting aside aliquots for potency and safety release assays, and resuspending the remaining cells in cryopreservation medium. A single dose is $\leq 1 \times 10^{10}$ autologous cells enriched for the CD4⁺ T cell subset. The potency assay measures the frequency of CD4 T cells that respond to peptide stimulation by expressing interferon-gamma. Other release criteria include that the product must include $\geq 0.5 \times 10^7$ HIV-specific CD4 T cells that are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell must not exceed 3. Five days before infusion with AGT103-T subjects receive one dose of busulfuram (or Cytosan) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ enriched and genetically modified CD4 T cell.

[0293] A Phase II study will evaluate efficacy of AGT103-T cell therapy. Phase II study participants include individuals enrolled previously in our Phase I study who were judged to have successful and stable engraftment of genetically modified, autologous, HIV-specific CD4 T cells and clinical responses defined as positive changes in parameters monitored as described in efficacy assessments (1.3.). Study participants will be asked to add Maraviroc to their existing regimen of antiretroviral medication. Maraviroc is a CCR5 antagonist that will enhance the effectiveness of genetic therapy directed at reducing CCR5 levels. Once the Maraviroc regimen is in place subjects will be asked to discontinue the previous antiretroviral drug regimen and only maintain Maraviroc monotherapy for 28 days or until plasma viral RNA levels exceed 10,000 per ml on 2 sequential weekly blood draws. Persistently high viremia requires participants to return to their original antiretroviral drug

regimen with or without Maraviroc according to recommendations of their HIV care physician.

[0294] If participants remain HIV suppressed (below 2,000 vRNA copies per ml of plasma) for >28 days on Maraviroc monotherapy, they will be asked to gradually reduce Maraviroc dosing over a period of 4 weeks followed by intensive monitoring for an additional 28 days. Subjects who maintained HIV suppression with Maraviroc monotherapy are considered to have a functional cure. Subjects who maintain HIV suppression even after Maraviroc withdrawal also have a functional cure. Monthly monitoring for 6 months followed by less intensive monitoring will establish the durability of functional cure.

Example 4: Generating a Population of CD4+ T cells through Depletion of CD8+ T cells prior to Peptide Stimulation

[0295] Because CD8+ T cell overgrowth significantly impacted the expansion of target CD4+ T cells, CD8+ T cells were depleted at the beginning of cell expansion to determine whether it would improve CD4+ T cell expansion. Current CD8+ T cell depletion methods require that cells are passed through a magnetic column. To avoid possible impacts of that procedure on antigen presenting cells and CD4+ T cells, the cell depletion was performed after peptide stimulation and before lentivirus transduction when cells were better able to withstand the mechanical stresses. The objective was to increase the final yield of CD4+ T cells and also increase the yield of target cells (HIV gag-protein CD4+ T cells that are also transduced with the AGT103 lentivirus vector). Figure 8 depicts a schematic of a CD8+ T cell depletion protocol. The method used to deplete CD8+ T cells is described in Example 8.

[0296] As shown in Figures 9, 11 and 12, CD8+ T cell depletion resulted in a substantial increase in the percentage of Gag-specific CD4+ T cells relative to control treatments in which CD8+ T cells were not depleted; Gag-specific cells express interferon- γ and the target cells are interferon- γ + and CD4+ (upper right quadrants). For PTID: 01-006, after CD8+ depletion, the value was 1.7% of total T cells compared to 0.69% in the control treatments in which CD8+ T cells were not depleted (Figure 9, upper right quadrants of Day 15 GagPepMix). Referring to Figure 9, on day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 44.5%, 55.5%, 0.032%, and 0%, respectively compared to 44.2%, 55.3%, 0.48% and 0.053% for the peptide-stimulated culture (1 day). On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 79.8%, 20.1%,

0.12%, and 0.018%, respectively. On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix had a fluorescence intensity of 58.9%, 19.2%, 21.2%, and 0.69%, respectively. On day 15, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 64.4%, 35.0%, 0.44%, and 0.14%, respectively. On day 15, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 61.9%, 32.9%, 3.47%, and 1.70%, respectively. The proportion of CD4⁺ T cells without CD8 depletion was 20.1% (Day 15 Control, sum of upper right and lower right quadrants) compared to 35.1% with CD8 T cell depletion (Day 15 Control, sum of upper right and lower right quadrants). We identified the large population of CD3⁺/CD4-negative/CD8-negative cells present in the final product as $\gamma\delta$ T cells of the subclass V δ 1 (Figure 10). Thus, in addition to depleting CD8⁺ T cells it may be necessary to also delete $\gamma\delta$ T cells.

[0297] For PTID: 01-007, the CD8⁺ T cell depletion increased the yield of target cells by approximately 6-fold relative to control treated cells in which CD8⁺ T cells were not depleted (Figure 11). Referring to Figure 11, on day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 33.6%, 66.4%, 5.9E-4%, and 1.78E-3%, respectively. On day 0 (following 16 hours exposure to Gag peptides), the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 33.7%, 66.3%, 0.011%, and 0.016%, respectively. On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 78.4%, 21.2%, 0.30%, and 0.018%, respectively. On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix had a fluorescence intensity of 76.3%, 20.2%, 2.95%, and 0.61%, respectively. On day 15, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 50.9%, 48.7%, 0.36%, and 0.10%, respectively. On day 15, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 51.6%, 44.4%, 0.43%, and 3.60%, respectively.

[0298] For PTID: 01-008, depletion of CD8⁺ T cells resulted in increased target cell yield of approximately 3-fold compared to control treated cells in which CD8⁺ T cells were not depleted (Figure 12). Referring to Figure 12, on day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 65.4%, 34.5%, 0.096%, and 7.71E-4%, respectively. On day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 65.4%, 34.3%, 0.20%, and 0.10%, respectively. On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 87.9%, 12.1%, 0.028%, and 6.24E-3%, respectively. On day 15, without CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix had a fluorescence intensity of 82.3%, 12.1%, 5.38%, and 0.23%, respectively. On day 16, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 87.8%, 12.0%, 0.22%, and 0.013%, respectively. On day 16, with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 87.8%, 11.1%, 0.30%, and 0.78%, respectively. For PTID: 01-008, the contaminating cells present at the end of process were identified as CD3⁺/CD4⁻negative/CD56⁺, meaning they are most likely NK cells (Figure 13).

[0299] Together, these data show that CD8⁺ T cell depletion had a consistent effect of increasing the Gag-responding CD4⁺ T cells for HIV⁺ patient PBMC samples.

Example 5: CD8 Cell Depletion Increased the Percentage of Gag-specific CD4⁺ T Cells but Outgrowth of Vδ1 Cells and CD56⁺ NK cells Impeded Growth of the Target Cell Population

[0300] For PTID: 01-006, CD8⁺ T cell depletion using the protocol described in Example 8 resulted in a final product that was less than 50% CD4⁺ T cells and that contained an outgrowth of Vδ1⁺ γδ T cells (Figure 10). This result suggests that depleting both CD8⁺ T cells and Vδ1⁺ T cells may increase the yield of CD4⁺ T cells and may also increase the yield of target cells. Gating data for lymphocytes showed 77.6% fluorescence intensity (far left graph of Figure 10). Using the parameter of CD3 expression, the fluorescence intensity was 75.3% (middle, left graph of Figure 10). Using the parameter of CD4 expression, fluorescence was measured in 4 separate quadrants (middle graph of figure 10). The lower

left quadrant showed a fluorescence intensity of 45.3% (upper value) and 45.5% (lower value). The lower right quadrant showed a fluorescence intensity of 44.9%. The upper left quadrant showed a fluorescence intensity of 9.26%. The upper left quadrant showed a fluorescence intensity of 0.35%. Using the parameter of PAN $\gamma\delta$, the fluorescence intensity was 73.8% (middle, right graph of Figure 10). Using the parameter of V δ 1, fluorescence was measured in 4 separate quadrants. The lower left quadrant showed a fluorescence intensity of 16.9%. The lower right quadrant showed a fluorescence intensity of 82.8%. The upper left quadrant showed a fluorescence intensity of 0.14%. The upper right quadrant showed a fluorescence intensity of 0.12%.

[0301] For PTID: 01-008, depletion of CD8⁺ T cells resulted in low yield of CD4⁺ T cells, which was likely due to expansion of CD56⁺ NK cells (Figure 13). The fluorescence intensity in the left graph using the variables of CD3 and CD4 defined 83.1% of cells as negative for both CD3 and CD4 expression, most likely NK cells. The fluorescence intensity in the right graph using the variable of CD56 was 65.7%. Due to their expansion, the CD56⁺ NK cells competed with the CD4⁺ T cells in the culture medium. This result suggests that routine depletion of CD56⁺ NK cells may be another important step in manufacturing of CD4⁺ T cells.

Example 6: Generating a Population of CD4⁺ T cells using various Depletion Protocols

[0302] Because CD8⁺ T cell depletion alone resulted in outgrowth of V δ 1 and CD56⁺ NK cells, various depletion protocols were developed. A depletion protocol was developed in which CD8⁺ T cells, CD56 NK cells, CD19 B cells, and $\gamma\delta$ cells were all depleted. A flow diagram of this scheme is depicted in Figure 14.

[0303] Figure 15 shows comparisons of various cell depletion strategies, including “no depletion” control; CD8⁺ T cell depletion; CD8⁺ T cell and $\gamma\delta$ T cell depletion; and CD8⁺ T cell, $\gamma\delta$ T cell, and B cell depletion. Target cells were identified by their response to peptide re-stimulation with subsequent intracellular accumulation of interferon-gamma. The *ex vivo* sample contained 0.055% of total T cells that were CD4⁺ and specific for responding to HIV gag peptides. The protocol for cell depletion in Example 8 was used to deplete the cells of different factors. Briefly, cells were cultured for 18 hours with PBMC plus Gag peptides followed by cell depletion (or the “no-depletion” control). After cell depletion, cells were cultured for an additional 12 days during which the cells were regularly fed supplementation with IL-7 and IL-15 cytokines. Depletion of CD8⁺ T cells, $\gamma\delta$ T cells, and CD19⁺ B cells produced the highest yield of target cells (CD4⁺ T cells and interferon-gamma⁺ cells)

(Figure 15).

[0304] Referring to Figure 15, on day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 56.4%, 43.5%, 0.034%, and 7.44E-4%, respectively. On day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 54.8%, 44.8%, 0.30%, and 0.055%, respectively. After 18 hours with no depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 83.9%, 16.0%, 0.061%, and 0.027%, respectively. After 18 hours with no depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 77.6%, 15.4%, 6.39%, and 0.54%, respectively. After 18 hours with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 41.9%, 57.9%, 0.094%, and 0.099%, respectively. After 18 hours with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 43.3%, 50.7%, 3.00%, and 2.98%, respectively. After 18 hours with CD8 and $\gamma\delta$ depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 40.4%, 59.3%, 0.12%, and 0.13%, respectively. After 18 hours with CD8 and $\gamma\delta$ depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 38.3%, 54.7%, 3.14%, and 3.86%, respectively. After 18 hours with CD8, $\gamma\delta$, and B depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 46.2%, 53.6%, 0.13%, and 0.080%, respectively. After 18 hours with CD8, $\gamma\delta$, and B depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 42.1%, 48.5%, 4.28%, and 5.06%, respectively.

[0305] Figure 16 shows comparison of various cell depletion strategies, include “no depletion control; CD8+ T cell depletion; and depletion of 4 cell types including CD8+ T cells, CD56+ NK cells, $\gamma\delta$ T cells, and B cells. After depletion of the 4 cell types, a substantial increase in the yield of CD4+ T cells/interferon-gamma+ target cells was observed.

[0306] Referring to Figure 16, on day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 42.6%, 57.4%, 2.71E-3%, and 0.0%, respectively. On day 0, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 42.5%, 57.4%, 0.031%, and 0.048%, respectively. After 18 hours with no depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 79.5%, 20.5%, 0.017%, and 9.73E-3%, respectively. After 18 hours with no depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 78.9%, 19.5%, 0.93%, and 0.65%, respectively. After 18 hours with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the control, had a fluorescence intensity of 51.4%, 48.4%, 0.11%, and 0.063%, respectively. After 18 hours with CD8 depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 51.7%, 43.0%, 0.22%, and 5.03%, respectively. After 18 hours with CD8, CD56, $\gamma\delta$, and B depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the cells that had no stimulation, had a fluorescence intensity of 12.8%, 87.0%, 0.14%, and 0.10%, respectively. After 18 hours with CD8, CD56, $\gamma\delta$, and B depletion, the lower left quadrant, the lower right quadrant, the upper left quadrant, and the upper right quadrant of the GagPepMix, had a fluorescence intensity of 13.2%, 79.4%, 0.27%, and 7.17%, respectively.

Example 7: Generating a Population of CD4+ T cells using a 4-Way Cell Depletion Protocol

[0307] Figure 17 depicts a flow diagram for an experiment using 4-way cell depletion, which depletes CD8+ T cells, CD56+ NK cells, CD19+ B cells, and $\gamma\delta$ T cells. The LV-GFP surrogate viral vector is used to transduce the cells. This vector has identical cell tropism to AGT103 but it expresses the green fluorescence protein marker so that transduction efficiency can be easily assessed. T cells were stimulated with CD3/CD28 beads to increase transduction efficiency. An assay that measures interferon-gamma expression was used to measure transduction efficiency in multiple cell subsets.

[0308] As shown in Figure 18, peptide stimulation alone followed by depletion of competing cells, was sufficient for efficient lentivirus transduction and efficiency was

highest in the CD4+/Gag-specific target cells. In this typical result, 68% of target cells were transduced with the LV-GFP vector. Referring to Figure 18, on the middle graph the lower right quadrant (68.6% fluorescence) and upper right quadrant (12.6% fluorescence), had a GFP transduction efficiency of 41.5% (lower, right graph), and 67.8%, respectively (upper, right graph). This is in contrast to the lower left quadrant (9.75% fluorescence) and the upper left quadrant (2.46% fluorescence) of the middle graph, which had a GFP transduction efficiency of 35.6% (lower, left graph) and 43.3%, respectively (upper, left graph).

Example 8: Laboratory Protocol for Cell Depletion

[0309] **Day 0:** Approximately 1×10^7 viable peripheral blood mononuclear cells (PBMCs) from venous blood of HIV-positive participants were cultured in 1mL of TexMACS GMP Medium (Miltenyi Biotec, Auburn, CA) containing 5% human serum (Gemini Bio Products, West Sacramento, CA), 10ng/mL IL7/IL15 (Miltenyi Biotec, Auburn, CA) and 100nM Saquinavir (NIH AIDS Reagent Program, Germantown, MD) in a 24-well plate. The cells were incubated with 1 μ g/mL PepMix HIV-1 (GAG) Ultra (JPT Peptide Technologies, Berlin, Germany) for 16 to 18 hours at 37°C; 1 μ g/mL was the concentration for each individual peptide in the final stimulation condition.

[0310] **Day 1:** After 16 to 18 hours incubation, CD8, CD56, CD19 and/or $\gamma\delta$ cells were depleted with PE anti-human CD8 Ab clone sk1 (BioLegend, San Diego, CA), PE anti-human CD56 Ab clone 5.1H11 (BioLegend, San Diego, CA), PE anti-human CD19 Ab clone HIB19 (BioLegend, San Diego, CA), and PE anti-human TCR $\gamma\delta$ Ab clone B1 (BioLegend, San Diego, CA) and anti-PE microbeads (for example, Anti-PE MicroBeads UltroPure, Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. Cells were stained with PE-conjugate antibodies for 10 minutes on ice. The labeled cells were washed and incubated with anti-PE MicroBeads for 15 minutes on ice, then separated in a magnetic field wherein cells expressing the target surface proteins were retained on the column and unlabeled (negatively-selected) cells passed through and were collected. Approximately 1×10^6 viable negatively-selected PBMCs were seeded in 0.5mL TexMACS GMP Medium containing 5% human serum, 10ng/mL IL7/IL15, and 100nM Saquinavir.

[0311] **Day 2:** Cells were transduced with lentivirus vector carrying GFP at an MOI of 5 to 10.

[0312] **Day 4:** Cells were fed with 0.5mL TexMACS GMP Medium containing 5% human serum, 20ng/mL IL7/IL15 (final 10ng/mL) and 200nM Saquinavir (final 100nM).

[0313] **Day 7:** Cells were fed with 1mL TexMACS GMP Medium containing 5% human

serum, 20ng/mL IL7/IL15 (final 10ng/mL) and 200nM Saquinavir (final 100nM).

[0314] **Day 10:** Cells were mixed thoroughly including the clumps of cells on the bottom of the well. After mixing, the entire volume of cells was transferred into a 12-well plate. Cells were then fed with 2mL TexMACS GMP Medium containing 5% human serum,
5 20ng/mL IL7/IL15 (final 10ng/mL) and 200nM Saquinavir (final 100nM).

[0315] **Days 14 to 16:** Cell counts were performed and cells were stimulated with peptide stimulation assay. 1×10^6 expanded cells were stimulated without or with 1 μ g/mL PepMix HIV-1 (GAG) Ultra for 4 hours in a 96-well plate.

[0316] To detect intracellular IFN- γ , Protein transport inhibitor GolgiPlug (Containing Brefeldin A) (BD Biosciences, San Jose, CA) was added. Cells were stained with FITC
10 anti-human CD3 Ab clone OKT3, PE anti-human CD4 Ab clone OKT4 and PerCP anti-human CD8 Ab clone SK1. After staining, cells were fixed, permeabilized, and incubated for 45 min at 4°C with APC anti-human IFN- γ clone B27. Intracellular staining solutions were obtained from the Cytotfix/Cytoperm Kit (BD Biosciences, San Jose, CA).

[0317] GFP expression was analyzed to determine the transduction efficiency of HIV-specific CD4 T cells. An IFN- γ Secretion Assay Cell Enrich and Detect Kit (PE) (Miltenyi Biotec, Auburn, CA) was used to detect IFN- γ secreting cells according to the manufacturer's instructions. Cells were labeled with IFN- γ Catch Reagent for 5 minutes on ice. The labeled cells were incubated in a closed tube for 45 minutes at 37 °C under slow
15 continuous rotation, then stained with PE anti-IFN- γ detection Ab, PerCP anti-human CD3 Ab clone OKT3 and APC anti-human CD4 Ab clone OKT4.

[0318] Data were acquired for at least 1×10^5 lymphocytes (gated on the basis of forward- and side-scatter profiles) from each sample, using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). All samples were analyzed using FlowJo software (FlowJo
20 10.1r1, Tree Star, San Carlos, CA).

Example 9: Commercial Protocol for Cell Depletion

[0319] In a commercial version of the protocol for manufacturing AGT103-T, PBMC purification, peptide stimulation and cell depletion are performed as described above, and cultured for 2 days before transferring the total culture fluid (200 ml) to a GREX 500M
30 static culture flask or similar container for cell culturing with no or minimal agitation, containing approximately 5 liters of culture medium including IL-7 and IL-15 cytokines. The filled flask is incubated at 37°C with 5% CO₂ for 7 days without agitation, then the entire volume is collected, cells are washed and resuspended in cryopreservation solution at

- approximately 1×10^8 total nucleated cells per mL, then frozen and stored in the vapor phase of liquid N₂. Inclusion of the static phase culture further increases the target cell yield, as this cell subset is demonstrated in laboratory studies to be fragile and susceptible to depletion during mechanical collection and/or culture agitation in a non-static culture system.

Sequences

The following sequences are referred to herein:

SEQ ID NO:	Description	Sequence
1	miR30 CCR5 coding sequence	AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTT
2	miR21 Vif coding sequence	CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGA ACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA
3	miR185 Tat coding sequence	GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCTCATAGCGTGGTCCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG
4	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGA TGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGG GTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTA CTTCCACGCCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGG AGCCCCCTTCGCTCGTGTGCTTGAAGTTCGAGGCCTGGCCTGGGCGCTG GGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGC TGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTTTGATGACCTGC TGCGACGCTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCA AGATCTGCACACTGGTATTTTCGGTTTTTTGGGGCCGCGGGCGGCG ACGGGGCCCCGTGCGTCCCAGCGCACATGTTCCGGCAGGCGGGGC CTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGC TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTCCGGCACCAGTTGCGT GAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA AAATGGAGGACGCGGCCTCGGGAGAGCGGGCGGGTGAGTCAC CCACACAAAGGAAAAGGGCCTTCCGTCCTCAGCCGTCGCTTCA TGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTA GTTCTCGAGCTTTTGGAGTACGTGCTCTTTAGGTTGGGGGGAGGG GTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTG AAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGAATTTG CCCTTTTTGAGTTTGGATCTTGGTTTATTCTCAAGCCTCAGACAG TGGTTCAAAGTTTTTTCTTCCATTTTCAGGTGTCGTGA
5	CCR5 miRNA target sequence	GAGCAAGCTCAGTTTACA
6	Vif miRNA	GGGATGTGTACTTCTGAACTT

	coding sequence	
7	Tat miRNA coding sequence	TCCGCTTCTTCCTGCCATAG
8	TAR decoy sequence	CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTCTCGACAGC GACCAGATCTGAGCCTGGGAGCTCTCTGGCTGTCAGTAAGCTGG TACAGAAGGTTGACGAAAATTCTTACTGAGCAAGAAA
9	Rev/Tat shRNA target sequence #1	GCGGAGACAGCGACGAAGAGC
10	Rev/Tat shRNA coding sequence #1	GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTG TCTCCGCTTTTT
11	Gag shRNA target sequence	GAAGAAATGATGACAGCAT
12	Gag shRNA coding sequence	GAAGAAATGATGACAGCATTTC AAGAGAATGCTGTCATCATTTC TTCTTTTT
13	Pol shRNA target sequence	CAGGAGCAGATGATACAG
14	Pol shRNA coding sequence	CAGGAGATGATACAGTTCAAGAGACTGTATCATCTGCTCCTGTTT TT
15	CCR5 shRNA target sequence #1	GTGTCAAGTCCAATCTATG
16	CCR5 shRNA coding sequence #1	GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGA CACTTTTT
17	CCR5 shRNA target sequence #2	GAGCATGACTGACATCTAC
18	CCR5 shRNA coding sequence #2	GAGCATGACTGACATCTACTTCAAGAGAGTAGATGTCAGTCATG CTCTTTTT
19	CCR5 shRNA target sequence #3	GTAGCTCTAACAGGTTGGA
20	CCR5 shRNA coding sequence #3	GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCTGTTAGAGC TACTTTTT
21	CCR5 shRNA target sequence #4	G TTCAGAAACTACCTCTTA
22	CCR5 shRNA coding sequence #4	G TTCAGAAACTACCTCTTATTCAAGAGATAAGAGGTAGTTTCTGA ACTTTTT
23	CCR5 shRNA target sequence #5	GAGCAAGCTCAGTTTACACC
24	CCR5 shRNA coding	GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAACTGAGCT TGCTCTTTTT

	sequence #5	
25	Homo sapiens CCR5 mRNA target sequence 1	ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTAT ACATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAATCGCAGC CCGCCTCCTGCCTCCGCTCTACTCACTGGTGTTCATCTTTGGTTTT GTGGGC
26	Homo sapiens CCR5 mRNA target sequence 2	AACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAA GAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCT GTTTTTCCTTCTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCC CAGTGGGACTTTGGAAATACAATGTGTCAACTCTTGACAGGGCT CTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATCCTCCTG ACAATCGATAGGTACCTGGCTGTCTGTCATGCTGTGTTTGCTTTA AAAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTGTGATCAC TTGGGTGGTGGCTGTGTTTGCGTCTCTCCAGGAATCATCTTTAC CAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCTCATTT TCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAA GATAGTCATCTTGGGGCTGGTCTGCCGCTGCTTGTCATGGTCAT CTGCTACTCGGAATCCTAAAAACTCTGCTTCGGTGTGAAATGA GAAGAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCATCATGA TTGTTTATTTTCTTCTTGGGCTCCCTACAACATTGTCCTTCTCCT GAAC
27	Homo sapiens CCR5 mRNA target sequence 3	ACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAAC AGGTTGGACCAAGCTATGCAGGTGA
28	Homo sapiens CCR5 mRNA target sequence 4	CAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCCATCATCT ATGCCTTTGTCTGGGGAGAAAGTTCAGAACTACCTCTTAGTCTTCT TCCAAAAGCACATTGCCAAACGCTTCTGCAATGCTGTTCTATTT TCCAG
29	Homo sapiens CCR5 mRNA target sequence 5	CAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACACCCGATCCAC TGGGGAGCAGGAAATATCTGTGGGCTTGTGA
30	miR30- CCR5/miR21- Vif/miR185 Tat microRNA cluster coding sequence	AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCT CTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGC TGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATG GCTGTACCACCTTGTCTGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGG CGAGGGATTCCGCTTCTTCCCTGCCATAGCGTGGTCCCCTCCCCTA TGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC
31	Long WPRE sequence	AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATT CTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAA TGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTC CTCCTTGTATAAATCCTGGTTGCTGTCTCTTATGAGGAGTTGTG GCCCCTTGTACAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGA CGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCT TTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACT CATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTT GGGCACTGACAATTCCGTGGTGTGTCGGGGAAATCATCGTCCTT TCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGAC GTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCC TTCCCGCGGCTGCTGCCGGCTCTGCGGCCTTTCGCGCTCTTCG

		CCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCC GCCT
32	Elongation Factor-1 alpha (EF1-alpha) promoter; miR30CCR5; miR21Vif; miR185 Tat cluster coding sequence	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGA TGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGG GTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCCTGCTTGAATTA CTTCCACGCCCCCTGGCTGCAGTACGTGATTCTTGATCCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTTCGAGGCCTTGCCTTAAGG AGCCCCCTTCGCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTG GGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGC TGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGC TGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCA AGATCTGCACACTGGTATTTTCGGTTTTTTGGGGCCCGGGCGGGC ACGGGGCCCGTGCGTCCCAGCGCACATGTTCCGGCGAGGCGGGGC CTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGC TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCCCCTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGT GAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCAC CCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCA TGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTA GTTCTCGAGCTTTTGAGTACGTCTCTTTAGGTTGGGGGGAGGG GTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTG AAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTG CCCTTTTTGAGTTTGATCTTGTTTCAATTCTCAAGCCTCAGACAG TGGTTCAAAGTTTTTTCTTCCATTTAGGTGTCGTGATGTACA AGGTATATTGCTGTTGACAGTGAGCGACTGTAACTGAGCTTGCT CTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGC TGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGCATCTCCATG GCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAAGTTGTGTTG AATCTCATGGAGTTTCAAGAACAACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGG CGAGGGATTCCGCTTCTTCTGCCATAGCGTGGTCCCCTCCCCTA TGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC
33	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGAT GAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCAT GCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGA AGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGAATT GCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAA TAAACG
34	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCT AACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGA GTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAAC TAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGC A
35	Psi Packaging signal	TACGCCAAAAATTTTGAAGTAGCGGAGGCTAGAAGGAGAGAG
36	Rev response element (RRE)	AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA TGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTA TTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATT GAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAA

		GCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGG ATCAACAGCTCC
37	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAA AGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAATT ACAAAAACAAATTACAAAATTCAAAATTTTA
38	3' delta LTR	TGGAAGGGCTAATTCACCTCCCAACGAAGATAAGATCTGCTTTTTG CTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCT TGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT CTGGTAACTAGAGATCCCTCAGACCCTTTAGTCAGTGTGGAAA ATCTCTAGCAGTAGTAGTTCATGTCA
39	CMV early (CAG) enhancer; Enhance Transcription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC ATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCCGCC TGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGA CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC AATGGGTGGACTATTTACGGTAACTGCCCACTTGGCAGTACAT CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC GGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATG GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
40	Chicken beta actin (CAG) promoter; Transcription	GCTATTACCATGGGTGCGAGGTGAGCCCCACGTTCTGCTTCACTCT CCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTATT TTTTAATTATTTTGTGACGCGATGGGGGCGGGGGGGGGGGGGC GCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG GAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTC CGAAAGTTTCCTTTTATGGCGAGGCGGCGGGCGGCGGCGGCCA TAAAAAGCGAAGCGCGCGGGCG
41	Chicken beta actin intron; Enhance gene expression	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCC TCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAG GTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCG CTTGGTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAG CCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGC TCGGGGGGGTGCGTGCGTGTGTGTGCGTGGGAGCGCCGCGTG CGCCCCGCGCTGCCGGCGGCTGTGAGCGCTGCGGGCGCGCGCG GGGGCTTTGTGCGCTCCGCGTGTGCGGAGGGGAGCGCGGCCGG GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAG GCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGTGTG GGCGCGGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCC GAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCG GGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGCGGCG AGGTGGGGGTGCCGGGCGGGGCGGGGCCCGCTCGGGCCGGGGA GGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGCGCCGGCGGCT GTCGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCG TGCGAGAGGGGCGCAGGGACTTCTTTTGTCCCAAATCTGGCGGAG CCGAAATCTGGGAGGCGCCGCCGCACCCCTCTAGCGGGCGCGG GCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGG GCCTTCGTGCGTCCCGCGCCGCGCTCCCTTCTCCATCTCCAGC CTCGGGGCTGCCGAGGGGGACGGCTGCCTTCGGGGGGGACGGG GCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGG
42	HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCG ATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAAAATAATAT AAATTAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATT CGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGAC AAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAA

		GAACCTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTG CATCAAAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACA AGATAGAGGAAGAGCAAAAACAAAAGTAAGAAAAAAGCACAGCA AGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGCCAAAATT ACCCTATAGTGCAGAACATCCAGGGGCAAATGGTACATCAGGCC ATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAGTAGAAGA GAAGGCTTTCAGCCCAGAAGTGATACCCATGTTTTTCAGCATTATC AGAAGGAGCCACCCCAAGATTTAAACACCATGCTAAACACAG TGGGGGGACATCAAGCAGCCATGCAAATGTAAAAAGAGACCATC AATGAGGAAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGC AGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGT GACATAGCAGGAACCTACTAGTACCCTTCAGGAACAAATAGGATG GATGACACATAATCCACCTATCCCAGTAGGAGAAATCTATAAAA GATGGATAATCCTGGGATTAAATAAAAATAGTAAGAATGTATAGC CCTACCAGCATTCTGGACATAAAGACAAGGACCAAGGAACCCTT TAGAGACTATGTAGACCGATTCTATAAAACTCTAAGAGCCGAGC AAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAACCTTGTTG GTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATT GGGACCAGGAGCGACACTAGAAGAAATGATGACAGCATGTCAG GGAGTGGGGGGACCCGGCCATAAAGCAAGAGTTTTGGCTGAAGC AATGAGCCAAGTAACAAATCCAGCTACCATAATGATACAGAAAG GCAATTTTAGGAACCAAGAAAGACTGTTAAGTGTTCATTGT GGCAAAGAAGGGCACATAGCCAAAAATTGCAGGGGCCCTAGGA AAAAGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATGAA AGATTGTACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGC CTTCCACAAAGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCA GAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAAG AGACAACAACCTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGA ACTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACCC CTCGTCACAATAA
43	HIV Pol; Protease and reverse transcriptase	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAA TTGGAGGTTTTATCAAAGTAGGACAGTATGATCAGATACTCATA GAAATCTGCGGACATAAAGCTATAGGTACAGTATTAGTAGGACC TACACCTGTCAACATAAATTGGAAGAAATCTGTTGACTCAGATTG GCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCAG TAAATTTAAAGCCAGGAATGGATGGCCCAAAGTTAAACAATGG CCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTAC AGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAA AATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAG TACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAGA GAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCT GCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGG GCGATGCATATTTTTCAGTTCCTTAGATAAAGACTTCAGGAAGT ATACTGCATTTACCATACCTAGTATAAACAATGAGACACCAGGG ATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATC ACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTT TTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGAT GATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAAC AAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTA CCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCTTTGG ATGGGTATGAACTCCATCCTGATAAATGGACAGTACAGCCTAT AGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGA AATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGG ATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACCAAAGC

		ACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTAGAA CTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAG TGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAG CAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATT TAAAAATCTGAAAACAGGAAAATATGCAAGAATGAAGGGTGCC CACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAAT AGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTA AATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGA GTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCAATAC CCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCA TAATAGGAGCAGAACTTTCTATGTAGATGGGGCAGCCAATAGG GAACTAAATTAGGAAAAGCAGGATATGTAAGTACAGAGGAA GACAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAAGACT GAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGA AGTAAACATAGTGACAGACTCACAATATGCATTGGGAATCATT AAGCACACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATA ATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT ACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATGGG TTGGTCAGTGCTGGAATCAGGAAAGTACTA
44	HIV Integrase; Integration of viral RNA	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATA TCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCAC CTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAG CTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAG GAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATC TTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGT AATTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCTCTTAA AATTAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAA TGGCAGCAATTTACCAGTACTACAGTTAAGGCCGCTGTTGGTG GGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAA GTCAAGGAGTAATAGAATCTATGAATAAAGAATTAAGAAAATT ATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGT ACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGA TTGGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC AACAGACATACAACTAAAGAATTACAAAAACAAATTACAAAA ATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTT TGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAG TAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGA AAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAGGTG ATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA
45	HIV RRE; Binds Rev element	AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTA TTGTCTGGTATAGTGACAGCAGCAGAACAAATTTGCTGAGGGCTATT GAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAA GCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGG ATCAACAGCTCCT
46	HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTCAAGG CAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCA ATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAG GTGGAGAGAGAGACAGAGACAGATCCATTTCGATTAGTGAACGG ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTT CAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGAT TGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAATATT GGTGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAG

47	Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCC CCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCAT TGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGAC ATATGGGAGGGCAAATCATTTAAAAACATCAGAATGAGTATTTGG TTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAA AGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGC TGTCCATTCCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGAT TTTTTTTATATTTTGTGTTTGTGTTATTTTTTTCTTTAACATCCCTAA AATTTTCCTTACATGTTTTACTAGCCAGATTTTTTCCTCCTCTCCTG ACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC
48	Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCC CCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCAT TGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGAC ATATGGGAGGGCAAATCATTTAAAAACATCAGAATGAGTATTTGG TTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACA AAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCT GCTGTCCATTCCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAG ATTTTTTTTATATTTTGTGTTTGTGTTATTTTTTTCTTTAACATCCCT AAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTTCCTCCTCTCC TGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATC
49	CMV promoter; Transcription	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTAC GGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCCAT TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGG ACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCC CACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT ATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCA GTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGT ATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACAT CAATGGGCGTGGATAGCGGTTTGAATCACGGGGATTTCGAAGTC TCCACCCCATTTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATC AACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACG CAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
50	Beta globin intron; Enhance gene expression	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTCGCTATTGTAA AATTCATGTTATATGGAGGGGGCAAAGTTTTTCAGGGTGTGTTTA GAATGGGAAGATGTCCCTTGATCACCATGGACCCTCATGATAA TTTTGTTTCTTTCACCTTCTACTCTGTTGACAACCATTGTCTCCTCT TATTTTCTTTTCATTTTCTGTAACCTTTTTCGTTAACTTTAGCTTGC ATTTGTAACGAATTTTTAAATTCACCTTTTGTGTTATTTGTCAGATTG TAAGTACTTTCTCTAATCACTTTTTTTTTCAAGGCAATCAGGGTAT ATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAAT TAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGGCTGGCG TGGAAATATTCTTATTGGTAGAAACAACCTACACCCTGGTCATCAT CCTGCCTTTCTCTTTATGGTTACAATGATATACACTGTTTGAGAT GAGGATAAAATACTCTGAGTCCAAACCGGGCCCCCTCTGCTAACC ATGTTTCATGCCTTCTTCTTTCTTCTACAG
51	VSV-G; Glycoprotein envelope-cell entry	ATGAAGTGCTTTTGTACTTAGCCTTTTTATTTCATTGGGGTGAATT GCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAACTGG AAAAATGTTCTTCTAATTACCATTATTGCCCGTCAAGCTCAGAT TTAAATTGGCATAATGACTTAATAGGCACAGCCTTACAAGTCAA AATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGGTTGGATGT GTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATG GACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTG

		TAGAACAATGCAAGGAAAGCATTGAACAAACGAAACAAGGAAC TTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATGCAAC TGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCACC ATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTACAG TTCATCAACGGAATAATGCAGCAATTACATATGCCCCACTGTCCAT AACTCTACAACCTGGCATTCTGACTATAAAGGTCAAAGGGCTATG TGATTCTAACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGA CGGAGAGCTATCATCCCTGGGAAAGGAGGGCACAGGGTTCAGA AGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAAT GCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCT GGTTGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCC CTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCT CAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGAT TATCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCT TCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCC AGGAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAAT ACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCC TCTCAAGAATGGTCGGAATGATCAGTGGAACCTACCACAGAAAGG GAACTGTGGGATGACTGGGCACCATATGAAGACGTGGAAATTGG ACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTTT ATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAG CTCAAAGGCTCAGGTGTTTGAACATCCTCACATTCAAGACGCTG CTTGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTG GGCTATCCAAAAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGT AGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGTTA ATCATTGGACTATTCTTGTTCTCCGAGTTGGTATCCATCTTTGCA TTAAATTAAAGCACACCAAGAAAAGACAGATTTATACAGACATA GAGATGA
52	Promoter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGC GCGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCG CCGACCCTGGGTCTCGCACATTCTTCACGTCCGTTTCGACGCTCA CCCGGATCTTCGCCGCTACCCTTGTGGGCCCCCGGCGACGCTTC CTGCTCCGCCCCCTAAGTCGGGAAGGTTCTTGCGGTTCGCGGCGT GCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTC GCAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGC GATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAG AGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGG CGGTAGTGTGGGCCCTGTTCTGCCCCGCGCGGTGTTCCGCATTCT GCAAGCCTCCGGAGCGCACGTCCGCAGTCGGCTCCCTCGTTGAC CGAATCACCGACCTCTCTCCCCAG
53	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCCTCACGGC GAGCGCTGCCACGTACAGACGAAGGGCGCAGGAGCGTTCTGATC CTTCCGCCCCGACGCTCAGGACAGCGGCCCGCTGCTCATAAGAC TCGGCCTTAGAACCCCAAGTATCAGCAGAAGGACATTTTAGGACG GGAATTGGGTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGG AACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGG GATCTCCGTGGGGCGGTGAACGCCGATGATTATATAAGGACGCG CCGGGTGTGGCACAGCTAGTTCGTCGACCGGGGATTGTTGGGTC GCGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTG CGGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCGCT CGGTGGGACGGAAGCGTGTGGAGAGACCGCAAGGGCTGTAGT CTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTGGGGGGA GCGCACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGC TTGTAAGGCGGGCTGTGAGGTCGTTGAAACAAGGTGGGGGGCAT

		GGTGGGCGGCAAGAACCCAAGGTCTTGAGGCCTTCGCTAATGCG GGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGGG GACCTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGGTTTGT CGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGCCGTTGGGCAGT GCACCCGTACCTTTGGGAGCGCGCGCCTCGTCGTGTCGTGACGTC ACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCGGT AGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCCG GCCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGGACCTCTGG TGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTT ATGTACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTGAACAT GCGCTCGGGGTGGCGAGTGTGTTTTGTGAAGTTTTTAGGCACC TTTTGAAATGTAATCATTTGGGTCAATATGTAATTTTCAGTGTA GACTAGTAAA
54	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCAC AAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGG TTTGTCCAAACTCATCAATGTATCTTATCA
55	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCC GTGCCCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCTTTCC TAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCA TTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAG GATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTC TATGG
56	HIV Gag; Bal	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGGAGAATTAGATAG GTGGGAAAAAATTCGGTTAAGGCCAGGGGGGAAAGAAAAAATAT AGATTAAAACATATAGTATGGGCAAGCAGGGAACTAGAAAGATT CGCAGTCAATCCTGGCCTGTTAGAAACATCAGAAGGCTGCAGAC AAATACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAA GAACTTAGATCATTATATAATACAGTAGCAACCCTCTATTGTGTA CATCAAAAGATAGAGGTAAAAGACACCAAGGAAGCTTTAGACA AAATAGAGGAAGAGCAAAACAAATGTAAGAAAAAGGCACAGCA AGCAGCAGCTGACACAGGAAACAGCGGTCAGGTCAGCCAAAAT TTCCCTATAGTGCAGAACCTCCAGGGGCAAATGGTACATCAGGC CATATCACCTAGAACTTTAAATGCATGGGTAAAAGTAATAGAAG AGAAAGCTTTCAGCCAGAAAGTAATACCCATGTTTTTCAGCATTAT CAGAAGGAGCCACCCACAAAGATTTAAACACCATGCTAAACACA GTGGGGGGACATCAAGCAGCCATGCAATGTTAAAAGAACCCAT CAATGAGGAAGCTGCAAGATGGGATAGATTGCATCCCGTGCAGG CAGGGCCTGTTGCACCAGGCCAGATAAGAGATCCAAGGGGAAGT GACATAGCAGGAACTACCAGTACCCTTCAGGAACAAATAGGATG GATGACAAGTAATCCACCTATCCCAGTAGGAGAAATCTATAAAA GATGGATAATCCTGGGATTAAATAAAATAGTAAGGATGTATAGC CCTACCAGCATTTTGGACATAAGACAAGGACCAAAGGAACCCCTT TAGAGACTATGTAGACCGGTTCTATAAAACTCTAAGAGCCGAGC AAGCTTCACAGGAGGTAAAAAATTGGATGACAGAAACCTTGTTG GTCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATT GGGACCAGCAGCTACACTAGAAGAAATGATGACAGCATGTCAG GGAGTGGGAGGACCCAGCCATAAAGCAAGAATTTTGGCAGAAG CAATGAGCCAAGTAACAAATTCAGCTACCATAATGATGCAGAAA GGCAATTTTAGGAACCAAAGAAAGATTGTTAAATGTTTCAATTG TGGCAAAGAAGGGCACATAGCCAGAACTGCAGGGCCCCTAGG AAAAGGGGCTGTTGGAAATGTGGAAAGGAAGGACACCAAATGA AAGACTGTACTGAGAGACAGGCTAATTTTTTAGGGAAAATCTGG CCTTCCCACAAAGGAAGGCCAGGGAATTTCTTCAGAGCAGACC

		AGAGCCAACAGCCCCACCAGCCCCACCAGAAGAGAGCTTCAGGT TTGGGGAAGAGACAACAACCTCCCTCTCAGAAGCAGGAGCTGATA GACAAGGAAGTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGC AACGACCCCTCGTCAACAATAA
57	HIV Pol; Bal	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAA TTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATA GAAATCTGTGGACATAAAGCTATAGGTACAGTATTAATAGGACC TACACCTGTCAACATAAATTGGAAGAAATCTGTTGACTCAGATTG GTTGCACTTTAAATTTTCCCATTAGTCCTATTGAAACTGTACCAG TAAATTTAAAACCAGGAATGGATGGCCCAAAAGTTAAACAATGG CCACTGACAGAAGAAAAAATAAAAAGCATTAAATGGAAATCTGTAC AGAAATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAA AATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAG TACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAGA AAACTCAAGACTTCTGGGAAGTACAATTAGGAATACACATCCCG CAGGGGTAAAAAAGAAAAAATCAGTAACAGTACTGGATGTGG GTGATGCATATTTTTCAGTTCCCTTAGATAAAGAATTCAGGAAGT ATACTGCATTTACCATACCTAGTATAAACAATGAAACACCAGGG ATCAGATATCAGTACAATGTACTTCCACAGGGATGGAAAGGATC ACCAGCAATATTTCAAAGTAGCATGACAAGAATCTTAGAGCCTT TTAGAAAACAAAATCCAGAAATAGTGATCTATCAATACATGGAT GATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAAC AAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTA CCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGG ATGGGTATGAACTCCATCCTGATAAATGGACAGTACAGCCTAT AGTGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGA AGTTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTACCCAGGA ATTAAGTAAAGCAATTATGTAGGCTCCTTAGGGGAACCAAGGC ATTAACAGAAAGTAATACCACTAACAAAAGAAACAGAGCTAGAA CTGGCAGAGAACAGGGGAAATTCTAAAAGAACAGTACATGGGG TGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAG CAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATT TAAAAATCTGAAAACAGGAAAATATGCAAGAATGAGGGGTGCC CACACTAATGATGTAAAACAATTAACAGAGGCAGTGCAAAAAAT AACCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTA AACTACCCATACAAAAAGAAACATGGGAAACATGGTGGACAGA GTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATAC CCCTCCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCCA TAATAGGAGCAGAAACATTCTATGTAGATGGAGCAGCTAACCGG GAGACTAAATTAGGAAAAGCAGGATATGTTACTAACAGAGGAA GACAAAAAGTTGTCTCCCTAACTGACACAACAAATCAGAAGACT GAGTTACAAGCAATTCATCTAGCTTTACAAGATTCAGGATTAGA AGTAAACATAGTAACAGACTCACAATATGCATTAGGAATCATT AAGCACAACCAGATAAAAGTGAATCAGAGTTAGTCAGTCAAATA ATAGAACAGTTAATAAAAAAGGAAAAGGTCTACCTGGCATGGGT ACCAGCGCACAAAGGAATTGGAGGAAATGAACAAGTAGATAAA TTAGTCAGTACTGGAATCAGGAAAGTACTA
58	HIV Integrase; Bal	TTTTTAGATGGAATAGATATAGCCCAAGAAGAACATGAGAAATA TCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTGCCAC CTGTGGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAG CTAAAAGGAGAAGCCATGCATGGACAAGTAGACTGTAGTCCAGG AATATGGCAACTAGATTGTACACATTTAGAAGGAAAAATTATCC TGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTT ATTCCAGCAGAGACAGGGCAGGAAACAGCATACTTTCTCTTAA

		ATTAGCAGGAAGATGGCCAGTAAAAACAATACATACAGACAAT GGCAGCAATTTCACTAGTACTACAGTCAAGGCCGCCTGTTGGTG GGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAA GTCAGGGAGTAGTAGAATCTATAAAATAAAGAATTAAGAAAATT ATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAACAGCAGT ACAAATGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGGA TTGGGGGGTATAGTGCAGGGGAAAGAATAGTAGACATAATAGC AACAGACATACAACTAAAGAATTACAAAAACAATTACAAAA ATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCACTT TGGAAAGGACCAGCAAAGCTTCTCTGGAAAGGTGAAGGGGCAG TAGTAATACAAGATAATAGTGACATAAAAGTAGTACCAAGAAGA AAAGCAAAGATCATTAGGGATTATGGAAAACAGATGGCAGGTG ATGATTGTGTGGCAAGTAGACAGGATGAGGATTAG
59	Envelope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAATAAT AGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCTATCGCATTAG TACAAAAACAACATGGTAAACCATGCGAATGCAGCGGAGGGCA GGTATCCGAGGCCCCACCGAACTCCATCCAACAGGTAACCTGCC CAGGCAAGACGGCCTACTTAATGACCAACCAAAAATGGAAATGC AGAGTCACTCCAAAAAATCTCACCCCTAGCGGGGGGAGAACTCCA GAACTGCCCCCTGTAACTTTCCAGGACTCGATGCACAGTTCTTG TTATACTGAATACCGGCAATGCAGGGCGAATAATAAGACATACT ACACGGCCACCTTGCTTAAAATACGGTCTGGGAGCCTCAACGAG GTACAGATATTACAAAACCCCAATCAGCTCCTACAGTCCCCTTGT AGGGGCTCTATAAATCAGCCCGTTTGCTGGAGTGCCACAGCCCC CATCCATATCTCCGATGGTGGAGGACCCCTCGATACTAAGAGAG TGTGGACAGTCCAAAAAAGGCTAGAACAAATTCATAAGGCTATG CATCCTGAACCTCAATACCACCCCTTAGCCCTGCCCAAAGTCAGA GATGACCTTAGCCTTGATGCACGGACTTTTGATATCCTGAATACC ACTTTTAGGTTACTCCAGATGTCCAATTTTAGCCTTGCCCAAGAT TGTTGGCTCTGTTTAAACTAGGTACCCCTACCCCTCTTGCGATA CCCACTCCCTCTTTAACCTACTCCCTAGCAGACTCCCTAGCGAAT GCCTCCTGTCAGATTATACCTCCCCTCTTGGTTCAACCGATGCAG TTCTCCAACCTCGTCCTGTTTATCTTCCCCTTTCATTAACGATACGG AACAAATAGACTTAGGTGCAGTCACCTTTACTAACTGCACCTCTG TAGCCAATGTCAGTAGTCCTTTATGTGCCCTAAACGGGTCAGTCT TCCTCTGTGGAAATAACATGGCATAACACCTATTTACCCCAAACT GGACAGGACTTTGCGTCCAAGCCTCCCTCCTCCCCGACATTGACA TCATCCCGGGGGATGAGCCAGTCCCCATTCTGCCATTGATCATT ATATACATAGACCTAAACGAGCTGTACAGTTCATCCCTTTACTAG CTGGACTGGGAATCACCGCAGCATTACACACCGGAGCTACAGGC CTAGGTGTCTCCGTCACCCAGTATACAAAATTATCCCATCAGTTA ATATCTGATGTCCAAGTCTTATCCGGTACCATAACAAGATTTACAA GACCAGGTAGACTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAG GGGACTGGACCTACTAACGGCAGAACAAAGGAGGAATTTGTTTAG CTTACAAGAAAAATGCTGTTTTTATGCTAACAAGTCAGGAATTG TGAGAAACAAAATAAGAACCCTACAAGAAGAATTACAAAAACG CAGGGAAAGCCTGGCATCCAACCCCTCTCTGGACCGGGCTGCAGG GCTTTCTTCCGTACCTCCTACCTCTCCTGGGACCCCTACTACCCCT CCTACTCATACTAACCATTGGGCCATGCGTTTTCAATCGATTGGT CCAATTTGTTAAAGACAGGATCTCAGTGGTCCAGGCTCTGGTTTT GACTCAGCAATATCACCAGCTAAAACCCATAGAGTACGAGCCAT GA
60	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACTTCGGCACCAGATGAGT CCTGGGAGCTGGAAAAGACTGATCATCCTCTTAAGCTGCGTATTC

		GGAGACGGCAAAACGAGTCTGCAGAATAAGAACCCCCACCAGC CTGTGACCCTCACCTGGCAGGTACTGTCCCAAACCTGGGGACGTT GTCTGGGACAAAAAGGCAGTCCAGCCCCCTTTGGACTTGGTGGCC CTCTCTTACACCTGATGTATGTGCCCTGGCGGCCGGTCTTGAGTC CTGGGATATCCCGGGATCCGATGTATCGTCCTCTAAAAGAGTTA GACCTCCTGATTCAGACTATACTGCCGCTTATAAGCAAATCACCT GGGGAGCCATAGGGTGCAGCTACCCTCGGGCTAGGACCAGGATG GCAAATTCCCCCTTCTACGTGTGTCCCCGAGCTGGCCGAACCCAT TCAGAAGCTAGGAGGTGTGGGGGGCTAGAATCCCTATACTGTAA AGAATGGAGTTGTGAGACCACGGGTACCGTTTATTGGCAACCCA AGTCCTCATGGGACCTCATAACTGTAAAATGGGACCAAAATGTG AAATGGGAGCAAAAATTTCAAAAGTGTGAACAAACCGGCTGGTG TAACCCCTCAAGATAGACTTCACAGAAAAAGGAAAACCTCTCCA GAGATTGGATAACGGAAAAAACCTGGGAATTAAGGTTCTATGTA TATGGACACCCAGGCATACAGTTGACTATCCGCTTAGAGGTCAC TAACATGCCGGTTGTGGCAGTGGGCCCAGACCCTGTCCTTGCGG AACAGGGACCTCCTAGCAAGCCCCCTCACTCTCCCTCTCTCCCCAC GGAAAGCGCCGCCACCCCTCTACCCCCGGCGGCTAGTGAGCAA ACCCCTGCGGTGCATGGAGAACTGTTACCCTAAACTCTCCGCCT CCCACCAGTGGCGACCGACTCTTTGGCCTTGTGCAGGGGGCCCTTC CTAACCTTGAATGCTACCAACCCAGGGGGCCACTAAGTCTTGCTG GCTCTGTTTGGGCATGAGCCCCCTTATTATGAAGGGATAGCCTC TTCAGGAGAGGTGCTTATACCTCCAACCATAACCGATGCCACTG GGGGGCCCAAGGAAAGCTTACCCTCACTGAGGTCTCCGGACTCG GGTCATGCATAGGGAAGGTGCCTCTTACCCATCAACATCTTTGCA ACCAGACCTTACCCATCAATTCCCTCTAAAAACCATCAGTATCTGC TCCCCTCAAACCATAGCTGGTGGGCCTGCAGCACTGGCCTCACCC CCTGCCTCTCCACCTCAGTTTTTAATCAGTCTAAAGACTTCTGTGT CCAGGTCCAGCTGATCCCCCGCATCTATTACCATTCTGAAGAAAC CTTGTTACAAGCCTATGACAAATCACCCCCCAGGTTTAAAAGAG AGCCTGCCTCACTTACCCTAGCTGTCTTCTGGGGTTAGGGATTG CGGCAGGTATAGGTACTGGCTCAACCGCCCTAATTAAGGGGCC ATAGACCTCCAGCAAGGCCTAACCAGCCTCCAAATCGCCATTGA CGCTGACCTCCGGGCCCTTCAGGACTCAATCAGCAAGCTAGAGG ACTCACTGACTTCCCTATCTGAGGTAGTACTCCAAAATAGGAGA GGCCTTGACTTACTATTCCCTAAAGAAGGAGGCCTCTGCGCGGCC CTAAAAGAAGAGTGCTGTTTTTATGTAGACCACTCAGGTGCAGT ACGAGACTCCATGAAAAAACTTAAAGAAAGACTAGATAAAAGA CAGTTAGAGCGCCAGAAAAACCAAACTGGTATGAAGGGTGGTT CAATAACTCCCCTTGGTTTACTACCCTACTATCAACCATCGCTGG GCCCCATTGCTCCTCCTTTTGTACTCACTCTTGGGCCCTGCATC ATCAATAAATTAATCCAATTCATCAATGATAGGATAAGTGCAGT CAAAATTTTAGTCCTTAGACAGAAATATCAGACCCTAGATAACG AGGAAAACCTTTAA
61	Envelope; FUG	ATGGTTCCGCAGGTTCTTTTGTGTGTACTCCTTCTGGGTTTTTCGT TGTGTTTCGGGAAGTTCCCCATTTACACGATACCAGACGAACTTG GTCCCTGGAGCCCTATTGACATACACCATCTCAGCTGTCCAAATA ACCTGGTTGTGGAGGATGAAGGATGTACCAACCTGTCCGAGTTC TCCTACATGGAACCTCAAAGTGGGATACATCTCAGCCATCAAAGT GAACGGGTTCACTTGCACAGGTGTTGTGACAGAGGCAGAGACCT ACACCAACTTTGTTGGTTATGTCACAACCACATTCAAGAGAAAG CATTTCCGCCCCACCCAGACGCATGTAGAGCCGCGTATAACTG GAAGATGGCCGGTGACCCAGATATGAAGAGTCCCTACACAATC CATACCCCGACTACCACTGGCTTCGAACTGTAAGAACCACCAAA

		GAGTCCCTCATTATCATATCCCCAAGTGTGACAGATTTGGACCCA TATGACAAATCCCTTCACTCAAGGGTCTTCCCTGGCGGAAAGTGC TCAGGAATAACGGTGTCTCTACCTACTGCTCAACTAACCATGAT TACACCATTTGGATGCCCCGAGAATCCGAGACCAAGGACACCTTG TGACATTTTTTACCAATAGCAGAGGGAAGAGAGCATCCAACGGGA ACAAGACTTGCGGCTTTGTGGATGAAAGAGGCCTGTATAAGTCT CTAAAAGGAGCATGCAGGCTCAAGTTATGTGGAGTTCTTGGACT TAGACTTATGGATGGAACATGGGTGCGGATGCAAACATCAGATG AGACCAAATGGTGCCCTCCAGATCAGTTGGTGAATTTGCACGAC TTTCGCTCAGACGAGATCGAGCATCTCGTTGTGGAGGAGTTAGTT AAGAAAAGAGAGGAATGTCTGGATGCATTAGAGTCCATCATGAC CACCAAGTCAGTAAGTTTCAGACGTCTCAGTCACCTGAGAAAAC TTGTCCCAGGGTTTGGAAAAGCATATAACCATATTCAACAAAACC TTGATGGAGGCTGATGCTCACTACAAGTCAGTCCGGACCTGGAA TGAGATCATCCCCTCAAAAAGGGTGTTTGAAAGTTGGAGGAAGGT GCCATCCTCATGTGAACGGGGTGTTTTCAATGGTATAATATTAG GGCCTGACGACCATGTCCTAATCCCAGAGATGCAATCATCCCTCC TCCAGCAACATATGGAGTTGTTGGAATCTTCAGTTATCCCCCTGA TGCACCCCCTGGCAGACCCTTCTACAGTTTTTCAAAGAAGGTGATG AGGCTGAGGATTTTGTGTAAGTTCACCTCCCCGATGTGTACAAAC AGATCTCAGGGGTTGACCTGGGTCTCCCGAACTGGGGAAAGTAT GTATTGATGACTGCAGGGGCCATGATTGGCCTGGTGTGATATTT TCCCTAATGACATGGTGCAGAGTTGGTATCCATCTTTGCATTA TTAAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGAT GAACCGACTTGGAAGTAA
62	Envelope; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACATCATC GATGAGGTGATCAACATTGTCATTATTGTGCTTATCGTGATCACG GGTATCAAGGCTGTCTACAATTTTGCCACCTGTGGGATATTCGCA TTGATCAGTTTCCTACTTCTGGCTGGCAGGTCCTGTGGCATGTAC GGTCTTAAGGGACCCGACATTTACAAAGGAGTTTACCAATTTAA GTCAGTGGAGTTTGATATGTCACATCTGAACCTGACCATGCCCCA CGCATGTTCAGCCAACAACCTCCCACCATTACATCAGTATGGGGA CTTCTGGACTAGAATTGACCTTCACCAATGATTCCATCATCAGTC ACAACTTTTGCAATCTGACCTCTGCCTTCAACAAAAAGACCTTTG ACCACACACTCATGAGTATAGTTTCGAGCCTACACCTCAGTATCA GAGGGAACCTCAACTATAAGGCAGTATCCTGCGACTTCAACAAT GGCATAACCATCCAATACAACCTTGACATTCTCAGATCGACAAAG TGCTCAGAGCCAGTGTAGAACCTTCAGAGGTAGAGTCCTAGATA TGTTTAGAACTGCCTTCGGGGGGAAATACATGAGGAGTGGCTGG GGCTGGACAGGCTCAGATGGCAAGACCACCTGGTGTAGCCAGAC GAGTTACCAATACCTGATTATACAAAATAGAACCTGGGAAAACC ACTGCACATATGCAGGTCTTTTTGGGATGTCCAGGATTCTCCTTT CCCAAGAGAAGACTAAGTTCTTCACTAGGAGACTAGCGGGCACA TTCACCTGGACTTTGTCAGACTCTTCAGGGGTGGAGAATCCAGGT GGTTATTGCCTGACCAAATGGATGATTCTTGCTGCAGAGCTTAAG TGTTTCGGGAACACAGCAGTTGCGAAATGCAATGTAAATCATGA TGCCGAATTCTGTGACATGCTGCGACTAATTGACTACAACAAGG CTGCTTTGAGTAAGTTCAAAGAGGACGTAGAATCTGCCTTGCACT TATTCAAACAACAGTGAATTCTTTGATTTCAGATCAACTACTGA TGAGGAACCACTTGAGAGATCTGATGGGGGTGCCATATTGCAAT TACTCAAAGTTTTGGTACCTAGAACATGCAAAGACCGGCGAAAC TAGTGTCCCCAAGTGCTGGCTTGTACCAATGGTTCTTACTTAAA TGAGACCCACTTCAGTGATCAAATCGAACAGGAAGCCGATAACA TGATTACAGAGATGTTGAGGAAGGATTACATAAAGAGGCAGGG

		GAGTACCCCCCTAGCATTGATGGACCTTCTGATGTTTTCCACATC TGCATATCTAGTCAGCATCTTCCTGCACCTTGTCAAAATACCAAC ACACAGGCACATAAAAAGGTGGCTCATGTCCAAAGCCACACCGAT TAACCAACAAAGGAATTTGTAGTTGTGGTGCATTAAAGGTGCCT GGTGTAAAAACCGTCTGGAAAAGACGCTGA
63	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTTCGCCCTTGTTGGCAGTCATCCCC ACAAATGCAGACAAAATTTGTCTTGGACATCATGCTGTATCAA TGGCACCAAAGTAAACACACTCACTGAGAGAGGAGTAGAAGTTG TCAATGCAACGGAAACAGTGGAGCGGACAAACATCCCCAAAATT TGCTCAAAGGGAAAAGAACCCTGATCTTGGCCAATGCGGACT GTTAGGGACCATTACCGGACCACCTCAATGCGACCAATTTCTAG AATTTTCAGCTGATCTAATAATCGAGAGACGAGAAGGAAATGAT GTTTGTTACCCGGGGAAGTTTGTTAATGAAGAGGCATTGCGACA AATCCTCAGAGGATCAGGTGGGATTGACAAAGAAACAATGGGAT TCACATATAGTGGAATAAGGACCAACGGAACAACCTAGTGCATGT AGAAGATCAGGGTCTTCATTCTATGCAGAAATGGAGTGGCTCCT GTCAAATACAGACAATGCTGCTTTCCCACAAATGACAAAATCAT ACAAAAACACAAGGAGAGAATCAGCTCTGATAGTCTGGGGAATC CACCATTGAGGATCAACCACCGAACAGACCAAACTATATGGGAG TGGAAATAAACTGATAACAGTCGGGAGTTCCAAATATCATCAAT CTTTTGTGCCGAGTCCAGGAACACGACCGCAGATAAATGGCCAG TCCGGACGGATTGATTTTCATTGGTTGATCTTGGATCCCAATGAT ACAGTTACTTTTAGTTTCAATGGGGCTTTTCATAGCTCCAAATCGT GCCAGCTTCTTGAGGGGAAAGTCCATGGGGATCCAGAGCGATGT GCAGGTTGATGCCAATTGCGAAGGGGAATGCTACCACAGTGGAG GGACTATAACAAGCAGATTGCCTTTTCAAACATCAATAGCAGA GCAGTTGGCAAATGCCCAAGATATGTAACACAGGAAAGTTTATT ATTGGCAACTGGGATGAAGAACGTTCCCGAACCTTCCAAAAAAA GGAAAAAAGAGGCCTGTTTGGCGCTATAGCAGGGTTTATTGAA AATGGTTGGGAAGGTCTGGTCGACGGGTGGTACGGTTTCAGGCA TCAGAATGCACAAGGAGAAGGAACTGCAGCAGACTACAAAAGC ACCAATCGGCAATTGATCAGATAACCGGAAAGTTAAATAGACT CATTGAGAAAACCAACCAGCAATTTGAGCTAATAGATAATGAAT TCACTGAGGTGGAAAAGCAGATTGGCAATTTAATTAAGTGGACC AAAGACTCCATCACAGAAGTATGGTCTTACAATGCTGAACCTTCTT GTGGCAATGGAAAACCAGCACACTATTGATTGGCTGATTCAGA GATGAACAAGCTGTATGAGCGAGTGAGGAAACAATTAAGGGAA AATGCTGAAGAGGATGGCACTGGTTGCTTTGAAATTTTTCATAAA TGTGACGATGATTGTATGGCTAGTATAAGGAACAATACTTATGA TCACAGCAAATACAGAGAAGAAGCGATGCAAAATAGAATACAA ATTGACCCAGTCAAATTGAGTAGTGGCTACAAAGATGTGATACT TTGGTTTAGCTTCGGGGCATCATGCTTTTTGCTTCTTGCCATTGCA ATGGGCCTTGTTTTCATATGTGTGAAGAACGGAAACATGCGGTG CACTATTTGTATATAA
64	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGACCATA CCTAGCACATTGCGCCGATTGCGGGGACGGGTACTTCTGCTATA GCCCAGTTGCTATCGAGGAGATCCGAGATGAGGCGTCTGATGGC ATGCTTAAGATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGGC AGGCACCCACGCCCACACGAAGCTCCGATATATGGCTGGTCATG ATGTTGAGGAATCTAAGAGAGATTCTTGAGGGTGTACACGTCC GCAGCGTGCTCCATACATGGGACGATGGGACACTTCATCGTCGC ACACTGTCCACCAGGCGACTACCTCAAGGTTTCGTTTCGAGGACG CAGATTCGCACGTGAAGGCATGTAAGGTCCAATACAAGCACAAT CCATTGCCGGTGGGTAGAGAGAAGTTCGTGGTTAGACCACACTT

		TGGCGTAGAGCTGCCATGCACCTCATACCAGCTGACAACGGCTC CCACCGACGAGGAGATTGACATGCATACACCGCCAGATATACCG GATCGCACCCCTGCTATCACAGACGGCGGGCAACGTCAAAATAAC AGCAGGCGGCAGGACTATCAGGTACAACGTGACCTGCGGCCGTG ACAACGTAGGCACTACCAGTACTGACAAGACCATCAACACATGC AAGATTGACCAATGCCATGCTGCCGTCACCAGCCATGACAAATG GCAATTTACCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTAG GAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTAACGTACCT GCCGAGTGCCGTTGGCTCGAGCGCCGGATGCCACCTATGGTAAG AAGGAGGTGACCCTGAGATTACACCCAGATCATCCGACGCTCTT CTCCTATAGGAGTTTAGGAGCCGAACCGCACCCGTACGAGGAAT GGGTTGACAAGTTCTCTGAGCGCATCATCCAGTGACGGAAGAA GGGATTGAGTACCAGTGGGGCAACAACCCGCCGGTCTGCCTGTG GGCGCAACTGACGACCGAGGGCAAAACCCCATGGCTGGCCACATG AAATCATTTCAGTACTATTATGGACTATACCCCGCCGCCACTATTG CCGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAACTCTGGCG GCCACATGCTGCATGCTGGCCACCGCGAGGAGAAAGTGCCTAAC ACCGTACGCCCTGACGCCAGGAGCGGTGGTACCGTTGACACTGG GGCTGCTTTGCTGCGCACCGAGGGCGAATGCA
65	Envelope; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAGATTAA CCCGTGGAAGTCCTTAATGGTCATGGGGGTCTATTTAAGAGTAG GGATGGCAGAGAGCCCCCATCAGGTCTTTAATGTAACCTGGAGA GTCACCAACCTGATGACTGGGCGTACCGCCAATGCCACCTCCCTT TTAGGAACTGTACAAGATGCCTTCCCAAGATTATATTTTGATCTA TGTGATCTGGTCGGAGAAGAGTGGGACCCTTCAGACCAGGAACC ATATGTCGGGTATGGCTGCAAATACCCCGGAGGGAGAAAGCGGA CCCGGACTTTTGACTTTTACGTGTGCCCTGGGCATACCGTAAAAT CGGGGTGTGGGGGGCCAAGAGAGGGCTACTGTGGTGAATGGGG TTGTGAAACCACCGGACAGGCTTACTGGAAGCCACATCATCAT GGGACCTAATCTCCCTTAAGCGCGGTAACACCCCTGGGACACG GGATGCTCCAAAATGGCTTGTGGCCCTGCTACGACCTCTCCAAA GTATCCAATTCTTCCAAGGGGCTACTCGAGGGGGGAGATGCAA CCTCTAGTCCTAGAAATCACTGATGCAGGAAAAAAGGCTAATT GGGACGGGCCCCAAATCGTGGGGACTGAGACTGTACCGGACAGG AACAGATCCTATTACCATGTTCTCCCTGACCCGCCAGGTCTCTCAA TATAGGGCCCCGCATCCCCATTGGGCCTAATCCCGTGATCACTGG TCAACTACCCCCCTCCCGACCCGTGCAGATCAGGCTCCCCAGGCC TCCTCAGCCTCCTCCTACAGGCGCAGCCTCTATAGTCCCTGAGAC TGCCCCACCTTCTCAACAACCTGGGACGGGAGACAGGCTGCTAA ACCTGGTAGAAGGAGCCTATCAGGCGCTTAACCTACCAATCCC GACAAGACCCAAGAATGTTGGCTGTGCTTAGTGTGCGGACCTCC TTATTACGAAGGAGTAGCGGTCGTGGGCACTTATACCAATCATTC TACCGCCCCGGCCAGCTGTACGGCCACTTCCCAACATAAGCTTAC CCTATCTGAAGTGACAGGACAGGGCCTATGCATGGGAGCACTAC CTAAACTCACCAGGCCTTATGTAACACCACCCAAAGTGCCGGC TCAGGATCCTACTACCTTGCAGCACCCGCTGGAACAATGTGGGC TTGTAGCACTGGATTGACTCCCTGCTTGTCCACCACGATGCTCAA TCTAACCACAGACTATTGTGTATTAGTTGAGCTCTGGCCCAGAAT AATTTACCACTCCCCGATTATATGTATGGTCAGCTTGAACAGCG TACCAAATATAAGAGGGAGCCAGTATCGTTGACCCTGGCCCTTC TGCTAGGAGGATTAACCATGGGAGGGATTGCAGCTGGAATAGGG ACGGGGACCACTGCCCTAATCAAAACCCAGCAGTTTGAGCAGCT TCACGCCGCTATCCAGACAGACCTCAACGAAGTCGAAAAATCAA TTACCAACCTAGAAAAGTCACTGACCTCGTTGTCTGAAGTAGTCC

		TACAGAACCGAAGAGGCCTAGATTTGCTCTTCCTAAAAGAGGGA GGTCTCTGCGCAGCCCTAAAAGAAGAATGTTGTTTTATGCAGAC CACACGGGACTAGTGAGAGACAGCATGGCCAACTAAGGGAAA GGCTTAATCAGAGACAAAACTATTTGAGTCAGGCCAAGGTTGG TTCGAAGGGCAGTTTAATAGATCCCCCTGGTTTACCACCTTAATC TCCACCATCATGGGACCTCTAATAGTACTCTTACTGATCTTACTC TTTGGACCCTGCATTCTCAATCGATTGGTCCAATTTGTAAAGAC AGGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAACAATATCAC CAGCTAAAACCTATAGAGTACGAGCCATGA
66	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGATTCAAG AGGACATCATTCTTTCTTTGGGTAATTATCCTTTTCCAAAGAACA TTTTCCATCCCCTTGGAGTCATCCACAATAGCACATTACAGGTT AGTGATGTCGACAACTGGTTTGCCGTGACAACTGTCATCCAC AAATCAATTGAGATCAGTTGGACTGAATCTCGAAGGGAATGGAG TGGCAACTGACGTGCCATCTGCAACTAAAAGATGGGGCTTCAGG TCCGGTGTCCACCAAAGGTGGTCAATTATGAAGCTGGTGAATG GGCTGAAAACCTGCTACAATCTTGAAATCAAAAAACCTGACGGGA GTGAGTGTCTACCAGCAGCGCCAGACGGGATTTCGGGGCTTCCCC CGGTGCCGGTATGTGCACAAAGTATCAGGAACGGGACCGTGTGC CGGAGACTTTGCCTTCCACAAAGAGGGTGCTTTCTTCCTGTATGA CCGACTTGCTTCCACAGTTATCTACCGAGGAACGACTTTTCGCTGA AGGTGTCGTTGCATTTCTGATACTGCCCCAAGCTAAGAAGGACTT CTTCAGCTCACACCCCTTGAGAGAGCCGGTCAATGCAACGGAGG ACCCGTCTAGTGGCTACTATTCTACCACAATTAGATATCAAGCTA CCGGTTTTGGAACCAATGAGACAGAGTATTTGTTTCGAGGTTGAC AATTTGACCTACGTCCAACCTGAATCAAGATTCACACCACAGTTT CTGCTCCAGCTGAATGAGACAATATATAAAGTGGGAAAAGGAG CAATACCACGGGAAAACCTAATTTGGAAGGTCAACCCCGAAATTG ATACAACAATCGGGGAGTGGGCCTTCTGGGAACTAAAAAACC TACTAGAAAAATTTCGAGTGAAGAGTTGTCTTTCACAGCTGTAT CAAACAGAGCCAAAACATCAGTGGTCAGAGTCCGGCGCGAACT TCTTCCGACCCAGGGACCAACACAACAACCTGAAGACCACAAAAT CATGGCTTCAGAAAATTCCTCTGCAATGGTTCAAGTGCACAGTCA AGGAAGGGAAGCTGCAGTGTGCATCTGACAACCTTGCCACAA TCTCCACGAGTCCTCAACCCCCCACAACCAAACAGGTCCGGAC AACAGCACCCACAATACACCCGTGTATAAACTTGACATCTCTGA GGCAACTCAAGTTGAACAACATCACCGCAGAACAGACAACGAC AGCACAGCCTCCGACACTCCCCCGCCACGACCGCAGCCGGACC CCTAAAAGCAGAGAACACCAACACGAGCAAGGGTACCGACCTC CTGGACCCCGCCACCACAACAAGTCCCCAAAACACAGCGAGAC CGCTGGCAACAACAACACTCATCACCAGATACCGGAGAAGAG AGTGCCAGCAGCGGGAAGCTAGGCTTAATTACCAATACTATTGC TGGAGTCGCAGGACTGATCACAGGCGGGAGGAGAGCTCGAAGA GAAGCAATTGTCAATGCTCAACCCAAATGCAACCCTAATTTACA TACTGGACTACTCAGGATGAAGGTGCTGCAATCGGACTGGCCT GGATACCATATTTCCGGCCAGCAGCCGAGGGAATTTACATAGAG GGGCTGATGCACAATCAAGATGGTTAATCTGTGGGTTGAGACA GCTGGCCAACGAGACGACTCAAGCTCTTCAACTGTTCTGAGAG CCACAACCGAGCTACGCACCTTTTCAATCCTCAACCGTAAGGCA ATTGATTTCTTGCTGCAGCGATGGGGCGGCACATGCCACATTTTG GGACCGGACTGCTGTATCGAACCACATGATTGGACCAAGAACAT AACAGACAAAATTGATCAGATTATTCATGATTTTGTGATAAAAC CCTTCCGGACCGGGGGACAATGACAATTGGTGGACAGGATGGA GACAATGGATACCGGCAGGTATTGGAGTTACAGGCGTTATAATT

		GCAGTTATCGCTTTATTCTGTATATGCAAATTTGTCTTTTAG
67	Short WPRE sequence	AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACCTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTAATGCCTCTGTATCATGCTATTGCTTCCCGTACGGCTTTTCGTTTTCTCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAAGTATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTGTC
68	Helper Plasmid Forward Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
69	Helper Plasmid Reverse Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
70	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCAGTAAATTAAGCCAGGAATGGATGGCCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACC AAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCATATCCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCATAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAACTAAATTAGGAAAAGCAGGATATGTAAGTACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCTGGATTAGAAGTAAACATAGTGACAGACTCACAAATATGCATTGGG

		AATCATTCAAGCACAAACCAGATAAGAGTGAATCAGAGTTAGTCA GTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTG GCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAG TAGATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTAA GATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCACA GTAATTGGAGAGCAATGGCTAGTGATTTTAAACCTACCACCTGTA GTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAA AGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATAT GGCAGCTAGATTGTACACATTTAGAAGGAAAAAGTTATCTTGTA GCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCC AGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAATTAG CAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGCAG CAATTTACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGG GGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAA GGAGTAATAGAATCTATGAATAAAGAATTAAAGAAAATTATAGG ACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAA TGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGG GGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAG ACATACAACTAAAGAATTACAAAAACAAATTACAAAAATTCAA AATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGAA AGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTA ATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAG CAAAGATCATCAGGGATTATGGAAAACAGATGGCAGGTGATGAT TGTGTGGCAAGTAGACAGGATGAGGATTAA
71	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCA TCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCA CCTCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAA GAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTTCGATTAG TGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCTGT GCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAA CGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTC AAATATTGGTGGAAATCTCCTACAATATTGGAGTCAGGAGCTAAA GAATAGAGGAGCTTTGTTCCCTTGGGTTCTTGGGAGCAGCAGGAA GCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGA CAATTATTGTCTGGTATAGTGCAGCAGCAGAACAAATTTGCTGAG GGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGG GCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATAC CTAAAGGATCAACAGCTCCTAGATCTTTTCCCTCTGCCAAAAT TATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAA TAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTGTG TGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCAT ATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAG TATATGAAACAGCCCCCTGCTGTCCATTCTTATTCCATAGAAAA GCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTGTTTAT TTTTTCTTTAACATCCCTAAAATTTTCTTACATGTTTTACTAGC CAGATTTTTCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCT CTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTA ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCAC AATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCT GGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT CACTGCCCCGCTTTCAGTCGGGAAACCTGTCGTGCCAGCGGATCC GCATCTCAATTAGTCAGCAACCATAGTCCCCGCCCTAACTCCGCC CATCCCGCCCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCA

		TGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTC GGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG GCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAA TGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAG CATTTTTTTCCTGCACTTCTAGTTGTGGTTTGTCCAACTCATCAA TGTATCTTATCAGCGGCCGCCCGGG
72	DNA fragment containing the CAG enhancer/promoter/intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCA TAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGG CCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAA TAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT GACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCA GTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGAC CTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCAT CGCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTTCACTC TCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTAT TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGG CGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGG CGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCT CCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCT ATAAAAAGCGAAGCGCGCGGCGGGGAGTCGCTGCGTTGCCT TCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCCGCCCGCCCCGG CTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGG CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCT CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGA GGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTG TGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCG CGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGT GCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTG TGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTGCGGC TGTAACCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGC CCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGCGCGGGGGCT CGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGC GGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGC GCGGCGGGCCCGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGC CGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGG ACTTCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGGC CCGCCGACCCCCCTAGCGGGCGCGGGCGAAGCGGTGCGGCGC CGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCG CGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGAGG GGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTTCG CTTCTGGCGTGTGACCGGCGGGAATTC
73	DNA fragment containing VSV-G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTATTGGG GTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGG AACTGGAAAAATGTTCTTCTAATTACCATTATTGCCCGTCAAG CTCAGATTTAAATTGGCATAATGACTTAATAGGCACAGCCTTACA AGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGGTT GGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCT GGTATGGACCGAAGTATATAACACATTCCATCCGATCCTTCACTC CATCTGTAGAACAATGCAAGGAAAGCATTGAACAAACGAAACA AGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGATA TGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTC CTACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGAT

		TCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCAC TGTCCATAACTCTACAACCTGGCATTCTGACTATAAGGTCAAAGG GCTATGTGATTCTAACCTCATTTCCATGGACATCACCTTCTTCTCA GAGGACGGAGAGCTATCATCCCTGGGAAAGGAGGGCACAGGGT TCAGAAGTAACTACTTTTGCTTATGAAACTGGAGGCAAGGCCTGC AAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGG TGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAG ATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCA GACCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCT TGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCG GGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAA AACCAGGAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTA AAATACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCC AATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACCTACCACAG AAAGGGAACGTGGGGATGACTGGGCACCATATGAAGACGTGGA AATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGT TTCCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTC ATCTTAGCTCAAAGGCTCAGGTGTTTGAACATCCTCACATTCAAG ACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTG ATACTGGGCTATCCAAAAATCCAATCGAGCTTGTTAGAAGGTTGG TTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAG GGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATC TTTGCATTAAATTAAGCACACCAAGAAAAGACAGATTTATACA GACATAGAGATGAGAATTC
74	Helper plasmid containing RRE and rabbit beta globin poly A	TCTAGAAGGAGCTTTGTTCCCTTGGGTTCTTGGGAGCAGCAGGAA GCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGA CAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAG GGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGG GCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATAC CTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAAT TATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAA TAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTG TGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCAT ATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAG TATATGAAACAGCCCCCTGCTGTCCATTCTTATTCCATAGAAAA GCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTGTTAT TTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGC CAGATTTTTCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCT CTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTA ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCAC AATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCT GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT CACTGCCCCGCTTTCAGTCGGGAAACCTGTCGTGCCAGCGGATCC GCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCC CATCCCGCCCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCA TGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTC GGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG GCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAA TGGTTACAAATAAAGCAATAGCATCACAAATTCACAAATAAAG CATTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA TGTATCTTATCACCCGGG
75	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACT AGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGTT

		AGGAGTCCCCCTCAGGATATAGTAGTTTCGCTTTTGCATAGGGAG GGGGAAATGTAGTCTTATGCAATACACTTGTAGTCTTGAACATG GTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGC ACCGTGATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGCC TTATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAAC CACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTAGC TCGATACAATAAACGCCATTTGACCATTACCACATTGGTGTGCA CCTCCAAGCTCGAGCTCGTTTGTAGTGAACCGTCAGATCGCCTGGA GACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGAC CGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGG CAGGAAGAAGCGGAGACAGCGACGAAGAAGTCTCAAGGCAGT CAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCC CGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGG AGAGAGAGACAGAGACAGATCCATTTCGATTAGTGAACGGATCCT TAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCT ACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGG AACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGG AATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA
76	Rev/Tat shRNA target sequence #2	ATGGCAGGAAGAAGCGGAG
77	Rev/Tat shRNA coding sequence #2	ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCTTCTTCCTGC CATTTTTT
78	H1 promoter and shRT sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAG TGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGA AGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGC ATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATC CGCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCT GTCTCCGCTTTTT
79	H1 CCR5 sequence	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAG TGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGA AGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGC ATGTCGCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACTTGGATC CGTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTG ACACTTTTT
80	CAG promoter	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCC ATATATGGAGTTCCGCGTTACATAAATTACGGTAAATGGCCCCGCC TGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGA CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC AATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACAT CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC GGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATG GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTAT TACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTACTCTCCCCA TCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTT ATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCG CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGC GGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAA GTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAA AGCGAAGCGCGCGGCGGGCG

81	pRSV Rev	AGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATT CATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGG CACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTG GAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGAC CATGATTACGAATTCGATGTACGGGCCAGATATACGCGTATCTG AGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGT ACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGCTTTTGCA TAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTAGTCTT GCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAG AAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGA TCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATTG GACGAACCACTGAATTCGCGATTGCAGAGATAATTGTATTTAAG TGCCTAGCTCGATAACAATAAACGCCATTTGACCATTACCCACATT GGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATC GCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACA CCGGGACCGATCCAGCCTCCCCTCGAAGCTAGTCGATTAGGCAT CTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTC AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACC TCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGA AGAAGGTGGAGAGAGAGACAGAGACAGATCCATTTCGATTAGTG AACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTG CCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAAC GAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCA AATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAG AATAGTGCTGTTAGCTTGCTCAATGCCACAGCTATAGCAGTAGCT GAGGGGACAGATAGGGTTATAGAAGTAGTACAAGAAGCTTGGC ACTGGCCGTCGTTTTACATGATCTGAGCCTGGGAGATCTCTGGCT AACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGA GTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAAC TAGAGATCACAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCA TTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTAC ACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCC TTTCTCGCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGG GGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGAC CCCAAAAACTTGATTGGGTGATGGTTCACGTAGTGGGCCATC GCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTT CTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCC TATCTCGGGCTATTCTTTTGATTATAAAGGGATTTTGCCGATTTTCG GCCTATTGGTTAAAAAATGAGCTGATTAAACAAAAATTTAACGC GAATTTTAACAAAATATTAACGTTTACAATTTTATGGTGCACCTCT CAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGAC ACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCC CGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGC ATGTGTCAGAGGTTTTACCGTCATCACCGAAACGCGCGAGACG AAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGAT ATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGT GCGCGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATAT GTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCCGC CTTATTCCCTTTTTTGCGGCATTTTGCTTCCTGTTTTTGCTCACCC AGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTG CACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATC CTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACT
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		TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCC GGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGA CTTGTTGAGTACTACCAAGTCACAGAAAAGCATCTTACGGATG GCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGT GATAAACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACC GAAGGAGCTAACCCTTTTTTGCACAACATGGGGGATCATGTAA CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCA AACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAAC GTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCG GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGAC CACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATA AATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACG ACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCG CTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAGTGTACAGAC CAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTT AATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGA CCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACC CCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGC GCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCG GTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAG GTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCT AGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCAC CGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTC GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGA GATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAA GGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTTCGAA CAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCG ATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACG CCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTT TTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAA CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCG AACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAG
82	pCMV-VSV-G	GAGCTTGGCCCATTCATACGTTGTATCCATATCATAATATGTAC ATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGA TTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTT CATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAAT GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC AATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTG GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC GTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACAT GACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGT CATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGG GCGTGGATAGCGGTTTGAATCACGGGGATTTCCAAGTCTCCACCC CATTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGG ACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAATG GGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCG TTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTT TGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGTCTGA CCGATCCTGAGAAGTTTCAAGGGTGAGTTTGGGGACCCCTTGATTGTT

	CTTTCTTTTTTCGCTATTGTAAAATTCATGTTATATGGAGGGGGCA AAGTTTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATC ACCATGGACCCTCATGATAATTTTGTTCCTTTCTACTCTG TTGACAACCATTGTCTCCTCTTATTTTCTTTTCATTTTCTGTAACCT TTTCGTTAAACTTTAGCTTGCATTTGTAACGAATTTTAAATTCAC TTTTGTTTATTGTGTCAGATTGTAAGTACTTTCTCTAATCACTTTTT TTTCAAGGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTT TAGAGAACAATTGTTATAATTAATGATAAGGTAGAAATATTTCT GCATATAAATTCTGGCTGGCGTGGAAATATTCTTATTGGTAGAAA CAACTACACCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACA ATGATATACACTGTTTGAGATGAGGATAAAAATACTCTGAGTCCA AACC GGCCCTCTGCTAACCATGTTTCATGCCTTCTTCTCTTTCT ACAGCTCCTGGGCAACGTGCTGGTTGTTGTGCTGTCTCATCATTT TGGCAAAGAATTCTCGACGGATCCGCCATGAAGTGCCTTTTGTA CTTAGCCTTTTTATTCAATTGGGGTGAATTGCAAGTTCACCATAGT TTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCTTCTA ATTACCATTATTGCCCCGTCAAGCTCAGATTTAAATTGGCATAATG ACTTAATAGGCACAGCCTTACAAGTCAAATGCCCAAGAGTCAC AAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATG GGTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAAC ACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGA AAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCT TCCCTCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAA GCAGTGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAA TACACAGGAGAATGGGTTGATTCACAGTTCATCAACGGAAAAATG CAGCAATTACATATGCCCCACTGTCCATAACTCTACAACCTGGCA TTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCT CATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCT GGGAAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATG AACTGGAGGCAAGGCCTGCAAAATGCAATACTGCAAGCATTGG GGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATAA GGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTC AAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAAT TCAGGACGTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAA CCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGGAT CTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCTGCTTTC ACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACAT CAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAAT GATCAGTGGAATACCACAGAAAGGGAATGTGGGATGACTGG GCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAG GACCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACATGG TATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTT CGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCTGATGA TGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATCCAAT CGAGCTTGTAAGAAGTTGGTTCAGTAGTTGGAAAAGCTCTATTG CCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGT TCTCCGAGTTGGTATCCATCTTTGCATTAAATTAAGCACACCAA GAAAAGACAGATTTATACAGACATAGAGATGAACCGACTTGGAA AGTGATAAGGATCCGTCGAGGAATTCCTCCTCAGGTGCAGGCT GCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCA CAAATACCACTGAGATCTTTTCCCTCTGCCAAAAATTATGGGGA CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGA AATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGCTCTC ACTCGGAAGGACATATGGGAGGGCAAATCATTTAAACATCAGA
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	ATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCATATGCTGG CTGCCATGAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATG AAACAGCCCCCTGCTGTCCATTCCCTATTCCATAGAAAAGCCTTG ACTTGAGGTTAGATTTTTTTTATATTTTGTGTTTGTGTTATTTTTTC TTAAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTT TTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCT TATGGAGATCCCTCGACGGATCGGCCGCAATTCGTAATCATGTC ATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACA CAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCTT AATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCG CTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCG GCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCC GCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTGCGCTGCGG CGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCAC AGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAAGGC CAGCAAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTT TTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGAC GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC CAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCG ACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGA AGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTTCG GTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCC GTTCAGCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAG TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCAC TGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAG AGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACA GTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA AGAGTTGGTAGCTCTTGATCCGGCAAACAACACCGCTGGTAG CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAA AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG CTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGA TTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGA AGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGAC AGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT CTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATA ACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAAT GATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAA TAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGC AACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC TAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGC CATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGC TTCATTAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATC CCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGAT CGTTGTCAGAAGTAAGTTGGCCGCAAGTGTATCACTCATGGTTAT GGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATG CTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATA GTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGG ATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATT GGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCAACTGATC TTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAAC AGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG AAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGC ATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGT
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		ATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCG AAAAGTGCCACCTAAATTGTAAGCGTTAATATTTTGTTAAAATTC GCGTTAAATTTTTGTAAATCAGCTCATTTTTTAACCAATAGGCC GAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT AGGGTTGAGTGTGTTCCAGTTTGGAACAAGAGTCCACTATTAA AGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAG GGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTG GGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAAGGGA GCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCG AGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCG CTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCACCCCGC CGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTGCGCATTCAG GCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGC TATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTA AGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAC GACGGCCAGTGAGCGCGCGTAATACGACTCACTATAGGGCGAAT TGGAGCTCCACCGCGGTGGCGGCCGCTCTAGA
83	PSPAX2 delta Rev	GTCGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGG GGTCAATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAAC TTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA GGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACT GCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCC CCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA CGTATTAGTCATCGCTATTACCATGGGTGAGGTGAGCCCCACGT TCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTT GTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGG GGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGG GCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAG AGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCG GCGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTC GCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGC CGCCCCCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAG CGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGT TTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAA AGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGG GGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCAGCCCC GCGCTGCCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCT TTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGG TGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCG TGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGC GGCGGTGCGGCTGTAACCCCCCTGCACCCCCCTCCCCAGTTG CTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGT GGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGG GGGTGCCGGGCGGGGCGGGGCCGCTCGGGCCGGGAGGGGCTC GGGGAGGGGCGCGGCGGCCCGGAGCGCCGGCGGCTGTGAG GCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGCGAG AGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAA TCTGGGAGGCGCCGCCGACCCCTCTAGCGGGCGCGGGCGAAG CGGTGCGGCGCCGCCAGGAAGGAAATGGGCGGGGAGGGCCTTC GTGCGTCGCCGCGCCCGCTCCCTTCTCCATCTCCAGCCTCGGG GCTGCCGCGAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGG GCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCT

		GCTAACCATGTTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCA ACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTCTG GGCCGGCCGCGTTGACGCGCACGGCAAGAGGCGAGGGGCGGCG ACTGGTGAGAGATGGGTGCGAGAGCGTCAGTATTAAGCGGGGG AGAATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGA AAGAAAAAATATAAATTAACATATAGTATGGGCAAGCAGGG AGCTAGAACGATTTCGCAGTTAATCCTGGCCTGTTAGAAACATCA GAAGGCTGTAGACAAATACTGGGACAGCTACAACCATCCCTTCA GACAGGATCAGAAGAACTTAGATCATTATATAATACAGTAGCAA CCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAG GAAGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGA AAAAAGCACAGCAAGCAGCAGCTGACACAGGACACAGCAATCA GGTCAGCCAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAA TGGTACATCAGGCCATATCACCTAGAACTTTAAATGCATGGGTA AAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGATACCCAT GTTTTTCAGCATTATCAGAAGGAGCCACCCACAAAGATTTAAACA CCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATG TTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAG TGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGA GAACCAAGGGGAAGTGACATAGCAGGAAGTACTAGTACCCTTCA GGAACAAATAGGATGGATGACACATAATCCACCTATCCCAGTAG GAGAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATA GTAAGAATGTATAGCCCTACCAGCATTCTGGACATAAGACAAGG ACCAAAGGAACCCCTTAGAGACTATGTAGACCGATTCTATAAAA CTCTAAGAGCCGAGCAAGCTTCACAAGAGGTAAAAAATTGGATG ACAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATTGTAAGAC TATTTTAAAAGCATTGGGACCAGGAGCGACACTAGAAGAAATGA TGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAAGCAAG AGTTTTGGCTGAAGCAATGAGCCAAGTAACAAATCCAGCTACCA TAATGATACAGAAAGGCAATTTTAGGAACCAAAGAAAGACTGTT AAGTGTTTCAATTGTGGCAAAGAAGGGCACATAGCCAAAAATTG CAGGGCCCCTAGGAAAAAGGGCTGTTGGAAATGTGGAAAGGAA GGACACCAAATGAAAGATTGTACTGAGAGACAGGCTAATTTTTT AGGGAAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAATTTTC TTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTC AGGTTTGGGGAAGAGACAACAACCTCCCTCTCAGAAGCAGGAGCC GATAGACAAGGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTT TGGCAGCGACCCCTCGTCACAATAAAGATAGGGGGGCAATTAAG GGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAG AAATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGG AATTGGAGGTTTTATCAAAGTAGGACAGTATGATCAGATACTCA TAGAAATCTGCGGACATAAAGCTATAGGTACAGTATTAGTAGGA CCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATT GGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCA GTAAAATTAAAGCCAGGAATGGATGGCCCAAAGTTAAACAATG GCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTA CAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGA AAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACA GTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAG AGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCC TGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGG GCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGT ATACTGCATTTACCATACCTAGTATAAACAATGAGACACCAGGG ATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATC
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84	Vif miRNA target sequence	AAGTTCAGAAGTACACATCCC
85	Tat miRNA target sequence	CTATGGCAGGAAGAAGCGGA
86	Elongation Factor-1 alpha (EF1-alpha) promoter with 3' restriction recognition site	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGAAAGTGA TGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACC GTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGG GTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCCTGCTTGAATTA CTTCCACGCCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTT CGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCCTTAAGG AGCCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTG GGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGC TGCTTTTCGATAAGTCTCTAGCCATTTAAAATTTTTTGATGACCTGC TGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAATGCGGGCCA AGATCTGCACACTGGTATTTTCGGTTTTTTGGGGCCGCGGGCGGCG ACGGGGCCCCGTGCGTCCCAGCGCACATGTTCCGGCAGGCGGGGC CTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGC TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGC CCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCACCAAGTTGCGT GAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCAC CCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCA TGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTA GTTCTCGAGCTTTTGGAGTACGTCTGTTTAGGTTGGGGGGAGGG GTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTG AAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGAATTTG CCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAG TGGTTCAAAGTTTTTTTCTTCCATTTCAAGGTGTCGTGATGTACA
87	miR30 CCR5 coding sequence with 5' and 3' restriction recognition sites	TGTACAAGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGA GCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGT TTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTGCTAGC

88	miR21 Vif coding sequence with 5' restriction recognition site	CCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTAC TTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCC GCACTGACATTTTGGTATCTTTCATCTGACCA
89	miR185 Tat coding sequence with 5' and 3' restriction recognition sites	GCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCT TCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGG CACCTTCCCTCCCAATGACCGCGTCTTCGTCCCCGGG

[0320] While certain of the preferred embodiments of the present disclosure have been described and specifically exemplified above, it is not intended that the disclosure be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present disclosure.

CLAIMS**WHAT IS CLAIMED IS:**

1. A method comprising:
 - purifying peripheral blood mononuclear cells (PBMC) from a source;
 - stimulating the PBMC with at least one HIV-specific peptide;
 - depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells;
 - transducing the depleted PBMC with a viral delivery system encoding at least one genetic element;
 - culturing the transduced PBMC for at least one day; and
 - harvesting the cultured PBMC.
2. The method of claim 1, wherein the culturing occurs in a static culture system or a semi-static culture system.
3. The method of claim 1, wherein the at least one HIV-specific peptide comprises a pool of synthetic, overlapping peptides.
4. The method of claim 3, wherein the pool of synthetic, overlapping peptides represents the HIV Gag polypeptide.
5. The method of claim 1, wherein the depleting comprises separating the at least one subset of cells from the depleted PBMC.
6. The method of claim 5, wherein the separating comprises magnetic bead separation.
7. The method of claim 1, wherein the at least one genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence.
8. The method of claim 7, wherein the HIV RNA sequence comprises an HIV Vif sequence, an HIV Tat sequence, or a variant thereof.
9. The method of claim 7, wherein the at least one genetic element comprises a microRNA or a shRNA.

10. The method of claim 7, wherein the at least one genetic element comprises a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% identity with at least one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 30.

11. A genetically-modified lymphocyte for treatment of a subject infected with HIV prepared by a process comprising the steps of:

purifying peripheral blood mononuclear cells (PBMC) from the subject, wherein the PBMC comprise at least one lymphocyte;

stimulating the at least one lymphocyte with at least one HIV-specific peptide;

depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells;

transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element;

culturing the PBMC for at least one day; and

harvesting the cultured PBMC.

12. A genetically-modified lymphocyte for treatment of a subject immunized with a preventive or therapeutic HIV vaccine prepared by a process comprising the steps of:

purifying peripheral blood mononuclear cells (PBMC) from the subject, wherein the PBMC comprise at least one lymphocyte;

stimulating the at least one lymphocyte with at least one HIV-specific peptide;

depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells;

transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element;

culturing the PBMC for at least one day; and

harvesting the cultured PBMC.

13. A genetically-modified lymphocyte for treatment of a subject exposed to, but not infected with, HIV prepared by a process comprising the steps of:

purifying peripheral blood mononuclear cells (PBMC) from the subject, wherein the PBMC comprise at least one lymphocyte;

stimulating the PBMC with at least one HIV-specific peptide;
depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, CD4⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells;
transducing the at least one lymphocyte with a viral delivery system encoding at least one genetic element;
culturing the PBMC for at least one day; and
harvesting the cultured PBMC.

14. A method, comprising:

stimulating PBMC with at least one HIV-specific peptide;
depleting at least one subset of cells from the PBMC, wherein the at least one subset of cells comprises any one or more of CD8⁺ T cells, $\gamma\delta$ cells, NK cells, B cells, T regulatory cells, and NKT cells; and
transducing the depleted PBMC with a viral delivery system encoding at least one genetic element.

15. The method of claim 14, wherein the at least one HIV-specific peptide comprises an HIV gag peptide.

16. The method of claim 14, wherein the at least one genetic element comprises a small RNA capable of inhibiting production of chemokine receptor CCR5 or at least one small RNA capable of targeting an HIV RNA sequence.

17. The method of claim 16, wherein the HIV RNA sequences comprises an HIV Vif sequence, an HIV Tat sequence, or a variant thereof.

1/18

Enrich (vaccine) and protect (AGT-LV) the HIV-specific CD4 T cells.

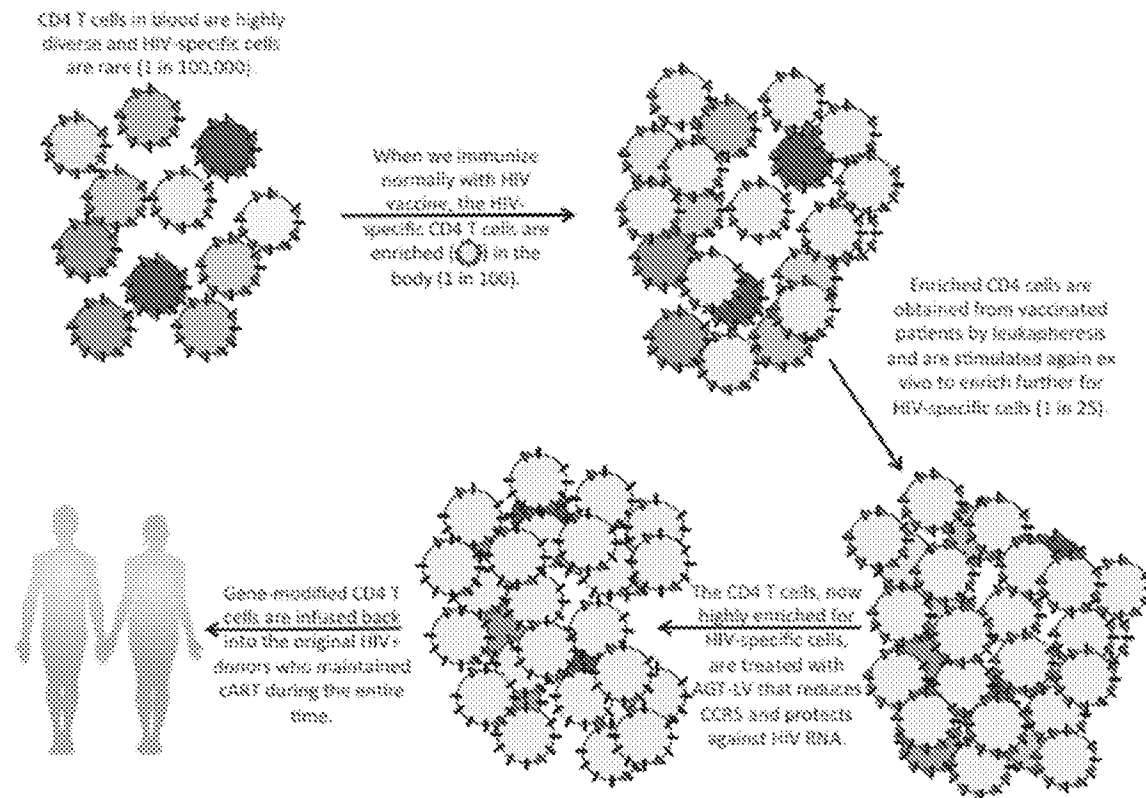


Figure 1

2/18

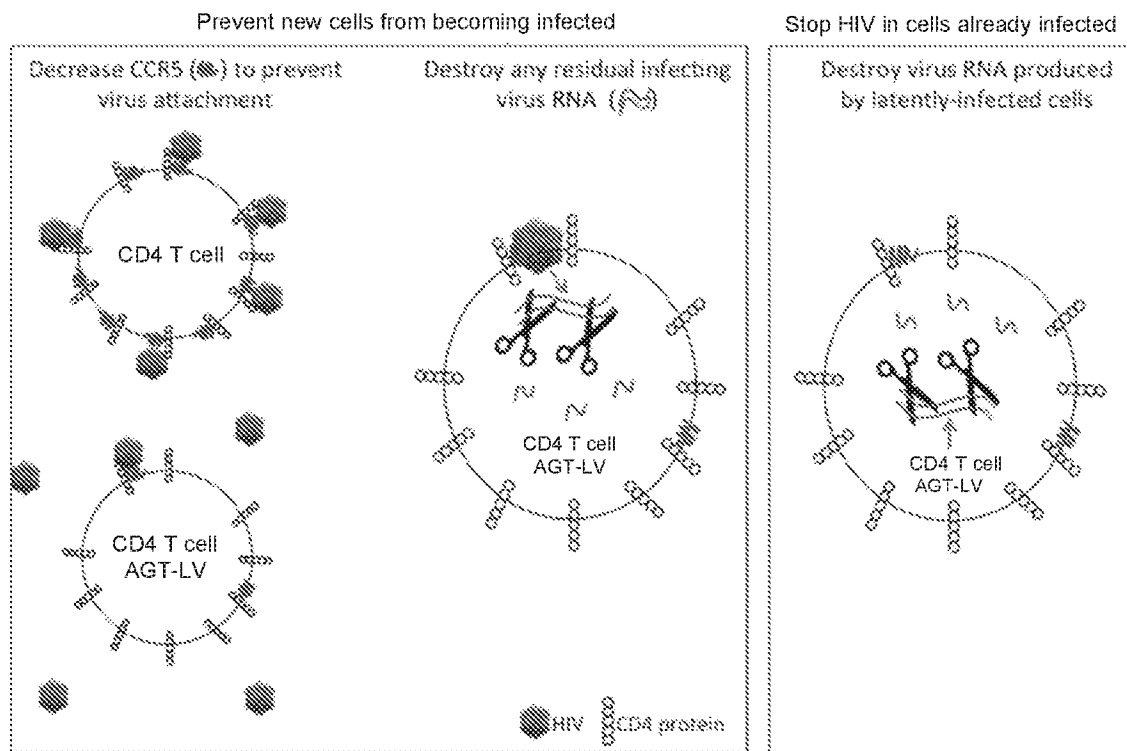


Figure 2

3/18

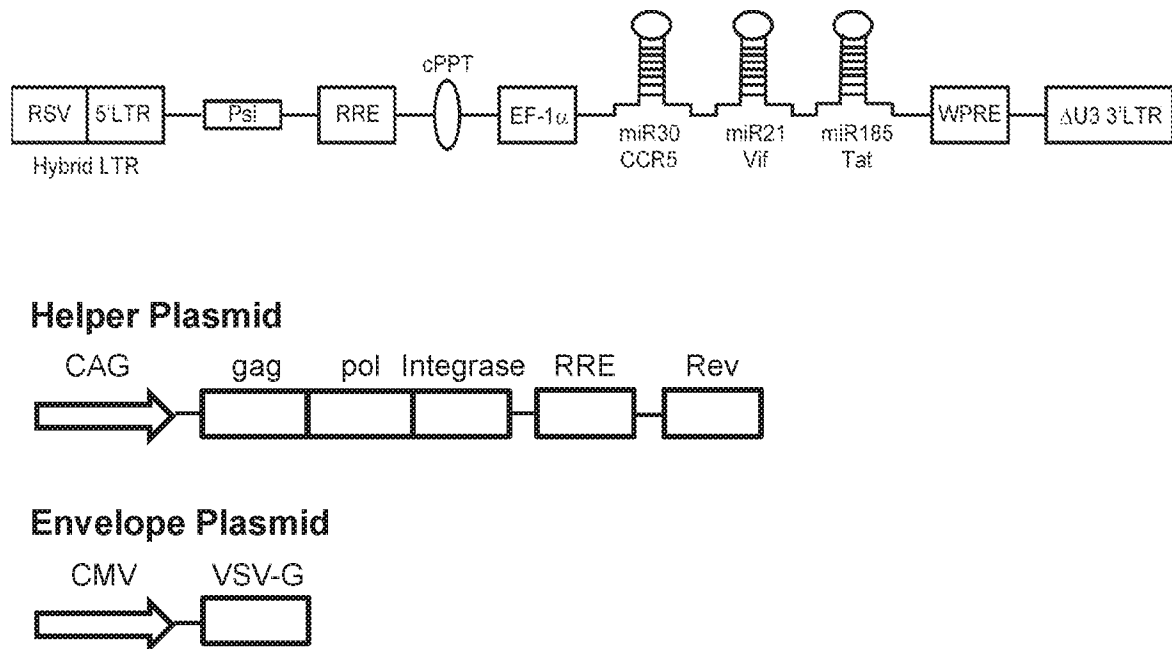
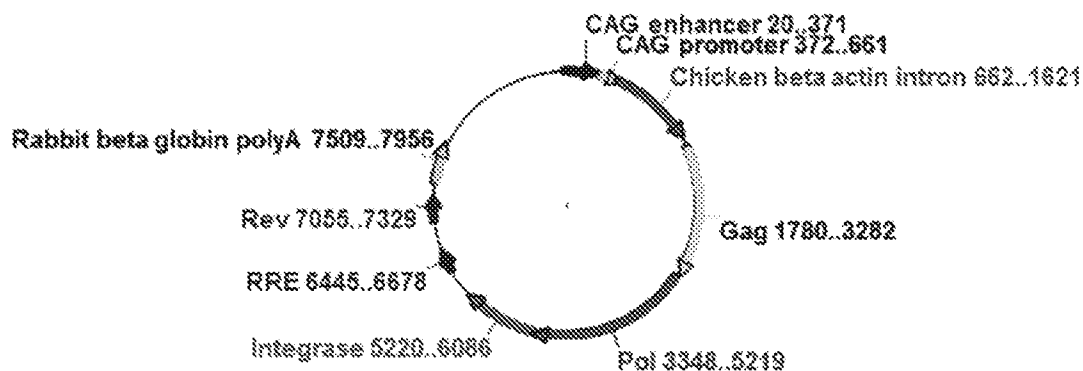
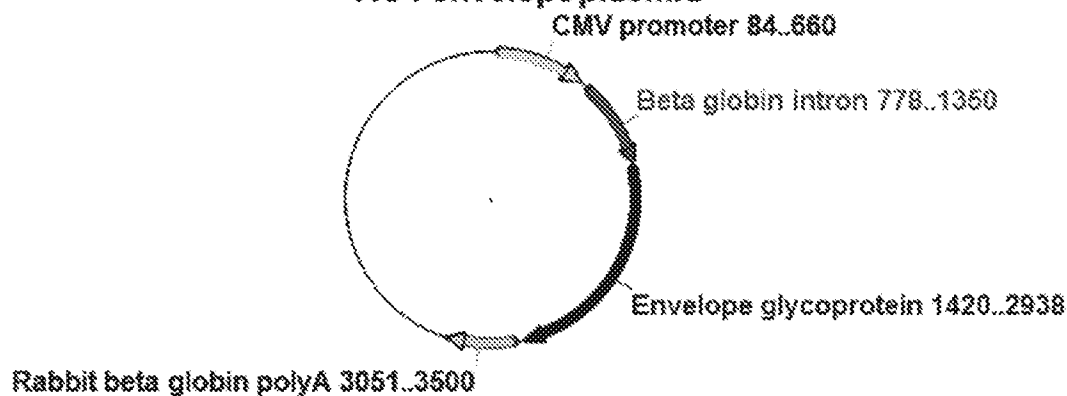
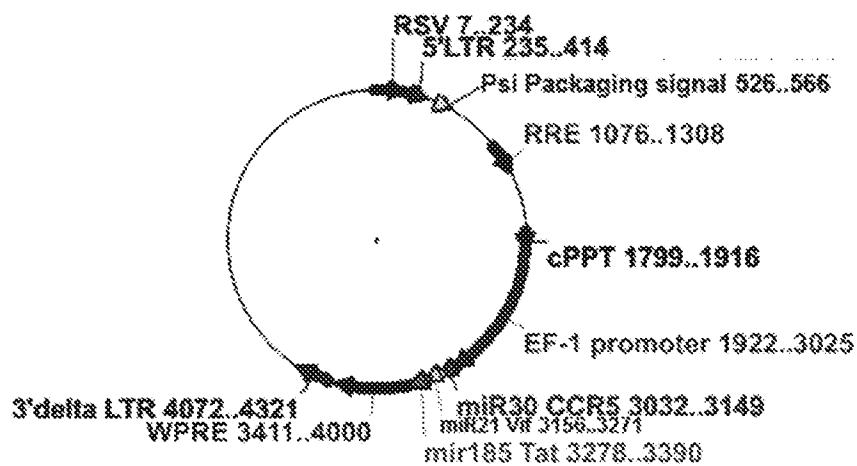


Figure 3

4/18

AGT Helper plus Rev plasmid**AGT envelope plasmid****AGT103 lentivirus plasmid****Figure 4**

5/18

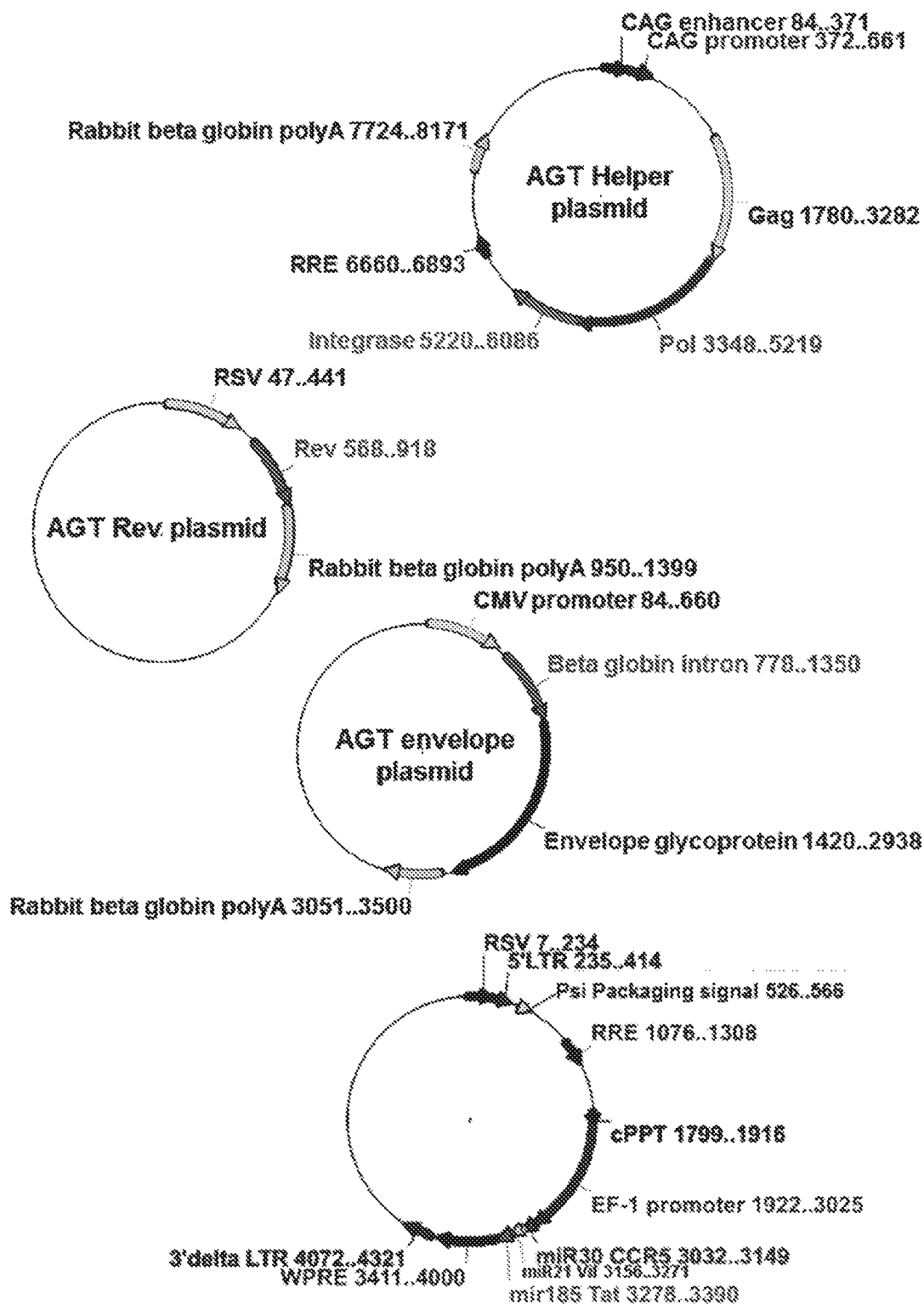
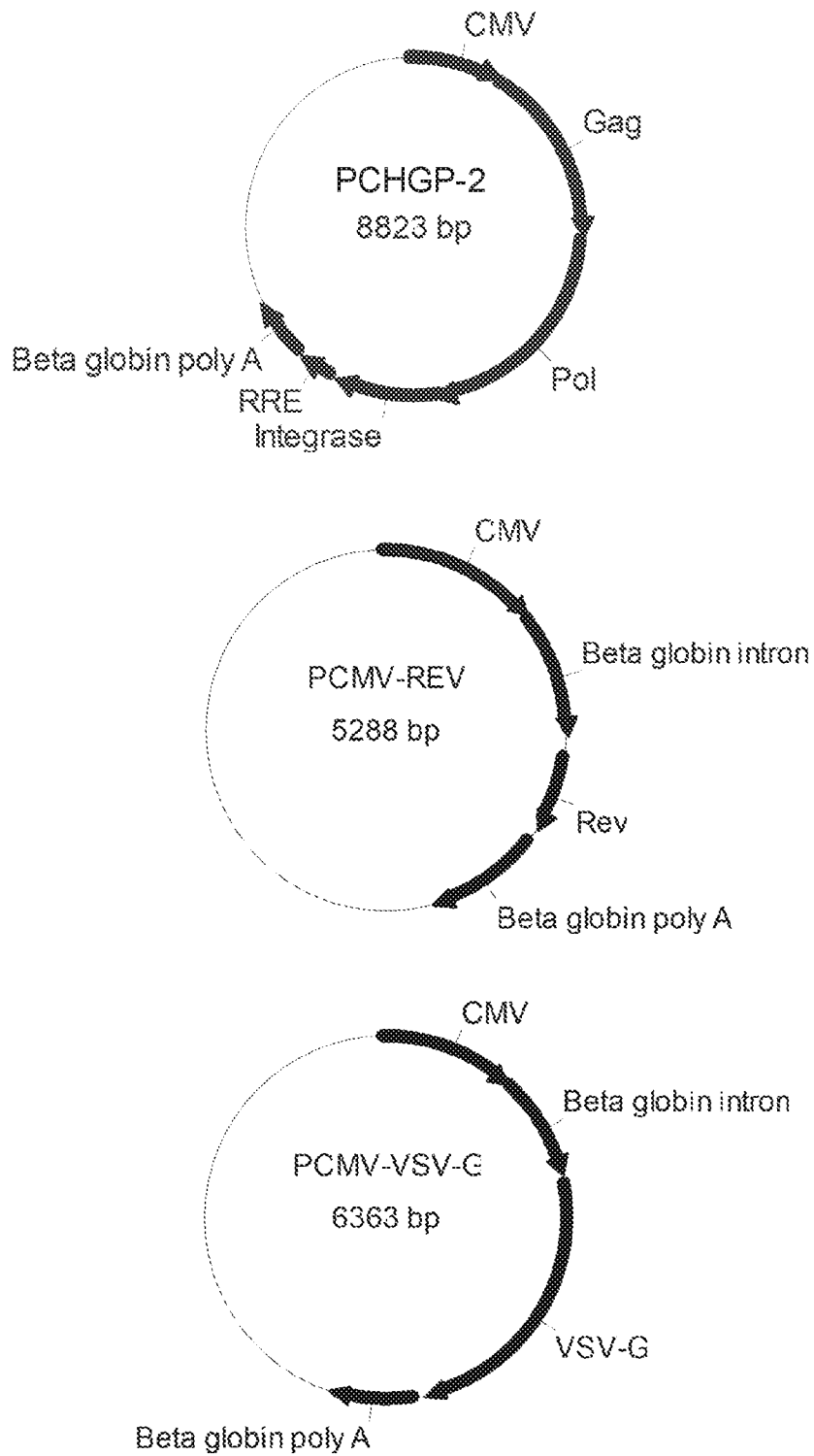


Figure 5

6/18

**Figure 6**

7/18

Elongation Factor-1 alpha (EF1-alpha) promoter (SEQ ID NO: 86)

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 CCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTTCAGGTGTCGTGATGTACA

miR30 CCR5 (SEQ ID NO: 87)

TGTACAAGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCAC
 AGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTGCTAGC

miR21 Vif (SEQ ID NO: 88)

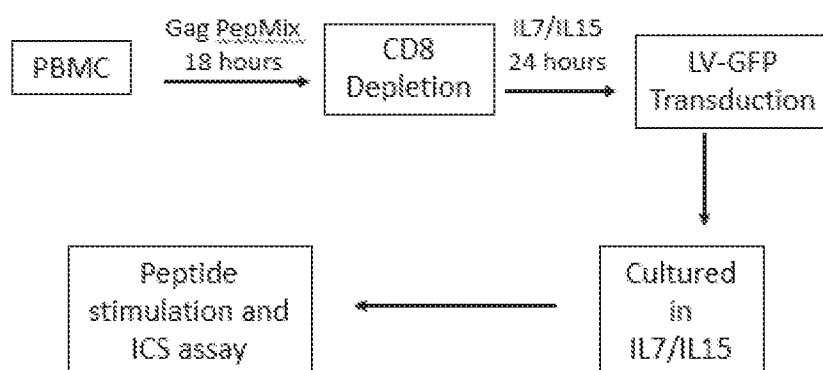
CCCGGGCATCTCCATGGCTGTACCACCTTGTGCGGGGATGTGTACTTCTGAACTTGTGTTGAATCT
 CATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA

miR185 Tat (SEQ ID NO: 89)

GCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCCT
 CCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCCCCGGG

Figure 7

8/18

**Figure 8**

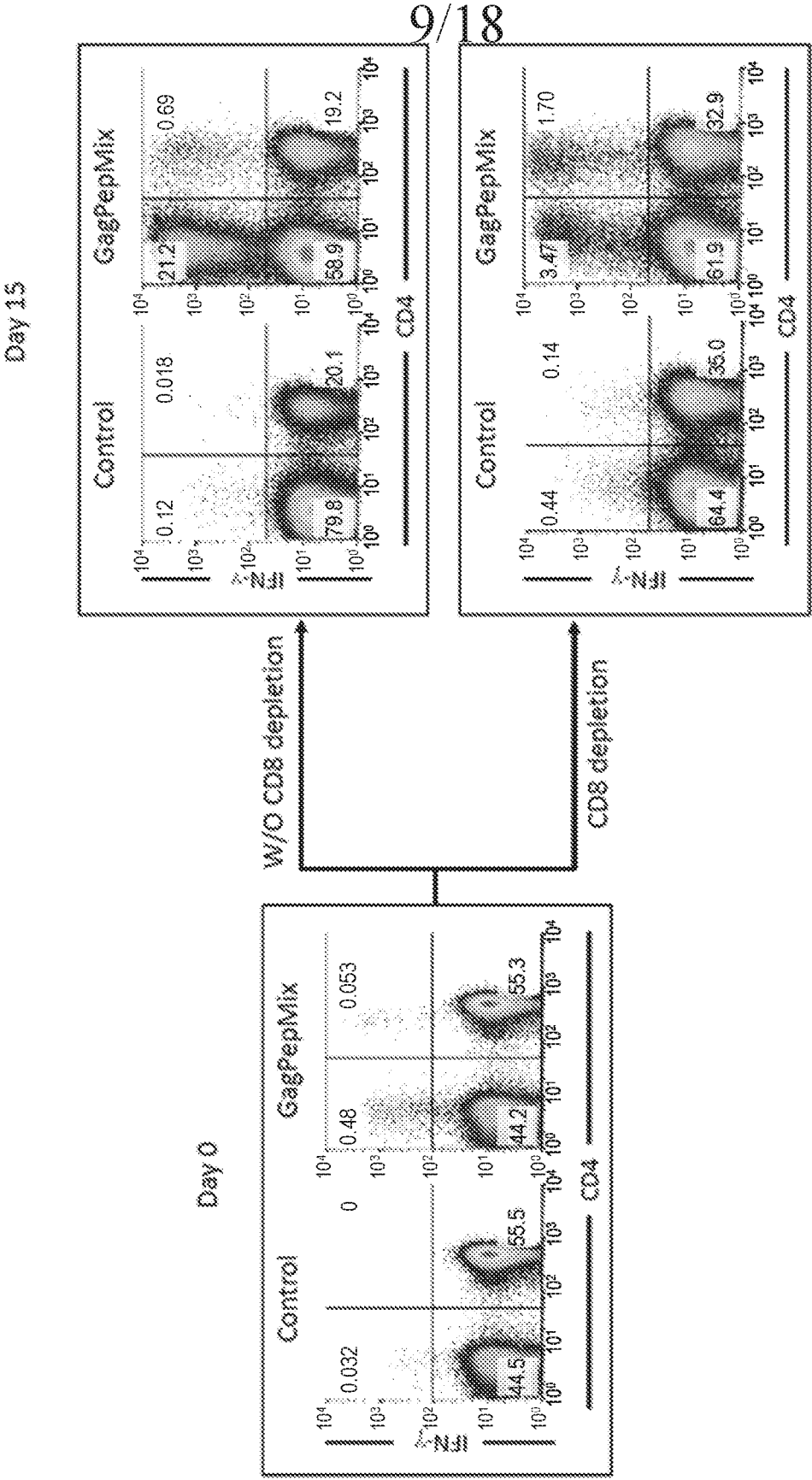


Figure 9

PTID: 01-006

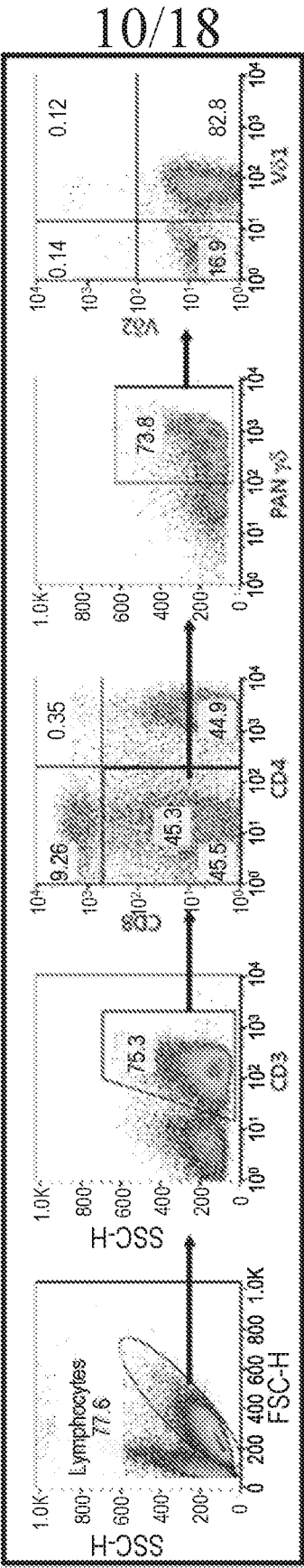


Figure 10

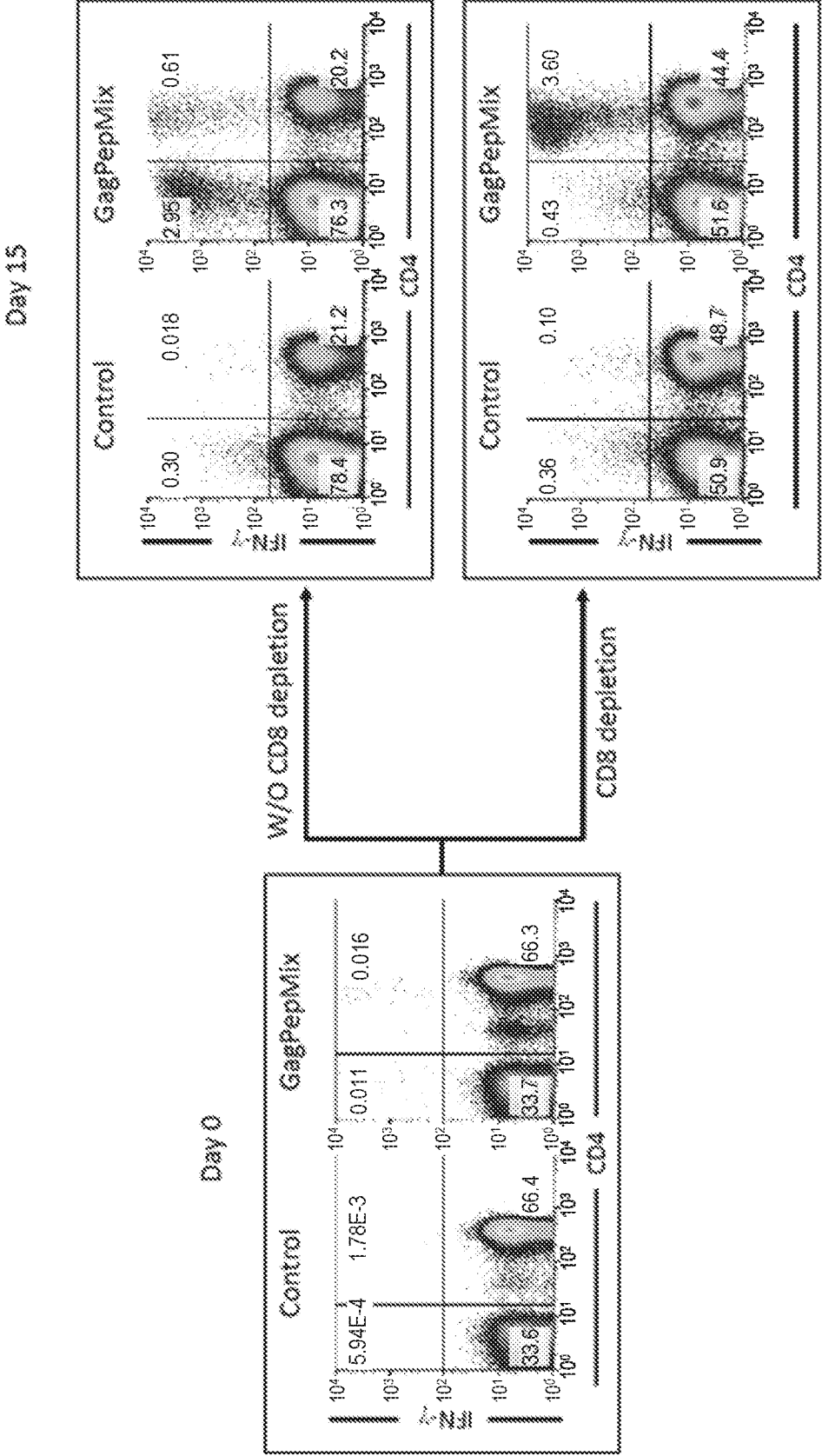


Figure 11

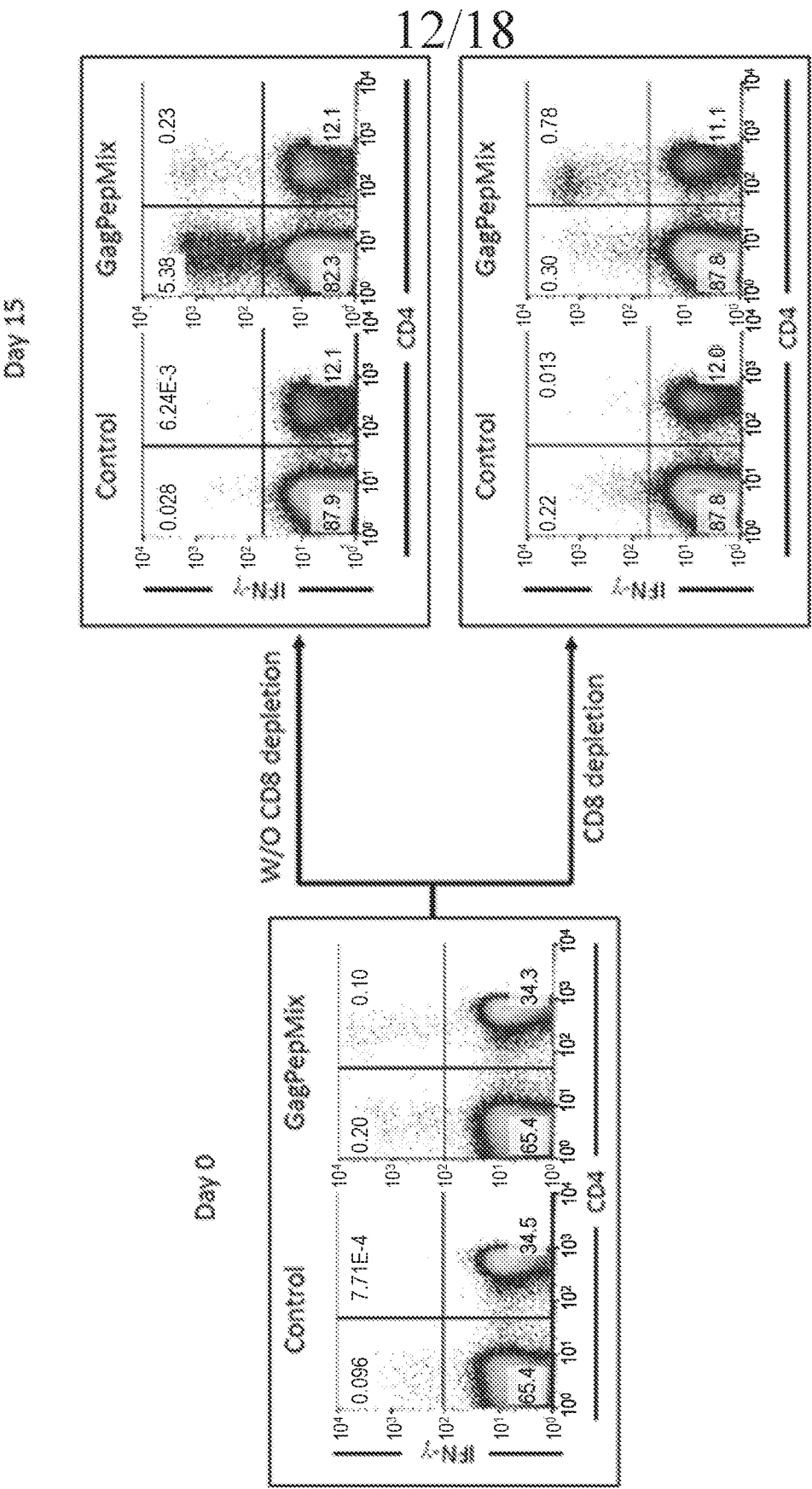


Figure 12

13/18

PTID: 01-008

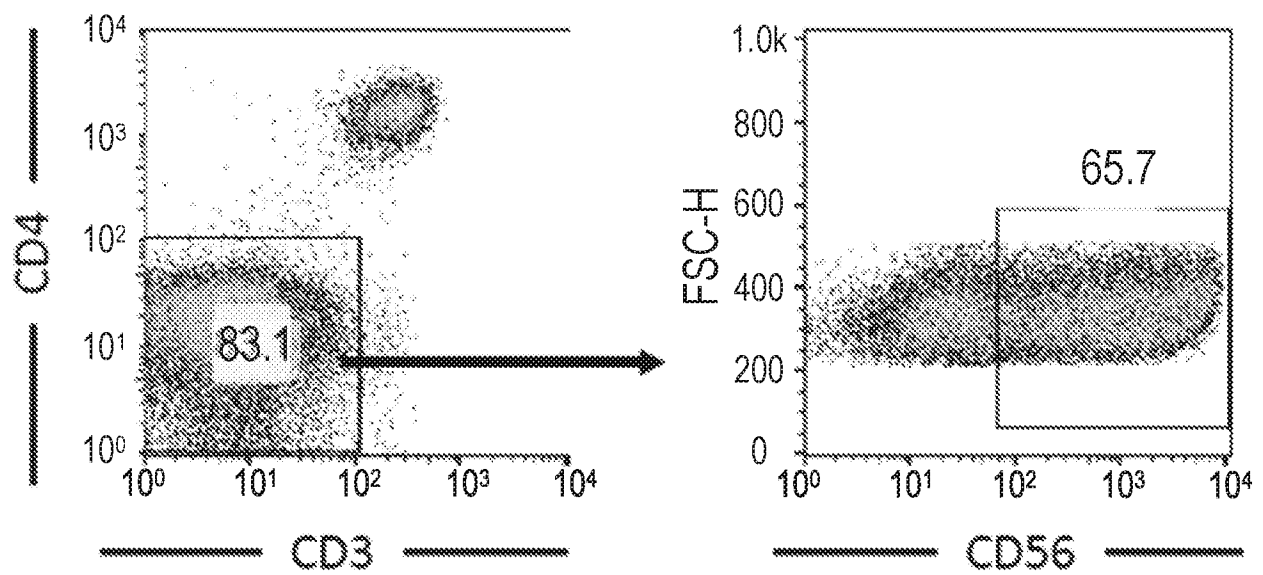
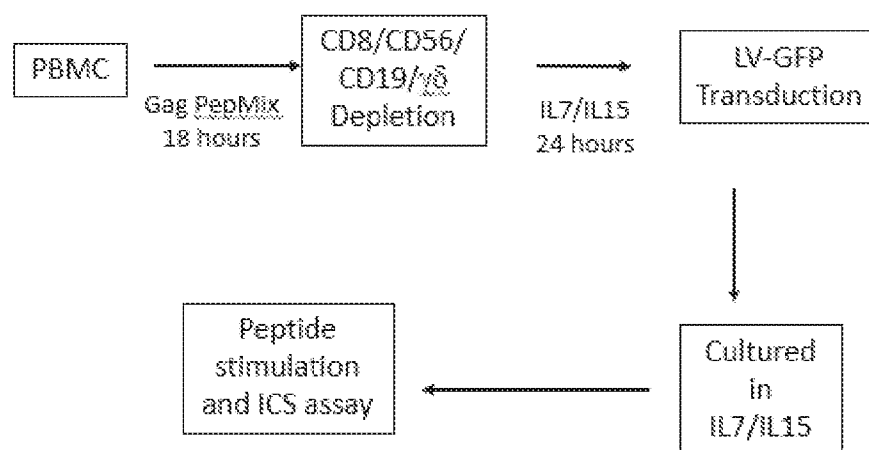


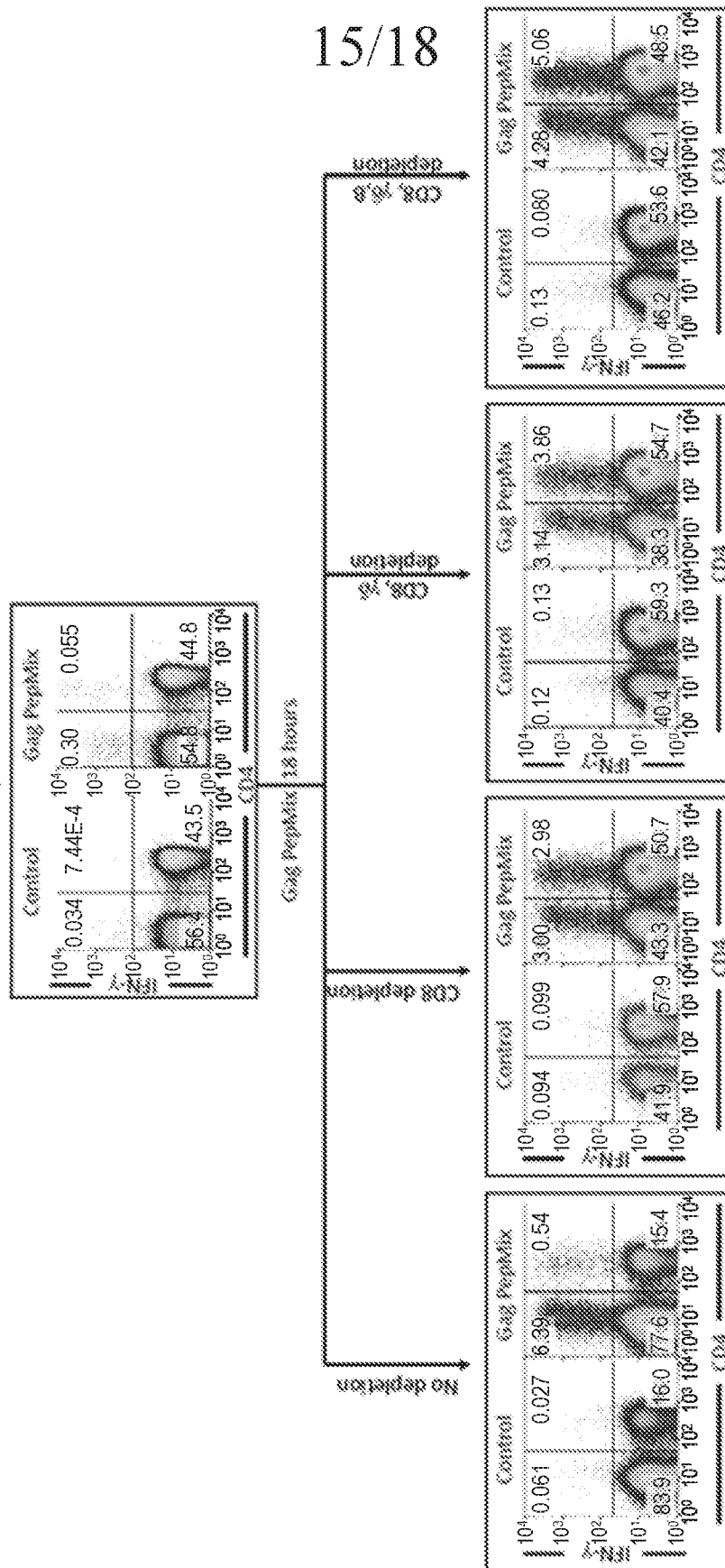
Figure 13

14/18

**Figure 14**

PTID: Precision For Medicine 11677

03



151617

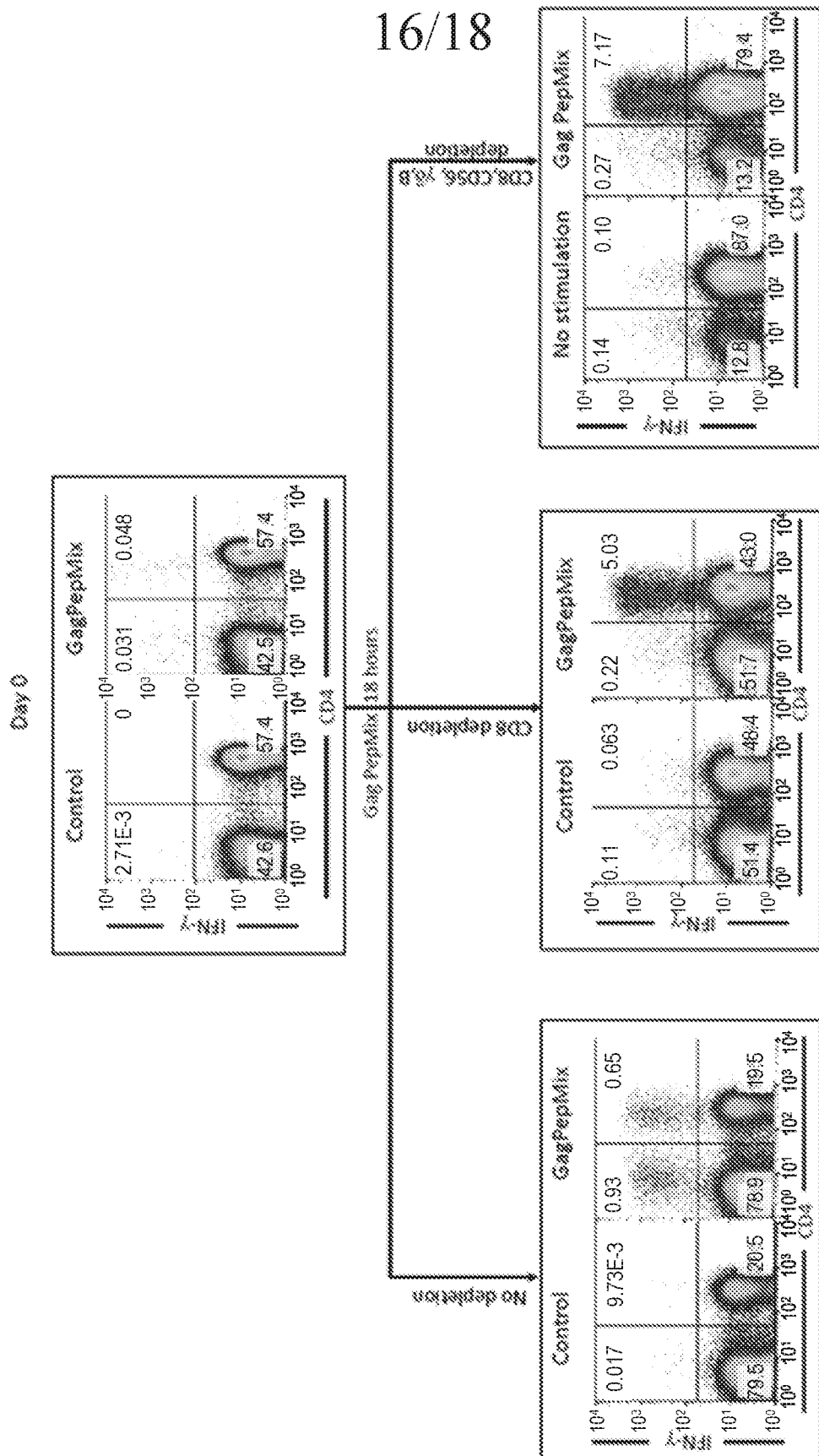
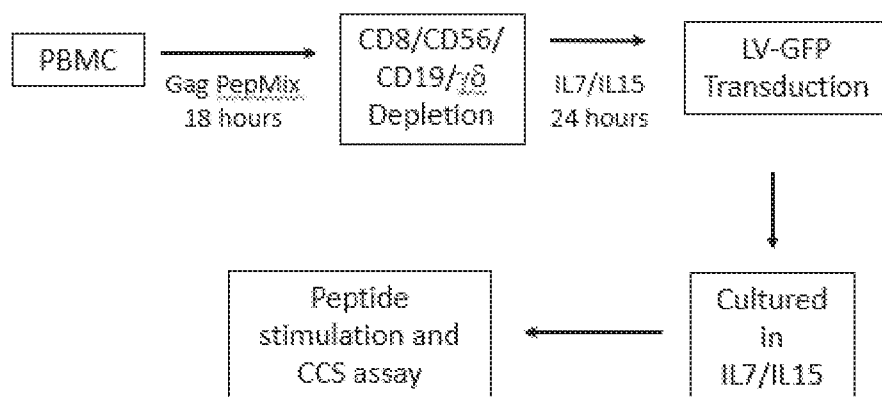


Figure 16

17/18

**Figure 17**

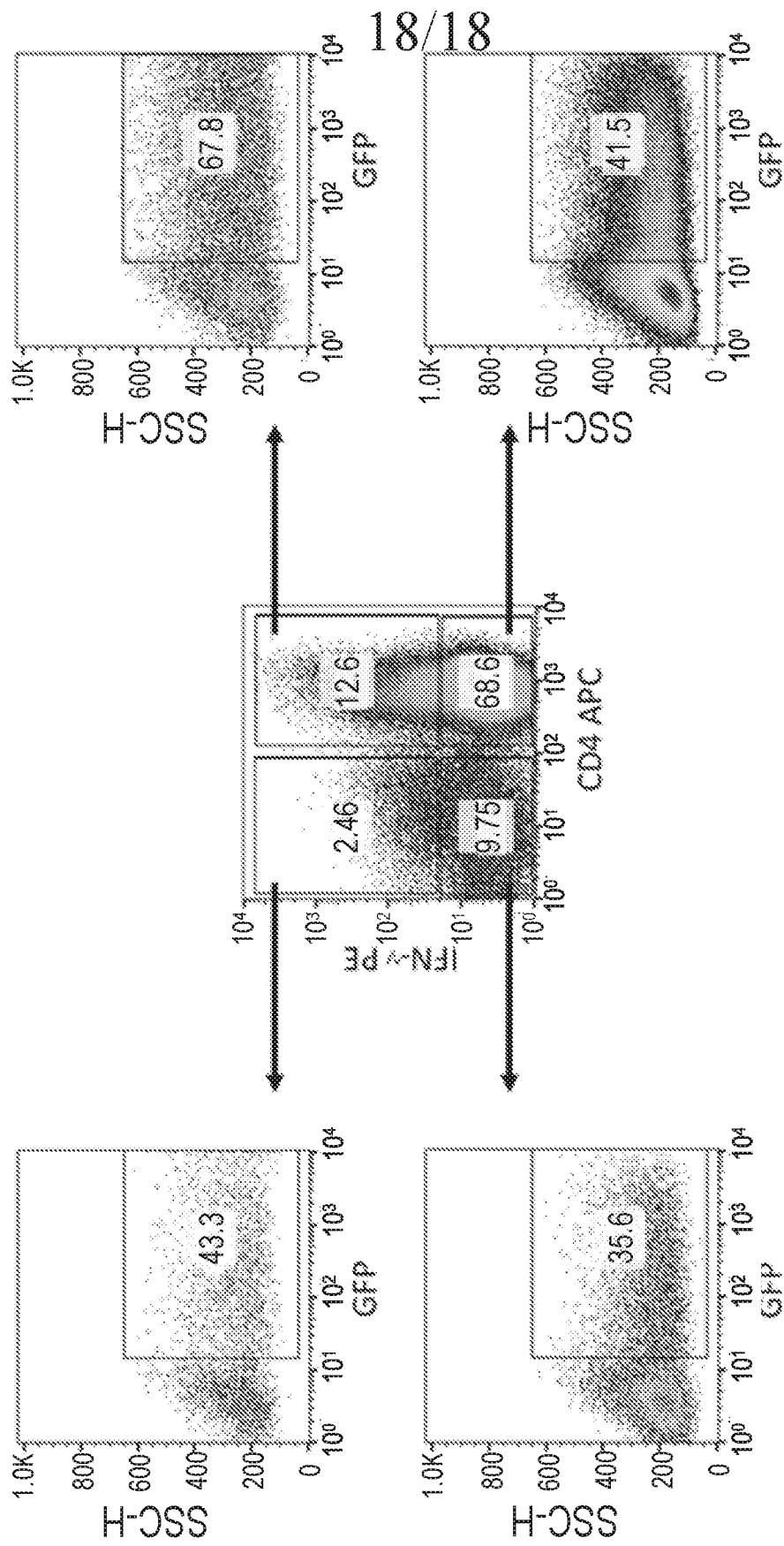


Figure 18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/024410

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/12; A61K 35/15; A61K 35/17; A61K 39/21; C07K 14/155; C12N 5/0783 (2019.01)

CPC - A61K 35/17; A61K 39/12; A61K 39/21; A61K 2035/124; A61K 2039/5156; C12N 15/1132; C12N 15/1138; C12N 2310/141; C12N 2740/16011 (2019.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/188.1; 424/208.1; 435/375; 514/44A; 514/3.8; 536/24.5 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/139065 A1 (AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.) 17 August 2017 (17.08.2017) entire document	11-13 ----- 1-10, 14-17
Y	US 2017/0037369 A1 (JUNO THERAPEUTICS, INC.) 09 February 2017 (09.02.2017) entire document	1-10, 14-17
Y	WO 2016/189159 A1 (GE HEALTHCARE BIO-SCIENCES AB) 01 December 2016 (01.12.2016) entire document	2
A	WO 2017/139065 A1 (AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.) 17 August 2017 (17.08.2017) entire document	1-17
A	WO 2017/007994 A1 (AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.) 12 January 2017 (12.01.2017) entire document	1-17

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09 July 2019

Date of mailing of the international search report

22 JUL 2019

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