**(12) STANDARD PATENT** (11) Application No. **AU 2017292582 C1**
**(19) AUSTRALIAN PATENT OFFICE**

(54) Title
**HIV pre-immunization and immunotherapy**

(51) International Patent Classification(s)
***A61K 39/12*** (2006.01) ***C07H 21/02*** (2006.01)
***A61K 35/12*** (2006.01) ***C12N 15/85*** (2006.01)
***A61K 35/17*** (2015.01) ***C12N 15/86*** (2006.01)
***A61P 31/18*** (2006.01)

(21) Application No: **2017292582** (22) Date of Filing: **2017.01.11**

(87) WIPO No: **WO18/009246**

(30) Priority Data

| (31) Number | (32) Date | (33) Country |
|---|---|---|
| **62/409,270** | **2016.10.17** | **US** |
| **62/385,864** | **2016.09.09** | **US** |
| **62/360,185** | **2016.07.08** | **US** |

(43) Publication Date: **2018.01.11**
(44) Accepted Journal Date: **2020.10.22**
(44) Amended Journal Date: **2021.11.11**

(71) Applicant(s)
**American Gene Technologies International Inc.**

(72) Inventor(s)
**Pauza, Charles David;Li, Haishan;Lahusen, Tyler**

(74) Agent / Attorney
**Davies Collison Cave Pty Ltd, Level 15 1 Nicholson Street, MELBOURNE, VIC, 3000, AU**

(56) Related Art
**WO 2017007994 A1**
**WO 2017139065 A1**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 January 2018 (11.01.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/009246 A1

(51) International Patent Classification:
*A61K 39/12* (2006.01) *C12N 15/86* (2006.01)
*A61K 35/12* (2015.01) *C12N 15/85* (2006.01)
*A61K 35/17* (2015.01) *C07H 21/02* (2006.01)
*C12N 5/06* (2006.01) *A61P 31/18* (2006.01)

(21) International Application Number:
PCT/US2017/013019

(22) International Filing Date:
11 January 2017 (11.01.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/360,185 08 July 2016 (08.07.2016) US
62/385,864 09 September 2016 (09.09.2016) US
62/409,270 17 October 2016 (17.10.2016) US

(71) Applicant: **AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.** [US/US]; 15010 Broschart Rd. #110, Rockville, MD 20850 (US).

(72) Inventors: **PAUZA, Charles David**; c/o American Gene Technologies International Inc., 15010 Broshart Rd. #110, Rockville, MA 20850 (US). **HAISHAN, Li**; c/o American Gene Technologies International Inc., 15010 Broshart Rd. #110, Rockville, MA 20850 (US). **LAHUSEN, Tyler**; c/o American Gene Technologies International Inc., 15010 Broshart Rd. #110, Rockville, MA 20850 (US).

(74) Agent: **MULHOLLAND, William F.**; Snell & Wilmer LLP, One Arizona Center, 400 E. Van Buren, Phoenix, AZ 85004 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY

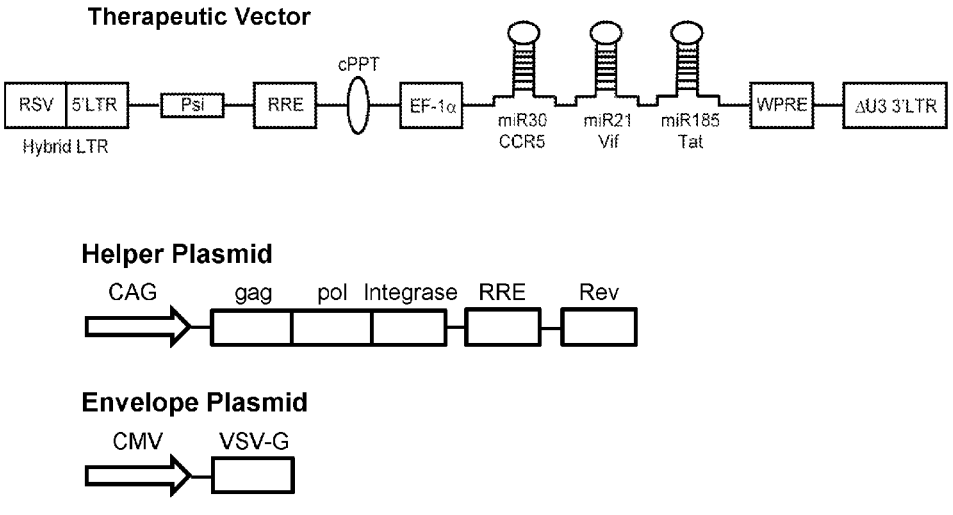


Figure 3

(57) Abstract: The present invention relates generally to immunization and immunotherapy for the treatment or prevention of HIV. In particular, the methods include *in vivo* and/or *ex vivo* enrichment of HIV-specific CD4+ T cells.

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EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published:**

— *with international search report (Art. 21(3))*

# HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to: U.S. Provisional Patent Application No. 62/360,185 filed on July 8, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", U.S. Provisional Patent Application No. 62/385,864 filed on September 9, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY", and U.S. Provisional Patent Application No. 62/409,270 filed on October 17, 2016 entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY," the disclosures of which are incorporated herein by reference.

## FIELD OF THE INVENTION

The present invention relates generally to the field of immunization and immunotherapy for the treatment and prevention of HIV. In particular, the disclosed methods of treatment and prevention relate to the administration of viral vectors and systems for the delivery of genes and other therapeutic, diagnostic, or research uses.

## BACKGROUND OF THE INVENTION

Combination antiretroviral therapy (cART) (also known as Highly Active Antiretroviral Therapy or HAART) limits HIV-1 replication and retards 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 functional cure. Alternative treatment strategies are needed.

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. Virus-specific T-helper cells, which are critical to maintenance of cytolytic T cell (CTL) function, likely play a role. Viremia is also influenced by neutralizing antibodies, but they are generally low in magnitude in HIV infection and do not keep up with evolving viral variants *in vivo*.

Together this data indicates that increasing the strength and breadth of HIV-specific cellular immune responses might have a clinical benefit through so-called HIV immunotherapy. 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.

Viral vectors can be used to transduce genes into target cells owing to specific virus envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for the transfer of genes into many different cell types including whole T cells or other immune cells as well as embryos, fertilized eggs, isolated tissue samples, tissue targets *in situ* and cultured cells. The ability to introduce and express foreign or altered genes in a cell is useful for therapeutic interventions such as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy.

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), vaccinia virus, and herpes virus have been proposed as therapeutic gene transfer vectors.

There are many factors that must be considered when developing viral vectors, including tissue tropism, stability of virus preparations, stability and control of expression, genome packaging capacity, and construct-dependent vector stability. In addition, *in vivo* application of viral vectors is often limited by host immune responses against viral structural proteins and/or transduced gene products.

Thus, toxicity and safety are key hurdles that must be overcome for viral vectors to be used *in vivo* for the treatment of subjects. There are numerous historical examples of gene therapy applications in humans that have met with problems associated with the host immune responses against the gene delivery vehicles or the therapeutic gene products. Viral vectors (*e.g.,* adenovirus) which co-transduce several viral genes together with one or more therapeutic gene(s) are particularly problematic.

Although lentiviral vectors do not generally induce cytotoxicity and do not elicit strong host immune responses, some lentiviral vectors such as HIV-1, which carry several immunostimulatory gene products, have the potential to cause cytotoxicity and induce strong immune responses *in vivo*. However, this may not be a concern for lentiviral derived

transducing vectors that do not encode multiple viral genes after transduction. Of course, this may not always be the case, as sometimes the purpose of the vector is to encode a protein that will provoke a clinically useful immune response.

Another important issue related to the use of lentiviral vectors is that of possible cytopathogenicity upon exposure to some cytotoxic viral proteins. Exposure to certain HIV-1 proteins may induce cell death or functional unresponsiveness in T cells. Likewise, the possibility of generating replication-competent, virulent virus by recombination is often a concern. Accordingly, there remains a need for improved treatments of HIV.

**SUMMARY OF THE INVENTION**

In one aspect, a method of treating cells infected with HIV is provided. The method variously includes contacting peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of a stimulatory agent, wherein the contacting is carried out *ex vivo*; transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. The method may further include infusing the transduced PBMC into a subject. The subject may be a human. The stimulatory agent may include any agent suitable for stimulating a T cell response in a subject. In embodiments, the stimulatory agent is a peptide or mixture of peptides, and in embodiments includes a gag peptide. The stimulatory agent may also include a vaccine. The vaccine may be a HIV vaccine, and in embodiments, the HIV vaccine is a MVA/HIV62B vaccine or a variant 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 further embodiments, the at least one genetic element includes at least one small RNA capable of targeting an HIV RNA sequence. In further embodiments, the at least one genetic element may include 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 includes any HIV sequence suitable for targeting by a viral delivery system. In embodiments, the HIV RNA sequence includes one or more of a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element includes any genetic element capable of being expressed by a viral delivery system. In embodiments, the at least one genetic element includes a microRNA

or a shRNA. In further embodiments, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes

CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC

ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA
GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA
GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT
CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG
ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG
CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG
GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA
GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a subject is disclosed. The method variously includes immunizing the subject with an effective amount of a first stimulatory agent; removing leukocytes from the subject and obtaining peripheral blood mononuclear cells (PBMC). The method further includes contacting the PBMC *ex vivo* with a therapeutically effective amount of a second stimulatory agent; transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; and culturing the transduced PBMC for a sufficient period of time to ensure adequate transduction. In embodiments, the transduced PBMC may be cultured from about 1 to about 35 days. In embodiments, the method further involves infusing the transduced PBMC into a subject. The subject may be a human. The first and second stimulatory agents may be the same or different. The first and second stimulatory agents may include one or more of a peptide or mixture of peptides. In embodiments, at least one of the first and second stimulatory agents includes a gag peptide. The at least one of the first and second stimulatory agents may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, 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. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or a variant thereof. The at least one genetic element may

include a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes

CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC

ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA
GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA
GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT
CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG
ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG
CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG
GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA
GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

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. The at least one encoded genetic element may also comprise at least one small RNA capable of targeting an HIV RNA sequence. In another aspect, 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, 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.

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA
AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT
CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA
AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT
CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG

AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, the microRNA cluster includes a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

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 preferably optimized for infecting a cell; and at least one

helper plasmid for expressing genes of interest. In embodiments, the genes of interest include one or more of gag, pol, and rev genes. In embodiments, the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line. In further embodiments, a lentiviral particle is produced by the packaging cell line. In embodiments, the lentiviral particle is capable of modulating production of a target of interest. In embodiments, the target of interest is any of chemokine receptor CCR5 or an HIV RNA sequence. The system may further include a first helper plasmid and a second helper plasmid. In embodiments, a first helper plasmid expresses the gag and pol genes, and a second helper plasmid expressesthe rev gene.

In another aspect, a lentiviral particle capable of infecting a cell is provided. The lentiviral particle includes an envelope protein preferably optimized for infecting a cell, and a lentiviral vector as described herein. In embodiments, the envelope protein may be optimized for infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is provided. The modified cell includes any cell capable of being infected with a lentiviral vector system for use in accordance with present aspects and embodiments. In embodiments, the cell is a CD4+ T cell that is infected with a lentiviral particle. In embodiments, the CD4+ T cell also has been selected to recognize an HIV antigen. In embodiments, the HIV antigen includes a gag antigen. In embodiments, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is provided. The method variously 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 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 higher than the first quantifiable measurement, the subject is selected for the treatment regimen. The at least one factor may include any of T cell proliferation or IFN gamma production.

The foregoing general description and following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further

explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following brief description of the drawings and detailed description of the invention.

**BRIEF DESCRIPTION OF THE DRAWINGS**

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

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

**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.

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

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

**Figure 6** 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. Sequences that are not underlined comprise the EF-1alpha promoter of transcription that was selected as being a preferable promoter for this miR cluster. Sequences that are underlined show the miR cluster consisting of miR30 CCR5, miR21 Vif, and miR185 Tat (as shown collectively in SEQ ID NO: 33).

**Figure 7** depicts exemplary lentiviral vector constructs according to various aspects of this disclosure.

**Figure 8** shows knockdown of CCR5 by an experimental vector and corresponding prevention of R5-tropic HIV infection in AGTc120 cells. (**A**) shows CCR5 expression in AGTc120 cells with or without AGT103 lentivirus vector. (**B**) shows the sensitivity of transduced AGTc120 cells to infection with a HIV BaL virus stock that was expressing green fluorescent protein (GFP) fused to the Nef gene of HIV.

**Figure 9** depicts data demonstrating regulation of CCR5 expression by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. **(A)** Screening data for potential candidates is shown. **(B)** CCR5 knock-down data following transduction with CCR5 shRNA-1 (SEQ ID NO: 16) is shown.

**Figure 10** depicts data demonstrating regulation of HIV components by shRNA inhibitor sequences in a lentiviral vector of the present disclosure. **(A)** Knock-down data for the rev/tat target gene is shown. **(B)** Knock-down data for the gag target gene is shown.

**Figure 11** depicts data demonstrating that AGT103 reduces expression of Tat protein expression in cells transfected with an HIV expression plasmid, as described herein.

**Figure 12** depicts data demonstrating regulation of HIV components by synthetic microRNA sequences in a lentiviral vector of the present disclosure. **(A)** Tat knock-down data is shown. **(B)** Vif knock-down data is shown.

**Figure 13** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure.

**Figure 14** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure containing either a long or short WPRE sequence.

**Figure 15** depicts data demonstrating regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector of the present disclosure with or without a WPRE sequence.

**Figure 16** depicts data demonstrating regulation of CCR5 expression by a CD4 promoter regulating synthetic microRNA sequences in a lentiviral vector of the present disclosure.

**Figure 17** depicts data demonstrating detection of HIV Gag-specific CD4 T cells.

**Figure 18** depicts data demonstrating HIV-specific CD4 T cell expansion and lentivirus transduction. **(A)** An exemplary schedule of treatment is shown. **(B)** IFN-gamma production in CD4-gated T cells is shown, as described herein. **(C)** IFN-gamma production and GFP expression in CD4-gated T cells is shown, as described herein. **(D)** Frequency of HIV-specific CD4+ T cells is shown, as described herein. **(E)** IFN-gamma production from PBMCs post-vaccination is shown, as described herein.

**Figure 19** depicts data demonstrating a functional assay for a dose response of increasing AGT103-GFP and inhibition of CCR5 expression. **(A)** Dose response data for increasing amounts of AGT103-GFP is shown. **(B)** Normally distributed populations in terms of CCR5 expression are shown. **(C)** Percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP is shown.

**Figure 20** depicts data demonstrating AGT103 transduction efficiency for primary human CD4+ T cells. **(A)** Frequency of transduced cells (GFP-positive) is shown by FACS, as described herein. **(B)** Number of vector copies per cell is shown, as described herein.

**Figure 21** depicts data demonstrating AGT103 inhibition of HIV replication in primary CD4+ T cells, as described herein.

**Figure 22** depicts data demonstrating AGT103 protection of primary human $CD4^+$ T cells from HIV-induced depletion.

**Figure 23** depicts data demonstrating generation of a CD4+ T cell population that is highly enriched for HIV-specific, AGT103-transduced CD4 T cells. **(A)** shows CD4 and CD8 expression profiles for cell populations, as described herein. **(B)** shows CD4 and CD8 expression profiles for cell populations, as described herein. **(C)** shows IFN-gamma and CD4 expression profiles for cell populations, as described herein. **(D)** shows IFN-gamma and GFP expression profiles for cell populations, as described herein.

## DETAILED DESCRIPTION

### Overview

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.

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, the various aspects and embodiments 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 first stimulation event enriches the fraction of HIV-specific CD4 T cells. This is followed by (1) isolating peripheral leukocytes by leukapheresis or purifying PBMC from venous blood, (2) a second stimulating event, for example re-stimulating CD4 T cells *ex vivo* with a suitable stimulatory agent, such as any vaccine or protein, for example, HIV or HIV-related peptides, (3) performing therapeutic lentivirus transduction, *ex vivo* T cell culture, and (4) re-infusion back into the original patient.

The various methods and compositions can be used to prevent new cells, such as CD4+ T cells, from becoming infected with HIV. For example as illustrated in Figure 2, to prevent new cells from becoming infected, CCR5 expression can be targeted to prevent virus attachment. 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 have already become infected with HIV. To stop the HIV viral cycle, viral RNA produced by latently-infected cells, such as latently-infected CD4+ T cells, is targeted.

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 functional 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, and also followed by return to progression of disease despite prior genetic therapy. By employing therapeutic immunization in accordance with the compositions and methods described herein, a new HIV treatment regimen has been developed including, in various embodiments, a functional cure.

**Definitions and Interpretation**

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.

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.

As used herein, the terms "administration of" or "administering" an active agent means providing an active agent of the invention 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.

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.

As used herein, the term "AGT103T" refers to a cell that has been transduced with a lentivirus that contains the AGT103 lentiviral vector.

Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Further, as used herein, the term "includes" means includes without limitation.

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 of a cellular source (see for *e.g.*: Rosenberg et al., *N. Engl. J. Med.* 323:570-578 (1990); Dudley el 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)).

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.

The term "functional cure", as referenced above, and 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, or discontinued dosing of such HIV therapies. An individual 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.

The term "HIV vaccine" encompasses immunogens plus vehicle 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 a recombinant virus vectors capable of expressing HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides, in addition to recombinant bacterial vectors, 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 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 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 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: for 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.

**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) |
| 96-I-0050 | APL 400-003 GENEVAX-HIV |
| ACTG 326; PACTG 326 | ALVAC vCP1452 |
| Ad26.ENVA.01 | Ad26.EnvA-01 |
| Ad26.ENVA.01 Mucosal/IPCAVD003 | Ad26.EnvA-01 |
| Ad5HVR48.ENVA.01 | Ad5HVR48.ENVA.01 |
| ANRS VAC 01 | ALVAC vCP125 |
| ANRS VAC 02 | rgp 160 + peptide V3 ANRS VAC 02 |
| ANRS VAC 03 | ALVAC-HIV MN120TMG strain (vCP205) |
| ANRS VAC 04 | LIPO-6 |
| ANRS VAC 04 bis | LIPO-6 |
| ANRS VAC 05 | ALVAC vCP125 |
| ANRS VAC 06 | ALVAC vCP125 |
| ANRS VAC 07 | ALVAC vCP300 |
| ANRS VAC 08 | ALVAC-HIV MN120TMG strain (vCP205) |
| ANRS VAC 09 | ALVAC-HIV MN120TMG strain (vCP205) |
| ANRS VAC 09 bis | LIPO-6 |
| ANRS VAC 10 | ALVAC vCP1452 |
| ANRS VAC 12 | LPHIV1 |
| ANRS VAC 14 | gp160 MN/LAI |
| ANRS VAC 16 | LPHIV1 |
| ANRS VAC 17 | LIPO-6 |
| ANRS VAC 18 | LIPO-5 |
| APL 400-003RX101 | APL 400-003 GENEVAX-HIV |
| AVEG 002 | HIVAC-1e |

| | |
|---|---|
| AVEG 002A | HIVAC-1e |
| AVEG 002B | HIVAC-1e |
| AVEG 003 | VaxSyn gp160 Vaccine (MicroGeneSys) |
| AVEG 003A | VaxSyn gp160 Vaccine (MicroGeneSys) |
| AVEG 003B | VaxSyn gp160 Vaccine (MicroGeneSys) |
| AVEG 004 | gp160 Vaccine (Immuno-AG) |
| AVEG 004A | gp160 Vaccine (Immuno-AG) |
| AVEG 004B | gp160 Vaccine (Immuno-AG) |
| AVEG 005A/B | Env 2-3 |
| AVEG 005C | Env 2-3 |
| AVEG 006X; VEU 006 | MN rgp120 |
| AVEG 007A/B | rgp120/HIV-1 SF-2 |
| AVEG 007C | rgp120/HIV-1 SF-2 |
| AVEG 008 | HIVAC-1e |
| AVEG 009 | MN rgp120 |
| AVEG 010 | HIVAC-1e |
| AVEG 011 | UBI HIV-1 Peptide Immunogen, Multivalent |
| AVEG 012A/B | ALVAC vCP125 |
| AVEG 013A | gp160 Vaccine (Immuno-AG) |
| AVEG 013B | gp160 Vaccine (Immuno-AG) |
| AVEG 014A/B | TBC-3B |
| AVEG 014C | TBC-3B |
| AVEG 015 | rgp120/HIV-1 SF-2 |
| AVEG 016 | MN rgp120 |
| AVEG 016A | MN rgp120 |
| AVEG 016B | MN rgp120 |
| AVEG 017 | UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent |
| AVEG 018 | UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent |
| AVEG 019 | p17/p24:Ty- VLP |
| AVEG 020 | gp120 C4-V3 |
| AVEG 021 | P3C541b Lipopeptide |

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|---|---|
| AVEG 022 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 022A | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 023 | UBI HIV-1 Peptide Immunogen, Multivalent |
| AVEG 024 | rgp120/HIV-1 SF-2 |
| AVEG 026 | ALVAC vCP300 |
| AVEG 027 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 028 | Salmonella typhi CVD 908-HIV-1 LAI gp 120 |
| AVEG 029 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 031 | APL 400-047 |
| AVEG 032 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 033 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 034/034A | ALVAC vCP1433 |
| AVEG 036 | MN rgp120 |
| AVEG 038 | ALVAC-HIV MN120TMG strain (vCP205) |
| AVEG 201 | rgp120/HIV-1 SF-2 |
| AVEG 202/HIVNET 014 | ALVAC-HIV MN120TMG strain (vCP205) |
| 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 |
| CM235gp120 and SF2gp120 | CM235 (ThaiE) gp120 plus SF2(B) gp120 |
| CombiHIVvac (KombiVIChvak) | CombiHIVvac |
| CRC282 | P2G12 |
| CRO2049/ CUT*HIVAC001 | GTU-MultiHIV |
| CUTHIVAC002 | DNA-C CN54ENV |
| DCVax-001 | DCVax-001 |
| DNA-4 | DNA-4 |
| DP6?001 | DP6?001 DNA |
| DVP-1 | EnvDNA |
| EN41-UGR7C | EN41-UGR7C |
| EnvDNA | EnvDNA |

| EnvPro | EnvPro |
|---|---|
| EuroNeut41 | EN41-FPA2 |
| EV01 | NYVAC-C |
| EV02 (EuroVacc 02) | DNA-C |
| EV03/ANRSVAC20 | 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 |
| HIVIS 03 | HIVIS-DNA |
| HIVIS 05 | HIVIS-DNA |
| HIVIS06 | HIVIS-DNA |
| HIVIS07 | HIVIS-DNA |
| HIVNET 007 | ALVAC-HIV MN120TMG strain (vCP205) |
| HIVNET 026 | ALVAC vCP1452 |
| HPTN 027 | ALVAC-HIV vCP1521 |
| HVRF-380-131004 | Vichrepol |
| HVTN 039 | ALVAC vCP1452 |
| HVTN 040 | AVX101 |
| HVTN 041 | rgp120w61d |
| HVTN 042 / ANRS VAC 19 | ALVAC vCP1452 |
| 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 |

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|---|---|
| HVTN 054 | VRC-HIVADV014-00-VP |
| HVTN 055 | TBC-M335 |
| HVTN 056 | MEP |
| HVTN 057 | VRC-HIVDNA009-00-VP |
| HVTN 059 | AVX101 |
| HVTN 060 | HIV-1 gag DNA |
| HVTN 063 | HIV-1 gag DNA |
| HVTN 064 | EP HIV-1043 |
| HVTN 065 | pGA2/JS7 DNA |
| HVTN 067 | EP-1233 |
| HVTN 068 | VRC-HIVADV014-00-VP |
| HVTN 069 | VRC-HIVDNA009-00-VP |
| HVTN 070 | PENNVAX-B |
| HVTN 071 | MRKAd5 HIV-1 gag |
| 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 080 | PENNVAX-B |
| HVTN 082 | VRC-HIVDNA016-00-VP |
| HVTN 083 | VRC-HIVADV038-00-VP |
| HVTN 084 | VRC-HIVADV054-00-VP |
| HVTN 085 | VRC-HIVADV014-00-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 098 | PENNVAX-GP |
| HVTN 100 | ALVAC-HIV-C (vCP2438) |
| HVTN 101 | DNA-HIV-PT123 |
| HVTN 102 | 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 114; GOVX-B11 | AIDSVAX B/E |
| HVTN 116 | VRC-HIVMAB060-00-AB |
| HVTN 203 | ALVAC vCP1452 |
| HVTN 204 | VRC-HIVDNA016-00-VP |
| HVTN 205 | pGA2/JS7 DNA |
| HVTN 502/Merck 023 (Step Study) | MRKAd5 HIV-1 gag/pol/nef |
| HVTN 503 (Phambili) | MRKAd5 HIV-1 gag/pol/nef |
| HVTN 505 | VRC-HIVDNA016-00-VP |
| HVTN 702 | ALVAC-HIV-C (vCP2438) |
| HVTN 703 AMP | VRC-HIVMAB060-00-AB |
| HVTN 908 | pGA2/JS7 DNA |
| IAVI 001 | DNA.HIVA |
| IAVI 002 | DNA.HIVA |
| IAVI 003 | MVA.HIVA |
| IAVI 004 | MVA.HIVA |
| IAVI 005 | DNA.HIVA |
| IAVI 006 | DNA.HIVA |
| IAVI 008 | MVA.HIVA |
| IAVI 009 | DNA.HIVA |
| IAVI 010 | DNA.HIVA |
| IAVI 011 | MVA.HIVA |
| IAVI 016 | MVA.HIVA |
| IAVI A001 | tgAAC09 |

| | |
|---|---|
| IAVI A002 | 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 C003 | ADMVA |
| IAVI C004/DHO-614 | ADVAX |
| IAVI D001 | TBC-M4 |
| IAVI N004 HIV-CORE 004 | Ad35-GRIN |
| IAVI P001 | ADVAX |
| IAVI P002 | ADVAX |
| IAVI R001 | rcAd26.MOS1.HIVEnv |
| IAVI S001 | SeV-G |
| IAVI V001 | VRC-HIVDNA016-00-VP |
| IAVI V002 | VRC-HIVDNA016-00-VP |
| 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 |
| IPCAVD010 | Ad26.Mos.HIV Trivalent |
| ISS P-001 | Tat vaccine |
| ISS P-002 | Tat vaccine |
| LFn-p24 vaccine | LFn-p24 |
| MCA-0835 | 3BNC117 |
| Merck V520-007 | Ad-5 HIV-1 gag (Merck) |
| MRC V001 | rgp120w61d |
| MRK Ad5 | Ad-5 HIV-1 gag (Merck) |

| | |
|---|---|
| MRKAd5 + ALVAC | MRKAd5 HIV-1 gag |
| Mucovac2 | CN54gp140 |
| MV1-F4 | Measles Vector - GSK |
| MYM-V101 | Virosome-Gp41 |
| NCHECR-AE1 | pHIS-HIV-AE |
| PACTG 230 | AIDSVAX B/E |
| PAVE100 | VRC-HIVDNA016-00-VP |
| PEACHI-04 | ChAdV63.HIVconsv |
| PedVacc001 & PedVacc002 | MVA.HIVA |
| PolyEnv1 | PolyEnv1 |
| PXVX-HIV-100-001 | Ad4-mgag |
| RISVAC02 | MVA-B |
| RisVac02 boost | MVA-B |
| RV 124 | ALVAC-HIV MN120TMG strain (vCP205) |
| RV 132 | ALVAC-HIV vCP1521 |
| RV 135 | ALVAC-HIV vCP1521 |
| RV 138; B011 | ALVAC-HIV MN120TMG strain (vCP205) |
| RV 144 | ALVAC-HIV vCP1521 |
| RV 151 / WRAIR 984 | LFn-p24 |
| RV 156 | VRC-HIVDNA009-00-VP |
| RV 156A | VRC-HIVDNA009-00-VP |
| RV 158 | MVA-CMDR |
| RV 172 | VRC-HIVDNA016-00-VP |
| RV 305 | ALVAC-HIV vCP1521 |
| RV 306 | ALVAC-HIV vCP1521 |
| RV 328 | AIDSVAX B/E |
| RV 365 | MVA-CMDR |
| RV262 | Pennvax-G |
| SG06RS02 | HIV gp140 ZM96 |
| TAB9 | TAB9 |
| TaMoVac II | HIVIS-DNA |
| TAMOVAC-01-MZ | HIVIS-DNA |

| | |
|---|---|
| Tiantan vaccinia HIV Vaccine | Chinese DNA |
| Tiantan vaccinia HIV Vaccine and DNA | Chinese DNA |
| TMB-108 | Ibalizumab |
| UBI HIV-1 MN China | UBI HIV-1 Peptide Immunogen, Multivalent |
| UBI HIV-1MN octameric - Australia study | UBI HIV-1 Peptide Immunogen, Multivalent |
| UBI V106 | UBI HIV-1 Peptide Vaccine, Microparticulate Monovalent |
| UCLA MIG-001 | TBC-3B |
| UCLA MIG-003 | ALVAC-HIV MN120TMG strain (vCP205) |
| UKHVCSpoke003 | DNA - CN54ENV and ZM96GPN |
| V24P1 | HIV p24/MF59 Vaccine |
| V3-MAPS | V3-MAPS |
| V520-016 | MRKAd5 HIV-1 gag/pol/nef |
| V520-027 | MRKAd5 HIV-1 gag/pol/nef |
| V526-001 MRKAd5 and MRKAd6 HIV-1 Trigene Vaccines | MRKAd5 HIV-1 gag/pol/nef |
| VAX 002 | AIDSVAX B/B |
| VAX 003 | AIDSVAX B/E |
| VAX 004 | AIDSVAX B/B |
| VRC 004 (03-I-0022) | VRC-HIVDNA009-00-VP |
| VRC 006 (04-I-0172) | VRC-HIVADV014-00-VP |
| VRC 007 (04-I-0254) | VRC-HIVDNA016-00-VP |
| VRC 008 (05-I-0148) | VRC-HIVDNA016-00-VP |
| VRC 009 (05-I-0081) | VRC-HIVDNA009-00-VP |
| VRC 010 (05-I-0140) | VRC-HIVADV014-00-VP |
| VRC 011(06-I-0149) | VRC-HIVDNA016-00-VP |
| VRC 012 (07-I-0167) | VRC-HIVADV027-00-VP |
| VRC 015 (08-I-0171) | VRC-HIVADV014-00-VP |
| VRC 016 | VRC-HIVDNA016-00-VP |
| VRC 602 | VRC-HIVMAB060-00-AB |
| VRC 607 | VRCHIVMAB080-00-AB |
| VRC01LS | VRCHIVMAB080-00-AB |

| | |
|---|---|
| VRI01 | MVA-B |
| X001 | CN54gp140 |

*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.

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.

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 situate on a vector in close proximity to each other and are co-expressed.

The term "packaging cell line" refers to any cell line that can be used to express a lentiviral particle.

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.

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).

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.

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.

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 invention. 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 invention. 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.

As used herein, "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.

As used herein, a "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).

As used herein, the term "SEQ ID NO" is synonymous with the term "Sequence ID No."

As used herein, "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.

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.

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.

The term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present invention, 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.

As used herein, the term "therapeutic vector" is synonymous with a lentiviral vector such as the AGT103 vector.

The term "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 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.

The term "vaccine", which is used interchangeably with the term "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 fragents, or virus-like particles (VLPs).

**Description of Aspects of the Disclosure**

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 (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*; 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. The method may further include infusing the transduced PBMC into a subject. The subject may be a human. The stimulatory agent may include a peptide or mixture of peptides, and in a preferred embodiment includes a gag peptide. The stimulatory agent may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In a preferred embodiment, the viral delivery system includes a lentiviral particle. In embodiments, the at least one genetic element may include 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 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. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or variants thereof. The at least one genetic element may include at least one of a microRNA or a shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, 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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or 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 GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes

CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC
CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes:
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT
CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG
ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG
CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG
GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA
GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

In another aspect, a method of treating HIV infection in a subject 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). The method further includes contacting the PBMC *ex vivo* with a therapeutically effective amount of a second stimulatory agent; 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 transduction. The method may further include further enrichment of the PBMC, for example, by preferably enriching the PBMC for CD4+ T cells. In embodiments, the transduced PBMC are cultured from about 1 to about 35 days. The method may further involve infusing the transduced PBMC into a subject. The subject may be a human. The first and second stimulatory agents may be the

same or different from each other. The at least one of the first and second stimulatory agents may include a peptide or mixture of peptides. In embodiments, at least one of the first and second stimulatory agents includes a gag peptide. The at least one of the first and second stimulatory agents may include a vaccine. The vaccine may be a HIV vaccine, and in a preferred embodiment, the HIV vaccine is a MVA/HIV62B vaccine or a variant thereof. In embodiments, the first stimulatory agent is a HIV vaccine and the second stimulatory agent is a gag peptide.

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. The HIV RNA sequence may include a HIV Vif sequence, a HIV Tat sequence, or variants thereof. The at least one genetic element may include a microRNA or a shRNA, or a cluster thereof. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, the at least one genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or 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 GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes

CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or

GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, 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

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

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. 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, 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.

In another aspect, 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT (SEQ ID NO: 1).

In another aspect, 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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA (SEQ ID NO: 2); or 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 GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes
CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG

AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC
TGACCA (SEQ ID NO: 2); or
GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCC
CAATGACCGCGTCTTCGTC (SEQ ID NO: 3).

In another aspect, 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
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA
AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT
CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA
CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC
ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA
GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAA
GCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes:
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCT
CGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGG
ATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCG
CACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAG
GGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA
GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31).

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 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.

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 infecting a T cell. In a preferred embodiment, the envelope protein is optimized for infecting a CD4+ T cell.

In another aspect, a modified cell is disclosed. In embodiments, the modified cell is a CD4+ T cell. In embodiments, the CD4+ T cell isinfected 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 preferred embodiment, the HIV antigen that is recognized by the CD4+ T cell includes a gag antigen. In a further preferred embodiment, the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

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 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.

**Human Immunodeficiency Virus (HIV)**

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. 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. HIV may be present in an infected individual as both free virus particles and within infected immune cells.

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 CD8 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.

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.

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 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 highly active antiretroviral therapy (HAART). In the vast majority of cases, HIV infection causes fatal disease although survival may be prolonged by HAART.

A major goal in the fight against HIV is to develop strategies for curing disease. Prolonged HAART 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.

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 (chemokine receptor), which plays a critical role in viral penetration of host cells. However, many investigators are optimistic that genetic therapy holds the best promise for eventually achieving an HIV cure.

As disclosed herein, the methods and compositions of the invention are able to achieve a functional cure that may or may not include complete eradication of all HIV from the body.

As mentioned above, a functional cure is defined as a state or condition wherein HIV+ individuals who previously required HAART, may survive with low or undetectable virus replication and using lower or intermittent doses of HAART, or are potentially able to discontinue HAART altogether. As used herein, a functional cure may still possibly require 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 HIV to prevent all possibility of recurrence.

The primary obstacles to achieving a functional 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.

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 HAART; however, when HAART is interrupted the rebounding virus infection repeats the process and again deletes the virus-specific cells, resetting the clock on disease progression.

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

In one embodiment of the invention, 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**

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

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 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.

There are multiple ongoing efforts to utilize genetic therapy in the treatment of 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).

Lentivirus-delivered nucleases or other mechanisms for gene 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 terms of performance for the individual viral vector construct and for improved use of the vector through a strategy for achieving functional HIV cure.

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 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.

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**

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.

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.

In some embodiments of the disclosed invention, 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 some embodiments of the disclosed invention, 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 yet another embodiment of the invention, 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**

In one aspect, the disclosure provides methods for using viral vectors to achieve a functional 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.

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.

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.

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, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), or biological or chemical adjuvants including cytokines and/or chemokines.

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 administration of the disclosed viral vectors to achieve a functional cure.

In embodiments, peripheral blood mononuclear cells (PBMCs) are obtained by leukapheresis and treated *ex vivo* to obtain about $1x10^{10}$ CD4 T cells of which about 0.1%, about 1%, about 5% or about 10% or about 30% 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 $1x10^{7}$, about $1x10^{8}$, about $1x10^{9}$, about $1x10^{10}$, about $1x10^{11}$, or about $1x10^{12}$ CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells are isolated for *ex vivo* re-stimulation.

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 is 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 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 5, about 10, 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold.

The methods additionally include combining in vivo therapeutic immunization and *ex vivo* re-stimulation of CD4 T cells with *ex vivo* lentiviral transduction and culturing.

Thus, in one embodiment, the re-stimulated PBMC fraction that has been enriched for HIV-specific CD4 T cells can 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.

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.

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.

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 functional cure are described herein.

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 invention 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.

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.

In some embodiments, a patient may be undergoing cART or HAART concurrently while being treated according to the methods of the invention. In other embodiments, a patient may undergo cART or HAART before or after being treated according to the methods of the invention. In some embodiments, cART or HAART is maintained throughout treatment according to the methods of the invention 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 invention is able to discontinue or reduce cART or HAART following treatment according to the methods of the invention.

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**

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.

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 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.

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 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 and spreading infection. An exemplary structure is diagrammed in Figure 3.

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;
- 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.

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.

Vectors of the invention 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 invention 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) for the purpose of tracking the vectors or antibiotic resistance genes for the purposes of selectively maintaining genetically-modified cells *in vivo*.

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.

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**

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.

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.

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.

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 such as that of the influenza virus, VSV-G, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia Virus such as MLV-E, MLV- A and GALV. These latter envelopes are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the 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 a filovirus envelope. For example, the GP of Ebola, which by post-transcriptional modification become the GP, and $GP_2$ glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S.

Patent No. 5,654,195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

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 and 4-vector 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.

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.

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: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1α 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: 32 or 80), and ΔU3 3' LTR (SEQ ID NO: 39). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, a helper plasmid includes the following elements: CAG promoter (SEQ ID NO: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO:

44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47). 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.

In another aspect, an envelope plasmid includes the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

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. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

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**

In various aspects, the present invention 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.

Thus, in various embodiments, following application of a treatment according to the invention (e.g., (a) immunization, (b) *ex vivo* leukocyte/lymphocyte culture; (c) re-stimulation with purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles; and (d) 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 $1x10^8$ HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus. Alternatively, the threshold value may be about $1x10^5$, about $1x10^6$, about $1x10^7$, about $1x10^8$, about $1x10^9$, or about $1x10^{10}$ CD4 T cells in the body of the patient.

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 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.

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 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**

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 the condition of the patient and the method of administration.

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, or about 100 μg to 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, 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, bucally, at genital mucosal or intranasally, including some virally-vectored or bacterially-vectored vaccines, fusion proteins, liposome formulations or similar preparations, may contain higher amounts 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.

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.

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.

In one embodiment, HIV-vaccines for immunization are administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising an HIV vaccineis 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.

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.

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.

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.

In a further embodiment, 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.

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 invention comprise solution, suspension, and gel compositions for nasal delivery.

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.

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 propyleneglycol, polyethyleneglycol, 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.

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.

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 $10^6$ 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 $10^6$ cells in culture.

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.

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. 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**

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 $p24^{gag}$ ("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.

This approach allows the inhibition of gene expression driven by the HIV LTR promoter region in a cell population by the culture of cells in a medium comprising the CM. Culture in CM4 likely inhibits HIV LTR driven gene expression by altering one or more

interactions between transcription mediating proteins and HIV gene expression regulatory elements. Transcription-mediating proteins of interest include host cell encoded proteins such as AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp1, and the HIV encoded protein Tat. HIV gene expression regulatory elements of interest include binding sites for AP-1, NFkappaB, NF-AT, IRF, LEF-1 and Sp1, as well as the transacting responsive element ("TAR") which interacts with Tat.

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.

Another method of enriching T Cells utilizes immunoaffinity-based selection. This method includes the simultaneous enrichment or selection of a first and second population of cells, such as a CD4+ and CD8+ cell population. Cells containing primary human T cells are contacted with a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8 in an incubation composition, under conditions whereby the immunoaffinity reagents specifically bind to CD4 and CD8 molecules, respectively, on the surface of cells in the sample. Cells bound to the first and/or the second immunoaffinity reagent are recovered, thereby generating an enriched composition comprising CD4+ cells and CD8+ cells. This approach may include incubation of the composition with a concentration of the first and/or second immunoaffinity reagent that is at a sub-optimal yield concentration. Notably, in some embodiments, transduced cells are a mixed T cell population, and in other embodiments transduced cells are not a mixed T cell population.

In some embodiments, immunoaffinity-based selection is used where the solid support is a sphere, such as a bead, such as a microbead or nanobead. In other embodiments, the bead can be a magnetic bead. In another embodiment, the antibody contains one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on the solid surface, such as a sphere or chromatography matrix, wherein the antibody is reversibly mobilized to the solid surface. In some embodiments, cells expressing a cell surface marker bound by the antibody on said solid surface are capable of being recovered from the matrix by disruption of the reversible binding between the binding reagent and binding partner. In some embodiments, the binding reagent is streptavidin or is a streptavidin analog or mutant.

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.

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.

The following examples are given to illustrate aspects of the present invention. It should be understood, however, that the invention 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.

## Examples

### Example 1: Development of a Lentiviral Vector System

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 elements being from left to right: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 34-35), Psi sequence (RNA packaging site) (SEQ ID NO: 36), RRE (Rev-response element) (SEQ ID NO: 37), cPPT (polypurine tract) (SEQ ID NO: 38), EF-1α 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: 32 or 80), and ΔU3 3' LTR (SEQ ID NO: 39). The therapeutic vector detailed in Figure 3 is also referred to herein as AGT103.

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: 41); HIV component gag (SEQ ID NO: 43); HIV component pol (SEQ ID NO: 44); HIV Int (SEQ ID NO: 45); HIV RRE (SEQ ID NO: 46); and HIV Rev (SEQ ID NO: 47).

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: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62).

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 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 infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

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 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.

Referring more specifically to Figure 4, the Helper plus Rev plasmid includes a CAG enhancer (SEQ ID NO: 40); a CAG promoter (SEQ ID NO: 41); a chicken beta actin intron

(SEQ ID NO: 42); a HIV gag (SEQ ID NO: 43); a HIV Pol (SEQ ID NO: 44); a HIV Int (SEQ ID NO: 45); a HIV RRE (SEQ ID NO: 46); a HIV Rev (SEQ ID NO: 47); and a rabbit beta globin poly A (SEQ ID NO: 48).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

*Synthesis of a 2-vector lentiviral packaging system including Helper (plus Rev) and Envelope plasmids.*

*Materials and Methods:*

*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 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'-TAAGCAGAATTC ATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 81) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 82). The sequence for the Gag, Pol, Integrase fragment was as follows:

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGG
AGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACA
TAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAG
AAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAG
ACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATG
GCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGG
AAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCA
GTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTT
CAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACC
ACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCG
ATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTAC
CATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCT
TCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAA
TCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGG
ATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATA
GAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAA

ACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAA
ATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATG
ACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGG
ATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGA
AGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAG
ATTCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTTAATA
GCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGA
GCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCACA
CTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGC
ATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAAC
ATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGG
AGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAAC
CCATAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACT
AAATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCC
CCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTT
GCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATATGCATTGG
GAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATA
ATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACA
CAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTGGTCAGTGCTGGAATCA
GGAAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAA
TATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCACCTGTAGTA
GCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCAT
GCATGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTT
AGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGC
AGAAGTAATTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAAT
TAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTC
ACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTT
GGCATTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGA
ATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAG
CAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGG
GGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAAC
TAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAG
GGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTG

AAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGA
AAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGT
GGCAAGTAGACAGGATGAGGATTAA (SEQ ID NO: 83)

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:

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGT
CAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGAC
CCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAG
ATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAG
CCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGG
ATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAT
CTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCCTTGGG
TTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGT
ACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAG
GGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCT
AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCA
TCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT
TTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACAT
CAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCA
TGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGT
CCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTT
GTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTA
GCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTT
ATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGT
TTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAA
GCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTG
CGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCC
GCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCC
CCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTT
ATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGA

GGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGC
TTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATT
TTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGC
GGCCGCCCCGGG (SEQ ID NO: 84)

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 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:

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCC
AACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA
ATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAAT
GACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTT
CCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTG
AGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTT
TGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGG
GGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCG
GAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG
GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGC
CCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGC
CCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCT
GTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGG
AGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGG
CCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCG
CTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGG
GGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGA
GCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCC
CCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGG
CGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCG
GGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCC

GGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGT
AATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAA
ATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGG
CGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCC
GTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCG
GGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATT
C (SEQ ID NO: 85)

*Construction of the VSV-G Envelope plasmid:*

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 sequence was as follows:

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCA
AGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTT
CTAATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAAT
AGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAG
ACGGTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTA
TGGACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACA
ATGCAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCT
TCCCTCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTG
TCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTG
ATTCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATA
ACTCTACAACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACC
TCATTTCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGG
GAAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGC
AAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGG
TGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGA
ATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAG
TCTAATTCAGGACGTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTG
GAGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGC
TCCTAAAAACCCAGGAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAA
ATACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAG

AATGGTCGGAATGATCAGTGGAACTACCACAGAAAGGGAACTGTGGGATGACT
GGGCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGT
TCAGGATATAAGTTTCCTTTATACATGATTGGACATGGTATGTTGGACTCCGATC
TTCATCTTAGCTCAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAGACGCTG
CTTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAA
AAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGC
CTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTG
GTATCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGACAGATTTATACAG
ACATAGAGATGAGAATTC (SEQ ID NO: 86)

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.

Referring, in part, to Figure 5, the Helper plasmid includes a CAG enhancer (SEQ ID NO: 49); a CAG promoter (SEQ ID NO: 50); a chicken beta actin intron (SEQ ID NO: 51); a HIV gag (SEQ ID NO: 52); a HIV Pol (SEQ ID NO: 53); a HIV Int (SEQ ID NO: 54); a HIV RRE (SEQ ID NO: 55); and a rabbit beta globin poly A (SEQ ID NO: 56).

The Rev plasmid includes a RSV promoter (SEQ ID NO: 57); a HIV Rev (SEQ ID NO: 58); and a rabbit beta globin poly A (SEQ ID NO: 59).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 60); a beta globin intron (SEQ ID NO: 61); a VSV-G (SEQ ID NO: 62); and a rabbit beta globin poly A (SEQ ID NO: 63).

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

*Materials and Methods:*

*Construction of the Helper plasmid without Rev:*

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:

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGG
CGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGT
GCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCA
ACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA
GATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATG
GGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTA
TTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATA
TGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGC
AACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATC
AGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGA
CTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCC
TAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTC
CCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGC
TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAA
TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAA
TGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGG
GAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCC
GCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCG
CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCT
CTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCA
AAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCAT
CACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCC
AAACTCATCAATGTATCTTATCACCCGGG (SEQ ID NO: 87)

*Construction of the Rev plasmid:*

The RSV promoter and HIV Rev sequence was synthesized as a single DNA 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:

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTT
TAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATA
GTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTG
TAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAA

AAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATT
AGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCA
TTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGA
CCATTCACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTC
AGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGG
ACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAG
AAGCGGAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTC
TCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGG
AATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGA
ACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCT
ACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG
GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGA
GTCAGGAGCTAAAGAATAGTCTAGA (SEQ ID NO: 88)

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:

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 64), phosphoglycerate kinase (PGK) (SEQ ID NO: 65), and ubiquitin C (UbC) (SEQ ID NO: 66) can replace the CMV (SEQ ID NO: 60) or CAG promoter (SEQ ID NO: 100). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 67) and bGH poly A (SEQ ID NO: 68) can replace the rabbit beta globin poly A (SEQ ID NO: 48). These sequences can also be further varied by addition, substitution, deletion or mutation.

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: 69); HIV Pol (SEQ ID NO: 70); and HIV Int (SEQ ID NO: 71) 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.

Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 72), gibbon ape leukemia virus (GALV) (SEQ ID NO: 73), Rabies (FUG) (SEQ ID NO: 74), lymphocytic choriomeningitis virus

(LCMV) (SEQ ID NO: 75), influenza A fowl plague virus (FPV) (SEQ ID NO: 76), Ross River alphavirus (RRV) (SEQ ID NO: 77), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 78), or Ebola virus (EboV) (SEQ ID NO: 79). 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.

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**

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

*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.

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 (rnadesigner.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

*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 6. For example, the shRNA sequences with the highest activity against CCR5, Tat or Vif gene expression were then assembled into a microRNA (miR) cluster under control of the EF-1alpha promoter. The promoter and miR sequences are depicted in Figure 6.

Further, and using standard molecular biology techniques (*e.g.,* Sambrook; Molecular Cloning: A Laboratory Manual, 4th Ed.) as well as the techniques described herein, a series of lentiviral vectors have been developed as depicted in Figure 7 herein.

Vector 1 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24-Y); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 2 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 3 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a H1 element (SEQ ID NO: 101); a shGag (SEQ ID NO: 12); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 4 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a 7SK element (SEQ ID NO: 103); a shRev/Tat (SEQ ID NO: 10); a H1 element (SEQ ID NO: 101); a shCCR5 (SEQ ID NOS: 16, 18, 20, 22, or 24); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 5 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 6 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1);

MiR21Vif (SEQ ID NO: 2); miR155Tat (SEQ ID NO: 104); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 7 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 8 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); and a long terminal repeat portion (SEQ ID NO: 102).

Vector 9 was developed and contains, from left to right: a long terminal repeat (LTR) portion (SEQ ID NO: 35); a CD4 element (SEQ ID NO: 30); miR30CCR5 (SEQ ID NO: 1); miR21Vif (SEQ ID NO: 2); miR185Tat (SEQ ID NO: 3); a posttranscriptional regulatory element of woodchuck hepatitis virus (WPRE) (SEQ ID NOS: 32, 80); and a long terminal repeat portion (SEQ ID NO: 102).

*Development of Vectors*

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.

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.

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.

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.

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.

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.

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. 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 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 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 system and proved to have negligible toxicity for mammalian cells. The miR 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**

| | **Internal Code** | **Material** | **Description** | **Remarks** | **Decision** |
|---|---|---|---|---|---|
| 1 | SIH-H1-shRT-1,3 | Lentiviral vector | shRNA construct for RT of LAI strain | Wrong target, lab virus, no virus test | Abandon |
| 2 | SIH-H1-shRT43 (Tat/Rev NL4-3) | Lentiviral vector | H1 promoter shRNA Tat/Rev overlap | Tat protein knock-down >90% | Lead |
| *Vector Construction*: For Rev/Tat (RT) shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Two different Rev/Tat target sequences were tested for their ability to decrease Tat mRNA expression. | | | | | |

The RT1,3 target sequence is (5'-ATGGCAGGAAGAAGCGGAG-3') (SEQ ID NO: 89) and shRNA sequence is (5'-ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCTTCTTCCTGCCATTTTTT-3') (SEQ ID NO: 90). The RT43 sequence is (5'-GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and shRNA sequence is (5'-GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 10). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

*Functional test for shRNA against Rev/Tat*: The ability of the vector to reduce Tat expression was tested using a luciferase reporter plasmid which contained the Rev/Tat target sequences inserted into the 3'-UTR (untranslated region of the mRNA). Either the shRT1,3 or shRT43 plasmid was co-transfected with the plasmid containing luciferase and the Rev/Tar target sequence. There was a 90% reduction in light emission indicating strong function of the shRT43 shRNA sequence but less than 10% with the shRT1,3 plasmid.

*Conclusion*: The SIH-H1-shRT43 was superior to SIH-H1-shRT-1,3 in terms of reducing mRNA levels in the Luciferase assay system. This indicates potent inhibitory activity of the shRT43 sequence and it was selected as a lead candidate for further development.

| | | | | | |
|---|---|---|---|---|---|
| 3 | SIH-H1-shGag-1 | Lentiviral vector | shRNA construct for LAI Gag | Inhibits Gag expression but will inhibit packaging | Abandon |

*Vector Construction*: For Gag shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. A Gag target sequence was tested for their ability to decrease Gag mRNA expression. The Gag target sequence is (5'- GAAGAAATGATGACAGCAT -3') (SEQ ID NO: 11) and shRNA sequence is (5'-GAAGAAATGATGACAGCATTTCAAGAGAATGCTGTCATCATTTCTTCTTTTT-3') (SEQ ID NO: 12). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

*Functional test for shRNA against Gag*: The ability of the vector to reduce Gag expression was tested using a luciferase reporter plasmid which contained the Gag target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Gag

plasmid was co-transfected with the plasmid containing luciferase and the Gag target sequence. There was nearly a 90% reduction in light emission indicating a strong effect of the shGag shRNA sequence.

*Conclusion*: This shRNA sequence 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 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 | SIH-H1-shPol-1 | Lentiviral vector | shRNA construct for Pol | Inhibits Pol expression but will inhibit packaging | Abandon |

*Vector Construction*: A Pol shRNA was constructed with oligonucleotide sequences containing BamHI and EcoRI restriction sites that were synthesized by MWG Operon. A Pol target sequence was tested for its ability to decrease Pol mRNA expression. The Pol target sequence is (5'- CAGGAGCAGATGATACAG -3') (SEQ ID NO: 13) and shRNA sequence is (5'-CAGGAGATGATACAGTTCAAGAGACTGTATCATCTGCTCCTGTTTTT-3') (SEQ ID NO: 14). Oligonucleotide sequences were inserted into the pSIH lentiviral vector (System Biosciences).

*Functional tests for shRNA against HIV Pol*: The ability of the vector to reduce Pol expression was tested using a luciferase reporter plasmid which contained the Pol target sequences inserted into the 3'-UTR (untranslated region of the mRNA). The Pol plasmid was co-transfected with the plasmid containing luciferase and the Pol target sequence. There was a 60% reduction in light emission indicating a strong effect of the shPol shRNA sequence.

*Conclusion*: This shRNA sequence 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 | SIH-H1-shCCR5-1 | Lentiviral vector | shRNA construct for CCR5 | Best of 5 candidates, Extracellular CCR5 protein reduction >90% | Lead |

*Vector Construction*: A CCR5 shRNA was 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). The CCR5 target sequence #1, which focuses on CCR5 gene sequence 1 (SEQ ID NO: 25), is (5'-GTGTCAAGTCCAATCTATG-3') (SEQ ID NO: 15) and the shRNA sequence is (5'-GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACACTTTTT-3') (SEQ ID NO: 16). The CCR5 target sequence #2, which focuses on CCR5 gene sequence 2 (SEQ ID NO: 26), is (5'-GAGCATGACTGACATCTAC-3') (SEQ ID NO: 17) and the shRNA sequence is (5'-GAGCATGACTGACATCTACTTCAAGAGAGTAGATGTCAGTCATGCTCTTTTT-3') (SEQ ID NO: 18). The CCR5 target sequence #3, which focuses on CCR5 gene sequence 3 (SEQ ID NO: 27), is (5'-GTAGCTCTAACAGGTTGGA-3') (SEQ ID NO: 19) and the shRNA sequence is (5'-GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCTGTTAGAGCTACTTTTT-3') (SEQ ID NO: 20). The CCR5 target sequence #4, which focuses on CCR5 gene sequence 4 (SEQ ID NO: 28, is (5'-GTTCAGAAACTACCTCTTA-3') (SEQ ID NO: 21) and the shRNA sequence is (5'-GTTCAGAAACTACCTCTTATTCAAGAGATAAGAGGTAGTTTCTGAACTTTTT-3') (SEQ ID NO: 22). The CCR5 target sequence #5, which focuses on CCR5 gene sequence 5 (SEQ ID NO: 29), is (5'-GAGCAAGCTCAGTTTACACC-3') (SEQ ID NO: 23) and the shRNA sequence is (5'-GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAAACTGAGCTTGCTCTTTT T-3') (SEQ ID NO: 24).

*Functional test for shRNA against CCR5*: The ability of a CCR5 shRNA sequence to knock-down CCR5 RNA expression was initially tested by co-transfecting each of the

lentiviral plasmids, in separate experiments for each plasmid, containing one of the five CCR5 target sequences with a plasmid expressing the human CCR5 gene. CCR5 mRNA expression was then assessed by qPCR analysis using CCR5-specific primers.

*Conclusion*: Based on the reduction in CCR5 mRNA levels the shRNACCR5-1 was most potent for 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-shCCR5 | Lentiviral vector | U6 promoter-TAR-H1-shCCR5 | Toxic to cells | Abandon |
| 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'-CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTCTCGACAGCGACCAGATCTGAGCCTGGGAGCTCTCTGGCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAATTCTTACTGAGCAAGAAA-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 shRT sequence is (5'-GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAAT

CACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGA GACCACTTGGATCCGCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCG TCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 91). 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'-GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTA GGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGG ACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAAT CACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGA GACCACTTGGATCCGTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGA CTTGACACTTTTT-3') (SEQ ID NO: 92). 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 | shCCR5 | Lentiviral vector | microRNA sequence | Extracellular CCR5 protein reduction >90% | Lead |

*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'-CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAG

TCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGT
GCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT
GCCTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAG
CTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCC
CTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTG
CGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC
CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCT
TGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGG
GCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCT
GCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT
GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAG
GCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGC
CCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCG
GGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTC
ATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGA
GCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAG
TTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT
GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAA
GCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA-3')
(SEQ ID NO: 4).

*Functional test for lentivirus CDH-shCCR5-1*: The ability of the miR CCR5 sequences to knock-down CCR5 expression was determined by transducing CEM-CCR5 T cells and measuring cell surface CCR5 expression after staining with a fluorescently-labeled monoclonal antibody against CCR5 and measuring the intensity of staining, that is directly proportional to the number of cell surface CCR5 molecules, by analytical flow cytometry. The most effective shRNA sequence for targeting CCR5 was CCR5 shRNA sequence #1. However, the most effective CCR5 targeting sequence for constructing the synthetic microRNA sequence was overlapping with CCR5 sequence #5; 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 sequence which is (5'-

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTG

| | | | | | |
|---|---|---|---|---|---|
| AAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGA CTTCAAGGGGCTT-3') (SEQ ID NO: 1). The miR CCR5 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 reduce by ≥ 90% indicating potent inhibition of CCR5 mRNA by the miR30CCR5 micro RNA construct in a lentivirus vector.<br><br>*Conclusion*: The miR30CCR5 construct is potent for reducing CCR5 cell surface expression and is a lead candidate for a therapeutic lentivirus for HIV. | | | | | |
| 13 | shVif | Lentiviral vector | microRNA sequence | Vif protein reduction>80% | Lead |
| *Vector Construction*: 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 sequence which is (5'-CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTT GAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTC ATCTGACCA-3') (SEQ ID NO: 2). The miR Vif target sequence is (5'-GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6).<br><br>*Functional test for potency of miR21Vif*: The ability of the miR Vif sequence to knock-down Vif expression was determined by measuring Vif protein expression by immunoblot analysis using an anti-Vif monoclonal antibody to identify the Vif protein.<br><br>*Conclusion*: the miR21Vif reduced Vif protein expression by ≥ 10-fold as determined by quantitative image analysis of immunoblot data. This was sufficient to justify miR21Vif as a lead candidate for our therapeutic lentivirus. | | | | | |
| 14 | shTat | Lentiviral vector | microRNA sequence | Tat RNA reduction>80% | Lead |
| *Vector Construction*: A Tat microRNA was constructed with oligonucleotide sequences containing BsrGI and NotI restriction sites that were synthesized by MWG Operon. The microRNA cluster 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 sequence which is (5'-GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC-3'). The miR Tat target sequence is (5'-TCCGCTTCTTCCTGCCATAG-3') (SEQ ID NO: 3).

*Functional test for potency of miR185Tat*: The ability of miR Tat to knock-down Tat expression was determined by measuring Tat mRNA expression by RT-PCR analysis using Tat specific primers. We compared the miR185Tat with a similar miR155Tat on the basis of reducing the relative levels of Tat mRNA.

*Conclusion*: The miR185Tat was approximately twice as potent for reducing Tat mRNA compare to miR155Tat, and was selected as the lead candidate for our therapeutic lentivirus.

| | | | | | |
|---|---|---|---|---|---|
| 15 | shCCR5-shVif-shTat | Lentiviral vector | microRNA cluster sequence | CCR5 reduction>90%, Vif protein reduction>80%, Tat RNA reduction>80%, >95% inhibition of HIV replication | Candidate |

*Vector Construction*: A miR30CCR5 miR21Vif miR185Tat microRNA cluster sequence 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'-AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCA

GGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC-3') (SEQ ID NO: 31) 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 microRNA cluster of miR30CCR5, miR21Vif and miR185Tat*: 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 miRNA cluster 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.

*Functional Assays*. Individual lentivirus vectors containing CCR5, Tat or Vif shRNA sequences 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.

*Starting Testing of Lentivirus*. T cell culture medium was made using RPMI 1640 supplemented with 10% FBS and 1% penicillin–streptomycin. Cytokine stocks of IL2 10,000 units/ml, IL-12 1µg/ml, IL-7 1µg/ml, IL-15 1µg/ml were also prepared in advance.

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).

*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 $2x10^6$/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 |

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.

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.

*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 1x106/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.

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 $0.5x10^6$ viable cells per ml.

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

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 $1x10^6$/ml. Assays were performed to determine the frequencies of antigen-specific T cells and lentivirus transduced cells.

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.

*Examine antigen-specific T cells by intracellular cytokine staining for IFN-gamma.* Cultured cells after peptide stimulation or after lentivirus transduction at $1x10^6$ cells/ml were stimulated with medium alone (negative control), Gag peptides (5μg/ml individual peptide), or PHA (5μg/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 Cytofix/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.

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.

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 4: CCR5 Knockdown with Experimental Vectors**

AGTc120 is a Hela cell line that stably expresses large amounts of CD4 and CCR5. AGTc120 was transduced with or without LV-CMV-mCherry (the red fluorescent protein mCherry expressed under control of the CMV Immediate Early Promoter) or AGT103/CMV-mCherry. Gene expression of the mCherry fluorescent protein was controlled by a CMV (cytomegalovirus immediate early promoter) expression cassette. The LV-CMV-mCherry vector lacked a microRNA cluster, while AGT103/CMV-mCherry expressed therapeutic miRNA against CCR5, Vif, and Tat.

As shown in Figure 8A, transduction efficiency was >90%. After 7 days, cells were collected and stained with fluorescent monoclonal antibody against CCR5 and subjected to analytical flow cytometry. Isotype controls are shown in gray on these histograms plotting Mean Fluorescence Intensity of CCR5 APC (x axis) versus cell number normalized to mode (y axis). After staining for cell surface CCR5, cells treated with no lentivirus or control lentivirus (expressing only the mCherry marker) showed no changes in CCR5 density while AGT103 (right section) reduced CCR5 staining intensity to nearly the levels of isotype control. After 7 days, cells were infected with or without R5-tropic HIV reporter virus Bal-GFP. 3 days later, cells were collected and analyzed by flow cytometry. More than 90% of cells were transduced. AGT103-CMV/CMVmCherry reduced CCR5 expression in transduced AGTc120 cells and blocked R5-tropic HIV infection compared with cells treated with the Control vector.

Figure 8B shows the relative insensitivity of transfected AGTc120 cells to infection with HIV. As above, the lentivirus vectors express mCherry protein and a transduced cell that was also infected with HIV (expressing GFP) would appear as a double positive cell in the upper right quadrant of the false color flow cytometry dot plots. In the absence of HIV (upper panels), there were no GFP+ cells under any condition. After HIV infection (lower panels), 56% of cells were infected in the absence of lentivirus transduction and 53.6% of cells became infected in AGTc120 cells transduced with the LV-CMV-mCherry. When cells were transduced with the therapeutic AGT103/CMV-mCherry vector, only 0.83% of cells appeared in the double positive quadrant indicating they were transduced and infected.

Dividing 53.62 (proportion of double positive cells with control vector) by 0.83 (the proportion of double positive cells with the therapeutic vector) shows that AGT103 provided greater than 65-fold protection against HIV in this experimental system.

**Example 5: Regulation of CCR5 Expression by shRNA Inhibitor Sequences in a Lentiviral Vector**

*Inhibitory RNA Design.* The sequence of *Homo sapiens* chemokine receptor CCR5 (CCR5, NC 000003.12) 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 RNA iDesigner from Thermo Scientific. A shRNA sequence may be inserted into a plasmid immediately after a RNA polymerase III promoter such as H1, U6,

or 7SK to regulate shRNA expression. The shRNA sequence may also be inserted into a lentiviral vector using similar promoters or embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and utilized independently of a plasmid or lentiviral vector.

*Plasmid Construction*. For CCR5 shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG Operon. Oligonucleotide sequences were annealed by incubating at 70ºC then cooled to room temperature. Annealed oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37ºC, then the enzymes were inactivated at 70ºC for 20 minutes. In parallel, plasmid DNA was digested with the restriction enzymes BamHI and EcoRI for one hour at 37ºC. The digested plasmid DNA was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentration was determined and the plasma to oligonucleotide sequence was ligated in the ratio 3:1 insert to vector. The ligation reaction was done with T4 DNA ligase for 30 minutes at room temperature. 2.5 µL of the ligation mix were added to 25 µL of STBL3 competent bacterial cells. Transformation required heat shock at 42ºC. Bacterial cells were spread on agar plates containing ampicillin and colonies were expanded in L broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacterial cultures using the Invitrogen DNA Miniprep kit and tested by restriction enzyme digestion. Insertion of the shRNA sequence into the plasmid was verified by DNA sequencing using a primer specific for the promoter used to regulate shRNA expression.

*Functional Assay for CCR5 mRNA Reduction*: The assay for inhibition of CCR5 expression required co-transfection of two plasmids. The first plasmid contains one of five different shRNA sequences directed against CCR5 mRNA. The second plasmid contains the cDNA sequence for human CCR5 gene. Plasmids were co-transfected into 293T cells. After 48 hours, cells were lysed and RNA was extracted using the RNeasy kit from Qiagen. cDNA was synthesized from RNA using a Super Script Kit from Invitrogen. The samples were then analyzed by quantitative RT-PCR using an Applied Biosystems Step One PCR machine. CCR5 expression was detected with SYBR Green from Invitrogen using the forward primer (5'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 93) and reverse primer (5'-CCCCAAAGAAGGTCAAGGTAATCA-3') (SEQ ID NO: 94) with standard conditions for

polymerase chain reaction analysis. The samples were normalized to the mRNA for beta actin gene expression using the forward primer (5'-AGCGCGGCTACAGCTTCA-3') (SEQ ID NO: 95) and reverse primer (5'-GGCGACGTAGCACAGCTTCP-3') (SEQ ID NO: 96) with standard conditions for polymerase chain reaction analysis. The relative expression of CCR5 mRNA was determined by its Ct value normalized to the level of actin messenger RNA for each sample. The results are shown in Figure 9.

As shown in Figure 9A, CCR5 knock-down was tested in 293T cells by co-transfection of the CCR5 shRNA construct and a CCR5-expressing plasmid. Control samples were transfected with a scrambled shRNA sequence that did not target any human gene and the CCR5-expressing plasmid. After 60 hours post-transfection, samples were harvested and CCR5 mRNA levels were measured by quantitative PCR. Further, as shown in Figure 9B, CCR5 knock-down after transduction with lentivirus expressing CCR5 shRNA-1 (SEQ ID NO: 16).

**Example 6: Regulation of HIV Components by shRNA Inhibitor Sequences in a Lentiviral Vector**

*Inhibitory RNA Design.*

The sequences of HIV type 1 Rev/Tat (5'- GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and Gag (5'-GAAGAAATGATGACAGCAT-3') (SEQ ID NO: 11) were used to design:

Rev/Tat:

(5'GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT-3') (SEQ ID NO: 10) and

Gag:

(5'GAAGAAATGATGACAGCATTTCAAGAGAATGCTGTCATCATTTCTTCTTTTT-3') (SEQ ID NO: 12) shRNA that were synthesized and cloned into plasmids as described above.

*Plasmid Construction.* The Rev/Tat or Gag target sequences were inserted into the 3'UTR (untranslated region) of the firefly luciferase gene used commonly as a reporter of gene expression in cells or tissues. Additionally, one plasmid was constructed to express the Rev/Tat shRNA and a second plasmid was constructed to express the Gag shRNA. Plasmid constructions were as described above.

*Functional assay for shRNA targeting of Rev/Tat or Gag mRNA*: Using plasmid co-transfection we tested whether a shRNA plasmid was capable of degrading luciferase messenger RNA and decreasing the intensity of light emission in co-transfected cells. A shRNA control (scrambled sequence) was used to establish the maximum yield of light from luciferase transfected cells. When the luciferase construct containing a Rev/Tat target sequence inserted into the 3'-UTR (untranslated region of the mRNA) was co-transfected with the Rev/Tat shRNA sequence there was nearly a 90% reduction in light emission indicating strong function of the shRNA sequence. A similar result was obtained when a luciferase construct containing a Gag target sequence in the 3'-UTR was co-transfected with the Gag shRNA sequence. These results indicate potent activity of the shRNA sequences.

As shown in Figure 10A, knock-down of the Rev/Tat target gene was measured by a reduction of luciferase activity, which was fused with the target mRNA sequence in the 3'UTR, by transient transfection in 293T cells. As shown in Figure 10B, knock-down of the Gag target gene sequence fused with the luciferase gene. The results are displayed as the mean ± SD of three independent transfection experiments, each in triplicate.

**Example 7: AGT103 decreases expression of Tat and Vif**

Cells were transfected with exemplary vector AGT103/CMV-GFP. AGT103 and other exemplary vectors are defined in Table 3 below.

**Table 3**

| Vector Designation | Composition |
|---|---|
| AGT103 | EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE |
| Control-mCherry | CMV-mCherry |
| AGT103/CMV-mCherry | CMV-mCherry-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE- |
| Control-GFP | CMV-mCherry |
| AGT103/CMV-GFP | CMV-GFP-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE- |
| Abbreviations:<br>EF-1: elongation factor 1 transcriptional promoter<br>miR30CCR5 – synthetic microRNA capable of reducing CCR5 protein on cell surfaces<br>miR21Vif – synthetic microRNA capable of reducing levels of HIV RNA and Vif protein expression | |

| |
|---|
| miR185Tat – synthetic micro RNA capable of reducing levels of HIV RNA and Tat protein expression |
| CMV – Immediate early transcriptional promoter from human cytomegalovirus |
| mCherry – coding region for the mCherry red fluorescent protein |
| GFP – coding region for the green fluorescent protein |
| WPRE – Woodchuck hepatitis virus post transcriptional regulatory element |

A T lymphoblastoid cell line (CEM; CCRF-CEM; American Type Culture Collection Catalogue number CCL119) was transduced with AGT103/CMV-GFP. 48 hours later the cells were transfected with an HIV expression plasmid encoding the entire viral sequence. After 24 hours, RNA was extracted from cells and tested for levels of intact Tat sequences using reverse transcriptase polymerase chain reaction. Relative expression levels for intact Tat RNA were reduced from approximately 850 in the presence of control lentivirus vector, to approximately 200 in the presence of AGT103/CMV-GFP for a total reduction of > 4 fold, as shown in Figure 11.

**Example 8: Regulation of HIV Components by Synthetic MicroRNA Sequences in a Lentiviral Vector**

*Inhibitory RNA Design.* The sequence of HIV-1 Tat and Vif genes were used to search for potential siRNA or shRNA candidates to knockdown Tat or Vif 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 RNA iDesigner from Thermo Scientific. The selected shRNA sequences most potent for Tat or Vif knockdown were embedded within a microRNA backbone to allow for expression by an RNA polymerase II promoter such as CMV or EF-I alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and used independently of a plasmid or lentiviral vector.

*Plasmid Construction.* The Tat target sequence (5'-TCCGCTTCTTCCTGCCATAG-3') (SEQ ID NO: 7) was incorporated into the miR185 backbone to create a Tat miRNA (5'-GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCT TCGTC-3') (SEQ ID NO: 3) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. Similarly, the Vif target sequence (5'-

GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6) was incorporated into the miR21 backbone to create a Vif miRNA (5'-CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA-3') (SEQ ID NO: 2) that was inserted into a lentivirus vector and expressed under control of the EF-1 alpha promoter. The resulting Vif/Tat miRNA-expressing lentivirus vectors were produced in 293T cells using a lentiviral vector packaging system. The Vif and Tat miRNA were embedded into a microRNA cluster consisting of miR CCR5, miR Vif, and miR Tat all expressed under control of the EF-1 promoter.

*Functional assay for miR185Tat inhibition of Tat mRNA accumulation.* A lentivirus vector expressing miR185 Tat (LV-EF1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000 under standard conditions. 24 hours later RNA was extracted and levels of Tat messenger RNA were tested by RT-PCR using Tat-specific primers and compared to actin mRNA levels for a control.

*Functional assay for miR21 Vif inhibition of Vif protein accumulation.* A lentivirus vector expressing miR21 Vif (LV-EF1-miR-CCR5-Vif-Tat) was used at a multiplicity of infection equal to 5 for transducing 293T cells. 24 hours after transduction, the cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine2000. 24 hours later cells were lysed and total soluble protein was tested to measure the content of Vif protein. Cell lysates were separated by SDS-PAGE according to established techniques. The separated proteins were transferred to nylon membranes and probed with a Vif-specific monoclonal antibody or actin control antibody.

As shown in Figure 12A, Tat knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing either synthetic miR185 Tat or miR155 Tat microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000 for 24 hours and then RNA was extracted for qPCR analysis with primers for Tat. As shown in Figure 12B, Vif knock-down was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing a synthetic miR21 Vif microRNA. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine2000

for 24 hours and then protein was extracted for immunoblot analysis with an antibody for HIV Vif.

**Example 9: Regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector**

CEM-CCR5 cells were transduced with a lentiviral vector containing a synthetic miR30 sequence for CCR5 (AGT103: TGTAAACTGAGCTTGCTCTA (SEQ ID NO: 97), AGT103-R5-1: TGTAAACTGAGCTTGCTCGC (SEQ ID NO: 98), or AGT103-R5-2: CATAGATTGGACTTGACAC (SEQ ID NO: 99). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified by mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The target sequence of AGT103 and AGT103-R5-1 is in the same region as CCR5 target sequence #5. The target sequence of AGT103-R5-2 is the same as CCR5 target sequence #1. AGT103 (2% of total CCR5) is most effective at reducing CCR5 levels as compared with AGT103-R5-1 (39% of total CCR5) and AGT103-R5-2 which does not reduce CCR5 levels. The data is demonstrated in Figure 13 herein.

**Example 10: Regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector containing either a long or short WPRE sequence.**

*Vector Construction.* Lentivirus vectors often require an RNA regulatory element for optimal expression of therapeutic genes or genetic constructs. A common choice is to use the Woodchuck hepatitis virus post transcriptional regulatory element (WPRE). We compared AGT103 that contains a full-length WPRE:

(5'AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTAT
GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTAT
TGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTC
TTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGT
TTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTC
CGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGC
CTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTG
TTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGA
TTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCT

TCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGC
CCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCT-3') (SEQ ID NO: 32)

with a modified AGT103 vector containing a shortened WPRE element (5'AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTA
TGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTAATGCCTCTGTATCATGCTA
TTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT
CTTTATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGT
TTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTC
TGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGC
CTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTG
TTGTC-3') (SEQ ID NO: 80).

*Functional assay for modulating cell surface CCR5 expression as a function of long versus short WPRE element in the vector sequence.* AGT103 containing long or short WPRE elements were used for transducing CEM-CCR5 T cells a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of detecting cell surface CCR5 protein. The antibody was conjugated to a fluorescent marker and the intensity of staining is directly proportional to the level of CCR5 on the cell surface. A control lentivirus had no effect on cell surface CCR5 levels resulting in a single population with a mean fluorescence intensity of 73.6 units. The conventional AGT103 with a long WPRE element reduced CCR5 expression to a mean fluorescence intensity level of 11 units. AGT103 modified to incorporate a short WPRE element resulted in a single population of cells with mean fluorescence intensity of 13 units. Accordingly, substituting a short WPRE element had little or no effect on the capacity for AGT103 to reduce cell surface CCR5 expression.

As shown in Figure 14, CEM-CCR5 cells were transduced with AGT103 containing either a long or short WPRE sequence. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with either the short (5.5% of total CCR5) or long (2.3% of total CCR5) WPRE sequence.

**Example 11: Regulation of CCR5 expression by synthetic microRNA sequences in a lentiviral vector with or without a WPRE sequence**

*Vector construction.* In order to test whether WPRE was required for AGT103 down regulation of CCR5 expression we constructed a modified vector without WPRE element sequences.

Functional assay for modulating cell surface CCR5 expression as a function of including or not including a long WPRE element in the AGT103 vector. In order to test whether WPRE was required for AGT103 modulation of CCR5 expression levels we transduced CEM-CCR5 T cells with AGT103 or a modified vector lacking WPRE using a multiplicity of infection equal to 5. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was directly conjugated to a fluorescent marker and the intensity of staining is directly proportional to the number of CCR5 molecules per cell surface. A lentivirus control vector had no effect on cell surface CCR5 levels resulting in a uniform population with mean fluorescence intensity of 164. The lentivirus vector (AGT103 with a long WPRE and also expressing GFP marker protein), AGT103 lacking GFP but containing a long WPRE element, or AGT103 lacking both GFP and WPRE all were similarly effective for modulating cell surface CCR5 expression. After removing GFP, AGT103 with or without WPRE elements were indistinguishable in terms of their capacity for modulating cell surface CCR5 expression.

CEM-CCR5 cells were transduced with AGT103 with or without GFP and WPRE. After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. The reduction in CCR5 levels was similar for AGT103 with (0% of total CCR5) or without (0% of total CCR5) the WPRE sequence. This data is demonstrated in Figure 15.

**Example 12: Regulation of CCR5 expression by a CD4 promoter regulating synthetic microRNA sequences in a lentiviral vector.**

*Vector Construction.* A modified version of AGT103 was constructed to test the effect of substituting alternate promoters for expressing the microRNA cluster that suppresses CCR5, Vif and Tat gene expression. In place of the normal EF-1 promoter we

substituted the T cell-specific promoter for CD4 glycoprotein expression using the sequence:

(5'TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCCTCTGCAAAGAAAAAAA
AAAAAAAAAAAGAACAAAGGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAGA
TGAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGGAGGCGGATCCTGTCAGC
TTTGCTCTCTCTGTGGCTGGCAGTTTCTCCAAAGGGTAACAGGTGTCAGCTGGCT
GAGCCTAGGCTGAACCCTGAGACATGCTACCTCTGTCTTCTCATGGCTGGAGGC
AGCCTTTGTAAGTCACAGAAAGTAGCTGAGGGGCTCTGGAAAAAAGACAGCCA
GGGTGGAGGTAGATTGGTCTTTGACTCCTGATTTAAGCCTGATTCTGCTTAACTT
TTTCCCTTGACTTTGGCATTTTCACTTTGACATGTTCCCTGAGAGCCTGGGGGGT
GGGGAACCCAGCTCCAGCTGGTGACGTTTGGGGCCGGCCCAGGCCTAGGGTGT
GGAGGAGCCTTGCCATCGGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGCA
GA-3') (SEQ ID NO: 30).

*Functional assay comparing EF-1 and CD4 gene promoters in terms of potency for reducing cell surface CCR5 protein expression.* AGT103 modified by substituting the CD4 gene promoter for the normal EF-1 promoter was used for transducing CEM-CCR5 T cells. Six days after transduction cells were collected and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. The monoclonal antibody was conjugated to a fluorescent marker and staining intensity is directly proportional to the level of cell surface CCR5 protein. A control lentivirus transduction resulted in a population of CEM-CCR5 T cells that were stained with a CCR5-specific monoclonal antibody and produced a mean fluorescence intensity of 81.7 units. The modified AGT103 using a CD4 gene promoter in place of the EF-1 promoter for expressing microRNA showed a broad distribution of staining with a mean fluorescence intensity roughly equal to 17.3 units. Based on this result, the EF-1 promoter is at least similar and likely superior to the CD4 gene promoter for microRNA expression. Depending on the desired target cell population, the EF-1 promoter is universally active in all cell types and the CD4 promoter is only active in T-lymphocytes.

CEM-CCR5 cells were transduced with a lentiviral vector containing a CD4 promoter regulating a synthetic microRNA sequence for CCR5, Vif, and Tat (AGT103). After 6 days, CCR5 expression was determined by FACS analysis with an APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as % CCR5 with LV-Control set at 100%. In cells transduced with LV-CD4-AGT103, CCR5

levels were 11% of total CCR5. This is comparable to that observed for LV-AGT103 which contains the EF1 promoter. This data is demonstrated in Figure 16.

**Example 13: Detecting HIV Gag-Specific CD4 T Cells**

*Cells and reagents*. Viable frozen peripheral blood mononuclear cells (PBMC) were obtained from a vaccine company. Data were obtained with a representative specimen from an HIV+ individual who was enrolled into an early stage clinical trial (TRIAL REGISTRATION: clinicaltrials.gov NCT01378156) testing a candidate HIV therapeutic vaccine. Two specimens were obtained for the "Before vaccination" and "After vaccination" studies. Cell culture products, supplements and cytokines were from commercial suppliers. Cells were tested for responses to recombinant Modified Vaccinia Ankara 62B from Geovax Corporation as described in Thompson, M., S. L. Heath, B. Sweeton, K. Williams, P. Cunningham, B. F. Keele, S. Sen, B. E. Palmer, N. Chomont, Y. Xu, R. Basu, M. S. Hellerstein, S. Kwa and H. L. Robinson (2016). "DNA/MVA Vaccination of HIV-1 Infected Participants with Viral Suppression on Antiretroviral Therapy, followed by Treatment Interruption: Elicitation of Immune Responses without Control of Re-Emergent Virus." PLoS One 11(10): e0163164. Synthetic peptides representing the entire HIV-1 Gag polyprotein were obtained from GeoVax the HIV (GAG) Ultra peptide sets were obtained from JPT Peptide Technologies GmbH (www.jpt.com), Berlin, Germany. HIV (GAG) Ultra contains 150 peptides each being 15 amino acids in length and overlapping by 11 amino acids. They were chemically synthesized then purified and analyzed by liquid chromatography – mass spectrometry. Collectively these peptides represent major immunogenic regions of the HIV Gag polyprotein and are designed for average coverage of 57.8% among known HIV strains. Peptide sequences are based on the HIV sequence database from the Los Alamos National Laboratory (http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html). Peptides are provided as dried trifluoroacetate salts, 25 micrograms per peptide, and are dissolved in approximately 40 microliters of DMSO then diluted with PBS to final concentration. Monoclonal antibodies for detecting CD4 and cytoplasmic IFN-gamma were obtained from commercial sources and intracellular staining was done with the BD Pharmingen Intracellular Staining Kit for interferon-gamma. Peptides were resuspended in DMSO and we include a DMSO only control condition.

*Functional assay for detecting HIV-specific CD4+ T cells*. Frozen PBMC were thawed, washed and resuspended in RPMI medium containing 10% fetal bovine serum,

supplements and cytokines. Cultured PBMC collected before or after vaccination were treated with DMSO control, MVA GeoVax (multiplicity of infection equal to 1 plaque forming unit per cell), Peptides GeoVax (1 microgram/ml) or HIV (GAG) Ultra peptide mixture (1 microgram/ml) for 20 hours in the presence of Golgi Stop reagent. Cells were collected, washed, fixed, permeabilized and stained with monoclonal antibodies specific for cell surface CD4 or intracellular interferon-gamma. Stained cells were analyzed with a FACSCalibur analytical flow cytometer and data were gated on the CD4+ T cell subset. Cells highlighted within boxed regions are double-positive and designated HIV-specific CD4 T cells on the basis of interferon-gamma expression after MVA or peptide stimulation. Numbers within the boxed regions show the percentage of total CD4 that were identified as HIV-specific. We did not detect strong responses to DMSO or MVA. Peptides from GeoVax elicited fewer responding cells compared to HIV (GAG) Ultra peptide mixture from JPT but differences were small and not significant.

As shown in Figure 17, PBMCs from a HIV-positive patient before or after vaccination were stimulated with DMSO (control), recombinant MVA expressing HIV Gag from GeoVax (MVA GeoVax), Gag peptide from GeoVax (Pep GeoVax, also referred to herein as Gag peptide pool 1) or Gag peptides from JPT (HIV (GAG) Ultra, also referred to herein as Gag peptide pool 2) for 20 hours. IFNg production was detected by intracellular staining and flow cytometry using standard protocols. Flow cytometry data were gated on CD4 T cells. Numbers captured in boxes are the percentage of total CD4 T cells designated "HIV-specific" on the basis of cytokine response to antigen-specific stimulation.

**Example 14: HIV-specific CD4 T cell expansion and lentivirus transduction**

Designing and testing methods for enriching PBMC to increase the proportion of HIV-specific CD4 T cells and transducing these cells with AGT103 to produce the cellular product AGT103T.

The protocol was designed for ex vivo culture of PBMC (peripheral blood mononuclear cells) from HIV-positive patients who had received a therapeutic HIV vaccine. In this example, the therapeutic vaccine consisted of three doses of plasmid DNA expressing HIV Gag, Pol and Env genes followed by two doses of MVA 62-B (modified vaccinia Ankara number 62-B) expressing the same HIV Gag, Pol, and Env genes. The protocol is not specific for a vaccine product and only requires a sufficient level of HIV-specific CD4+ T cells after immunization. Venous blood was collected and PBMC were purified by Ficoll-Paque density

gradient centrifugation. Alternately, PBMC or defined cellular tractions can be prepared by positive or negative selection methods using antibody cocktails and fluorescence activated or magnetic bead sorting. The purified PBMC are washed and cultured in standard medium containing supplements, antibiotics and fetal bovine serum. To these cultures, a pool of synthetic peptides was added representing possible T cell epitopes within the HIV Gag polyprotein. Cultures are supplemented by adding cytokines interleukin-2 and interleukin-12 that were selected after testing combinations of interleukin-2 and interleukin-12, interleukin 2 and interleukin-7, interleukin 2 and interleukin-15. Peptide stimulation is followed by a culture interval of approximately 12 days. During the 12 days culture, fresh medium and fresh cytokine supplements were added approximately once every four days.

The peptide stimulation interval is designed to increase the frequency of HIV-specific CD4 T cells in the PBMC culture. These HIV-specific CD4 T cells were activated by prior therapeutic immunization and can be re-stimulated and caused to proliferate by synthetic peptide exposure. Our goal is to achieve greater than or equal to 1% of total CD4 T cells being HIV-specific by end of the peptide stimulation culture period.

On approximately day 12 of culture cells are washed to remove residual materials then stimulated with synthetic beads decorated with antibodies against CD4 T cell surface proteins CD3 and CD28. This well-established method for polyclonal stimulation of T cells will reactivate the cells and make them more susceptible for AGT103 lentivirus transduction. The lentivirus transduction is performed on approximately day 13 of culture and uses a multiplicity of infection between 1 and 5. After transduction cells are washed to remove residual lentivirus vector and cultured in media containing interleukin-2 and interleukin-12 with fresh medium and cytokines added approximately once every four days until approximately day 24 of culture.

Throughout the culture interval the antiretroviral drug Saquinavir is added at a concentration of approximately 100 nM to suppress any possible outgrowth of HIV.

On approximately day 24 of culture cells are harvested, washed, a sample is set aside for potency and release assay, then the remaining cells are suspended in cryopreservation medium before freezing in single aliquots of approximately $1\times10^{10}$ cells per dose that will contain approximately $1\times10^{8}$ HIV-specific CD4 T cells that are transduced with AGT103.

Potency of the cell product (AGT103T) is tested in one of two alternate potency assays. Potency assay 1 tests for the average number of genome copies (integrated AGT103 vector sequences) per CD4 T cell. The minimum potency is approximately 0.5 genome copies per CD4 T cell in order to release the product. The assay is performed by positive selection of CD3 positive/CD4 positive T cells using magnetic bead labeled monoclonal antibodies, extracting total cellular DNA and using a quantitative PCR reaction to detect sequences unique to the AGT103 vector. Potency assay 2 tests for the average number of genome copies of integrated AGT103 within the subpopulation of HIV-specific CD4 T cells. This essay is accomplished by first stimulating the PBMC with the pool of synthetic peptides representing HIV Gag protein. Cells are then stained with a specific antibody reagent capable of binding to the CD4 T cell and also capturing secreted interferon-gamma cytokine. The CD4 positive/interferon-gamma positive cells are captured by magnetic bead selection, total cellular DNA is prepared, and the number of genome copies of AGT103 per cell is determined with a quantitative PCR reaction. Release criterion based on potency using Assay 2 require that greater than or equal to 0.5 genome copies per HIV-specific CD4 T-cell are present in the AGT103 cell product.

*Functional test for enriching and transducing HIV-specific CD4 T cells from PBMC of HIV-positive patients that received a therapeutic HIV vaccine*. The impact of therapeutic vaccination on the frequency of HIV-specific CD4 T cells was tested by a peptide stimulation assay (figure 14 panel B). Before vaccination the frequency of HIV-specific CD4 T cells was 0.036% in this representative individual. After vaccination, the frequency of HIV-specific CD4 T cells was increased approximately 2-fold to the value of 0.076%. Responding cells (HIV-specific) identified by accumulation of cytoplasmic interferon-gamma, were only detected after specific peptide stimulation.

We also tested whether peptide stimulation to enrich for HIV-specific CD4 T cells followed by AGT103 transduction would reach our goal of generating approximately 1% of total CD4 T cells in culture that were both HIV-specific and transduced by AGT103. In this case, we used an experimental version of AGT103 that expresses green fluorescence protein (see GFP). In Figure 14, panel C the post-vaccination culture after peptide stimulation (HIV (GAG) Ultra) and AGT103 transduction demonstrated that 1.11% of total CD4 T cells were both HIV-specific (based on expressing interferon-gamma in response to peptide stimulation) and AGT103 transduced (based on expression of GFP).

Several patients from a therapeutic HIV vaccine study were tested to assess the range of responses to peptide stimulation and to begin defining eligibility criteria for entering a gene therapy arm in a future human clinical trial. Figure 18 Panel D show the frequency of HIV-specific CD4 T cells in 4 vaccine trial participants comparing their pre-and post-vaccination specimens. In three cases the post-vaccination specimens show a value of HIV-specific CD4 T cells that was greater than or equal to 0.076% of total CD4 T cells. The ability to reach this value was not predicted by the pre-vaccination specimens as patient 001-004 and patient 001-006 both started with pre-vaccination values of 0.02% HIV-specific CD4 T cells but one reached an eventual post-vaccination value of 0.12% HIV-specific CD4 T cells while the other individual fail to increase this value after vaccination. The same three patients that responded well to vaccine, in terms of increasing the frequency of HIV-specific CD4 T cells, also showed substantial enrichment of HIV-specific CD4 T cells after peptide stimulation and culture. In the three cases shown in Figure 18 Panel E, peptide stimulation and subsequent culture generated samples where 2.07%, 0.72% or 1.54% respectively of total CD4 T cells were HIV-specific. These values indicate that a majority of individuals responding to a therapeutic HIV vaccine will have a sufficiently large ex vivo response to peptide stimulation in order to enable our goal of achieving approximately 1% of total CD4 T cells that are HIV-specific and transduced with AGT103 in the final cell product.

As shown in Figure 18, Panel A describes the schedule of treatment. Panel B demonstrates that PBMCs were stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production was detected by intracellular staining by FACS. $CD4^+$ T cells were gated for analysis. Panel C demonstrates $CD4^+$ T cells were expanded and transduced with AGT103-GFP using the method as shown in Panel A. Expanded $CD4^+$ T cells were rested in fresh medium without any cytokine for 2 days and re-stimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production and GFP expression was detected by FACS. $CD4^+$ T cells were gated for analysis. Panel D demonstrates frequency of HIV-specific $CD4^+$ T cells (IFN gamma positive, pre- and post-vaccination) were detected from 4 patients. Panel E demonstrates Post-vaccination PBMCs from 4 patients were expanded and HIV-specific $CD4^+$ T cells were examined.

**Example 15: Dose Response**

*Vector Construction.* A modified version of AGT103 was constructed to test the dose response for increasing AGT103 and its effects on cell surface CCR5 levels. The AGT103

was modified to include a green fluorescent protein (GFP) expression cassette under control of the CMV promoter. Transduced cells expression the miR30CCR5 miR21Vif miR185Tat micro RNA cluster and emit green light due to expressing GFP.

*Functional assay for dose response of increasing AGT103-GFP and inhibition of CCR5 expression.* CEM-CCR5 T cells were transduced with AGT103-GFP using multiplicity of infection per cell from 0 to 5. Transduced cells were stained with a fluorescently conjugated (APC) monoclonal antibody specific for cell surface CCR5. The intensity of staining is proportional to the number of CCR5 molecules per cell surface. The intensity of green fluorescence is proportional to the number of integrated AGT103-GFP copies per cell.

As shown in Figure 19, Panel A demonstrates the dose response for increasing AGT103-GFP and its effects on cell surface CCR5 expression. At multiplicity of infection equal to 0.4 only 1.04% of cells are both green (indicating transduction) and showing significantly reduced CCR5 expression. At multiplicity of infection equal to 1 the number of CCR5low, GFP+ cells increases to 68.1%/ At multiplicity of infection equal to 5 the number of CCR5low, GFP+ cells increased to 95.7%. These data are presented in histogram form in Figure 19, Panel B that shows a normally distribution population in terms of CCR5 staining, moving toward lower mean fluorescence intensity with increasing doses of AGT103-GFP. The potency of AGT103-GFP is presented in graphical form in Figure 19, Panel C showing the percentage inhibition of CCR5 expression with increasing doses of AGT103-GFP. At multiplicity of infection equal to 5, there was greater than 99% reduction in CCR5 expression levels.

**Example 16: AGT103 efficiently transduces primary human CD4$^+$ T cells**

*Transducing primary CD4 T cells with AGT103 lentivirus vector.* A modified AGT103 vector containing the green fluorescence protein marker (GFP) was used at multiplicities of infection between 0.2 and 5 for transducing purified, primary human CD4 T cells.

*Functional assay for transduction efficiency of AGT103 in primary human CD4 T cells.* CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media containing interleukin-2 for 1 day before AGT103 transduction. The relationship between lentivirus vector dose (the multiplicity of infection) and transduction efficiency is demonstrated in Figure 20, Panel A showing that

multiplicity of infection equal to 0.2 resulted in 9.27% of CD4 positive T cells being transduced by AGT103 and that value was increased to 63.1% of CD4 positive T cells being transduced by AGT103 with a multiplicity of infection equal to 5. In addition to achieving efficient transduction of primary CD4 positive T cells it is also necessary to quantify the number of genome copies per cell. In Figure 20, Panel B total cellular DNA from primary human CD4 T cells transduced at several multiplicities of infection were tested by quantitative PCR to determine the number of genome copies per cell. In a multiplicity of infection equal to 0.2 we measured 0.096 genome copies per cell that was in good agreement with 9.27% GFP positive CD4 T cells in panel A. Multiplicity of infection equal to 1 generated 0.691 genome copies per cell and multiplicity of infection equal to 5 generated 1.245 genome copies per cell.

As shown in Figure 20, $CD4^+$ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations. After 2 days, beads were removed and $CD4^+$ T cells were collected. As shown in Panel A, frequency of transduced cells (GFP positive) were detected by FACS. As shown in Panel B, the number of vector copies per cell was determined by qPCR. At a multiplicity of infection (MOI) of 5, 63% of $CD4^+$ T cells were transduced with an average of 1 vector copy per cell.

**Example 17: AGT103 inhibits HIV replication in primary $CD4^+$ T cells**

*Protecting primary human CD4 positive T cells from HIV infection by transducing cells with AGT103.* Therapeutic lentivirus AGT103 was used for transducing primary human CD4 positive T cells at multiplicities of infection between 0.2 and 5 per cell. The transduced cells were then challenged with a CXCR4-tropic HIV strain NL4.3 that does not require cell surface CCR5 for penetration. This assay tests the potency of microRNA against Vif and Tat genes of HIV in terms of preventing productive infection in primary CD4 positive T cells, but uses an indirect method to detect the amount of HIV released from infected, primary human CD4 T cells.

*Functional assay for AGT103 protection against CXCR4-tropic HIV infection of primary human CD4 positive T cells.* CD4 T cells were isolated from human PBMC (HIV-negative donor) using magnetic bead labeled antibodies and standard procedures. The purified CD4 T cells were stimulated ex vivo with CD3/CD28 beads and cultured in media containing interleukin-2 for 1 day before AGT103 transduction using multiplicities of infection between 0.2 and 5. Two days after transduction the CD4 positive T cell cultures were challenged with

HIV strain NL4.3 that was engineered to express the green fluorescent protein (GFP). The transduced and HIV-exposed primary CD4 T cell cultures were maintained for 7 days before collecting cell-free culture fluids containing HIV. The cell-free culture fluids were used to infect a highly permissive T cell line C8166 for 2 days. The proportion of HIV-infected C8166 cells was determined by flow cytometry detecting GFP fluorescence. With a mock lentivirus infection, the dose of 0.1 multiplicity of infection for NL4.3 HIV resulted in an amount of HIV being released into culture fluids that was capable of establishing productive infection in 15.4% of C8166 T cells. With the dose 0.2 multiplicity of infection for AGT103, this value for HIV infection of C8166 cells is reduced to 5.3% and multiplicity of infection equal to 1 for AGT103 resulted in only 3.19% of C8166 T cells being infected by HIV. C8166 infection was reduced further to 0.62% after AGT103 transduction using a multiplicity of infection equal to 5. There is a clear dose response relationship between the amount of AGT103 used for transduction and the amount of HIV released into the culture medium.

As shown in Figure 21, $CD4^+$ T cells isolated from PBMC were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and $CD4^+$ T cells were infected with 0.1 MOI of HIV NL4.3-GFP. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30U/ml) for 7 days. At the end of the culture, supernatant was collected to infect the HIV permissive cell line C8166 for 2 days. HIV-infected C8166 cells (GFP positive) were detected by FACS. There was a reduction in viable HIV with an increase in the multiplicity of infection of AGT103 as observed by less infection of C8166 cells MOI 0.2=65.6%, MOI 1= 79.3%, and MOI 5=96%).

**Example 18: AGT103 protects primary human $CD4^+$ T cells from HIV-induced depletion**

*AGT103 transduction of primary human CD4 T cells to protect against HIV-mediated cytopathology and cell depletion.* PBMC were obtained from healthy, HIV-negative donors and stimulated with CD3/CD28 beads then cultured for 1 day in medium containing interleukin-2 before AGT103 transduction using multiplicities of infection between 0.2 and 5.

*Functional assay for AGT103 protection of primary human CD4 T cells against HIV-mediated cytopathology.* AGT103-transduced primary human CD4 T cells were infected with HIV NL 4.3 strain (CXCR4-tropic) that does not require CCR5 for cellular entry. When using

the CXCR4-tropic NL 4.3, only the effect of Vif and Tat microRNA on HIV replication is being tested. The dose of HIV NL 4.3 was 0.1 multiplicity of infection. One day after HIV infection, cells were washed to remove residual virus and cultured in medium plus interleukin-2. Cells were collected every three days during a 14-day culture then stained with a monoclonal antibody that was specific for CD4 and directly conjugated to a fluorescent marker to allow measurement of the proportion of CD4 positive T cells in PBMC. Untreated CD4 T cells or CD4 T cells transduced with the control lentivirus vector were highly susceptible to HIV challenge and the proportion of CD4 positive T cells in PBMC fell below 10% by day 14 culture. In contrast, there was a dose-dependent effect of AGT103 on preventing cell depletion by HIV challenge. With a AGT103 dose of 0.2 multiplicity of infection more than 20% of PBMC were CD4 T cells by day 14 of culture and this value increased to more than 50% of PBMC being CD4 positive T cells by day 14 of culture with a AGT103 dose of multiplicity of infection equal to 5. Again, there is a clear dose response effect of AGT103 on HIV cytopathogenicity in human PBMC.

As shown in Figure 22, PBMCs were stimulated with CD3/CD28 beads plus IL-2 for 1 day and transduced with AGT103 at various concentrations (MOI). After 2 days, beads were removed and cells were infected with 0.1 MOI of HIV NL4.3. 24 hours later, cells were washed 3 times with PBS and cultured with IL-2 (30U/ml). Cells were collected every 3 days and the frequency of $CD4^+$ T cells were analyzed by FACS. After 14 days of exposure to HIV, there was an 87% reduction in $CD4^+$ T cells transduced with LV-Control, a 60% reduction with AGT103 MOI 0.2, a 37% reduction with AGT103 MOI 1, and a 17% reduction with AGT103 MOI 5.

**Example 19: Generating a Population of CD4+ T cells enriched for HIV-Specificity and transduced with AGT103/CMV-GFP**

Therapeutic vaccination against HIV had minimal effect on the distribution of CD4+, CD8+ and CD4+/CD8+ T cells. As shown in Figure 23A, the CD4 T cell population is shown in the upper left quadrant of the analytical flow cytometry dot plots, and changes from 52% to 57% of total T cells after the vaccination series. These are representative data.

Peripheral blood mononuclear cells from a participant in an HIV therapeutic vaccine trial were cultured for 12 days in medium +/- interleukin-2/interleukin-12 or +/- interleukin-7/interleukin-15. Some cultures were stimulated with overlapping peptides representing the entire p55 Gag protein of HIV-1 (HIV (GAG) Ultra peptide mixture) as a source of epitope

peptides for T cell stimulation. These peptides are 10-20 amino acids in length and overlap by 20-50% of their length to represent the entire Gag precursor protein (p55) from HIV-1 BaL strain. The composition and sequence of individual peptides can be adjusted to compensate for regional variations in the predominant circulating HIV sequences or when detailed sequence information is available for an individual patient receiving this therapy. At culture end, cells were recovered and stained with anti-CD4 or anti-CD8 monoclonal antibodies and the CD3+ population was gated and displayed here. The HIV (GAG) Ultra peptide mixture stimulation for either pre- or post-vaccination samples was similar to the medium control indicating that HIV (GAG) Ultra peptide mixture was not toxic to cells and was not acting as a polyclonal mitogen. The results of this analysis can be found in Figure 23B.

HIV (GAG) Ultra peptide mixture and interleukin-2/interleukin-12 provided for optimal expansion of antigen-specific CD4 T cells. As shown in the upper panels of Figure 23C, there was an increase in cytokine (interferon-gamma) secreting cells in post-vaccination specimens exposed to HIV (GAG) Ultra peptide mixture. In the pre-vaccination sample, cytokine secreting cells increased from 0.43 to 0.69% as a result of exposure to antigenic peptides. In contrast, the post-vaccination samples showed an increase of cytokine secreting cells from 0.62 to 1.76% of total CD4 T cells as a result of peptide stimulation. These data demonstrate the strong impact of vaccination on the CD4 T cell responses to HIV antigen.

Finally, AGT103/CMV-GFP transduction of antigen-expanded CD4 T cells produced HIV-specific and HIV-resistant helper CD4 T cells that are needed for infusion into patients as part of a functional cure for HIV (in accordance with other various aspects and embodiments, AGT103 alone is used; for example, clinical embodiments may not include the CMV-GFP segment). The upper panels of Figure 23C show the results of analyzing the CD4+ T cell population in culture. The x axis of Figure 23C shows Green Fluorescent Protein (GFP) emission indicating that individual cells were transduced with the AGT103/CMV-GFP. In the post-vaccination samples 1.11% of total CD4 T cells that were both cytokine secreting was recovered, indicating that the cells are responding specifically to HIV antigen, and transduced with AGT103/CMV-GFP. This is the target cell population and the clinical product intended for infusion and functional cure of HIV. With the efficiency of cell expansion during the antigen stimulation and subsequent polyclonal expansion phases of ex vivo culture, $4x10^8$ antigen-specific, lentivirus transduced CD4 T cells can be produced. This exceeds the target for cell production by 4-fold and will allow achievement of a count of antigen-specific and

HIV-resistant CD4 T cells of approximately 40 cells/microliter of blood or around 5.7% of total circulating CD4 T cells.

Table 4 below shows the results of the *ex vivo* production of HIV-specific and HIV-resistant CD4 T cells using the disclosed vectors and methods.

**Table 4**

| Material/manipulation | Total CD4 T cells | Percentage HIV-specific | Percentage HIV-specific and HIV-resistant |
|---|---|---|---|
| Leukapheresis pack from HIV+ patient | ~$7x10^8$ | ~0.12 | N/A |
| Peptide expansion ex vivo | ~$8x10^8$ | ~2.4 | N/A |
| Mitogen expansion | ~$1.5x10^{10}$ | ~2.4 | N/A |
| Lentivirus transduction | ~$1.5x10^{10}$ | ~2.4 | ~1.6 |

**Example 20: Clinical Study for Treatment of HIV**

AGT103T is a genetically modified autologous PBMC containing > 5 x $10^7$ HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector.

A Phase I clinical trial will test the safety and feasibility of infusing *ex vivo* modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 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 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 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 $\leq$ $1x10^{10}$ 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 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 AGT103T subjects receive one dose of busulfuram (or Cytoxan) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ PBMC containing genetically modified CD4 T cells.

A Phase II study will evaluate efficacy of AGT103T 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.

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.

*Patient Selection*

*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 >600 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

*Safety assessments*

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

*Efficacy assessments – Phase I*

- Number and frequency of modified CD4 T cells.
- Durability of modified CD4 T cells.
- In vitro response to Gag peptide restimulation (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 stimulation.

*Efficacy assessments – Phase II*

- 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 $5x10^4$ vRNA copies per ml are permitted) with Maraviroc monotherapy.
- Continued virus suppression during and after Maraviroc withdrawal.
- Stable CD4 T cell count.

*AGT103T consists of up to 1 x $10^{10}$ genetically modified, autologous CD4+ T cells containing ≥ 5 x $10^7$ HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector.* A Phase I clinical trial will test the safety and feasibility of infusing *ex vivo* modified autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, CD4+ T-cell counts >600 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 (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 ≥ 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 $\leq 1x10^{10}$ 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 AGT103T subjects receive one dose of busulfuram (or Cytoxan) conditioning regimen followed by infusion of $\leq 1 \times 10^{10}$ enriched and genetically modified CD4 T cell.

A Phase II study will evaluate efficacy of AGT103T 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.

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.

**Sequences**

The following sequences are referred to herein:

| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 1 | miR30 CCR5 | AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACT GAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGA GCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT TCAAGGGGCTT |
| 2 | miR21 Vif | CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTG TACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAG AAGAACACATCCGCACTGACATTTTGGTATCTTTCA TCTGACCA |
| 3 | miR185 Tat | GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT TCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCC CTCCCAATGACCGCGTCTTCGTC |
| 4 | Elongation Factor-1 alpha (EF1-alpha) promoter | CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCC TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACAC |

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| | | TGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG<br>GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC<br>GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG<br>GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT<br>GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG<br>GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC<br>GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA<br>GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG<br>GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT<br>TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA<br>GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTC<br>GAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGA<br>GGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG<br>GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT<br>GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA<br>TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA<br>AGTTTTTTTCTTCCATTTCAGGTGTCGTGA |
| 5 | CCR5 target sequence | GAGCAAGCTCAGTTTACA |
| 6 | Vif target sequence | GGGATGTGTACTTCTGAACTT |
| 7 | Tat target sequence | TCCGCTTCTTCCTGCCATAG |
| 8 | TAR decoy sequence | CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTC<br>TCGACAGCGACCAGATCTGAGCCTGGGAGCTCTCTG<br>GCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAA<br>TTCTTACTGAGCAAGAAA |
| 9 | Rev/Tat target sequence | GCGGAGACAGCGACGAAGAGC |
| 10 | Rev/Tat shRNA sequence | GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCT<br>TCGTCGCTGTCTCCGCTTTTT |

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| 11 | Gag target sequence | GAAGAAATGATGACAGCAT |
| 12 | Gag shRNA sequence | GAAGAAATGATGACAGCATTTCAAGAGAATGCTGT CATCATTTCTTCTTTTT |
| 13 | Pol target sequence | CAGGAGCAGATGATACAG |
| 14 | Pol shRNA sequence | CAGGAGATGATACAGTTCAAGAGACTGTATCATCTG CTCCTGTTTTT |
| 15 | CCR5 target sequence #1 | GTGTCAAGTCCAATCTATG |
| 16 | CCR5 shRNA sequence #1 | GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATT GGACTTGACACTTTTT |
| 17 | CCR5 target sequence #2 | GAGCATGACTGACATCTAC |
| 18 | CCR5 shRNA sequence #2 | GAGCATGACTGACATCTACTTCAAGAGAGTAGATGT CAGTCATGCTCTTTTT |
| 19 | CCR5 target sequence #3 | GTAGCTCTAACAGGTTGGA |
| 20 | CCR5 shRNA sequence #3 | GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCT GTTAGAGCTACTTTTT |
| 21 | CCR5 target sequence #4 | GTTCAGAAACTACCTCTTA |
| 22 | CCR5 shRNA sequence #4 | GTTCAGAAACTACCTCTTATTCAAGAGATAAGAGGT AGTTTCTGAACTTTTT |
| 23 | CCR5 target sequence #5 | GAGCAAGCTCAGTTTACACC |
| 24 | CCR5 shRNA sequence #5 | GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTA AACTGAGCTTGCTCTTTTT |
| 25 | Homo sapiens CCR5 gene, sequence 1 | ATGGATTATCAAGTGTCAAGTCCAATCTATGACATC AATTATTATACATCGGAGCCCTGCCAAAAAATCAAT GTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTC TACTCACTGGTGTTCATCTTTGGTTTTGTGGGC |

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| 26 | Homo sapiens CCR5 gene, sequence 2 | AACATGCTGGTCATCCTCATCCTGATAAACTGCAAA AGGCTGAAGAGCATGACTGACATCTACCTGCTCAAC CTGGCCATCTCTGACCTGTTTTTCCTTCTTACTGTCC CCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACT TTGGAAATACAATGTGTCAACTCTTGACAGGGCTCT ATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCAT CCTCCTGACAATCGATAGGTACCTGGCTGTCGTCCA TGCTGTGTTTGCTTTAAAAGCCAGGACGGTCACCTT TGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGC TGTGTTTGCGTCTCTCCCAGGAATCATCTTTACCAG ATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTC TCATTTTCCATACAGTCAGTATCAATTCTGGAAGAA TTTCCAGACATTAAAGATAGTCATCTTGGGGCTGGT CCTGCCGCTGCTTGTCATGGTCATCTGCTACTCGGG AATCCTAAAAACTCTGCTTCGGTGTCGAAATGAGAA GAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCAT CATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAAC ATTGTCCTTCTCCTGAAC |
| 27 | Homo sapiens CCR5 gene, sequence 3 | ACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGT AGCTCTAACAGGTTGGACCAAGCTATGCAGGTGA |
| 28 | Homo sapiens CCR5 gene, sequence 4 | CAGAGACTCTTGGGATGACGCACTGCTGCATCAACC CCATCATCTATGCCTTTGTCGGGGAGAAGTTCAGAA ACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCA AACGCTTCTGCAAATGCTGTTCTATTTTCCAG |
| 29 | Homo sapiens CCR5 gene, sequence 5 | CAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACAC CCGATCCACTGGGGAGCAGGAAATATCTGTGGGCTT GTGA |
| 30 | CD4 promoter sequence | TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCC TCTGCAAAGAAAAAAAAAAAAAAAAAAGAACAAA GGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAGAT GAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGG |

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| | | AGGCGGATCCTGTCAGCTTTGCTCTCTCTGTGGCTG<br>GCAGTTTCTCCAAAGGGTAACAGGTGTCAGCTGGCT<br>GAGCCTAGGCTGAACCCTGAGACATGCTACCTCTGT<br>CTTCTCATGGCTGGAGGCAGCCTTTGTAAGTCACAG<br>AAAGTAGCTGAGGGGCTCTGGAAAAAAGACAGCCA<br>GGGTGGAGGTAGATTGGTCTTTGACTCCTGATTTAA<br>GCCTGATTCTGCTTAACTTTTTCCCTTGACTTTGGCA<br>TTTTCACTTTGACATGTTCCCTGAGAGCCTGGGGGG<br>TGGGGAACCCAGCTCCAGCTGGTGACGTTTGGGGCC<br>GGCCCAGGCCTAGGGTGTGGAGGAGCCTTGCCATC<br>GGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGC<br>AGA |
| 31 | miR30-CCR5/miR21-Vif/miR185 Tat microRNA cluster sequence | AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACT<br>GAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGA<br>GCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT<br>TCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCA<br>CCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG<br>AATCTCATGGAGTTCAGAAGAACACATCCGCACTG<br>ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGC<br>CTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC<br>CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAG<br>AAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT<br>C |
| 32 | Long WPRE sequence | AATCAACCTCTGATTACAAAATTTGTGAAAGATTGA<br>CTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATG<br>TGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT<br>GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATA<br>AATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGC<br>CCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGT<br>TTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCA<br>CCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCC<br>CCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTG<br>CCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGG |

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| | | CACTGACAATTCCGTGGTGTTGTCGGGGAAATCATC<br>GTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGG<br>ATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCG<br>GCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTG<br>CTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTC<br>GCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCT<br>CCCCGCCT |
| 33 | Elongation Factor-1 alpha (EF1-alpha) promoter; miR30CCR5; miR21Vif; miR185 Tat | CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG<br>GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC<br>GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT<br>CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC<br>CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG<br>GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC<br>CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG<br>ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG<br>GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG<br>CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG<br>GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCC<br>TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA<br>ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA<br>AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACAC<br>TGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG<br>GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC<br>GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG<br>GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT<br>GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG<br>GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC<br>GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA<br>GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG<br>GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT<br>TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA<br>GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTC<br>GAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGA |

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| | | GGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG<br>GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT<br>GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA<br>TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA<br>AGTTTTTTTCTTCCATTTCAGGTGTCGTGATGTACA<br>AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACT<br>GAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGA<br>GCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT<br>TCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCA<br>CCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG<br>AATCTCATGGAGTTCAGAAGAACACATCCGCACTG<br>ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGC<br>CTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC<br>CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAG<br>AAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT<br>C |
| 34 | Rous Sarcoma virus (RSV) promoter | GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACAT<br>GGTAACGATGAGTTAGCAACATGCCTTACAAGGAG<br>AGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTA<br>AGGTGGTACGATCGTGCCTTATTAGGAAGGCAACA<br>GACGGGTCTGACATGGATTGGACGAACCACTGAAT<br>TGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAG<br>CTCGATACAATAAACG |
| 35 | 5’ Long terminal repeat (LTR) | GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC<br>TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC<br>AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTG<br>CCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCC<br>TCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA |
| 36 | Psi Packaging signal | TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGG<br>AGAGAG |
| 37 | Rev response element (RRE) | AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG<br>AAGCACTATGGGCGCAGCCTCAATGACGCTGACGG |

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| | | TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC<br>AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA<br>CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG<br>CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA<br>CCTAAAGGATCAACAGCTCC |
| 38 | Central polypurine tract (cPPT) | TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTG<br>CAGGGGAAAGAATAGTAGACATAATAGCAACAGAC<br>ATACAAACTAAAGAATTACAAAAACAAATTACAAA<br>ATTCAAAATTTTA |
| 39 | 3’ delta LTR | TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT<br>CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACC<br>AGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGA<br>ACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAG<br>TGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT<br>CTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAG<br>TGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA |
| 40 | Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription | TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT<br>TCATAGCCCATATATGGAGTTCCGCGTTACATAACT<br>TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG<br>ACCCCCGCCCATTGACGTCAATAATGACGTATGTTC<br>CCATAGTAACGCCAATAGGGACTTTCCATTGACGTC<br>AATGGGTGGACTATTTACGGTAAACTGCCCACTTGG<br>CAGTACATCAAGTGTATCATATGCCAAGTACGCCCC<br>CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC<br>ATTATGCCCAGTACATGACCTTATGGGACTTTCCTA<br>CTTGGCAGTACATCTACGTATTAGTCATC |
| 41 | Helper/Rev; Chicken beta actin (CAG) promoter; Transcription | GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTG<br>CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCA<br>ATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGC<br>GATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGG<br>CGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG<br>AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG |

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| | | CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG<br>GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC<br>GGGCG |
| 42 | Helper/Rev;<br>Chicken beta<br>actin intron;<br>Enhance gene<br>expression | GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC<br>CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG<br>ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG<br>CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAA<br>TGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGC<br>CTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGG<br>GGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC<br>GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC<br>GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTG<br>TGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGG<br>GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG<br>GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGG<br>GGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTG<br>TAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGA<br>GCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGG<br>GGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG<br>TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCC<br>GCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCG<br>GCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGG<br>CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG<br>AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGC<br>GGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT<br>CTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCA<br>GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCG<br>CCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGG<br>GGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA<br>CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGAC<br>CGGCGG |

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| 43 | Helper/Rev; HIV Gag; Viral capsid | ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA<br>ATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG<br>GGGGAAAGAAAAAATATAAATTAAAACATATAGTA<br>TGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA<br>TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACA<br>AATACTGGGACAGCTACAACCATCCCTTCAGACAG<br>GATCAGAAGAACTTAGATCATTATATAATACAGTAG<br>CAACCCTCTATTGTGTGCATCAAAGGATAGAGATAA<br>AAGACACCAAGGAAGCTTTAGACAAGATAGAGGAA<br>GAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAG<br>CAGCAGCTGACACAGGACACAGCAATCAGGTCAGC<br>CAAAATTACCCTATAGTGCAGAACATCCAGGGGCA<br>AATGGTACATCAGGCCATATCACCTAGAACTTTAAA<br>TGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCA<br>GCCCAGAAGTGATACCCATGTTTTCAGCATTATCAG<br>AAGGAGCCACCCCACAAGATTTAAACACCATGCTA<br>AACACAGTGGGGGGACATCAAGCAGCCATGCAAAT<br>GTTAAAAGAGACCATCAATGAGGAAGCTGCAGAAT<br>GGGATAGAGTGCATCCAGTGCATGCAGGGCCTATT<br>GCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGA<br>CATAGCAGGAACTACTAGTACCCTTCAGGAACAAA<br>TAGGATGGATGACACATAATCCACCTATCCCAGTAG<br>GAGAAATCTATAAAAGATGGATAATCCTGGGATTA<br>AATAAAATAGTAAGAATGTATAGCCCTACCAGCATT<br>CTGGACATAAGACAAGGACCAAAGGAACCCTTTAG<br>AGACTATGTAGACCGATTCTATAAAACTCTAAGAGC<br>CGAGCAAGCTTCACAAGAGGTAAAAAATTGGATGA<br>CAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATT<br>GTAAGACTATTTTAAAAGCATTGGGACCAGGAGCG<br>ACACTAGAAGAAATGATGACAGCATGTCAGGGAGT<br>GGGGGGACCCGGCCATAAAGCAAGAGTTTTGGCTG<br>AAGCAATGAGCCAAGTAACAAATCCAGCTACCATA<br>ATGATACAGAAAGGCAATTTTAGGAACCAAAGAAA |

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| | | GACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCA<br>CATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGG<br>GCTGTTGGAAATGTGGAAAGGAAGGACACCAAATG<br>AAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGG<br>AAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAA<br>TTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACC<br>AGAAGAGAGCTTCAGGTTTGGGGAAGAGACAACAA<br>CTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAA<br>CTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCA<br>GCGACCCCTCGTCACAATAA |
| 44 | Helper/Rev; HIV Pol; Protease and reverse transcriptase | ATGAATTTGCCAGGAAGATGGAAACCAAAAATGAT<br>AGGGGGAATTGGAGGTTTTATCAAAGTAGGACAGT<br>ATGATCAGATACTCATAGAAATCTGCGGACATAAA<br>GCTATAGGTACAGTATTAGTAGGACCTACACCTGTC<br>AACATAATTGGAAGAAATCTGTTGACTCAGATTGGC<br>TGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTG<br>TACCAGTAAAATTAAAGCCAGGAATGGATGGCCCA<br>AAAGTTAAACAATGGCCATTGACAGAAGAAAAAAT<br>AAAAGCATTAGTAGAAATTTGTACAGAAATGGAAA<br>AGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAAT<br>CCATACAATACTCCAGTATTTGCCATAAAGAAAAAA<br>GACAGTACTAAATGGAGAAAATTAGTAGATTTCAG<br>AGAACTTAATAAGAGAACTCAAGATTTCTGGGAAG<br>TTCAATTAGGAATACCACATCCTGCAGGGTTAAAAC<br>AGAAAAAATCAGTAACAGTACTGGATGTGGGCGAT<br>GCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGG<br>AAGTATACTGCATTTACCATACCTAGTATAAACAAT<br>GAGACACCAGGGATTAGATATCAGTACAATGTGCTT<br>CCACAGGGATGGAAAGGATCACCAGCAATATTCCA<br>GTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAA<br>ACAAAATCCAGACATAGTCATCTATCAATACATGGA<br>TGATTTGTATGTAGGATCTGACTTAGAAATAGGGCA<br>GCATAGAACAAAAATAGAGGAACTGAGACAACATC |

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| | | TGTTGAGGTGGGGATTTACCACACCAGACAAAAAA<br>CATCAGAAAGAACCTCCATTCCTTTGGATGGGTTAT<br>GAACTCCATCCTGATAAATGGACAGTACAGCCTATA<br>GTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGA<br>CATACAGAAATTAGTGGGAAAATTGAATTGGGCAA<br>GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTAT<br>GTAAACTTCTTAGGGGAACCAAAGCACTAACAGAA<br>GTAGTACCACTAACAGAAGAAGCAGAGCTAGAACT<br>GGCAGAAAACAGGGAGATTCTAAAAGAACCGGTAC<br>ATGGAGTGTATTATGACCCATCAAAAGACTTAATAG<br>CAGAAATACAGAAGCAGGGGCAAGGCCAATGGACA<br>TATCAAATTTATCAAGAGCCATTTAAAAATCTGAAA<br>ACAGGAAAATATGCAAGAATGAAGGGTGCCCACAC<br>TAATGATGTGAAACAATTAACAGAGGCAGTACAAA<br>AAATAGCCACAGAAAGCATAGTAATATGGGGAAAG<br>ACTCCTAAATTTAAATTACCCATACAAAAGGAAACA<br>TGGGAAGCATGGTGGACAGAGTATTGGCAAGCCAC<br>CTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCC<br>CTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAAC<br>CCATAATAGGAGCAGAAACTTTCTATGTAGATGGG<br>GCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGG<br>ATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC<br>CCCTAACGGACACAACAAATCAGAAGACTGAGTTA<br>CAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA<br>GAAGTAAACATAGTGACAGACTCACAATATGCATT<br>GGGAATCATTCAAGCACAACCAGATAAGAGTGAAT<br>CAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATA<br>AAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGC<br>ACACAAAGGAATTGGAGGAAATGAACAAGTAGATG<br>GGTTGGTCAGTGCTGGAATCAGGAAAGTACTA |
| 45 | Helper Rev; HIV Integrase; | TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA<br>TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA<br>GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAA |

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| | Integration of viral RNA | TAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGG GAAGCCATGCATGGACAAGTAGACTGTAGCCCAGG AATATGGCAGCTAGATTGTACACATTTAGAAGGAA AAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGAT ATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGG CAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGA AGATGGCCAGTAAAAACAGTACATACAGACAATGG CAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTG TTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCC CTACAATCCCCAAAGTCAAGGAGTAATAGAATCTAT GAATAAAGAATTAAAGAAAATTATAGGACAGGTAA GAGATCAGGCTGAACATCTTAAGACAGCAGTACAA ATGGCAGTATTCATCCACAATTTTAAAAGAAAAGG GGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAG TAGACATAATAGCAACAGACATACAAACTAAAGAA TTACAAAAACAAATTACAAAAATTCAAAATTTTCGG GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAA AGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGG CAGTAGTAATACAAGATAATAGTGACATAAAAGTA GTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTA TGGAAAACAGATGGCAGGTGATGATTGTGTGGCAA GTAGACAGGATGAGGATTAA |
| 46 | Helper/Rev; HIV RRE; Binds Rev element | AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG AAGCACTATGGGCGCAGCGTCAATGACGCTGACGG TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA CCTAAAGGATCAACAGCTCCT |
| 47 | Helper/Rev; HIV Rev; Nuclear export and | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG |

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| | stabilize viral mRNA | AGAGACAGAGACAGATCCATTCGATTAGTGAACGG ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 48 | Helper/Rev; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT TTAGAGTTTGGCAACATATGCCATATGCTGGCTGCC ATGAACAAAGGTGGCTATAAAGAGGTCATCAGTAT ATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCAT AGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATA TTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAA ATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTC CTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTC TCTTATGAAGATC |
| 49 | Helper; CMV early (CAG) enhancer; Enhance transcription | TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT TCATAGCCCATATATGGAGTTCCGCGTTACATAACT TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG ACCCCCGCCCATTGACGTCAATAATGACGTATGTTC CCATAGTAACGCCAATAGGGACTTTCCATTGACGTC AATGGGTGGACTATTTACGGTAAACTGCCCACTTGG CAGTACATCAAGTGTATCATATGCCAAGTACGCCCC CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC ATTATGCCCAGTACATGACCTTATGGGACTTTCCTA CTTGGCAGTACATCTACGTATTAGTCATC |
| 50 | Helper; Chicken beta actin (CAG) promoter; Transcription | GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTG CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCA ATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGC GATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGG |

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| | | CGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG<br>AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG<br>CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG<br>GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC<br>GGGCG |
| 51 | Helper; Chicken beta actin intron; Enhance gene expression | GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC<br>CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG<br>ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG<br>CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAA<br>TGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGC<br>CTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGG<br>GGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC<br>GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC<br>GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTG<br>TGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGG<br>GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG<br>GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGG<br>GGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTG<br>TAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGA<br>GCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGG<br>GGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG<br>TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCC<br>GCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCG<br>GCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGG<br>CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG<br>AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGC<br>GGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT<br>CTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCA<br>GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCG<br>CCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGG<br>GGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA<br>CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGAC<br>CGGCGG |

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| 52 | Helper; HIV Gag; Viral capsid | ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA<br>ATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG<br>GGGGAAAGAAAAAATATAAATTAAAACATATAGTA<br>TGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA<br>TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACA<br>AATACTGGGACAGCTACAACCATCCCTTCAGACAG<br>GATCAGAAGAACTTAGATCATTATATAATACAGTAG<br>CAACCCTCTATTGTGTGCATCAAAGGATAGAGATAA<br>AAGACACCAAGGAAGCTTTAGACAAGATAGAGGAA<br>GAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAG<br>CAGCAGCTGACACAGGACACAGCAATCAGGTCAGC<br>CAAAATTACCCTATAGTGCAGAACATCCAGGGGCA<br>AATGGTACATCAGGCCATATCACCTAGAACTTTAAA<br>TGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCA<br>GCCCAGAAGTGATACCCATGTTTTCAGCATTATCAG<br>AAGGAGCCACCCCACAAGATTTAAACACCATGCTA<br>AACACAGTGGGGGGACATCAAGCAGCCATGCAAAT<br>GTTAAAAGAGACCATCAATGAGGAAGCTGCAGAAT<br>GGGATAGAGTGCATCCAGTGCATGCAGGGCCTATT<br>GCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGA<br>CATAGCAGGAACTACTAGTACCCTTCAGGAACAAA<br>TAGGATGGATGACACATAATCCACCTATCCCAGTAG<br>GAGAAATCTATAAAAGATGGATAATCCTGGGATTA<br>AATAAAATAGTAAGAATGTATAGCCCTACCAGCATT<br>CTGGACATAAGACAAGGACCAAAGGAACCCTTTAG<br>AGACTATGTAGACCGATTCTATAAAACTCTAAGAGC<br>CGAGCAAGCTTCACAAGAGGTAAAAAATTGGATGA<br>CAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATT<br>GTAAGACTATTTTAAAAGCATTGGGACCAGGAGCG<br>ACACTAGAAGAAATGATGACAGCATGTCAGGGAGT<br>GGGGGGACCCGGCCATAAAGCAAGAGTTTTGGCTG<br>AAGCAATGAGCCAAGTAACAAATCCAGCTACCATA<br>ATGATACAGAAAGGCAATTTTAGGAACCAAAGAAA |

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| | | GACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCA<br>CATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGG<br>GCTGTTGGAAATGTGGAAAGGAAGGACACCAAATG<br>AAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGG<br>AAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAA<br>TTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACC<br>AGAAGAGAGCTTCAGGTTTGGGGAAGAGACAACAA<br>CTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAA<br>CTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCA<br>GCGACCCCTCGTCACAATAA |
| 53 | Helper; HIV Pol; Protease and reverse transcriptase | ATGAATTTGCCAGGAAGATGGAAACCAAAAATGAT<br>AGGGGGAATTGGAGGTTTTATCAAAGTAGGACAGT<br>ATGATCAGATACTCATAGAAATCTGCGGACATAAA<br>GCTATAGGTACAGTATTAGTAGGACCTACACCTGTC<br>AACATAATTGGAAGAAATCTGTTGACTCAGATTGGC<br>TGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTG<br>TACCAGTAAAATTAAAGCCAGGAATGGATGGCCCA<br>AAAGTTAAACAATGGCCATTGACAGAAGAAAAAAT<br>AAAAGCATTAGTAGAAATTTGTACAGAAATGGAAA<br>AGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAAT<br>CCATACAATACTCCAGTATTTGCCATAAAGAAAAAA<br>GACAGTACTAAATGGAGAAAATTAGTAGATTTCAG<br>AGAACTTAATAAGAGAACTCAAGATTTCTGGGAAG<br>TTCAATTAGGAATACCACATCCTGCAGGGTTAAAAC<br>AGAAAAAATCAGTAACAGTACTGGATGTGGGCGAT<br>GCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGG<br>AAGTATACTGCATTTACCATACCTAGTATAAACAAT<br>GAGACACCAGGGATTAGATATCAGTACAATGTGCTT<br>CCACAGGGATGGAAAGGATCACCAGCAATATTCCA<br>GTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAA<br>ACAAAATCCAGACATAGTCATCTATCAATACATGGA<br>TGATTTGTATGTAGGATCTGACTTAGAAATAGGGCA<br>GCATAGAACAAAAATAGAGGAACTGAGACAACATC |

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| | | TGTTGAGGTGGGGATTTACCACACCAGACAAAAAA<br>CATCAGAAAGAACCTCCATTCCTTTGGATGGGTTAT<br>GAACTCCATCCTGATAAATGGACAGTACAGCCTATA<br>GTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGA<br>CATACAGAAATTAGTGGGAAAATTGAATTGGGCAA<br>GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTAT<br>GTAAACTTCTTAGGGGAACCAAAGCACTAACAGAA<br>GTAGTACCACTAACAGAAGAAGCAGAGCTAGAACT<br>GGCAGAAAACAGGGAGATTCTAAAAGAACCGGTAC<br>ATGGAGTGTATTATGACCCATCAAAAGACTTAATAG<br>CAGAAATACAGAAGCAGGGGCAAGGCCAATGGACA<br>TATCAAATTTATCAAGAGCCATTTAAAAATCTGAAA<br>ACAGGAAAATATGCAAGAATGAAGGGTGCCCACAC<br>TAATGATGTGAAACAATTAACAGAGGCAGTACAAA<br>AAATAGCCACAGAAAGCATAGTAATATGGGGAAAG<br>ACTCCTAAATTTAAATTACCCATACAAAAGGAAACA<br>TGGGAAGCATGGTGGACAGAGTATTGGCAAGCCAC<br>CTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCC<br>CTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAAC<br>CCATAATAGGAGCAGAAACTTTCTATGTAGATGGG<br>GCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGG<br>ATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC<br>CCCTAACGGACACAACAAATCAGAAGACTGAGTTA<br>CAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA<br>GAAGTAAACATAGTGACAGACTCACAATATGCATT<br>GGGAATCATTCAAGCACAACCAGATAAGAGTGAAT<br>CAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATA<br>AAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGC<br>ACACAAAGGAATTGGAGGAAATGAACAAGTAGATG<br>GGTTGGTCAGTGCTGGAATCAGGAAAGTACTA |
| 54 | Helper; HIV Integrase; | TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA<br>TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA<br>GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAA |

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| | Integration of viral RNA | TAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGG GAAGCCATGCATGGACAAGTAGACTGTAGCCCAGG AATATGGCAGCTAGATTGTACACATTTAGAAGGAA AAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGAT ATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGG CAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGA AGATGGCCAGTAAAAACAGTACATACAGACAATGG CAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTG TTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCC CTACAATCCCCAAAGTCAAGGAGTAATAGAATCTAT GAATAAAGAATTAAAGAAAATTATAGGACAGGTAA GAGATCAGGCTGAACATCTTAAGACAGCAGTACAA ATGGCAGTATTCATCCACAATTTTAAAAGAAAAGG GGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAG TAGACATAATAGCAACAGACATACAAACTAAAGAA TTACAAAAACAAATTACAAAAATTCAAAATTTTCGG GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAA AGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGG CAGTAGTAATACAAGATAATAGTGACATAAAAGTA GTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTA TGGAAAACAGATGGCAGGTGATGATTGTGTGGCAA GTAGACAGGATGAGGATTAA |
| 55 | Helper; HIV RRE; Binds Rev element | AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG AAGCACTATGGGCGCAGCGTCAATGACGCTGACGG TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA CCTAAAGGATCAACAGCTCCT |
| 56 | Helper; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG |

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| | | GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT<br>TTAGAGTTTGGCAACATATGCCATATGCTGGCTGCC<br>ATGAACAAAGGTGGCTATAAAGAGGTCATCAGTAT<br>ATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCAT<br>AGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATA<br>TTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAA<br>ATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTC<br>CTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTC<br>TCTTATGAAGATC |
| 57 | Rev; RSV promoter; Transcription | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC<br>TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC<br>AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA<br>CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG<br>AGAGACAGAGACAGATCCATTCGATTAGTGAACGG<br>ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT<br>GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT<br>CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG<br>CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC<br>TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 58 | Rev; HIV Rev; Nuclear export and stabilize viral mRNA | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC<br>TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC<br>AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA<br>CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG<br>AGAGACAGAGACAGATCCATTCGATTAGTGAACGG<br>ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT<br>GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT<br>CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG<br>CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC<br>TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 59 | Rev; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT<br>CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA<br>AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA<br>TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG |

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| | | GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT<br>TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGC<br>CATGAACAAAGGTTGGCTATAAAGAGGTCATCAGT<br>ATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCC<br>ATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTA<br>TATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTA<br>AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCC<br>TCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCT<br>TCTCTTATGGAGATC |
| 60 | Envelope; CMV promoter; Transcription | ACATTGATTATTGACTAGTTATTAATAGTAATCAAT<br>TACGGGGTCATTAGTTCATAGCCCATATATGGAGTT<br>CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG<br>CTGACCGCCCAACGACCCCCGCCCATTGACGTCAAT<br>AATGACGTATGTTCCCATAGTAACGCCAATAGGGAC<br>TTTCCATTGACGTCAATGGGTGGAGTATTTACGGTA<br>AACTGCCCACTTGGCAGTACATCAAGTGTATCATAT<br>GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA<br>ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT<br>ATGGGACTTTCCTACTTGGCAGTACATCTACGTATT<br>AGTCATCGCTATTACCATGGTGATGCGGTTTTGGCA<br>GTACATCAATGGGCGTGGATAGCGGTTTGACTCACG<br>GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGG<br>GAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCC<br>AAAATGTCGTAACAACTCCGCCCCATTGACGCAAAT<br>GGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA<br>GC |
| 61 | Envelope; Beta globin intron; Enhance gene expression | GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCG<br>CTATTGTAAAATTCATGTTATATGGAGGGGGCAAAG<br>TTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCT<br>TGTATCACCATGGACCCTCATGATAATTTTGTTTCTT<br>TCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTT<br>ATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTT<br>TAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTT |

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| | | GTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCAC<br>TTTTTTTTCAAGGCAATCAGGGTATATTATATTGTAC<br>TTCAGCACAGTTTTAGAGAACAATTGTTATAATTAA<br>ATGATAAGGTAGAATATTTCTGCATATAAATTCTGG<br>CTGGCGTGGAAATATTCTTATTGGTAGAAACAACTA<br>CACCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTA<br>CAATGATATACACTGTTTGAGATGAGGATAAAATAC<br>TCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTT<br>CATGCCTTCTTCTCTTTCCTACAG |
| 62 | Envelope; VSV-G; Glycoprotein envelope-cell entry | ATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTG<br>GGGTGAATTGCAAGTTCACCATAGTTTTTCCACACA<br>ACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAATT<br>ACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGC<br>ATAATGACTTAATAGGCACAGCCTTACAAGTCAAA<br>ATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGG<br>TTGGATGTGTCATGCTTCCAAATGGGTCACTACTTG<br>TGATTTCCGCTGGTATGGACCGAAGTATATAACACA<br>TTCCATCCGATCCTTCACTCCATCTGTAGAACAATG<br>CAAGGAAAGCATTGAACAAACGAAACAAGGAACTT<br>GGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT<br>ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCC<br>AGGTGACTCCTCACCATGTGCTGGTTGATGAATACA<br>CAGGAGAATGGGTTGATTCACAGTTCATCAACGGA<br>AAATGCAGCAATTACATATGCCCCACTGTCCATAAC<br>TCTACAACCTGGCATTCTGACTATAAGGTCAAAGGG<br>CTATGTGATTCTAACCTCATTTCCATGGACATCACCT<br>TCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAA<br>AGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTT<br>ATGAAACTGGAGGCAAGGCCTGCAAAATGCAATAC<br>TGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTC<br>TGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCA<br>GCCAGATTCCCTGAATGCCCAGAAGGGTCAAGTATC<br>TCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTA |

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| | | ATTCAGGACGTTGAGAGGATCTTGGATTATTCCCTC<br>TGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCT<br>TCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCT<br>AAAAACCCAGGAACCGGTCCTGCTTTCACCATAATC<br>AATGGTACCCTAAAATACTTTGAGACCAGATACATC<br>AGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATG<br>GTCGGAATGATCAGTGGAACTACCACAGAAAGGGA<br>ACTGTGGGATGACTGGGCACCATATGAAGACGTGG<br>AAATTGGACCCAATGGAGTTCTGAGGACCAGTTCA<br>GGATATAAGTTTCCTTTATACATGATTGGACATGGT<br>ATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCT<br>CAGGTGTTCGAACATCCTCACATTCAAGACGCTGCT<br>TCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTG<br>ATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG<br>AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCT<br>CTTTTTTCTTTATCATAGGGTTAATCATTGGACTATT<br>CTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAA<br>TTAAAGCACACCAAGAAAAGACAGATTTATACAGA<br>CATAGAGATGA |
| 63 | Envelope; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT<br>CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA<br>AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA<br>TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG<br>GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT<br>TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGC<br>CATGAACAAAGGTTGGCTATAAAGAGGTCATCAGT<br>ATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCC<br>ATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTA<br>TATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTA<br>AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCC<br>TCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCT<br>TCTCTTATGGAGATC |

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| 64 | Promoter; EF-1 | CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG<br>GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC<br>GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT<br>CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC<br>CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG<br>GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC<br>CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG<br>ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG<br>GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG<br>CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG<br>GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCC<br>TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA<br>ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA<br>AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACAC<br>TGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG<br>GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC<br>GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG<br>GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT<br>GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG<br>GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC<br>GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA<br>GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG<br>GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT<br>TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA<br>GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTC<br>GAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGA<br>GGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG<br>GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT<br>GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA<br>TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA<br>AGTTTTTTTCTTCCATTTCAGGTGTCGTGA |
| 65 | Promoter; PGK | GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGG<br>GTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTC |

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| | | CGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCA<br>CATTCTTCACGTCCGTTCGCAGCGTCACCCGGATCT<br>TCGCCGCTACCCTTGTGGGCCCCCCGGCGACGCTTC<br>CTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGT<br>TCGCGGCGTGCCGGACGTGACAAACGGAAGCCGCA<br>CGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAG<br>GGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGT<br>GGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGA<br>GCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGG<br>GTGTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGC<br>GCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCAC<br>GTCGGCAGTCGGCTCCCTCGTTGACCGAATCACCGA<br>CCTCTCTCCCCAG |
| 66 | Promoter; UbC | GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCT<br>CCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGC<br>GCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCTCA<br>GGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAG<br>AACCCCAGTATCAGCAGAAGGACATTTTAGGACGG<br>GACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCA<br>GAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCT<br>CGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTG<br>AACGCCGATGATTATATAAGGACGCGCCGGGTGTG<br>GCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCG<br>CGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGG<br>TGAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGT<br>GGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTG<br>GAGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCGA<br>GCAAGGTTGCCCTGAACTGGGGGTTGGGGGGAGCG<br>CACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAA<br>GACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAAC<br>AAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAG<br>GTCTTGAGGCCTTCGCTAATGCGGGAAAGCTCTTAT<br>TCGGGTGAGATGGGCTGGGGCACCATCTGGGGACC |

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| | | CTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGG<br>TTTGTCGTCTGGTTGCGGGGGCGGCAGTTATGCGGT<br>GCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCG<br>CGCCTCGTCGTGTCGTGACGTCACCCGTTCTGTTGG<br>CTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGTG<br>TGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTT<br>CGGGCCTAGGGTAGGCTCTCCTGAATCGACAGGCG<br>CCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCG<br>TCAGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTT<br>AAGTAGCTGAAGCTCCGGTTTTGAACTATGCGCTCG<br>GGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCA<br>CCTTTTGAAATGTAATCATTTGGGTCAATATGTAAT<br>TTTCAGTGTTAGACTAGTAAA |
| 67 | Poly A; SV40 | GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA<br>TAGCATCACAAATTTCACAAATAAAGCATTTTTTTC<br>ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA<br>TGTATCTTATCA |
| 68 | Poly A; bGH | GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC<br>CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC<br>ACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATT<br>GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG<br>GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGG<br>ATTGGGAAGACAATAGCAGGCATGCTGGGGATGCG<br>GTGGGCTCTATGG |
| 69 | HIV Gag; Bal | ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA<br>ATTAGATAGGTGGGAAAAAATTCGGTTAAGGCCAG<br>GGGGAAAGAAAAAATATAGATTAAAACATATAGTA<br>TGGGCAAGCAGGGAACTAGAAAGATTCGCAGTCAA<br>TCCTGGCCTGTTAGAAACATCAGAAGGCTGCAGAC<br>AAATACTGGGACAGCTACAACCATCCCTTCAGACA<br>GGATCAGAAGAACTTAGATCATTATATAATACAGTA<br>GCAACCCTCTATTGTGTACATCAAAAGATAGAGGTA<br>AAAGACACCAAGGAAGCTTTAGACAAAATAGAGGA |

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| | | AGAGCAAAACAAATGTAAGAAAAAGGCACAGCAA<br>GCAGCAGCTGACACAGGAAACAGCGGTCAGGTCAG<br>CCAAAATTTCCCTATAGTGCAGAACCTCCAGGGGCA<br>AATGGTACATCAGGCCATATCACCTAGAACTTTAAA<br>TGCATGGGTAAAAGTAATAGAAGAGAAAGCTTTCA<br>GCCCAGAAGTAATACCCATGTTTTCAGCATTATCAG<br>AAGGAGCCACCCCACAAGATTTAAACACCATGCTA<br>AACACAGTGGGGGGACATCAAGCAGCCATGCAAAT<br>GTTAAAAGAACCCATCAATGAGGAAGCTGCAAGAT<br>GGGATAGATTGCATCCCGTGCAGGCAGGGCCTGTTG<br>CACCAGGCCAGATAAGAGATCCAAGGGGAAGTGAC<br>ATAGCAGGAACTACCAGTACCCTTCAGGAACAAAT<br>AGGATGGATGACAAGTAATCCACCTATCCCAGTAG<br>GAGAAATCTATAAAAGATGGATAATCCTGGGATTA<br>AATAAAATAGTAAGGATGTATAGCCCTACCAGCATT<br>TTGGACATAAGACAAGGACCAAAGGAACCCTTTAG<br>AGACTATGTAGACCGGTTCTATAAAACTCTAAGAGC<br>CGAGCAAGCTTCACAGGAGGTAAAAAATTGGATGA<br>CAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATT<br>GTAAGACTATTTTAAAAGCATTGGGACCAGCAGCTA<br>CACTAGAAGAAATGATGACAGCATGTCAGGGAGTG<br>GGAGGACCCAGCCATAAAGCAAGAATTTTGGCAGA<br>AGCAATGAGCCAAGTAACAAATTCAGCTACCATAA<br>TGATGCAGAAAGGCAATTTTAGGAACCAAAGAAAG<br>ATTGTTAAATGTTTCAATTGTGGCAAAGAAGGGCAC<br>ATAGCCAGAAACTGCAGGGCCCCTAGGAAAAGGGG<br>CTGTTGGAAATGTGGAAAGGAAGGACACCAAATGA<br>AAGACTGTACTGAGAGACAGGCTAATTTTTTAGGGA<br>AAATCTGGCCTTCCCACAAAGGAAGGCCAGGGAAT<br>TTCCTTCAGAGCAGACCAGAGCCAACAGCCCCACC<br>AGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAAG<br>AGACAACAACTCCCTCTCAGAAGCAGGAGCTGATA |

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| | | GACAAGGAACTGTATCCTTTAGCTTCCCTCAGATCA<br>CTCTTTGGCAACGACCCCTCGTCACAATAA |
| 70 | HIV Pol; Bal | ATGAATTTGCCAGGAAGATGGAAACCAAAAATGAT<br>AGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGT<br>ATGATCAGATACTCATAGAAATCTGTGGACATAAA<br>GCTATAGGTACAGTATTAATAGGACCTACACCTGTC<br>AACATAATTGGAAGAAATCTGTTGACTCAGATTGGT<br>TGCACTTTAAATTTTCCCATTAGTCCTATTGAAACTG<br>TACCAGTAAAATTAAAACCAGGAATGGATGGCCCA<br>AAAGTTAAACAATGGCCACTGACAGAAGAAAAAAT<br>AAAAGCATTAATGGAAATCTGTACAGAAATGGAAA<br>AGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAAT<br>CCATACAATACTCCAGTATTTGCCATAAAGAAAAAA<br>GACAGTACTAAATGGAGAAAATTAGTAGATTTCAG<br>AGAACTTAATAAGAAAACTCAAGACTTCTGGGAAG<br>TACAATTAGGAATACACATCCCGCAGGGGTTAAAA<br>AAGAAAAAATCAGTAACAGTACTGGATGTGGGTGA<br>TGCATATTTTTCAGTTCCCTTAGATAAAGAATTCAG<br>GAAGTATACTGCATTTACCATACCTAGTATAAACAA<br>TGAAACACCAGGGATCAGATATCAGTACAATGTAC<br>TTCCACAGGGATGGAAAGGATCACCAGCAATATTTC<br>AAAGTAGCATGACAAGAATCTTAGAGCCTTTTAGA<br>AAACAAAATCCAGAAATAGTGATCTATCAATACAT<br>GGATGATTTGTATGTAGGATCTGACTTAGAAATAGG<br>GCAGCATAGAACAAAAATAGAGGAACTGAGACAAC<br>ATCTGTTGAGGTGGGGATTTACCACACCAGACAAA<br>AAACATCAGAAAGAACCTCCATTCCTTTGGATGGGT<br>TATGAACTCCATCCTGATAAATGGACAGTACAGCCT<br>ATAGTGCTGCCAGAAAAAGACAGCTGGACTGTCAA<br>TGACATACAGAAGTTAGTGGGAAAATTGAATTGGG<br>CAAGTCAGATTTACCCAGGAATTAAAGTAAAGCAA<br>TTATGTAGGCTCCTTAGGGGAACCAAGGCATTAACA<br>GAAGTAATACCACTAACAAAAGAAACAGAGCTAGA |

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| | | ACTGGCAGAGAACAGGGAAATTCTAAAAGAACCAG<br>TACATGGGGTGTATTATGACCCATCAAAAGACTTAA<br>TAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGG<br>ACATATCAAATTTATCAAGAGCCATTTAAAAATCTG<br>AAAACAGGAAAATATGCAAGAATGAGGGGTGCCCA<br>CACTAATGATGTAAAACAATTAACAGAGGCAGTGC<br>AAAAAATAACCACAGAAAGCATAGTAATATGGGGA<br>AAGACTCCTAAATTTAAACTACCCATACAAAAAGA<br>AACATGGGAAACATGGTGGACAGAGTATTGGCAAG<br>CCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCC<br>CTCCCTTAGTGAAATTATGGTACCAGTTAGAGAAAG<br>AACCCATAATAGGAGCAGAAACATTCTATGTAGAT<br>GGAGCAGCTAACCGGGAGACTAAATTAGGAAAAGC<br>AGGATATGTTACTAACAGAGGAAGACAAAAAGTTG<br>TCTCCCTAACTGACACAACAAATCAGAAGACTGAGT<br>TACAAGCAATTCATCTAGCTTTACAAGATTCAGGAT<br>TAGAAGTAAACATAGTAACAGACTCACAATATGCA<br>TTAGGAATCATTCAAGCACAACCAGATAAAAGTGA<br>ATCAGAGTTAGTCAGTCAAATAATAGAACAGTTAAT<br>AAAAAAGGAAAAGGTCTACCTGGCATGGGTACCAG<br>CGCACAAAGGAATTGGAGGAAATGAACAAGTAGAT<br>AAATTAGTCAGTACTGGAATCAGGAAAGTACTA |
| 71 | HIV Integrase; Bal | TTTTTAGATGGAATAGATATAGCCCAAGAAGAACAT<br>GAGAAATATCACAGTAATTGGAGAGCAATGGCTAG<br>TGATTTTAACCTGCCACCTGTGGTAGCAAAAGAAAT<br>AGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAG<br>AAGCCATGCATGGACAAGTAGACTGTAGTCCAGGA<br>ATATGGCAACTAGATTGTACACATTTAGAAGGAAA<br>AATTATCCTGGTAGCAGTTCATGTAGCCAGTGGATA<br>TATAGAAGCAGAAGTTATTCCAGCAGAGACAGGGC<br>AGGAAACAGCATACTTTCTCTTAAAATTAGCAGGAA<br>GATGGCCAGTAAAAACAATACATACAGACAATGGC<br>AGCAATTTCACTAGTACTACAGTCAAGGCCGCCTGT |

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| | | TGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCC<br>CTACAATCCCCAAAGTCAGGGAGTAGTAGAATCTAT<br>AAATAAAGAATTAAAGAAAATTATAGGACAGGTAA<br>GAGATCAGGCTGAACATCTTAAAACAGCAGTACAA<br>ATGGCAGTATTCATCCACAATTTTAAAAGAAAAGG<br>GGGGATTGGGGGGTATAGTGCAGGGGAAAGAATAG<br>TAGACATAATAGCAACAGACATACAAACTAAAGAA<br>TTACAAAAACAAATTACAAAAATTCAAAATTTTCGG<br>GTTTATTACAGGGACAGCAGAGATCCACTTTGGAAA<br>GGACCAGCAAAGCTTCTCTGGAAAGGTGAAGGGGC<br>AGTAGTAATACAAGATAATAGTGACATAAAAGTAG<br>TACCAAGAAGAAAAGCAAAGATCATTAGGGATTAT<br>GGAAAACAGATGGCAGGTGATGATTGTGTGGCAAG<br>TAGACAGGATGAGGATTAG |
| 72 | Envelope;<br>RD114 | ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGC<br>CTAATAATAGTTCGGGCAGGGTTTGACGACCCCCGC<br>AAGGCTATCGCATTAGTACAAAAACAACATGGTAA<br>ACCATGCGAATGCAGCGGAGGGCAGGTATCCGAGG<br>CCCCACCGAACTCCATCCAACAGGTAACTTGCCCAG<br>GCAAGACGGCCTACTTAATGACCAACCAAAAATGG<br>AAATGCAGAGTCACTCCAAAAAATCTCACCCCTAGC<br>GGGGGAGAACTCCAGAACTGCCCCTGTAACACTTTC<br>CAGGACTCGATGCACAGTTCTTGTTATACTGAATAC<br>CGGCAATGCAGGGCGAATAATAAGACATACTACAC<br>GGCCACCTTGCTTAAAATACGGTCTGGGAGCCTCAA<br>CGAGGTACAGATATTACAAAACCCCAATCAGCTCCT<br>ACAGTCCCCTTGTAGGGGCTCTATAAATCAGCCCGT<br>TTGCTGGAGTGCCACAGCCCCCATCCATATCTCCGA<br>TGGTGGAGGACCCCTCGATACTAAGAGAGTGTGGA<br>CAGTCCAAAAAAGGCTAGAACAAATTCATAAGGCT<br>ATGCATCCTGAACTTCAATACCACCCCTTAGCCCTG<br>CCCAAAGTCAGAGATGACCTTAGCCTTGATGCACGG<br>ACTTTTGATATCCTGAATACCACTTTTAGGTTACTCC |

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| | | AGATGTCCAATTTTAGCCTTGCCCAAGATTGTTGGC<br>TCTGTTTAAAACTAGGTACCCCTACCCCTCTTGCGA<br>TACCCACTCCCTCTTTAACCTACTCCCTAGCAGACTC<br>CCTAGCGAATGCCTCCTGTCAGATTATACCTCCCCT<br>CTTGGTTCAACCGATGCAGTTCTCCAACTCGTCCTG<br>TTTATCTTCCCCTTTCATTAACGATACGGAACAAAT<br>AGACTTAGGTGCAGTCACCTTTACTAACTGCACCTC<br>TGTAGCCAATGTCAGTAGTCCTTTATGTGCCCTAAA<br>CGGGTCAGTCTTCCTCTGTGGAAATAACATGGCATA<br>CACCTATTTACCCCAAAACTGGACAGGACTTTGCGT<br>CCAAGCCTCCCTCCTCCCCGACATTGACATCATCCC<br>GGGGGATGAGCCAGTCCCCATTCCTGCCATTGATCA<br>TTATATACATAGACCTAAACGAGCTGTACAGTTCAT<br>CCCTTTACTAGCTGGACTGGGAATCACCGCAGCATT<br>CACCACCGGAGCTACAGGCCTAGGTGTCTCCGTCAC<br>CCAGTATACAAAATTATCCCATCAGTTAATATCTGA<br>TGTCCAAGTCTTATCCGGTACCATACAAGATTTACA<br>AGACCAGGTAGACTCGTTAGCTGAAGTAGTTCTCCA<br>AAATAGGAGGGGACTGGACCTACTAACGGCAGAAC<br>AAGGAGGAATTTGTTTAGCCTTACAAGAAAAATGCT<br>GTTTTTATGCTAACAAGTCAGGAATTGTGAGAAACA<br>AAATAAGAACCCTACAAGAAGAATTACAAAAACGC<br>AGGGAAAGCCTGGCATCCAACCCTCTCTGGACCGG<br>GCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTCCTG<br>GGACCCCTACTCACCCTCCTACTCATACTAACCATT<br>GGGCCATGCGTTTTCAATCGATTGGTCCAATTTGTT<br>AAAGACAGGATCTCAGTGGTCCAGGCTCTGGTTTTG<br>ACTCAGCAATATCACCAGCTAAAACCCATAGAGTA<br>CGAGCCATGA |
| 73 | Envelope;<br>GALV | ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCAC<br>CAGATGAGTCCTGGGAGCTGGAAAAGACTGATCAT<br>CCTCTTAAGCTGCGTATTCGGAGACGGCAAAACGA<br>GTCTGCAGAATAAGAACCCCCACCAGCCTGTGACCC |

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| | | TCACCTGGCAGGTACTGTCCCAAACTGGGGACGTTG<br>TCTGGGACAAAAAGGCAGTCCAGCCCCTTTGGACTT<br>GGTGGCCCTCTCTTACACCTGATGTATGTGCCCTGG<br>CGGCCGGTCTTGAGTCCTGGGATATCCCGGGATCCG<br>ATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATT<br>CAGACTATACTGCCGCTTATAAGCAAATCACCTGGG<br>GAGCCATAGGGTGCAGCTACCCTCGGGCTAGGACC<br>AGGATGGCAAATTCCCCCTTCTACGTGTGTCCCCGA<br>GCTGGCCGAACCCATTCAGAAGCTAGGAGGTGTGG<br>GGGGCTAGAATCCCTATACTGTAAAGAATGGAGTT<br>GTGAGACCACGGGTACCGTTTATTGGCAACCCAAGT<br>CCTCATGGGACCTCATAACTGTAAAATGGGACCAA<br>AATGTGAAATGGGAGCAAAAATTTCAAAAGTGTGA<br>ACAAACCGGCTGGTGTAACCCCCTCAAGATAGACTT<br>CACAGAAAAAGGAAAACTCTCCAGAGATTGGATAA<br>CGGAAAAAACCTGGGAATTAAGGTTCTATGTATATG<br>GACACCCAGGCATACAGTTGACTATCCGCTTAGAGG<br>TCACTAACATGCCGGTTGTGGCAGTGGGCCCAGACC<br>CTGTCCTTGCGGAACAGGGACCTCCTAGCAAGCCCC<br>TCACTCTCCCTCTCTCCCCACGGAAAGCGCCGCCCA<br>CCCCTCTACCCCCGGCGGCTAGTGAGCAAACCCCTG<br>CGGTGCATGGAGAAACTGTTACCCTAAACTCTCCGC<br>CTCCCACCAGTGGCGACCGACTCTTTGGCCTTGTGC<br>AGGGGGCCTTCCTAACCTTGAATGCTACCAACCCAG<br>GGGCCACTAAGTCTTGCTGGCTCTGTTTGGGCATGA<br>GCCCCCCTTATTATGAAGGGATAGCCTCTTCAGGAG<br>AGGTCGCTTATACCTCCAACCATACCCGATGCCACT<br>GGGGGGCCCAAGGAAAGCTTACCCTCACTGAGGTC<br>TCCGGACTCGGGTCATGCATAGGGAAGGTGCCTCTT<br>ACCCATCAACATCTTTGCAACCAGACCTTACCCATC<br>AATTCCTCTAAAAACCATCAGTATCTGCTCCCCTCA<br>AACCATAGCTGGTGGGCCTGCAGCACTGGCCTCACC<br>CCCTGCCTCTCCACCTCAGTTTTTAATCAGTCTAAAG |

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| | | ACTTCTGTGTCCAGGTCCAGCTGATCCCCCGCATCT<br>ATTACCATTCTGAAGAAACCTTGTTACAAGCCTATG<br>ACAAATCACCCCCCAGGTTTAAAAGAGAGCCTGCCT<br>CACTTACCCTAGCTGTCTTCCTGGGGTTAGGGATTG<br>CGGCAGGTATAGGTACTGGCTCAACCGCCCTAATTA<br>AAGGGCCCATAGACCTCCAGCAAGGCCTAACCAGC<br>CTCCAAATCGCCATTGACGCTGACCTCCGGGCCCTT<br>CAGGACTCAATCAGCAAGCTAGAGGACTCACTGAC<br>TTCCCTATCTGAGGTAGTACTCCAAAATAGGAGAGG<br>CCTTGACTTACTATTCCTTAAAGAAGGAGGCCTCTG<br>CGCGGCCCTAAAAGAAGAGTGCTGTTTTTATGTAGA<br>CCACTCAGGTGCAGTACGAGACTCCATGAAAAAAC<br>TTAAAGAAAGACTAGATAAAAGACAGTTAGAGCGC<br>CAGAAAAACCAAAACTGGTATGAAGGGTGGTTCAA<br>TAACTCCCCTTGGTTTACTACCCTACTATCAACCATC<br>GCTGGGCCCCTATTGCTCCTCCTTTTGTTACTCACTC<br>TTGGGCCCTGCATCATCAATAAATTAATCCAATTCA<br>TCAATGATAGGATAAGTGCAGTCAAAATTTTAGTCC<br>TTAGACAGAAATATCAGACCCTAGATAACGAGGAA<br>AACCTTTAA |
| 74 | Envelope; FUG | ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGG<br>GTTTTTCGTTGTGTTTCGGGAAGTTCCCCATTTACAC<br>GATACCAGACGAACTTGGTCCCTGGAGCCCTATTGA<br>CATACACCATCTCAGCTGTCCAAATAACCTGGTTGT<br>GGAGGATGAAGGATGTACCAACCTGTCCGAGTTCTC<br>CTACATGGAACTCAAAGTGGGATACATCTCAGCCAT<br>CAAAGTGAACGGGTTCACTTGCACAGGTGTTGTGAC<br>AGAGGCAGAGACCTACACCAACTTTGTTGGTTATGT<br>CACAACCACATTCAAGAGAAAGCATTTCCGCCCCAC<br>CCCAGACGCATGTAGAGCCGCGTATAACTGGAAGA<br>TGGCCGGTGACCCCAGATATGAAGAGTCCCTACAC<br>AATCCATACCCCGACTACCACTGGCTTCGAACTGTA<br>AGAACCACCAAAGAGTCCCTCATTATCATATCCCCA |

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| | | AGTGTGACAGATTTGGACCCATATGACAAATCCCTT<br>CACTCAAGGGTCTTCCCTGGCGGAAAGTGCTCAGGA<br>ATAACGGTGTCCTCTACCTACTGCTCAACTAACCAT<br>GATTACACCATTTGGATGCCCGAGAATCCGAGACCA<br>AGGACACCTTGTGACATTTTTACCAATAGCAGAGGG<br>AAGAGAGCATCCAACGGGAACAAGACTTGCGGCTT<br>TGTGGATGAAAGAGGCCTGTATAAGTCTCTAAAAG<br>GAGCATGCAGGCTCAAGTTATGTGGAGTTCTTGGAC<br>TTAGACTTATGGATGGAACATGGGTCGCGATGCAA<br>ACATCAGATGAGACCAAATGGTGCCCTCCAGATCA<br>GTTGGTGAATTTGCACGACTTTCGCTCAGACGAGAT<br>CGAGCATCTCGTTGTGGAGGAGTTAGTTAAGAAAA<br>GAGAGGAATGTCTGGATGCATTAGAGTCCATCATG<br>ACCACCAAGTCAGTAAGTTTCAGACGTCTCAGTCAC<br>CTGAGAAAACTTGTCCCAGGGTTTGGAAAAGCATAT<br>ACCATATTCAACAAAACCTTGATGGAGGCTGATGCT<br>CACTACAAGTCAGTCCGGACCTGGAATGAGATCATC<br>CCCTCAAAAGGGTGTTTGAAAGTTGGAGGAAGGTG<br>CCATCCTCATGTGAACGGGGTGTTTTTCAATGGTAT<br>AATATTAGGGCCTGACGACCATGTCCTAATCCCAGA<br>GATGCAATCATCCCTCCTCCAGCAACATATGGAGTT<br>GTTGGAATCTTCAGTTATCCCCCTGATGCACCCCCT<br>GGCAGACCCTTCTACAGTTTTCAAAGAAGGTGATGA<br>GGCTGAGGATTTTGTTGAAGTTCACCTCCCCGATGT<br>GTACAAACAGATCTCAGGGGTTGACCTGGGTCTCCC<br>GAACTGGGGAAAGTATGTATTGATGACTGCAGGGG<br>CCATGATTGGCCTGGTGTTGATATTTTCCCTAATGA<br>CATGGTGCAGAGTTGGTATCCATCTTTGCATTAAAT<br>TAAAGCACACCAAGAAAAGACAGATTTATACAGAC<br>ATAGAGATGAACCGACTTGGAAAGTAA |
| 75 | Envelope;<br>LCMV | ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCT<br>CACATCATCGATGAGGTGATCAACATTGTCATTATT<br>GTGCTTATCGTGATCACGGGTATCAAGGCTGTCTAC |

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| | | AATTTTGCCACCTGTGGGATATTCGCATTGATCAGT |
| | | TTCCTACTTCTGGCTGGCAGGTCCTGTGGCATGTAC |
| | | GGTCTTAAGGGACCCGACATTTACAAAGGAGTTTAC |
| | | CAATTTAAGTCAGTGGAGTTTGATATGTCACATCTG |
| | | AACCTGACCATGCCCAACGCATGTTCAGCCAACAAC |
| | | TCCCACCATTACATCAGTATGGGGACTTCTGGACTA |
| | | GAATTGACCTTCACCAATGATTCCATCATCAGTCAC |
| | | AACTTTTGCAATCTGACCTCTGCCTTCAACAAAAAG |
| | | ACCTTTGACCACACACTCATGAGTATAGTTTCGAGC |
| | | CTACACCTCAGTATCAGAGGGAACTCCAACTATAAG |
| | | GCAGTATCCTGCGACTTCAACAATGGCATAACCATC |
| | | CAATACAACTTGACATTCTCAGATCGACAAAGTGCT |
| | | CAGAGCCAGTGTAGAACCTTCAGAGGTAGAGTCCT |
| | | AGATATGTTTAGAACTGCCTTCGGGGGGAAATACAT |
| | | GAGGAGTGGCTGGGGCTGGACAGGCTCAGATGGCA |
| | | AGACCACCTGGTGTAGCCAGACGAGTTACCAATAC |
| | | CTGATTATACAAAATAGAACCTGGGAAAACCACTG |
| | | CACATATGCAGGTCCTTTTGGGATGTCCAGGATTCT |
| | | CCTTTCCCAAGAGAAGACTAAGTTCTTCACTAGGAG |
| | | ACTAGCGGGCACATTCACCTGGACTTTGTCAGACTC |
| | | TTCAGGGGTGGAGAATCCAGGTGGTTATTGCCTGAC |
| | | CAAATGGATGATTCTTGCTGCAGAGCTTAAGTGTTT |
| | | CGGGAACACAGCAGTTGCGAAATGCAATGTAAATC |
| | | ATGATGCCGAATTCTGTGACATGCTGCGACTAATTG |
| | | ACTACAACAAGGCTGCTTTGAGTAAGTTCAAAGAG |
| | | GACGTAGAATCTGCCTTGCACTTATTCAAAACAACA |
| | | GTGAATTCTTTGATTTCAGATCAACTACTGATGAGG |
| | | AACCACTTGAGAGATCTGATGGGGGTGCCATATTGC |
| | | AATTACTCAAAGTTTTGGTACCTAGAACATGCAAAG |
| | | ACCGGCGAAACTAGTGTCCCCAAGTGCTGGCTTGTC |
| | | ACCAATGGTTCTTACTTAAATGAGACCCACTTCAGT |
| | | GATCAAATCGAACAGGAAGCCGATAACATGATTAC |
| | | AGAGATGTTGAGGAAGGATTACATAAAGAGGCAGG |

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| | | GGAGTACCCCCCTAGCATTGATGGACCTTCTGATGT<br>TTTCCACATCTGCATATCTAGTCAGCATCTTCCTGCA<br>CCTTGTCAAAATACCAACACACAGGCACATAAAAG<br>GTGGCTCATGTCCAAAGCCACACCGATTAACCAACA<br>AAGGAATTTGTAGTTGTGGTGCATTTAAGGTGCCTG<br>GTGTAAAAACCGTCTGGAAAAGACGCTGA |
| 76 | Envelope; FPV | ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCA<br>GTCATCCCCACAAATGCAGACAAAATTTGTCTTGGA<br>CATCATGCTGTATCAAATGGCACCAAAGTAAACAC<br>ACTCACTGAGAGAGGAGTAGAAGTTGTCAATGCAA<br>CGGAAACAGTGGAGCGGACAAACATCCCCAAAATT<br>TGCTCAAAAGGGAAAAGAACCACTGATCTTGGCCA<br>ATGCGGACTGTTAGGGACCATTACCGGACCACCTCA<br>ATGCGACCAATTTCTAGAATTTTCAGCTGATCTAAT<br>AATCGAGAGACGAGAAGGAAATGATGTTTGTTACC<br>CGGGGAAGTTTGTTAATGAAGAGGCATTGCGACAA<br>ATCCTCAGAGGATCAGGTGGGATTGACAAAGAAAC<br>AATGGGATTCACATATAGTGGAATAAGGACCAACG<br>GAACAACTAGTGCATGTAGAAGATCAGGGTCTTCAT<br>TCTATGCAGAAATGGAGTGGCTCCTGTCAAATACAG<br>ACAATGCTGCTTTCCCACAAATGACAAAATCATACA<br>AAAACACAAGGAGAGAATCAGCTCTGATAGTCTGG<br>GGAATCCACCATTCAGGATCAACCACCGAACAGAC<br>CAAACTATATGGGAGTGGAAATAAACTGATAACAG<br>TCGGGAGTTCCAAATATCATCAATCTTTTGTGCCGA<br>GTCCAGGAACACGACCGCAGATAAATGGCCAGTCC<br>GGACGGATTGATTTTCATTGGTTGATCTTGGATCCC<br>AATGATACAGTTACTTTTAGTTTCAATGGGGCTTTC<br>ATAGCTCCAAATCGTGCCAGCTTCTTGAGGGGAAAG<br>TCCATGGGGATCCAGAGCGATGTGCAGGTTGATGCC<br>AATTGCGAAGGGGAATGCTACCACAGTGGAGGGAC<br>TATAACAAGCAGATTGCCTTTTCAAAACATCAATAG<br>CAGAGCAGTTGGCAAATGCCCAAGATATGTAAAAC |

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| | | AGGAAAGTTTATTATTGGCAACTGGGATGAAGAAC<br>GTTCCCGAACCTTCCAAAAAAAGGAAAAAAAGAGG<br>CCTGTTTGGCGCTATAGCAGGGTTTATTGAAAATGG<br>TTGGGAAGGTCTGGTCGACGGGTGGTACGGTTTCAG<br>GCATCAGAATGCACAAGGAGAAGGAACTGCAGCAG<br>ACTACAAAAGCACCCAATCGGCAATTGATCAGATA<br>ACCGGAAAGTTAAATAGACTCATTGAGAAAACCAA<br>CCAGCAATTTGAGCTAATAGATAATGAATTCACTGA<br>GGTGGAAAAGCAGATTGGCAATTTAATTAACTGGA<br>CCAAAGACTCCATCACAGAAGTATGGTCTTACAATG<br>CTGAACTTCTTGTGGCAATGGAAAACCAGCACACTA<br>TTGATTTGGCTGATTCAGAGATGAACAAGCTGTATG<br>AGCGAGTGAGGAAACAATTAAGGGAAAATGCTGAA<br>GAGGATGGCACTGGTTGCTTTGAAATTTTTCATAAA<br>TGTGACGATGATTGTATGGCTAGTATAAGGAACAAT<br>ACTTATGATCACAGCAAATACAGAGAAGAAGCGAT<br>GCAAAATAGAATACAAATTGACCCAGTCAAATTGA<br>GTAGTGGCTACAAAGATGTGATACTTTGGTTTAGCT<br>TCGGGGCATCATGCTTTTTGCTTCTTGCCATTGCAAT<br>GGGCCTTGTTTTCATATGTGTGAAGAACGGAAACAT<br>GCGGTGCACTATTTGTATATAA |
| 77 | Envelope; RRV | AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACT<br>AGACCATACCTAGCACATTGCGCCGATTGCGGGGA<br>CGGGTACTTCTGCTATAGCCCAGTTGCTATCGAGGA<br>GATCCGAGATGAGGCGTCTGATGGCATGCTTAAGAT<br>CCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAG<br>GCACCCACGCCCACACGAAGCTCCGATATATGGCTG<br>GTCATGATGTTCAGGAATCTAAGAGAGATTCCTTGA<br>GGGTGTACACGTCCGCAGCGTGCTCCATACATGGGA<br>CGATGGGACACTTCATCGTCGCACACTGTCCACCAG<br>GCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATT<br>CGCACGTGAAGGCATGTAAGGTCCAATACAAGCAC<br>AATCCATTGCCGGTGGGTAGAGAGAAGTTCGTGGTT |

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| | | AGACCACACTTTGGCGTAGAGCTGCCATGCACCTCA<br>TACCAGCTGACAACGGCTCCCACCGACGAGGAGAT<br>TGACATGCATACACCGCCAGATATACCGGATCGCAC<br>CCTGCTATCACAGACGGCGGGCAACGTCAAAATAA<br>CAGCAGGCGGCAGGACTATCAGGTACAACTGTACC<br>TGCGGCCGTGACAACGTAGGCACTACCAGTACTGA<br>CAAGACCATCAACACATGCAAGATTGACCAATGCC<br>ATGCTGCCGTCACCAGCCATGACAAATGGCAATTTA<br>CCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTA<br>GGAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTA<br>ACGTCACCTGCCGAGTGCCGTTGGCTCGAGCGCCGG<br>ATGCCACCTATGGTAAGAAGGAGGTGACCCTGAGA<br>TTACACCCAGATCATCCGACGCTCTTCTCCTATAGG<br>AGTTTAGGAGCCGAACCGCACCCGTACGAGGAATG<br>GGTTGACAAGTTCTCTGAGCGCATCATCCCAGTGAC<br>GGAAGAAGGGATTGAGTACCAGTGGGGCAACAACC<br>CGCCGGTCTGCCTGTGGGCGCAACTGACGACCGAG<br>GGCAAACCCCATGGCTGGCCACATGAAATCATTCA<br>GTACTATTATGGACTATACCCCGCCGCCACTATTGC<br>CGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAAC<br>TCTGGCGGCCACATGCTGCATGCTGGCCACCGCGAG<br>GAGAAAGTGCCTAACACCGTACGCCCTGACGCCAG<br>GAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCT<br>GCGCACCGAGGGCGAATGCA |
| 78 | Envelope; MLV 10A1 | AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACT<br>AGACCATACCTAGCACATTGCGCCGATTGCGGGGA<br>CGGGTACTTCTGCTATAGCCCAGTTGCTATCGAGGA<br>GATCCGAGATGAGGCGTCTGATGGCATGCTTAAGAT<br>CCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAG<br>GCACCCACGCCCACACGAAGCTCCGATATATGGCTG<br>GTCATGATGTTCAGGAATCTAAGAGAGATTCCTTGA<br>GGGTGTACACGTCCGCAGCGTGCTCCATACATGGGA<br>CGATGGGACACTTCATCGTCGCACACTGTCCACCAG |

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| | | GCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATT<br>CGCACGTGAAGGCATGTAAGGTCCAATACAAGCAC<br>AATCCATTGCCGGTGGGTAGAGAGAAGTTCGTGGTT<br>AGACCACACTTTGGCGTAGAGCTGCCATGCACCTCA<br>TACCAGCTGACAACGGCTCCCACCGACGAGGAGAT<br>TGACATGCATACACCGCCAGATATACCGGATCGCAC<br>CCTGCTATCACAGACGGCGGGCAACGTCAAAATAA<br>CAGCAGGCGGCAGGACTATCAGGTACAACTGTACC<br>TGCGGCCGTGACAACGTAGGCACTACCAGTACTGA<br>CAAGACCATCAACACATGCAAGATTGACCAATGCC<br>ATGCTGCCGTCACCAGCCATGACAAATGGCAATTTA<br>CCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTA<br>GGAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTA<br>ACGTCACCTGCCGAGTGCCGTTGGCTCGAGCGCCGG<br>ATGCCACCTATGGTAAGAAGGAGGTGACCCTGAGA<br>TTACACCCAGATCATCCGACGCTCTTCTCCTATAGG<br>AGTTTAGGAGCCGAACCGCACCCGTACGAGGAATG<br>GGTTGACAAGTTCTCTGAGCGCATCATCCCAGTGAC<br>GGAAGAAGGGATTGAGTACCAGTGGGGCAACAACC<br>CGCCGGTCTGCCTGTGGGCGCAACTGACGACCGAG<br>GGCAAACCCCATGGCTGGCCACATGAAATCATTCA<br>GTACTATTATGGACTATACCCCGCCGCCACTATTGC<br>CGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAAC<br>TCTGGCGGCCACATGCTGCATGCTGGCCACCGCGAG<br>GAGAAAGTGCCTAACACCGTACGCCCTGACGCCAG<br>GAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCT<br>GCGCACCGAGGGCGAATGCA |
| 79 | Envelope; Ebola | ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGAT<br>CGATTCAAGAGGACATCATTCTTTCTTTGGGTAATT<br>ATCCTTTTCCAAAGAACATTTTCCATCCCACTTGGA<br>GTCATCCACAATAGCACATTACAGGTTAGTGATGTC<br>GACAAACTGGTTTGCCGTGACAAACTGTCATCCACA<br>AATCAATTGAGATCAGTTGGACTGAATCTCGAAGG |

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| | | GAATGGAGTGGCAACTGACGTGCCATCTGCAACTA |
| | | AAAGATGGGGCTTCAGGTCCGGTGTCCCACCAAAG |
| | | GTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAA |
| | | CTGCTACAATCTTGAAATCAAAAAACCTGACGGGA |
| | | GTGAGTGTCTACCAGCAGCGCCAGACGGGATTCGG |
| | | GGCTTCCCCCGGTGCCGGTATGTGCACAAAGTATCA |
| | | GGAACGGGACCGTGTGCCGGAGACTTTGCCTTCCAC |
| | | AAAGAGGGTGCTTTCTTCCTGTATGACCGACTTGCT |
| | | TCCACAGTTATCTACCGAGGAACGACTTTCGCTGAA |
| | | GGTGTCGTTGCATTTCTGATACTGCCCCAAGCTAAG |
| | | AAGGACTTCTTCAGCTCACACCCCTTGAGAGAGCCG |
| | | GTCAATGCAACGGAGGACCCGTCTAGTGGCTACTAT |
| | | TCTACCACAATTAGATATCAAGCTACCGGTTTTGGA |
| | | ACCAATGAGACAGAGTATTTGTTCGAGGTTGACAAT |
| | | TTGACCTACGTCCAACTTGAATCAAGATTCACACCA |
| | | CAGTTTCTGCTCCAGCTGAATGAGACAATATATACA |
| | | AGTGGGAAAAGGAGCAATACCACGGGAAAACTAAT |
| | | TTGGAAGGTCAACCCCGAAATTGATACAACAATCG |
| | | GGGAGTGGGCCTTCTGGGAAACTAAAAAAACCTCA |
| | | CTAGAAAAATTCGCAGTGAAGAGTTGTCTTTCACAG |
| | | CTGTATCAAACAGAGCCAAAAACATCAGTGGTCAG |
| | | AGTCCGGCGCGAACTTCTTCCGACCCAGGGACCAAC |
| | | ACAACAACTGAAGACCACAAAATCATGGCTTCAGA |
| | | AAATTCCTCTGCAATGGTTCAAGTGCACAGTCAAGG |
| | | AAGGGAAGCTGCAGTGTCGCATCTGACAACCCTTGC |
| | | CACAATCTCCACGAGTCCTCAACCCCCCACAACCAA |
| | | ACCAGGTCCGGACAACAGCACCCACAATACACCCG |
| | | TGTATAAACTTGACATCTCTGAGGCAACTCAAGTTG |
| | | AACAACATCACCGCAGAACAGACAACGACAGCACA |
| | | GCCTCCGACACTCCCCCCGCCACGACCGCAGCCGGA |
| | | CCCCTAAAAGCAGAGAACACCAACACGAGCAAGGG |
| | | TACCGACCTCCTGGACCCCGCCACCACAACAAGTCC |
| | | CCAAAACCACAGCGAGACCGCTGGCAACAACAACA |

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| | | CTCATCACCAAGATACCGGAGAAGAGAGTGCCAGC<br>AGCGGGAAGCTAGGCTTAATTACCAATACTATTGCT<br>GGAGTCGCAGGACTGATCACAGGCGGGAGGAGAGC<br>TCGAAGAGAAGCAATTGTCAATGCTCAACCCAAAT<br>GCAACCCTAATTTACATTACTGGACTACTCAGGATG<br>AAGGTGCTGCAATCGGACTGGCCTGGATACCATATT<br>TCGGGCCAGCAGCCGAGGGAATTTACATAGAGGGG<br>CTGATGCACAATCAAGATGGTTTAATCTGTGGGTTG<br>AGACAGCTGGCCAACGAGACGACTCAAGCTCTTCA<br>ACTGTTCCTGAGAGCCACAACCGAGCTACGCACCTT<br>TTCAATCCTCAACCGTAAGGCAATTGATTTCTTGCT<br>GCAGCGATGGGGCGGCACATGCCACATTTTGGGAC<br>CGGACTGCTGTATCGAACCACATGATTGGACCAAG<br>AACATAACAGACAAAATTGATCAGATTATTCATGAT<br>TTTGTTGATAAAACCCTTCCGGACCAGGGGGACAAT<br>GACAATTGGTGGACAGGATGGAGACAATGGATACC<br>GGCAGGTATTGGAGTTACAGGCGTTATAATTGCAGT<br>TATCGCTTTATTCTGTATATGCAAATTTGTCTTTTAG |
| 80 | Short WPRE sequence | AATCAACCTCTGGATTACAAAATTTGTGAAAGATTG<br>ACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGT<br>GTGGATATGCTGCTTTAATGCCTCTGTATCATGCTAT<br>TGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTAT<br>AAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGG<br>CCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTG<br>TTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCC<br>ACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCC<br>CCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCT<br>GCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGG<br>GCACTGATAATTCCGTGGTGTTGTC |
| 81 | Primer | TAAGCAGAATTC ATGAATTTGCCAGGAAGAT |
| 82 | Primer | CCATACAATGAATGGACACTAGGCGGCCGCACGAA<br>T |

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| 83 | Gag, Pol, Integrase fragment | GAATTCATGAATTTGCCAGGAAGATGGAAACCAAA<br>AATGATAGGGGGAATTGGAGGTTTTATCAAAGTAA<br>GACAGTATGATCAGATACTCATAGAAATCTGCGGA<br>CATAAAGCTATAGGTACAGTATTAGTAGGACCTACA<br>CCTGTCAACATAATTGGAAGAAATCTGTTGACTCAG<br>ATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTG<br>AGACTGTACCAGTAAAATTAAAGCCAGGAATGGAT<br>GGCCCAAAAGTTAAACAATGGCCATTGACAGAAGA<br>AAAAATAAAAGCATTAGTAGAAATTTGTACAGAAA<br>TGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT<br>GAAAATCCATACAATACTCCAGTATTTGCCATAAAG<br>AAAAAAGACAGTACTAAATGGAGAAAATTAGTAGA<br>TTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTG<br>GGAAGTTCAATTAGGAATACCACATCCTGCAGGGTT<br>AAAACAGAAAAAATCAGTAACAGTACTGGATGTGG<br>GCGATGCATATTTTTCAGTTCCCTTAGATAAAGACT<br>TCAGGAAGTATACTGCATTTACCATACCTAGTATAA<br>ACAATGAGACACCAGGGATTAGATATCAGTACAAT<br>GTGCTTCCACAGGGATGGAAAGGATCACCAGCAAT<br>ATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTT<br>TAGAAAACAAAATCCAGACATAGTCATCTATCAAT<br>ACATGGATGATTTGTATGTAGGATCTGACTTAGAAA<br>TAGGGCAGCATAGAACAAAAATAGAGGAACTGAGA<br>CAACATCTGTTGAGGTGGGGATTTACCACACCAGAC<br>AAAAAACATCAGAAAGAACCTCCATTCCTTTGGATG<br>GGTTATGAACTCCATCCTGATAAATGGACAGTACAG<br>CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGT<br>CAATGACATACAGAAATTAGTGGGAAAATTGAATT<br>GGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGG<br>CAATTATGTAAACTTCTTAGGGGAACCAAAGCACTA<br>ACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCT<br>AGAACTGGCAGAAAACAGGGAGATTCTAAAAGAAC<br>CGGTACATGGAGTGTATTATGACCCATCAAAAGACT |

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| | | TAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAA |
| | | TGGACATATCAAATTTATCAAGAGCCATTTAAAAAT |
| | | CTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGC |
| | | CCACACTAATGATGTGAAACAATTAACAGAGGCAG |
| | | TACAAAAAATAGCCACAGAAAGCATAGTAATATGG |
| | | GGAAAGACTCCTAAATTTAAATTACCCATACAAAA |
| | | GGAAACATGGGAAGCATGGTGGACAGAGTATTGGC |
| | | AAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATA |
| | | CCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGA |
| | | AAGAACCCATAATAGGAGCAGAAACTTTCTATGTA |
| | | GATGGGGCAGCCAATAGGGAAACTAAATTAGGAAA |
| | | AGCAGGATATGTAACTGACAGAGGAAGACAAAAAG |
| | | TTGTCCCCCTAACGGACACAACAAATCAGAAGACT |
| | | GAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCG |
| | | GGATTAGAAGTAAACATAGTGACAGACTCACAATA |
| | | TGCATTGGGAATCATTCAAGCACAACCAGATAAGA |
| | | GTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAG |
| | | TTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT |
| | | ACCAGCACACAAAGGAATTGGAGGAAATGAACAAG |
| | | TAGATAAATTGGTCAGTGCTGGAATCAGGAAAGTA |
| | | CTATTTTTAGATGGAATAGATAAGGCCCAAGAAGA |
| | | ACATGAGAAATATCACAGTAATTGGAGAGCAATGG |
| | | CTAGTGATTTTAACCTACCACCTGTAGTAGCAAAAG |
| | | AAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAA |
| | | GGGGAAGCCATGCATGGACAAGTAGACTGTAGCCC |
| | | AGGAATATGGCAGCTAGATTGTACACATTTAGAAG |
| | | GAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG |
| | | GATATATAGAAGCAGAAGTAATTCCAGCAGAGACA |
| | | GGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCA |
| | | GGAAGATGGCCAGTAAAAACAGTACATACAGACAA |
| | | TGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGC |
| | | CTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCA |
| | | TTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAAT |

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| | | CTATGAATAAAGAATTAAAGAAAATTATAGGACAG<br>GTAAGAGATCAGGCTGAACATCTTAAGACAGCAGT<br>ACAAATGGCAGTATTCATCCACAATTTTAAAAGAAA<br>AGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGA<br>ATAGTAGACATAATAGCAACAGACATACAAACTAA<br>AGAATTACAAAAACAAATTACAAAAATTCAAAATT<br>TTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTT<br>GGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAA<br>GGGGCAGTAGTAATACAAGATAATAGTGACATAAA<br>AGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGG<br>ATTATGGAAAACAGATGGCAGGTGATGATTGTGTG<br>GCAAGTAGACAGGATGAGGATTAA |
| 84 | DNA Fragment containing Rev, RRE and rabbit beta globin poly A | TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGA<br>AGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCT<br>CTATCAAAGCAACCCACCTCCCAATCCCGAGGGGA<br>CCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGG<br>AGAGAGAGACAGAGACAGATCCATTCGATTAGTGA<br>ACGGATCCTTGGCACTTATCTGGGACGATCTGCGGA<br>GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACT<br>TACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG<br>GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGG<br>AATCTCCTACAATATTGGAGTCAGGAGCTAAAGAAT<br>AGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCA<br>GGAAGCACTATGGGCGCAGCGTCAATGACGCTGAC<br>GGTACAGGCCAGACAATTATTGTCTGGTATAGTGCA<br>GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC<br>AACAGCATCTGTTGCAACTCACAGTCTGGGGCATCA<br>AGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGA<br>TACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCC<br>TCTGCCAAAAATTATGGGGACATCATGAAGCCCCTT<br>GAGCATCTGACTTCTGGCTAATAAAGGAAATTTATT<br>TTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCT<br>CACTCGGAAGGACATATGGGAGGGCAAATCATTTA |

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| | | AAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCA<br>ACATATGCCATATGCTGGCTGCCATGAACAAAGGTG<br>GCTATAAAGAGGTCATCAGTATATGAAACAGCCCC<br>CTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGA<br>CTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTT<br>ATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACAT<br>GTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTAC<br>TCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC<br>CCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGT<br>CATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCAC<br>AATTCCACACAACATACGAGCCGGAAGCATAAAGT<br>GTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTC<br>ACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAG<br>TCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCA<br>ATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGC<br>CCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATT<br>CTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGC<br>AGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCA<br>GAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTT<br>TTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAAT<br>GGTTACAAATAAAGCAATAGCATCACAAATTTCAC<br>AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT<br>TTGTCCAAACTCATCAATGTATCTTATCAGCGGCCG<br>CCCCGGG |
| 85 | DNA fragment containing the CAG enhancer/promoter/intron sequence | ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTC<br>ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTAC<br>ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC<br>CAACGACCCCCGCCCATTGACGTCAATAATGACGTA<br>TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTG<br>ACGTCAATGGGTGGACTATTTACGGTAAACTGCCCA<br>CTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC<br>GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC<br>CTGGCATTATGCCCAGTACATGACCTTATGGGACTT |

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| | | TCCTACTTGGCAGTACATCTACGTATTAGTCATCGC |
| | | TATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT |
| | | TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAAT |
| | | TTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCG |
| | | ATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGC |
| | | GGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGA |
| | | GGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGC |
| | | GCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGG |
| | | CGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCG |
| | | GGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCC |
| | | GCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTG |
| | | ACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGG |
| | | ACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGG |
| | | TTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGA |
| | | AAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCG |
| | | GGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGT |
| | | GTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCC |
| | | CGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGC |
| | | TTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC |
| | | CGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGA |
| | | GGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGG |
| | | GGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGG |
| | | CTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGC |
| | | TGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGC |
| | | GGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG |
| | | GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGG |
| | | CCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCG |
| | | CGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGC |
| | | GGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTG |
| | | CGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTG |
| | | GCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCC |
| | | CTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGG |
| | | CAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGT |

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| | | CGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTC<br>GGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGG<br>GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG<br>ACCGGCGGGAATTC |
| 86 | DNA fragment containing VSV-G | GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTAT<br>TCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTC<br>CACACAACCAAAAAGGAAACTGGAAAAATGTTCCT<br>TCTAATTACCATTATTGCCCGTCAAGCTCAGATTTA<br>AATTGGCATAATGACTTAATAGGCACAGCCTTACAA<br>GTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGC<br>AGACGGTTGGATGTGTCATGCTTCCAAATGGGTCAC<br>TACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT<br>AACACATTCCATCCGATCCTTCACTCCATCTGTAGA<br>ACAATGCAAGGAAAGCATTGAACAAACGAAACAAG<br>GAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTT<br>GTGGATATGCAACTGTGACGGATGCCGAAGCAGTG<br>ATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGAT<br>GAATACACAGGAGAATGGGTTGATTCACAGTTCATC<br>AACGGAAAATGCAGCAATTACATATGCCCCACTGTC<br>CATAACTCTACAACCTGGCATTCTGACTATAAGGTC<br>AAAGGGCTATGTGATTCTAACCTCATTTCCATGGAC<br>ATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCC<br>CTGGGAAAGGAGGGCACAGGGTTCAGAAGTAACTA<br>CTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAAT<br>GCAATACTGCAAGCATTGGGGAGTCAGACTCCCATC<br>AGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTT<br>TGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTC<br>AAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGT<br>AAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTA<br>TTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAG<br>CGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATC<br>TTGCTCCTAAAAACCCAGGAACCGGTCCTGCTTTCA<br>CCATAATCAATGGTACCCTAAAATACTTTGAGACCA |

| | | |
|---|---|---|
| | | GATACATCAGAGTCGATATTGCTGCTCCAATCCTCT<br>CAAGAATGGTCGGAATGATCAGTGGAACTACCACA<br>GAAAGGGAACTGTGGGATGACTGGGCACCATATGA<br>AGACGTGGAAATTGGACCCAATGGAGTTCTGAGGA<br>CCAGTTCAGGATATAAGTTTCCTTTATACATGATTG<br>GACATGGTATGTTGGACTCCGATCTTCATCTTAGCT<br>CAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAG<br>ACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTAT<br>TTTTTGGTGATACTGGGCTATCCAAAAATCCAATCG<br>AGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCT<br>CTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCAT<br>TGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTT<br>TGCATTAAATTAAAGCACACCAAGAAAAGACAGAT<br>TTATACAGACATAGAGATGAGAATTC |
| 87 | Helper plasmid containing RRE and rabbit beta globin poly A | TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGC<br>AGCAGGAAGCACTATGGGCGCAGCGTCAATGACGC<br>TGACGGTACAGGCCAGACAATTATTGTCTGGTATAG<br>TGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAG<br>GCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGC<br>ATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGA<br>AAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT<br>TCCCTCTGCCAAAAATTATGGGGACATCATGAAGCC<br>CCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATT<br>TATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGT<br>CTCTCACTCGGAAGGACATATGGGAGGGCAAATCA<br>TTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTT<br>GGCAACATATGCCATATGCTGGCTGCCATGAACAA<br>AGGTGGCTATAAAGAGGTCATCAGTATATGAAACA<br>GCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGC<br>CTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTT<br>GTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCT<br>TACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTG<br>ACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGA |

| | | |
|---|---|---|
| | | AGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATC<br>ATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCC<br>GCTCACAATTCCACACAACATACGAGCCGGAAGCA<br>TAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGC<br>TAACTCACATTAATTGCGTTGCGCTCACTGCCCGCT<br>TTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGC<br>ATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAA<br>CTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCG<br>CCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTAT<br>TTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCT<br>ATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCT<br>AGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCT<br>TATAATGGTTACAAATAAAGCAATAGCATCACAAA<br>TTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT<br>TGTGGTTTGTCCAAACTCATCAATGTATCTTATCACC<br>CGGG |
| 88 | RSV promoter and HIV Rev | CAATTGCGATGTACGGGCCAGATATACGCGTATCTG<br>AGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGGGC<br>TTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATAT<br>AGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTA<br>GTCTTATGCAATACACTTGTAGTCTTGCAACATGGT<br>AACGATGAGTTAGCAACATGCCTTACAAGGAGAGA<br>AAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGG<br>TGGTACGATCGTGCCTTATTAGGAAGGCAACAGAC<br>AGGTCTGACATGGATTGGACGAACCACTGAATTCCG<br>CATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCG<br>ATACAATAAACGCCATTTGACCATTCACCACATTGG<br>TGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCG<br>TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGA<br>CCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC<br>CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGA<br>AGAAGCGGAGACAGCGACGAAGAACTCCTCAAGGC<br>AGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC |

| | | |
|---|---|---|
| | | ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAA GGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAG ACAGATCCATTCGATTAGTGAACGGATCCTTAGCAC TTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCA GCTACCACCGCTTGAGAGACTTACTCTTGATTGTAA CGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGG GAAGCCCTCAAATATTGGTGGAATCTCCTACAATAT TGGAGTCAGGAGCTAAAGAATAGTCTAGA |
| 89 | Target sequence | ATGGCAGGAAGAAGCGGAG |
| 90 | shRNA sequence | ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCT TCTTCCTGCCATTTTTT |
| 91 | H1 promoter and shRT sequence | GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC ACTTGGATCCGCGGAGACAGCGACGAAGAGCTTCA AGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT |
| 92 | H1 CCR5 sequence | GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC ACTTGGATCCGTGTCAAGTCCAATCTATGTTCAAGA GACATAGATTGGACTTGACACTTTTT |
| 93 | Primer | AGGAATTGATGGCGAGAAGG |
| 94 | Primer | CCCCAAAGAAGGTCAAGGTAATCA |
| 95 | Primer | AGCGCGGCTACAGCTTCA |
| 96 | Primer | GGCGACGTAGCACAGCTTCP |

| | | |
|---|---|---|
| 97 | AGT103 CCR5 miR30 | TGTAAACTGAGCTTGCTCTA |
| 98 | AGT103-R5-1 | TGTAAACTGAGCTTGCTCGC |
| 99 | AGT103-R5-2 | CATAGATTGGACTTGACAC |
| 100 | CAG promoter | TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT TCATAGCCCATATATGGAGTTCCGCGTTACATAACT TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG ACCCCCGCCCATTGACGTCAATAATGACGTATGTTC CCATAGTAACGCCAATAGGGACTTTCCATTGACGTC AATGGGTGGACTATTTACGGTAAACTGCCCACTTGG CAGTACATCAAGTGTATCATATGCCAAGTACGCCCC CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC ATTATGCCCAGTACATGACCTTATGGGACTTTCCTA CTTGGCAGTACATCTACGTATTAGTCATCGCTATTA CCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACT CTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGT ATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGG GGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGC GGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGG AGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCT CCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCG GCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG |
| 101 | H1 element | GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC ACTT |
| 102 | 3’ LTR | TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACC AGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGA |

| | | |
|---|---|---|
| | | ACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAG<br>TGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT<br>CTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAG<br>TGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA |
| 103 | 7SK promoter | CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGC<br>ATTCTGGATAGTGTCAAAACAGCCGGAAATCAAGT<br>CCGTTTATCTCAAACTTTAGCATTTTGGGAATAAAT<br>GATATTTGCTATGCTGGTTAAATTAGATTTTAGTTA<br>AATTTCCTGCTGAAGCTCTAGTACGATAAGCAACTT<br>GACCTAAGTGTAAAGTTGAGATTTCCTTCAGGTTTA<br>TATAGCTTGTGCGCCGCCTGGCTACCTC |
| 104 | miR155 Tat | CTGGAGGCTTGCTGAAGGCTGTATGCTGTCCGCTTC<br>TTCCTGCCATAGGGTTTTGGCCACTGACTGACCCTA<br>TGGGGAAGAAGCGGACAGGACACAAGGCCTGTTAC<br>TAGCACTCACATGGAACAAATGGCC |

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

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

**CLAIMS**

1. A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO:31.

2. The lentiviral vector of claim 1, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO:31.

3. A lentiviral particle produced by a packaging cell and capable of infecting a target cell, the lentiviral particle comprising:

   a. an envelope protein capable of infecting the target cell; and

   b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO:31.

4. The lentiviral particle of claim 3, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO:31.

5. The lentiviral particle of claim 3 or claim 4, wherein the target cell is a CD4+ T cell.

6. A modified cell comprising a primary T cell infected with a lentiviral particle, wherein the lentiviral particle comprises:

   a. an envelope protein capable of infecting the target cell; and

   b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO:31.

7. The modified cell of claim 6, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO:31.

8. The modified cell of claim 6 or claim 7, wherein the primary T cell is a primary CD4+ T cell.

9. A method for the treatment of cells infected with HIV, the method comprising:

a. contact of peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of an *ex vivo* stimulatory agent, wherein the contact is conducted *ex vivo*;
b. transduction of the PBMC *ex vivo* with a lentiviral particle, wherein the lentiviral particle comprises:
    i. an envelope protein capable of infecting the PBMC; and
    ii. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 80% sequence identity with SEQ ID NO:31;

    and
c. culture of the transduced PBMC for at least about 1 day.

10. The method of claim 9, wherein the encoded microRNA cluster comprises a sequence having at least 85% sequence identity with SEQ ID NO:31.

11. The method of any one of claims 9 to 10, further comprising infusion of the transduced PBMC into a subject.

12. The method of any one of claims 9 to 11, further comprising positive selection of selected HIV-specific CD4+ T cells from the PBMC.

13. The method of any one of claims 9 to 12, further comprising immunization of the subject with an effective amount of an *in vivo* stimulatory agent, wherein the immunization occurs prior to contacting the peripheral blood mononuclear cells (PMBC) with the *ex vivo* stimulatory agent.

14. The method of claim 13, wherein each of the *in vivo* stimulatory agent and *ex vivo* stimulatory agent is independently selected from a peptide and a vaccine.

15. A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO:1, (ii) at least 90% sequence identity with SEQ ID NO:2, and (iii) at least 90% sequence identity with SEQ ID NO:3.

16. The lentiviral vector of claim 15, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO:1, (ii) at least 95% sequence identity with SEQ ID NO:2, or (iii) at least 95% sequence identity with SEQ ID NO:3.

17. The lentiviral vector of claim 15, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

18. A lentiviral particle capable of infecting a target cell, the lentiviral particle comprising:

a. an envelope protein capable of infecting the target cell; and

b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO:1, (ii) at least 90% sequence identity with SEQ ID NO:2, and (iii) at least 90% sequence identity with SEQ ID NO:3.

19. The lentiviral particle of claim 18, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO:1, (ii) at least 95% sequence identity with SEQ ID NO:2, or (iii) at least 95% sequence identity with SEQ ID NO:3.

20. The lentiviral particle of claim 19, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

21. The lentiviral particle of any one of claims 18 to 20, wherein the target cell is a CD4+ T cell.

22. A modified cell comprising a primary T cell infected with a lentiviral particle, wherein the lentiviral particle comprises:

a. an envelope protein capable of infecting the target cell; and

b. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO:1, (ii) at least 90% sequence identity with SEQ ID NO:2, and (iii) at least 90% sequence identity with SEQ ID NO:3.

23. The modified cell of claim 22, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO:1, (ii) at least 95% sequence identity with SEQ ID NO:2, or (iii) at least 95% sequence identity with SEQ ID NO:3.

24. The modified cell of claim 22, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

25. A method for the treatment of cells infected with HIV, the method comprising:

a. contact of peripheral blood mononuclear cells (PBMC) isolated from a subject infected with HIV with a therapeutically effective amount of an *ex vivo* stimulatory agent, wherein the contact is conducted *ex vivo*;

b. transduction of the PBMC ex vivo with a lentiviral particle,

wherein the lentiviral particle comprises:

i. an envelope protein capable of infecting the PBMC; and

ii. an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 90% sequence identity with SEQ ID NO:1, (ii) at least 90% sequence identity with SEQ ID NO:2, and (iii) at least 90% sequence identity with SEQ ID NO:3.

26. The method of claim 25, wherein the encoded microRNA cluster comprises a sequence comprising (i) at least 95% sequence identity with SEQ ID NO:1, (ii) at least 95% sequence identity with SEQ ID NO:2, or (iii) at least 95% sequence identity with SEQ ID NO:3.

27. The method of claim 25, wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

28. The method of any one of claims 25 to 27, further comprising infusion of the transduced PBMC into a subject.

29. The method of any one of claims 25 to 28, further comprising positive selection of selected HIV-specific CD4+ T cells from the PBMC.

30. The method of any one of claims 25 to 29, further comprising immunization of the subject with an effective amount of an *in vivo* stimulatory agent, wherein the immunization occurs prior to contacting the peripheral blood mononuclear cells (PBMC) with the *ex vivo* stimulatory agent.

31. The method of claim 32, wherein each of the *in vivo* stimulatory agent and *ex vivo* stimulatory agent is independently selected from a peptide and a vaccine.

32. The use of the lentiviral particle of any one of claims 3 to 5 and 18 to 21, in the manufacture of a medicament for the treatment of HIV infection in a subject in need thereof.

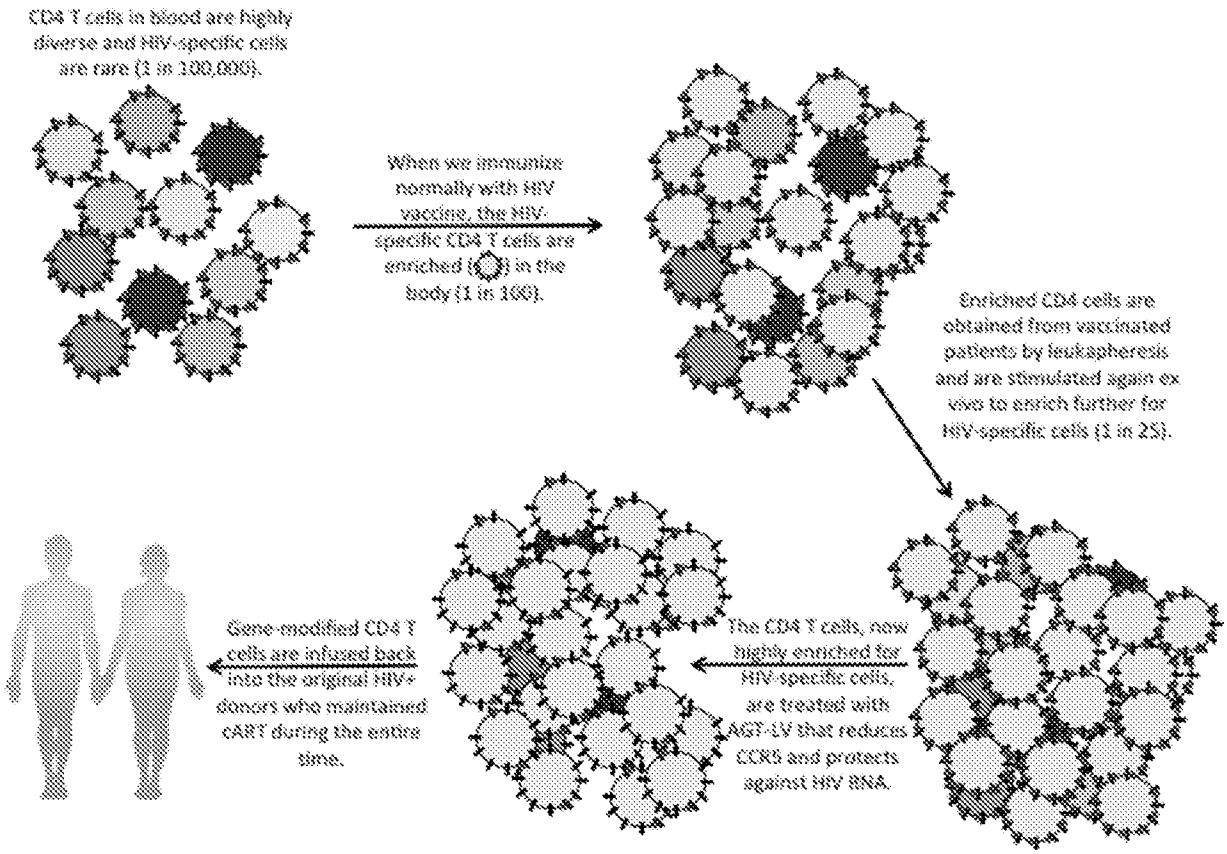

CD4 T cells in blood are highly diverse and HIV-specific cells are rare (1 in 100,000).
When we immunize normally with HIV vaccine, the HIV-specific CD4 T cells are enriched (○) in the body (1 in 100).
Enriched CD4 cells are obtained from vaccinated patients by leukapheresis and are stimulated again ex vivo to enrich further for HIV-specific cells (1 in 25).
The CD4 T cells, now highly enriched for HIV-specific cells, are treated with AGT-LV that reduces CCR5 and protects against HIV RNA.
Gene-modified CD4 T cells are infused back into the original HIV+ donors who maintained cART during the entire time.

**Figure 1**

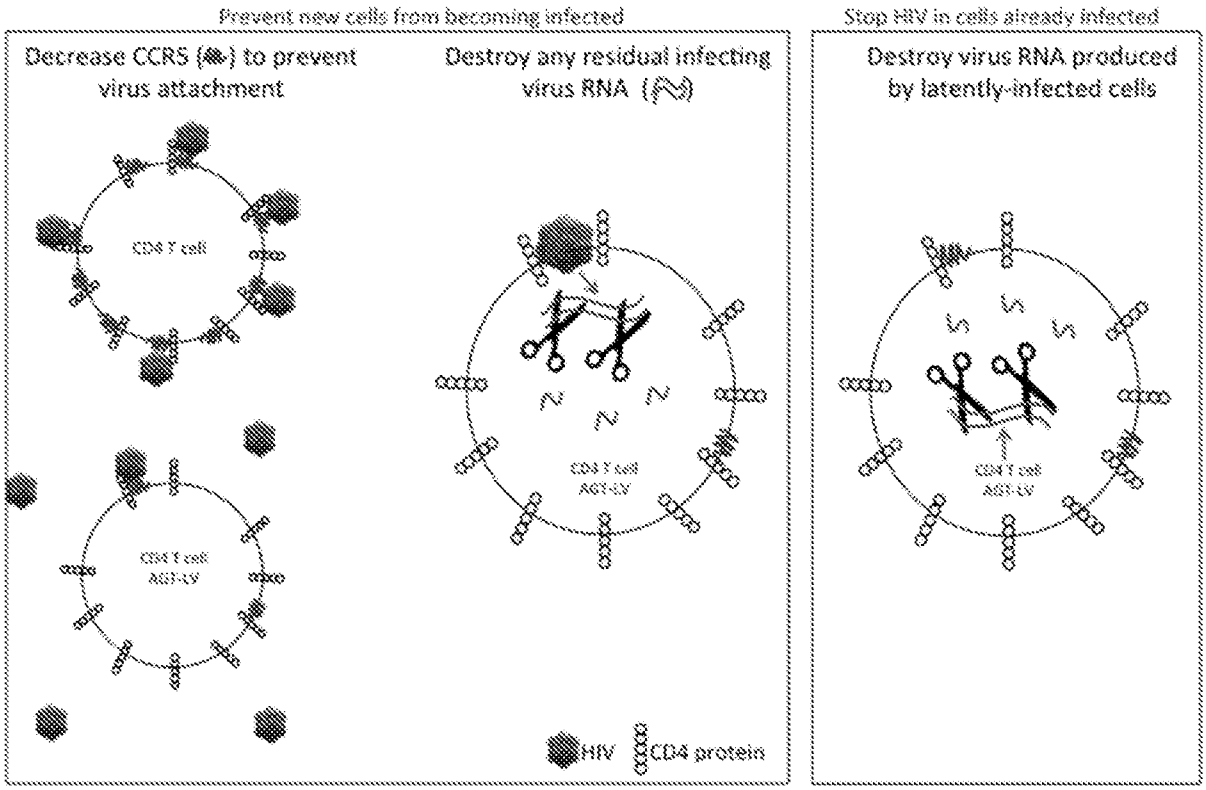

Prevent new cells from becoming infected
Stop HIV in cells already infected
Decrease CCR5 to prevent virus attachment
Destroy any residual infecting virus RNA
Destroy virus RNA produced by latently-infected cells
CD4 T cell
HIV
CD4 protein

Figure 2

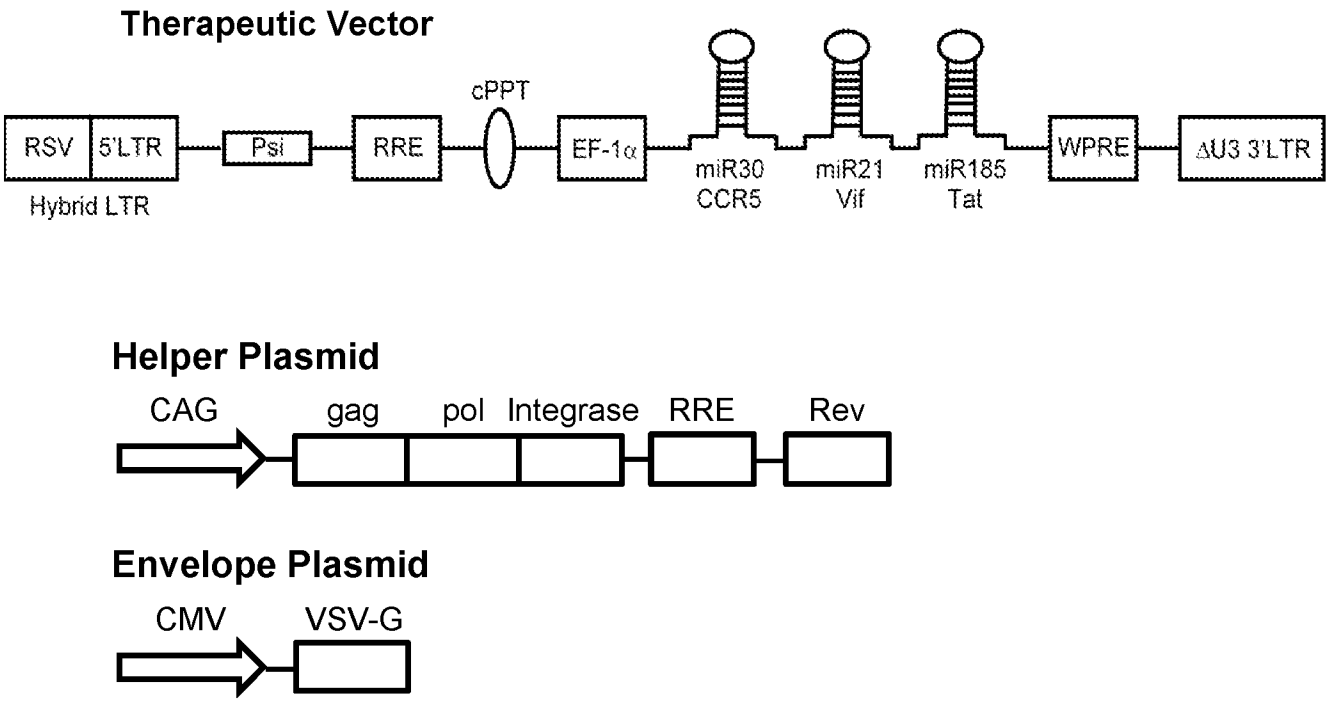
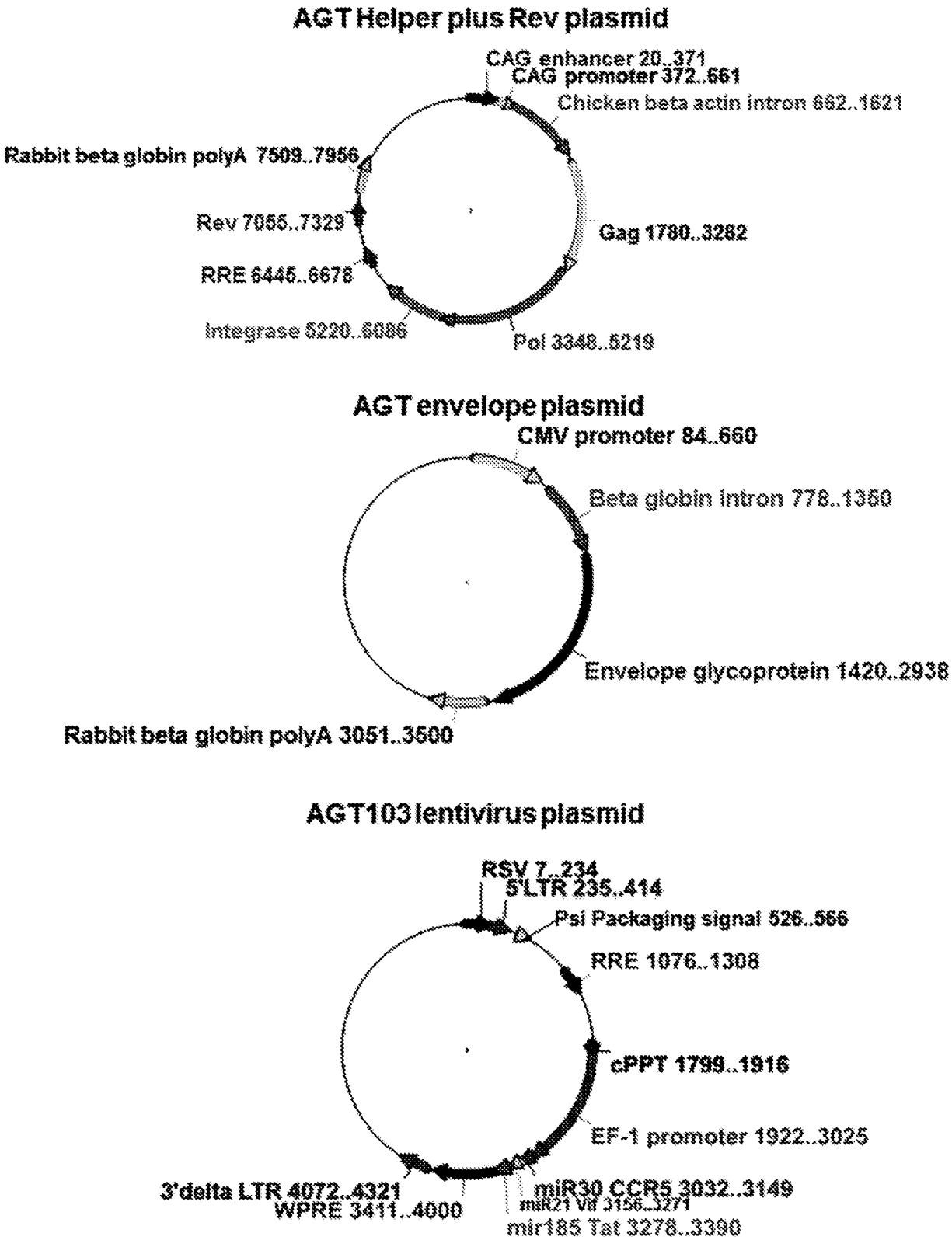

Therapeutic Vector
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
EF-1α
miR30
CCR5
miR21
Vif
miR185
Tat
WPRE
ΔU3 3'LTR
Helper Plasmid
CAG
gag
pol
Integrase
RRE
Rev
Envelope Plasmid
CMV
VSV-G

**Figure 3**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CAG enhancer 20..371
CAG promoter 372..661
Chicken beta actin intron 662..1621
Rabbit beta globin polyA 7509..7956
Rev 7055..7329
Gag 1780..3282
RRE 6445..6678
Integrase 5220..6086
Pol 3348..5219
AGT envelope plasmid
CMV promoter 84..660
Beta globin intron 778..1350
Envelope glycoprotein 1420..2938
Rabbit beta globin polyA 3051..3500
AGT103 lentivirus plasmid
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
RRE 1076..1308
cPPT 1799..1916
EF-1 promoter 1922..3025
3'delta LTR 4072..4321
WPRE 3411..4000
miR30 CCR5 3032..3149
miR21 Vif 3156..3271
mir185 Tat 3278..3390

Figure 4

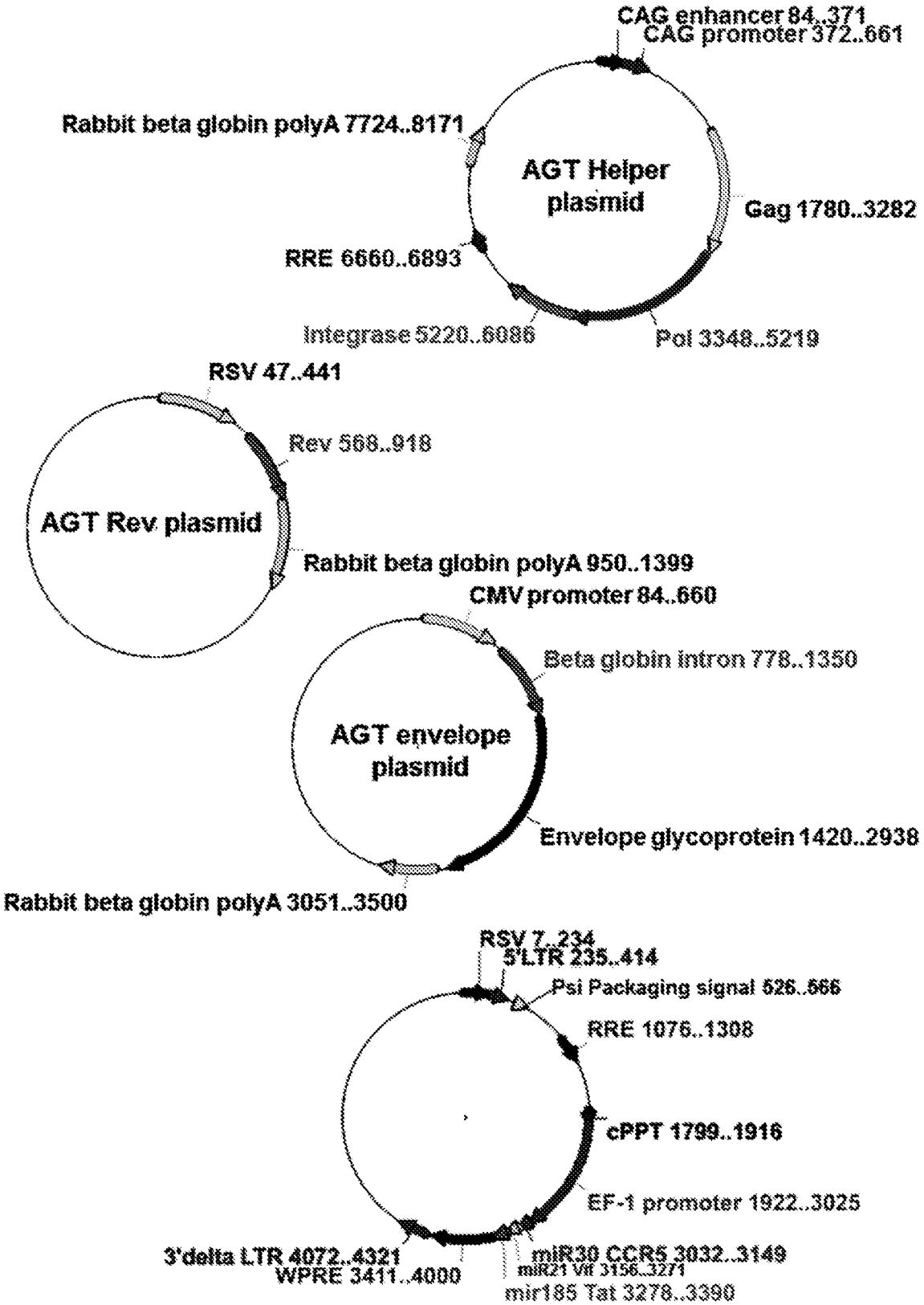

CAG enhancer 84..371
CAG promoter 372..661
Rabbit beta globin polyA 7724..8171
AGT Helper plasmid
Gag 1780..3282
RRE 6660..6893
Integrase 5220..6086
Pol 3348..5219
RSV 47..441
Rev 568..918
AGT Rev plasmid
Rabbit beta globin polyA 950..1399
CMV promoter 84..660
Beta globin intron 778..1350
AGT envelope plasmid
Envelope glycoprotein 1420..2938
Rabbit beta globin polyA 3051..3500
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
RRE 1076..1308
cPPT 1799..1916
EF-1 promoter 1922..3025
3'delta LTR 4072..4321
WPRE 3411..4000
miR30 CCR5 3032..3149
miR21 Vif 3156..3271
mir185 Tat 3278..3390

Figure 5

## Elongation Factor-1 alpha (EF1-alpha) promoter

*CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT*
*TTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA*
*CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGG*
*GTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCC*
*CGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTC*
*GTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGC*
*GCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCT*
*TTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTG*
*GGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGA*
*GCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCT*
*CGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAG*
*CGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA*
*GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATG*
*TGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGT*
*CGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACT*
*GAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATC*
*TTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA*T
GTACA

## miR30 CCR5

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATG
GGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTT

## miR21 Vif

CCCGGG*CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATC*
*TCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA*

## miR185 Tat

GCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCC
TCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC

**Figure 6**

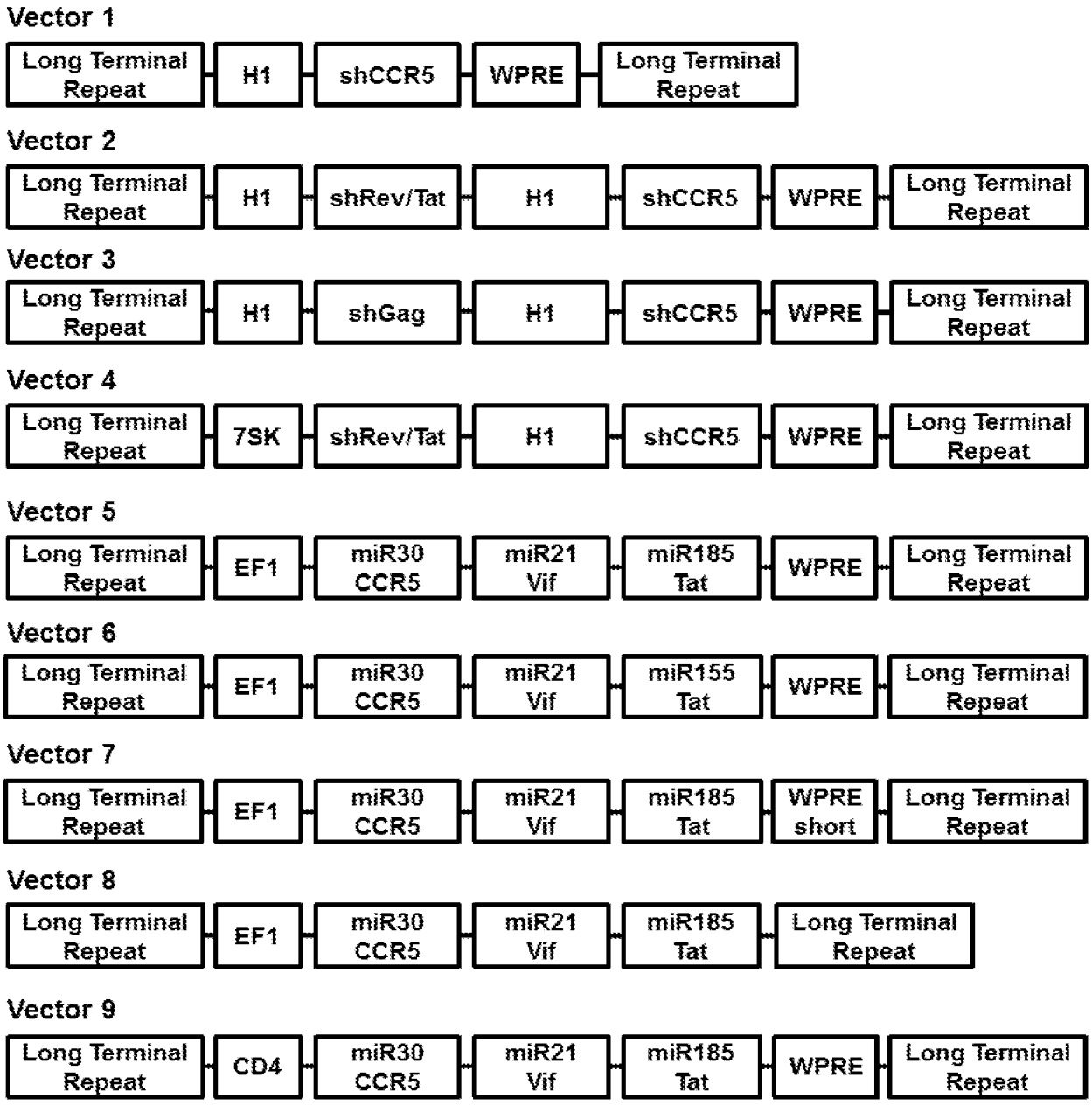

Vector 1
Long Terminal Repeat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 2
Long Terminal Repeat
H1
shRev/Tat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 3
Long Terminal Repeat
H1
shGag
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 4
Long Terminal Repeat
7SK
shRev/Tat
H1
shCCR5
WPRE
Long Terminal Repeat
Vector 5
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE
Long Terminal Repeat
Vector 6
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR155 Tat
WPRE
Long Terminal Repeat
Vector 7
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE short
Long Terminal Repeat
Vector 8
Long Terminal Repeat
EF1
miR30 CCR5
miR21 Vif
miR185 Tat
Long Terminal Repeat
Vector 9
Long Terminal Repeat
CD4
miR30 CCR5
miR21 Vif
miR185 Tat
WPRE
Long Terminal Repeat

**Figure 7**

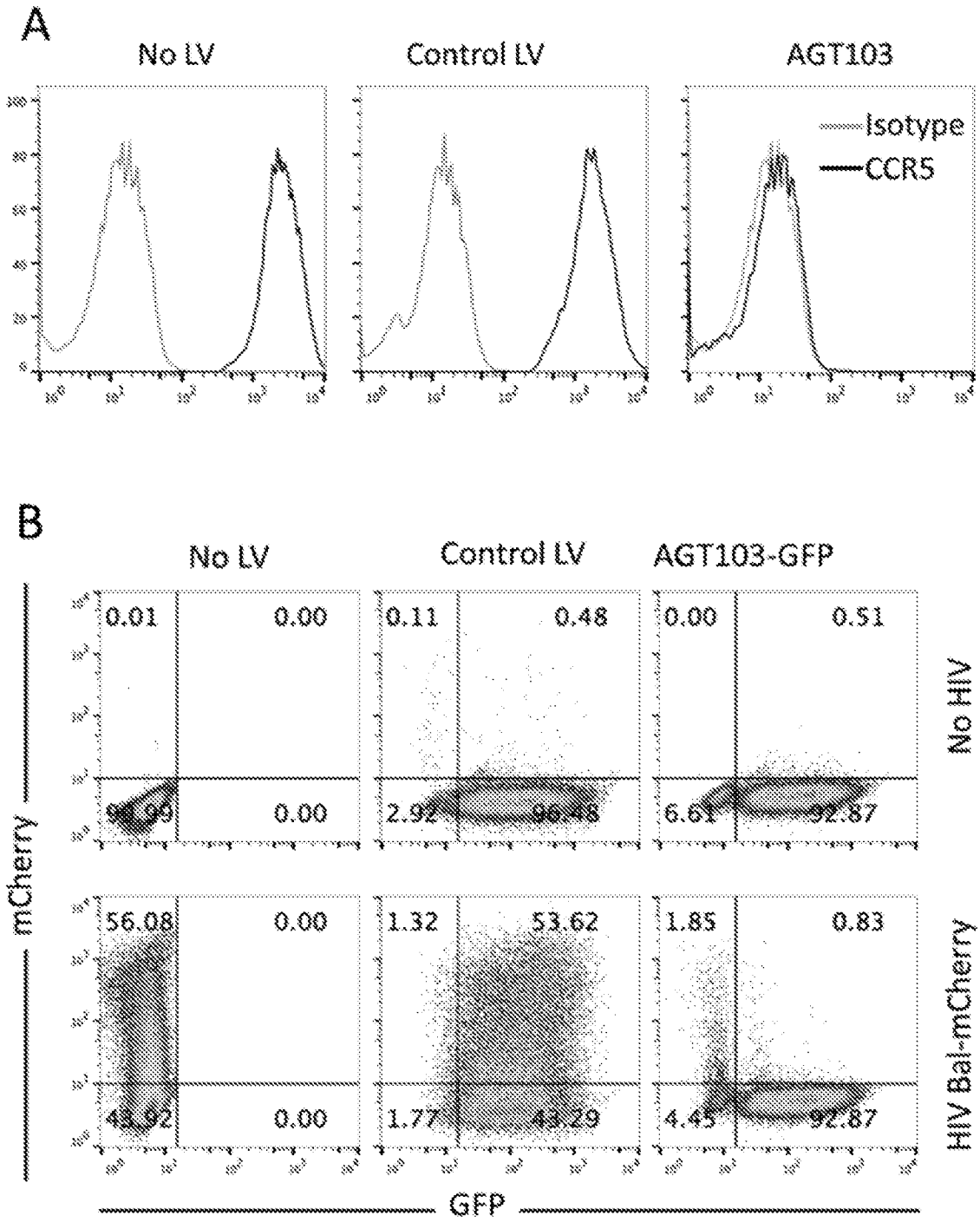

A
No LV
Control LV
AGT103
Isotype
CCR5
B
No LV
Control LV
AGT103-GFP
0.01
0.00
0.11
0.48
0.00
0.51
0.00
2.92
96.48
6.61
92.87
No HIV
56.08
0.00
1.32
53.62
1.85
0.83
43.92
0.00
1.77
43.29
4.45
92.87
HIV Bal-mCherry
mCherry
GFP

Figure 8

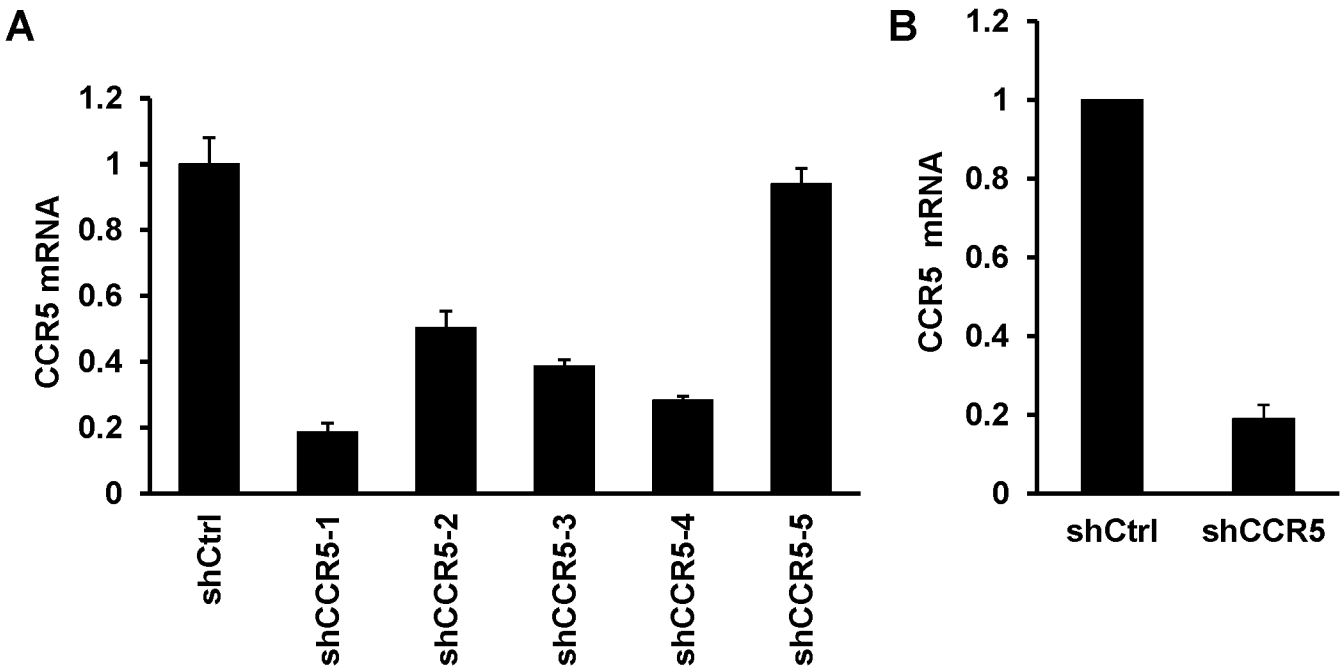

A
CCR5 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
shCtrl
shCCR5-1
shCCR5-2
shCCR5-3
shCCR5-4
shCCR5-5
B
CCR5 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
shCtrl
shCCR5

Figure 9

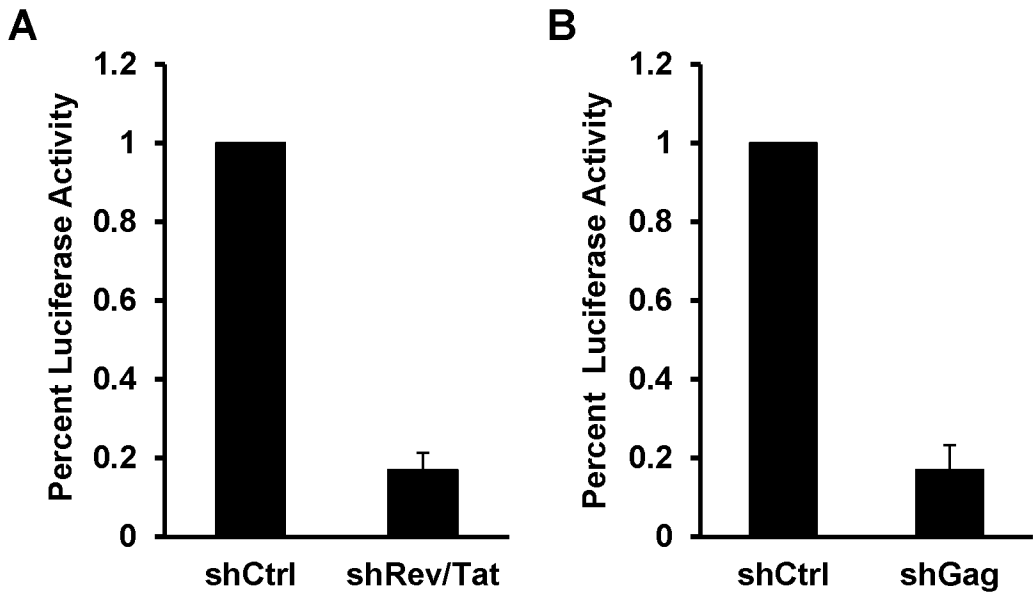

A
Percent Luciferase Activity
1.2
1
0.8
0.6
0.4
0.2
0
shCtrl
shRev/Tat
B
Percent Luciferase Activity
1.2
1
0.8
0.6
0.4
0.2
0
shCtrl
shGag

Figure 10

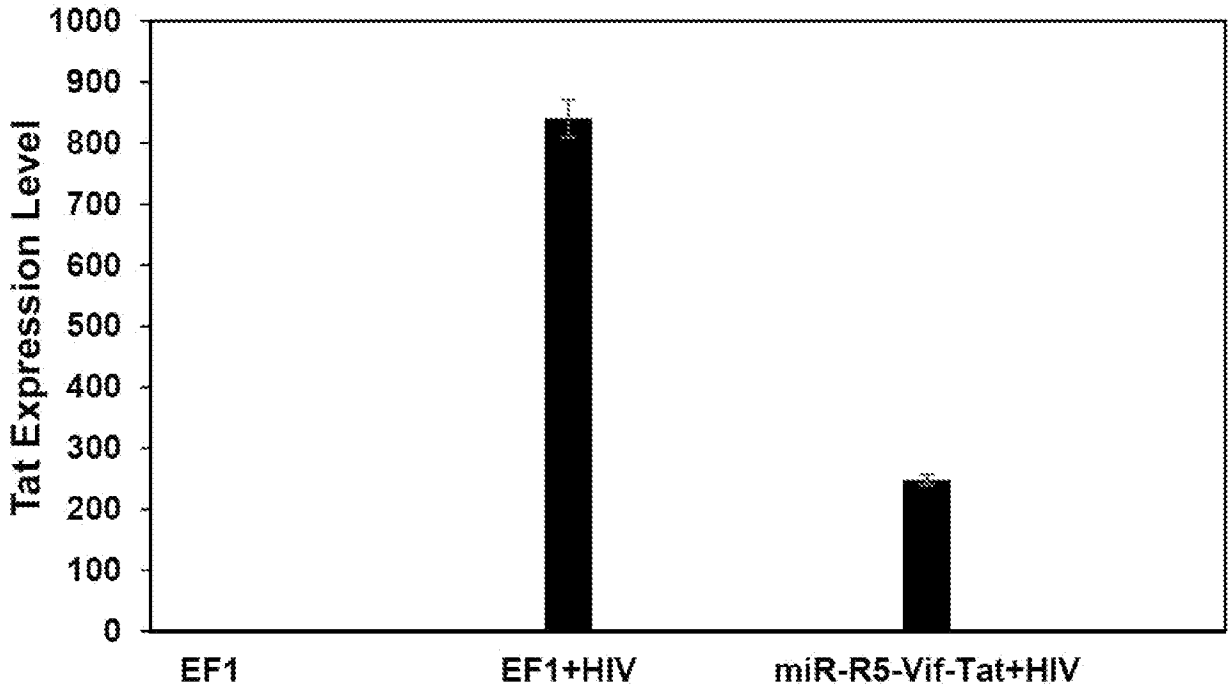
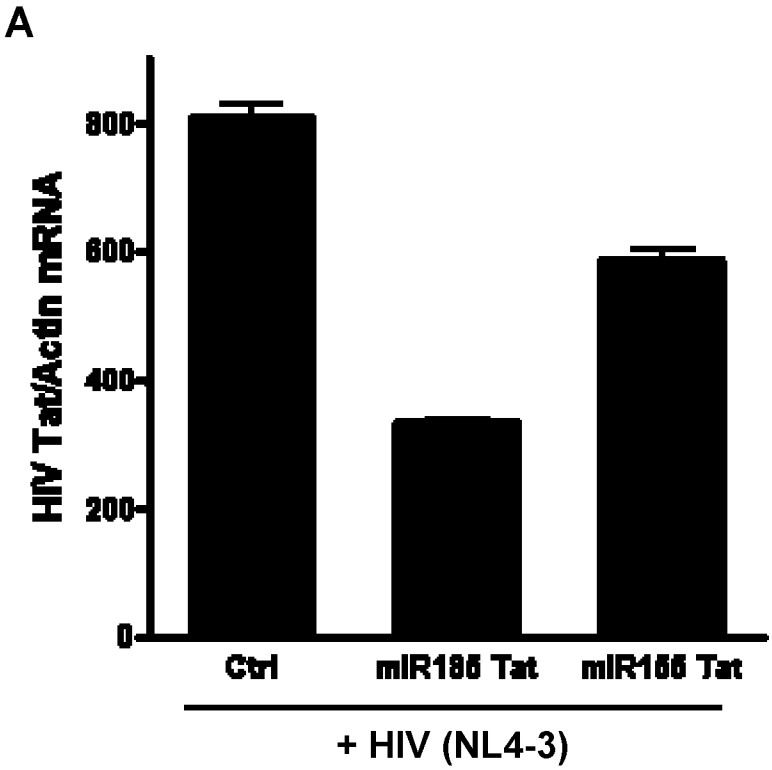

Tat Expression Level
1000
900
800
700
600
500
400
300
200
100
0
EF1
EF1+HIV
miR-R5-Vif-Tat+HIV

Figure 11


A
HIV Tat/Actin mRNA
800
600
400
200
0
Ctrl
miR186 Tat
miR166 Tat
+ HIV (NL4-3)

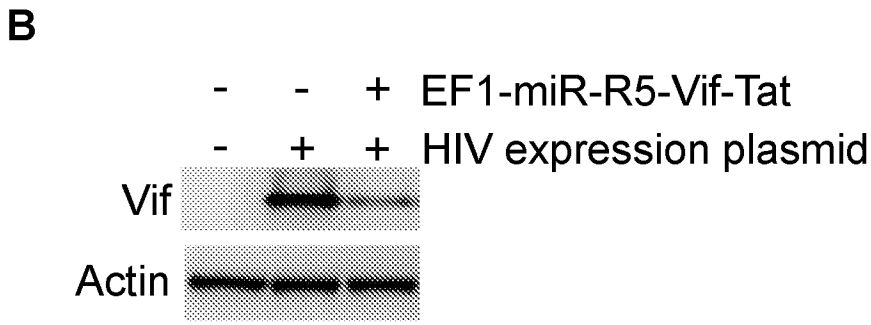

B
- - + EF1-miR-R5-Vif-Tat
- + + HIV expression plasmid
Vif
Actin

Figure 12

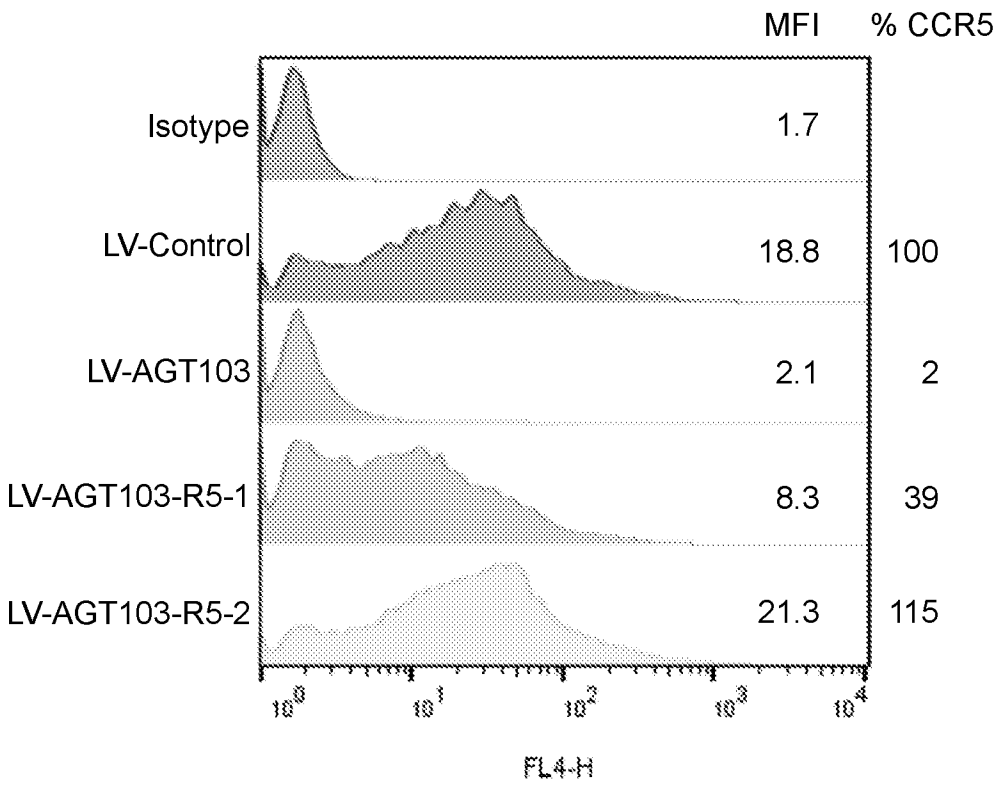

MFI
% CCR5
Isotype
1.7
LV-Control
18.8
100
LV-AGT103
2.1
2
LV-AGT103-R5-1
8.3
39
LV-AGT103-R5-2
21.3
115
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
FL4-H

**Figure 13**

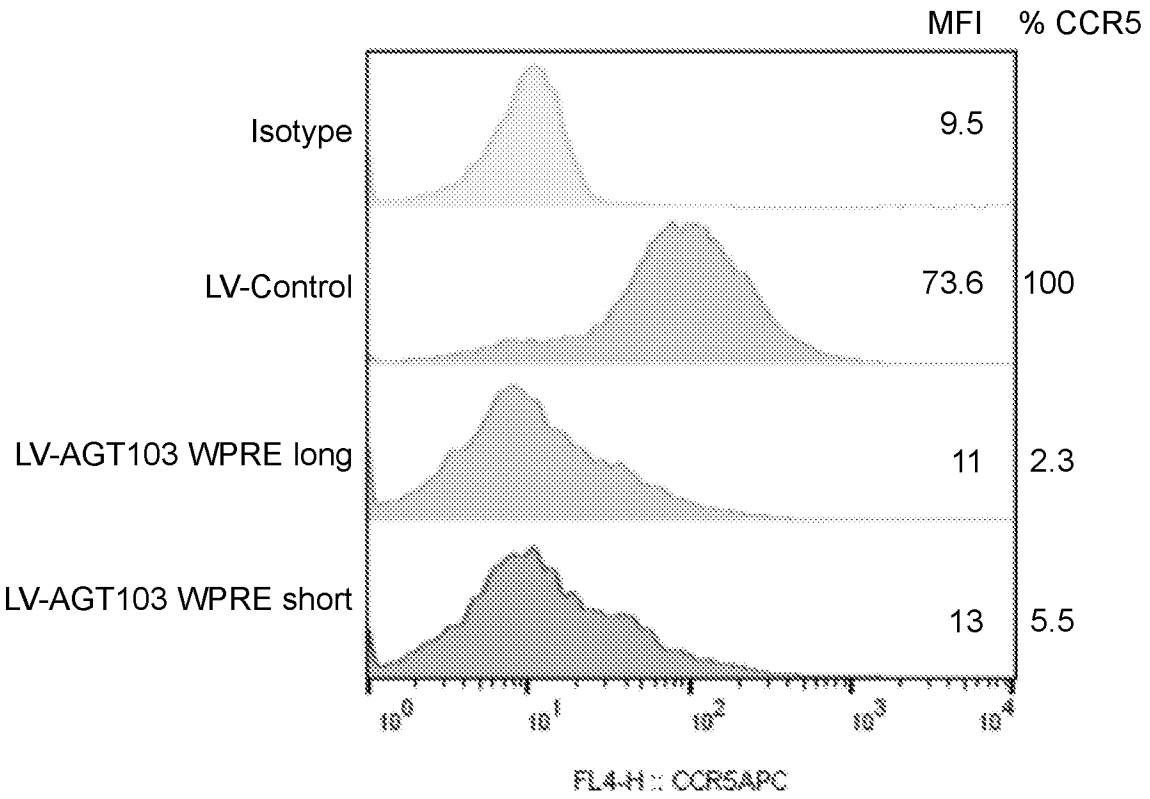

MFI
% CCR5
Isotype
9.5
LV-Control
73.6
100
LV-AGT103 WPRE long
11
2.3
LV-AGT103 WPRE short
13
5.5
FL4-H :: CCR5APC

**Figure 14**

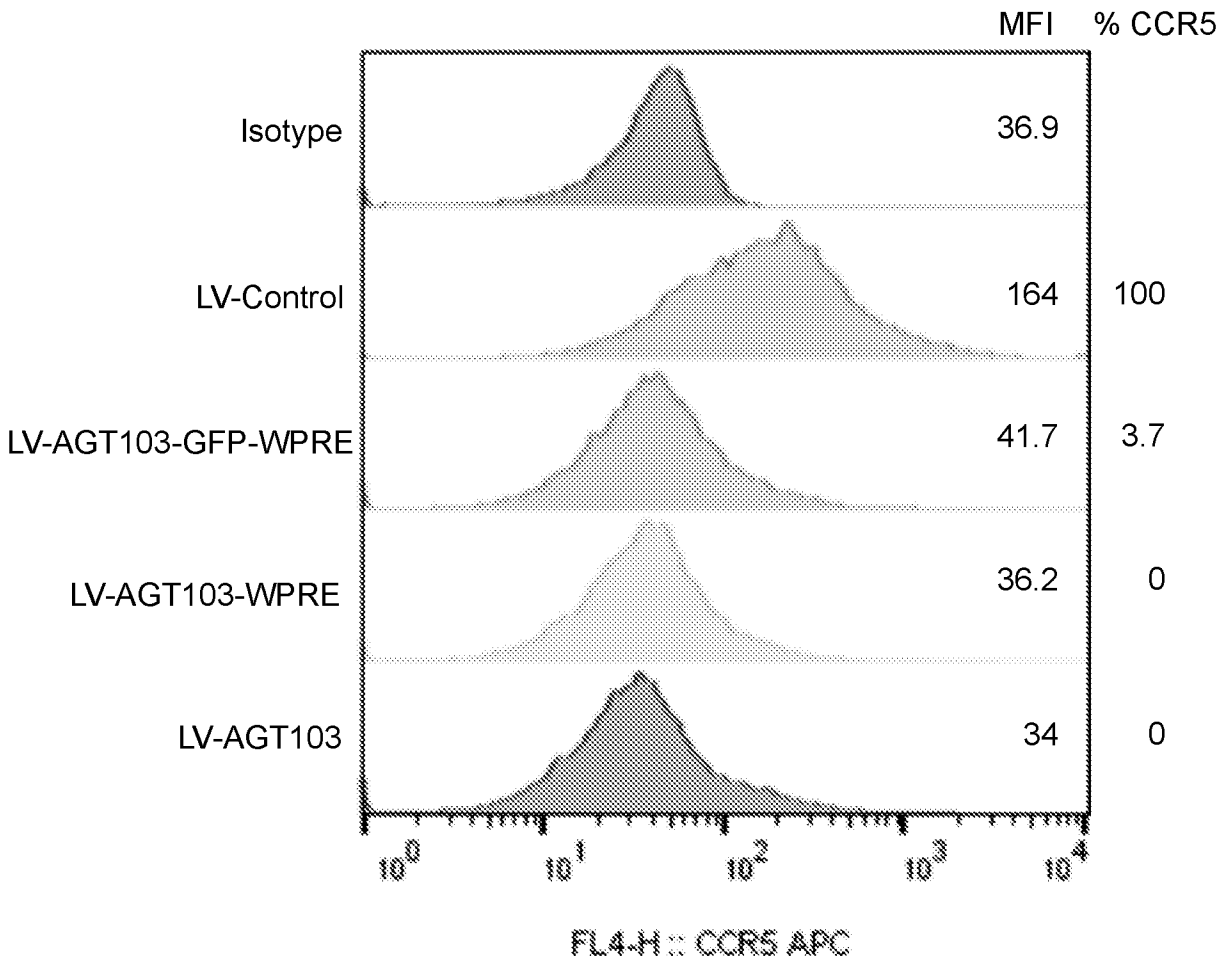

MFI
% CCR5
Isotype
36.9
LV-Control
164
100
LV-AGT103-GFP-WPRE
41.7
3.7
LV-AGT103-WPRE
36.2
0
LV-AGT103
34
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
FL4-H :: CCR5 APC

**Figure 15**

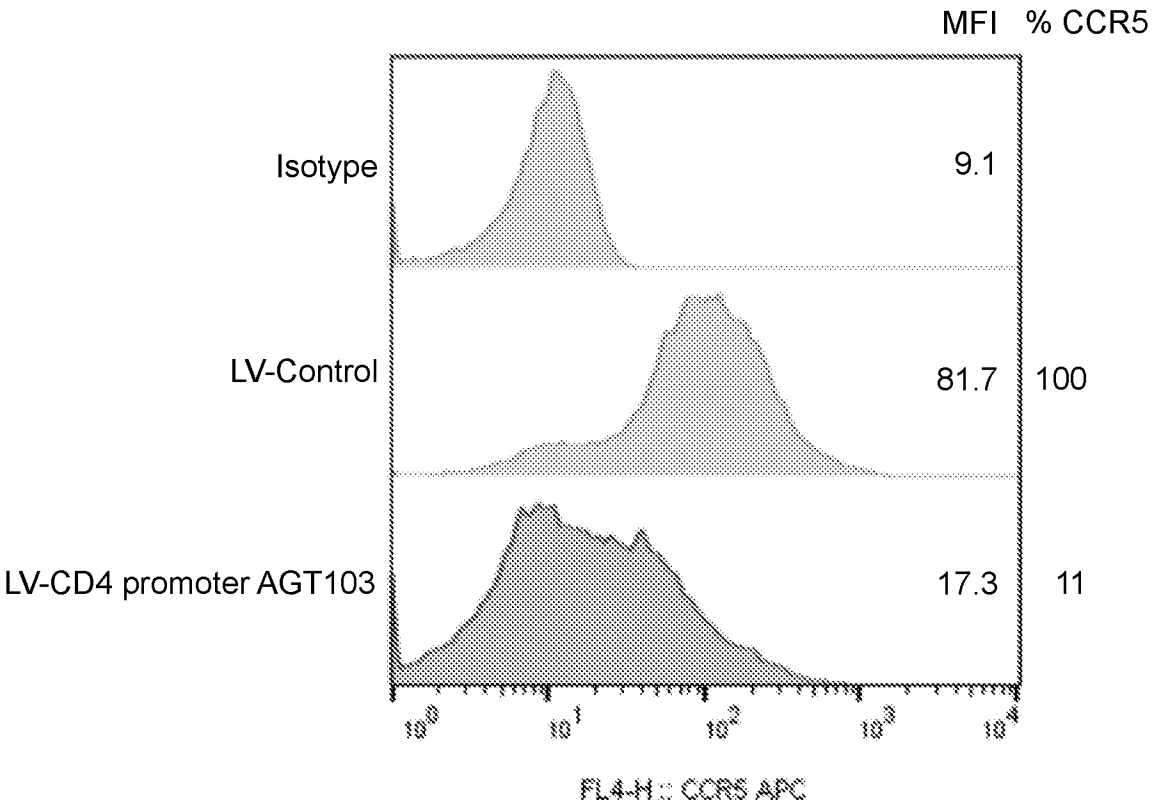

MFI
% CCR5
Isotype
9.1
LV-Control
81.7
100
LV-CD4 promoter AGT103
17.3
11
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
FL4-H :: CCR5 APC

**Figure 16**

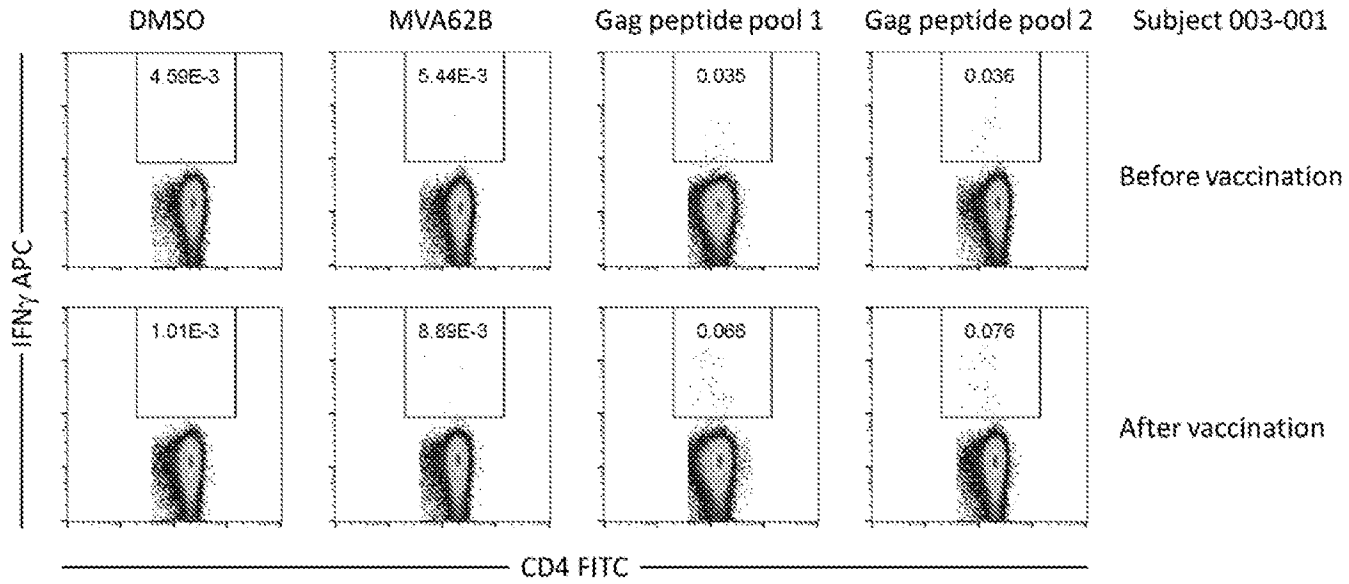

DMSO
MVA62B
Gag peptide pool 1
Gag peptide pool 2
Subject 003-001
4.59E-3
5.44E-3
0.035
0.036
Before vaccination
1.01E-3
8.89E-3
0.066
0.076
After vaccination
IFNγ APC
CD4 FITC

**Figure 17**

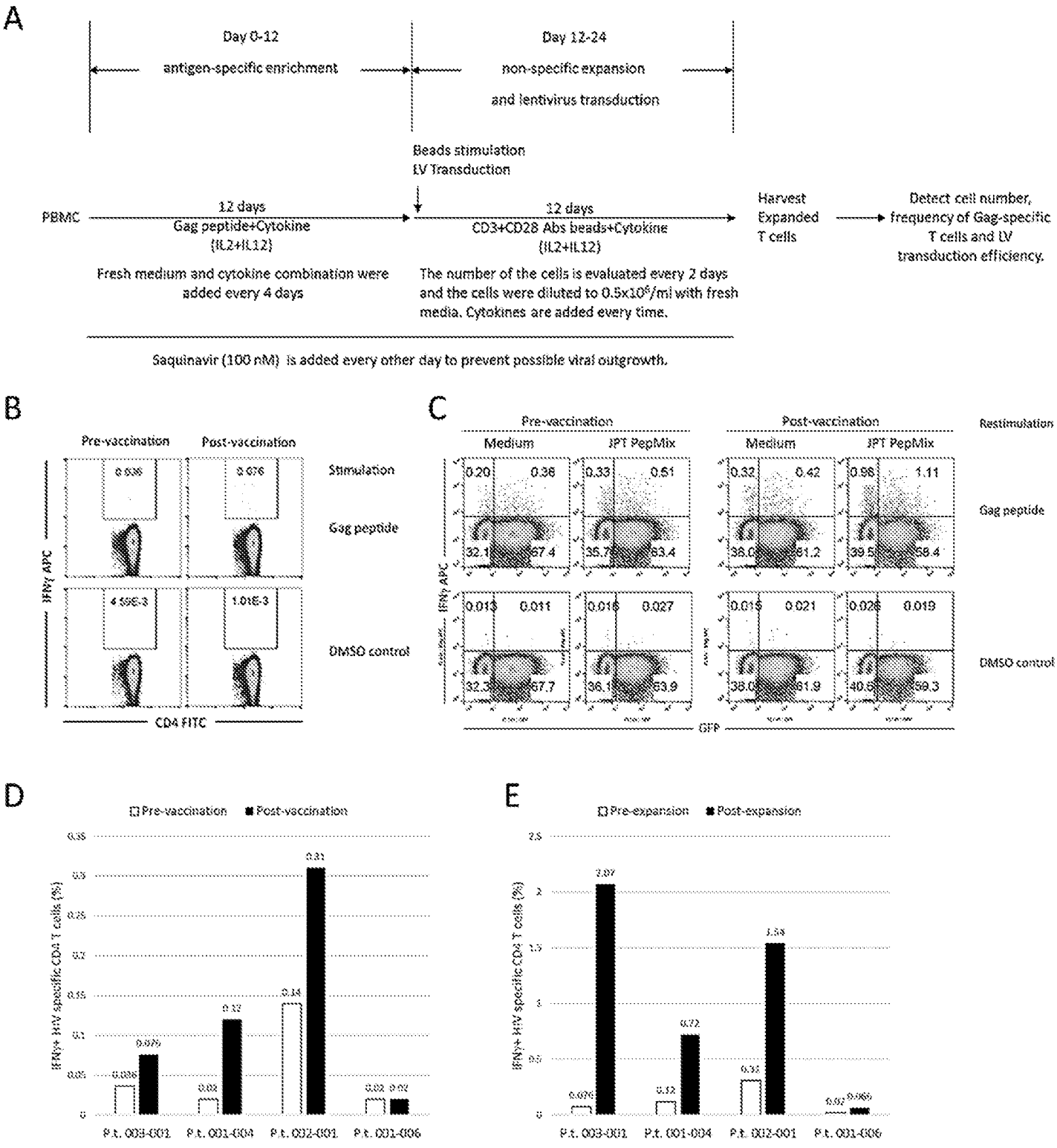

A
Day 0-12
antigen-specific enrichment
Day 12-24
non-specific expansion
and lentivirus transduction
Beads stimulation
LV Transduction
PBMC
12 days
Gag peptide+Cytokine
(IL2+IL12)
12 days
CD3+CD28 Abs beads+Cytokine
(IL2+IL12)
Harvest
Expanded
T cells
Detect cell number,
frequency of Gag-specific
T cells and LV
transduction efficiency.
Fresh medium and cytokine combination were added every 4 days
The number of the cells is evaluated every 2 days and the cells were diluted to 0.5x10^6/ml with fresh media. Cytokines are added every time.
Saquinavir (100 nM) is added every other day to prevent possible viral outgrowth.
B
Pre-vaccination
Post-vaccination
Stimulation
Gag peptide
DMSO control
IFNγ APC
CD4 FITC
C
Pre-vaccination
Post-vaccination
Restimulation
Medium
JPT PepMix
Gag peptide
DMSO control
IFNγ APC
GFP
D
Pre-vaccination
Post-vaccination
IFNγ+ HIV specific CD4 T cells (%)
P.t. 003-001
P.t. 001-004
P.t. 002-001
P.t. 001-006
E
Pre-expansion
Post-expansion
IFNγ+ HIV specific CD4 T cells (%)
P.t. 003-001
P.t. 001-004
P.t. 002-001
P.t. 001-006

Figure 18

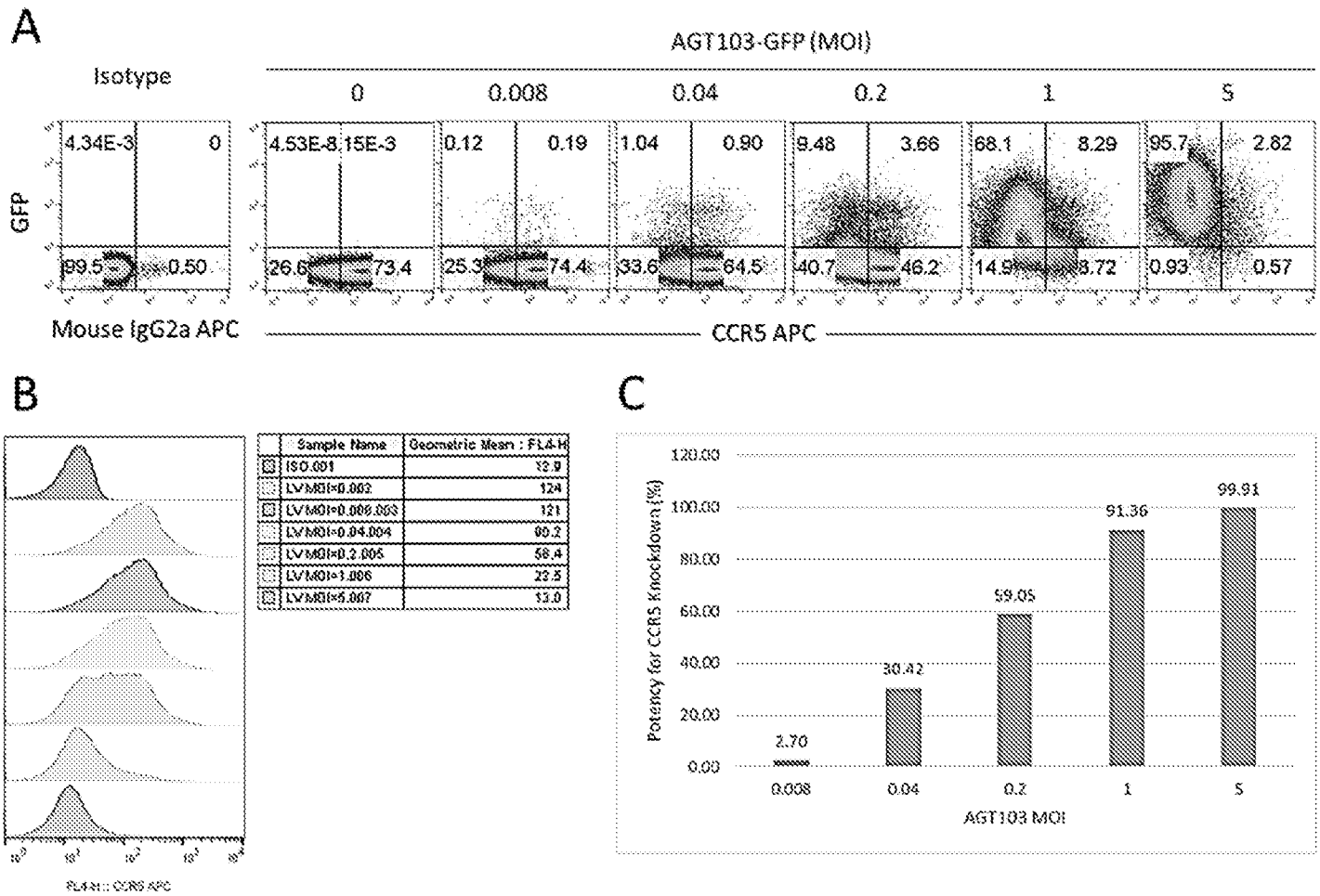

A
AGT103-GFP (MOI)
Isotype
0
0.008
0.04
0.2
1
5
GFP
4.34E-3
0
4.53E-8
15E-3
0.12
0.19
1.04
0.90
9.48
3.66
68.1
8.29
95.7
2.82
99.5
0.50
26.6
73.4
25.3
74.4
33.6
64.5
40.7
46.2
14.9
3.72
0.93
0.57
Mouse IgG2a APC
CCR5 APC
B
Sample Name
Geometric Mean : FL4-H
ISO.001
12.9
LVMOI=0.002
124
LVMOI=0.004.003
121
LVMOI=0.04.004
90.2
LVMOI=0.2.005
58.4
LVMOI=1.006
23.5
LVMOI=5.007
13.0
FL4-H :: CCR5 APC
C
Potency for CCR5 Knockdown (%)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
2.70
30.42
59.05
91.36
99.91
0.008
0.04
0.2
1
5
AGT103 MOI

**Figure 19**

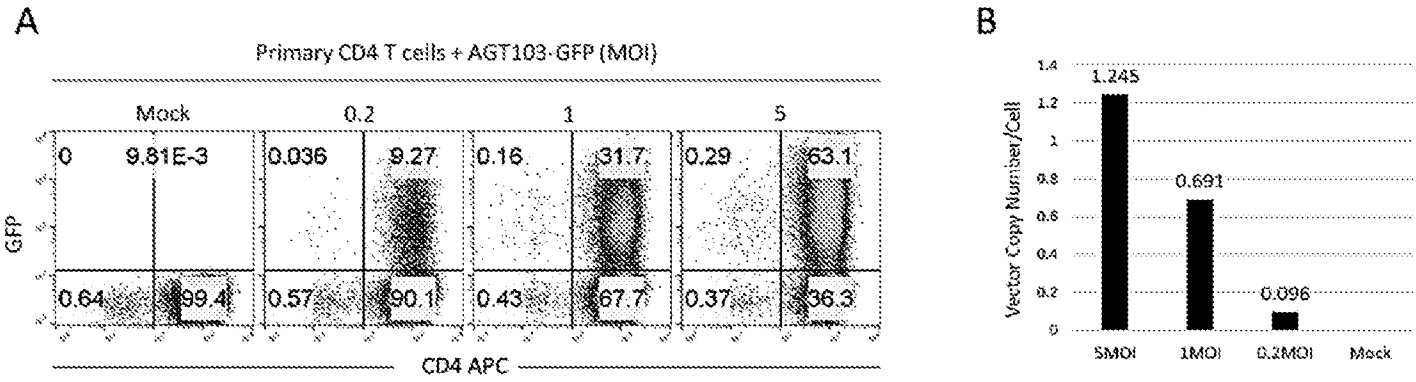

A
Primary CD4 T cells + AGT103-GFP (MOI)
Mock
0.2
1
5
0
9.81E-3
0.036
9.27
0.16
31.7
0.29
63.1
0.64
99.4
0.57
90.1
0.43
67.7
0.37
36.3
GFP
CD4 APC
B
Vector Copy Number/Cell
1.245
0.691
0.096
5MOI
1MOI
0.2MOI
Mock

**Figure 20**

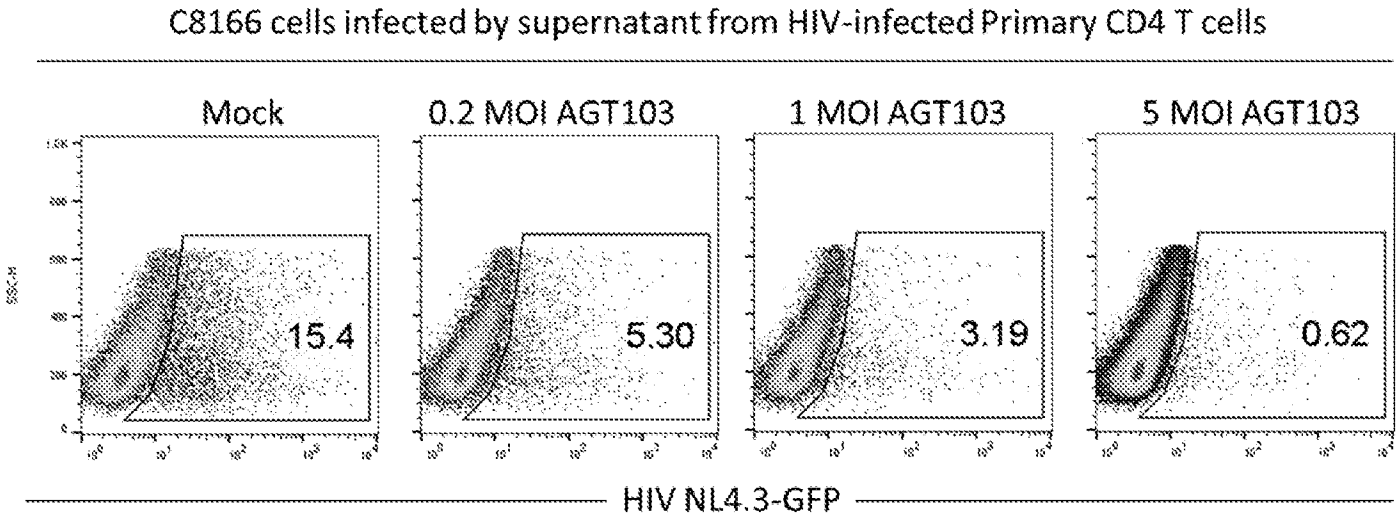

C8166 cells infected by supernatant from HIV-infected Primary CD4 T cells
Mock
0.2 MOI AGT103
1 MOI AGT103
5 MOI AGT103
15.4
5.30
3.19
0.62
HIV NL4.3-GFP

**Figure 21**

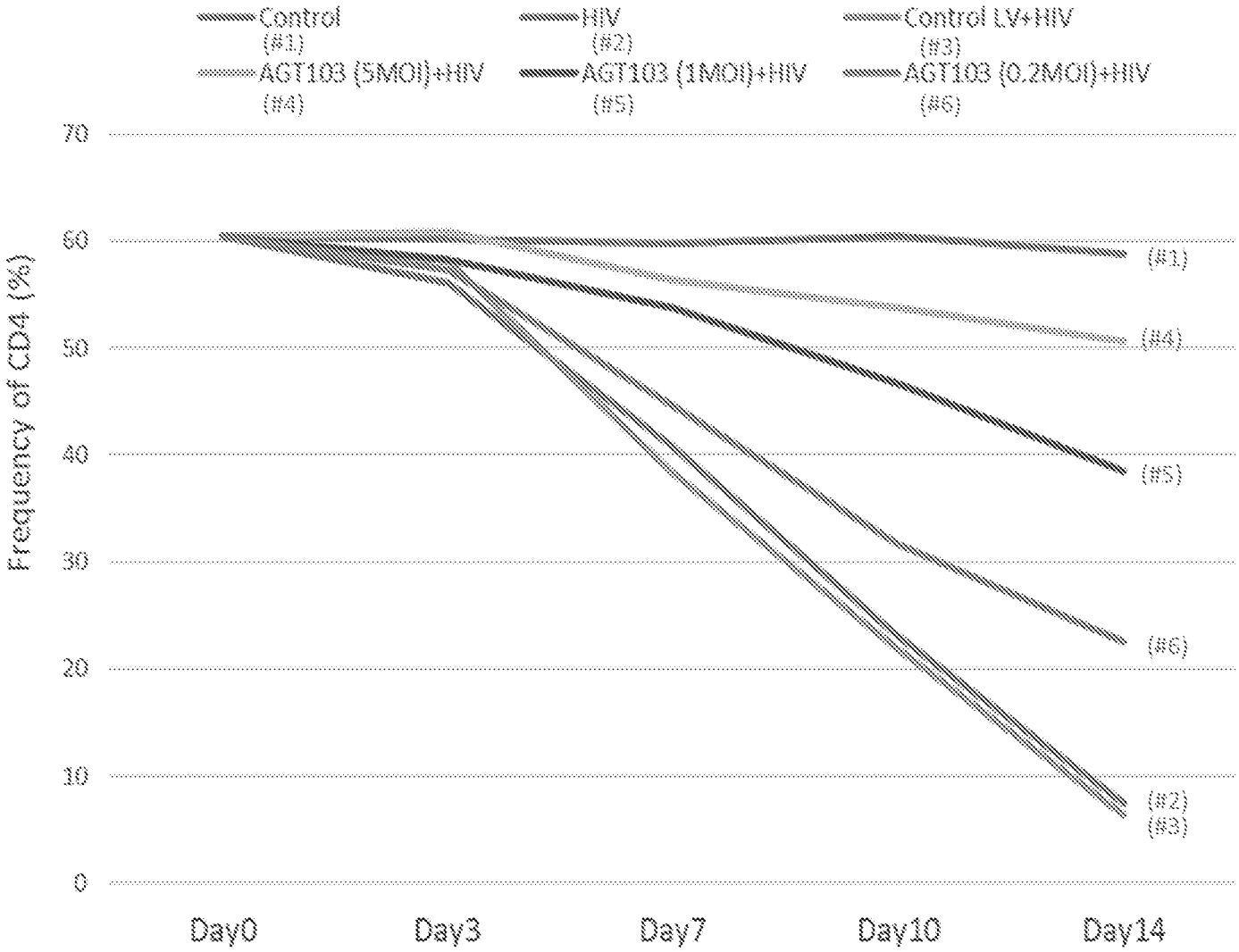

Control (#1)
HIV (#2)
Control LV+HIV (#3)
AGT103 (5MOI)+HIV (#4)
AGT103 (1MOI)+HIV (#5)
AGT103 (0.2MOI)+HIV (#6)
Frequency of CD4 (%)
70
60
50
40
30
20
10
0
Day0
Day3
Day7
Day10
Day14
(#1)
(#4)
(#5)
(#6)
(#2)
(#3)

**Figure 22**

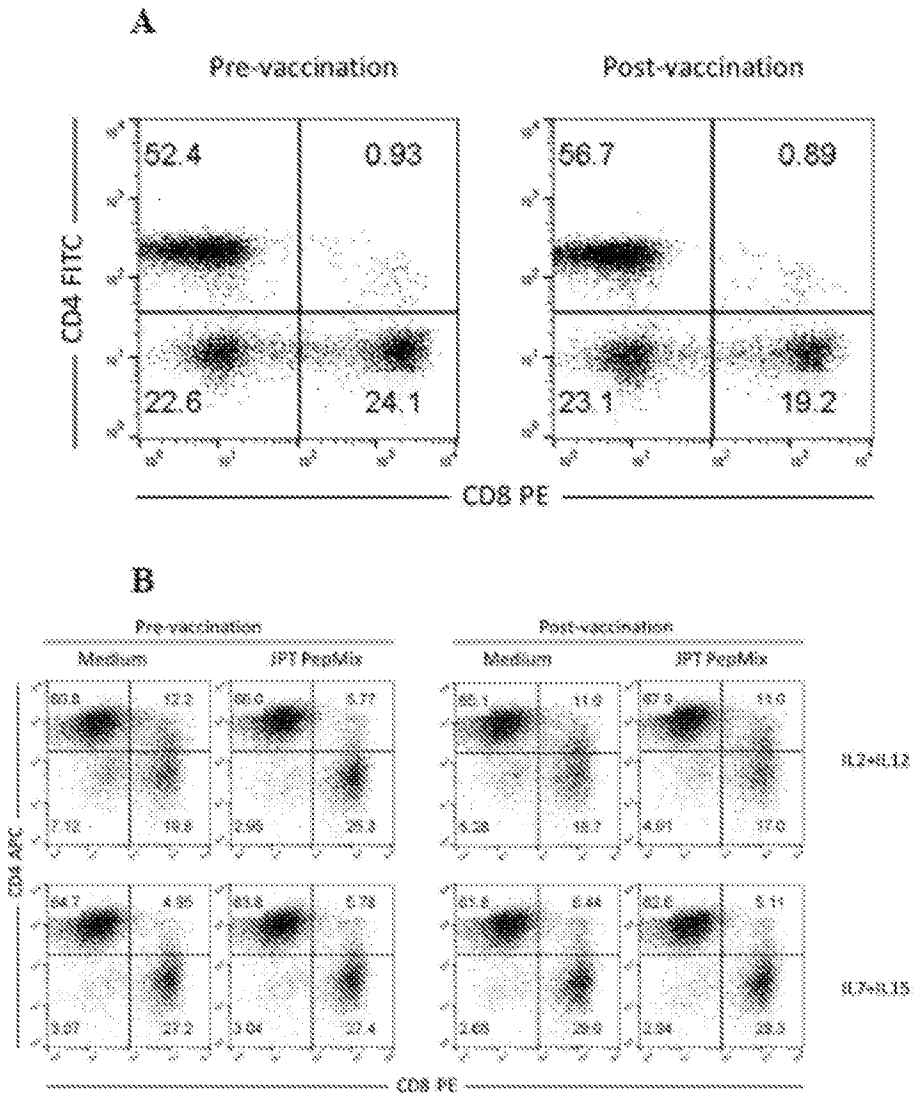

A
Pre-vaccination
Post-vaccination
52.4
0.93
22.6
24.1
56.7
0.89
23.1
19.2
CD4 FITC
CD8 PE
B
Pre-vaccination
Post-vaccination
Medium
JPT PepMix
CD4 APC
CD8 PE
IL2+IL12
IL7+IL15

**Figure 23**

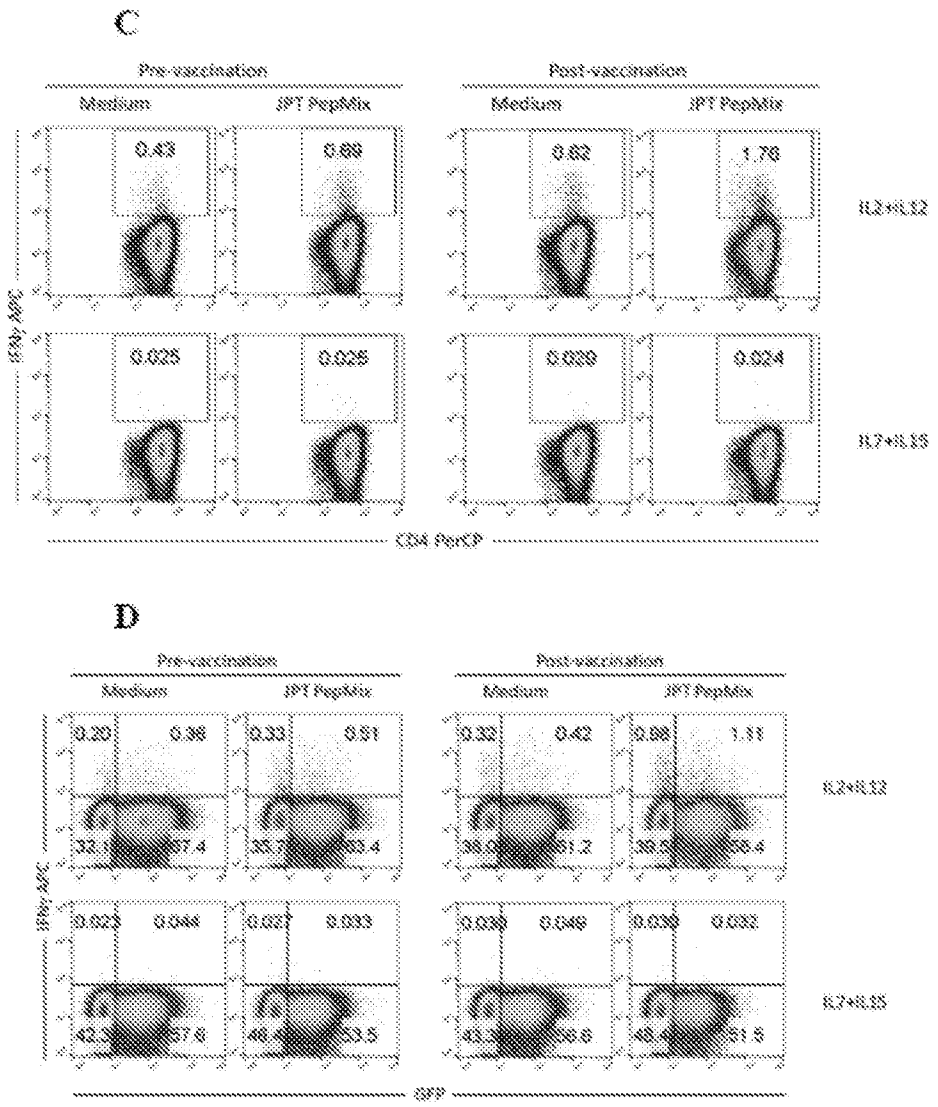



**Figure 23 Cont'd**