Hiv pre-immunization and immunotherapy

Classifications

A61K39/21 Retroviridae, e.g. equine infectious anemia virus

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Application IL276038A events

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Info: Patent citations (153), Cited by (16), Similar documents, Priority and Related Applications

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Claims (44)

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CLAIMS:

1. A lentiviral vector comprising at least one encoded genetic element, the at least one genetic element comprising: at least two of: (i) a sequence comprising at least 80% sequence identity with SEQ ID NO: 1, (ii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 2, and (iii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 3; or each of: (iv) a sequence comprising at least 80% sequence identity with SEQ ID NO: 97, (v) a sequence comprising at least 80% sequence identity with SEQ ID NO: 6, and (vi) a sequence comprising at least 80% sequence identity with SEQ ID NO: 7.

2. The lentiviral vector of claim 1, wherein the at least one encoded genetic element comprises a microRNA.

3. The lentiviral vector of claim 1, wherein the at least one encoded genetic element comprises: at least two of: SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3; or each of: SEQ ID NO: 97, SEQ ID NO: 6, and SEQ ID NO: 7.

4. A lentiviral vector system for expressing a lentiviral particle, the system comprising: a lentiviral vector according to any one of claims 1-3; 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 the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are capable of transfecting a packaging cell line, and wherein, upon such transfection, the packaging cell line is capable of producing a lentiviral particle capable of inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

5. The lentiviral vector system of claim 4, wherein the at least one helper plasmid comprises a first plasmid for expressing the gag and pol genes, and a second plasmid for expressing the rev gene.

6. A lentiviral particle capable of infecting a cell, the lentiviral particle comprising an envelope protein optimized for infecting a cell, and a lentiviral vector according to any one of claims 1-4. 159

7. The lentiviral particle of claim 6, wherein the envelope protein is optimized for infecting a T cell.

8. The lentiviral particle of claim 7, wherein the envelope protein is optimized for infecting a CD4+ T cell.

9. A modified cell comprising a CD4+ T cell, wherein the CD4+ T cell has been transfected with a lentiviral particle according to any one of claims 6-8.

10. The modified cell of claim 9, wherein the CD4+ T cell is capable of recognizing an HIV antigen.

11. The modified cell of claim 10, wherein the HIV antigen comprises a gag antigen.

12. The modified cell of claim 9, wherein the CD4+ T cell expresses a decreased level of CCR5 following infection with the lentiviral particle.

13. A method of treating cells, the method comprising: (a) obtaining, or having obtained, peripheral blood mononuclear cells (PBMC) from a subject, wherein the subject has been previously immunized with a therapeutically effective amount of a first stimulatory agent; (b) contacting, or having contacted, the PBMC with a therapeutically effective amount of a second stimulatory agent, wherein the contacting is carried out ex vivo; (c) transducing, or having transduced, the PBMC ex vivo with a viral delivery system encoding at least one genetic element, wherein at least one encoded genetic element comprises: at least two of: (i) a sequence comprising at least 80% sequence identity with SEQ ID NO: 1, (ii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 2, and (iii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 3; or each of: (iv) a sequence comprising at least 80% sequence identity with SEQ ID NO: 97, (v) a sequence comprising at least 80% sequence identity with SEQ ID NO: 6, and (vi) a sequence comprising at least 80% sequence identity with SEQ ID NO: 7; and (d) culturing, or having cultured, the transduced PBMC for at least 1 day.

14. The method of claim 13, wherein subject is infected with HIV.

15. The method of claim 13, wherein the transduced PBMC are cultured from about 1 to about 35 days. 160

16. Transduced PBMC produced by the method of claim 13, wherein the transduced PBMC are infused into a subject.

17. The transduced PBMC of claim 16, wherein the subject is a human.

18. The method of claim 13, wherein at least one of the first stimulatory agent and the second stimulatory agent comprises a peptide.

19. The method of claim 18, wherein the peptide comprises a gag peptide.

20. The method of claim 13, wherein at least one of the first stimulatory agent and the second stimulatory agent comprises a vaccine.

21. The method of claim 20, wherein the vaccine comprises an HIV vaccine.

22. The method of claim 21, wherein the HIV vaccine comprises a MVA/HIV62B vaccine or a variant thereof.

23. The method of claim 13, wherein the first stimulatory agent and the second stimulatory agent are the same.

24. The method of claim 13, wherein the viral delivery system comprises a lentiviral particle.

25. The method of claim 13, wherein the at least one genetic element, when expressed, is capable of targeting an HIV RNA sequence.

26. The method of claim 25, wherein, when the at least one encoded genetic element comprises any one of SEQ ID NO: 1, or SEQ ID NO: 97, the at least one genetic element, when expressed, is also capable of inhibiting production of chemokine receptor CCR5.

27. The method of claim 13, wherein the at least one encoded genetic element comprises a microRNA.

28. The method of claim 13, wherein the at least one encoded genetic element comprises: at least two of: SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3; or each of: SEQ ID NO: 97, SEQ ID NO: 6, and SEQ ID NO: 7.

29. Transduced peripheral blood mononuclear cells (PBMC) for use in treating a subject, wherein the transduced PBMC have been prepared by a method comprising: (a) removing, or having removed, leukocytes from the subject, wherein the subject has been previously immunized with a therapeutically effective amount of a first stimulatory agent; (b) purifying, or having purified, the PBMC ex vivo from the leukocytes; 161 (c) contacting, or having contacted, the PBMC ex vivo with a therapeutically effective amount of a second stimulatory agent; (d) transducing, of having transduced, the PBMC ex vivo with a viral delivery system encoding at least one genetic element, wherein the at least one genetic element comprises: at least two of: (i) a sequence comprising at least 80% sequence identity with SEQ ID NO: 1, (ii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 2, and (iii) a sequence comprising at least 80% sequence identity with SEQ ID NO: 3; or each of: (iv) a sequence comprising at least 80% sequence identity with SEQ ID NO: 97, (v) a sequence comprising at least 80% sequence identity with SEQ ID NO: 6, and (vi) a sequence comprising at least 80% sequence identity with SEQ ID NO: 7; and (e) culturing, or having cultured, the transduced PBMC for at least 1 day.

30. The transduced PBMC for use of claim 29, wherein the subject is infected with HIV.

31. The transduced PBMC for use of claim 29, wherein the transduced PBMC are cultured from about 1 to about 35 days.

32. The transduced PBMC for use of claim 29, further comprising infusing, or having infused, the transduced PBMC into the subject.

33. The transduced PBMC for use of any one of claims 29-32, wherein the subject is a human.

34. The transduced PBMC for use of claim 29, wherein at least one of the first stimulatory agent and the second stimulatory agent comprises a peptide.

35. The transduced PBMC for use of claim 34, wherein the peptide comprises a gag peptide.

36. The transduced PBMC for use of claim 29, wherein at least one of the first stimulatory agent and the second stimulatory agent comprises a vaccine.

37. The transduced PBMC for use of claim 36, wherein the vaccine comprises an HIV vaccine.

38. The transduced PBMC for use of claim 37, wherein the HIV vaccine comprises a MVA/HIV62B vaccine or a variant thereof. 162

39. The transduced PBMC for use of claim 29, wherein the first stimulatory agent and the second stimulatory agent are the same.

40. The transduced PBMC for use of claim 29, wherein the viral delivery system comprises a lentiviral particle.

41. The transduced PBMC for use of claim 29, wherein the at least one genetic element comprises at least one small RNA capable of targeting an HIV RNA sequence.

42. The transduced PBMC for use of claim 41, wherein, when the at least one genetic element comprises any one of SEQ ID NO: 1, or SEQ ID NO: 97, the at least one genetic element also comprises a small RNA capable of inhibiting production of chemokine receptor CCR5.

43. The transduced PBMC for use of claim 29, wherein the at least one genetic element comprises a microRNA.

44. The transduced PBMC for use of claim 29, wherein the at least one genetic element comprises: at least two of: SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3; or each of: SEQ ID NO: 97, SEQ ID NO: 6, and SEQ ID NO: 7. 163 276038/2 PCT PATENT APPLICATION HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY Inventors: Charles D. PAUZA Haishan LI Tyler LAHUSEN Assignee: American Gene Technologies International Inc. 15010 Broschart Road, #110 Rockville, MD 20850 Entity: Small entity Filed Electronically on: January 11, 2017 1 276038/2 HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY CROSS-REFERENCE TO RELATED APPLICATIONS This application claims priority to: U.S. Provisional Patent Application No. 62/360,185 5 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. 10

Description

HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY Filing Date 11 01 2017 FIELD AND BACKGROUND 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 2 276038/2 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. 3 276038/2 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 4 276038/2 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT 276038/2 ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA 6 276038/2 GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG 7 276038/2 TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises:

AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA 8 276038/2 ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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 9 276038/2 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. 276038/2 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 11 276038/2 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. 12 276038/2 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 13 276038/2 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, 14 276038/2 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 276038/2 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. 16 276038/2 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 -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 17 276038/2 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 18 276038/2 AVEG 021 P3C541b Lipopeptide 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 CN54gp140 Conjugate Vaccine (TL01) 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 19 276038/2 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 276038/2 HVTN 052 VRC-HIVDNA009-00-VP 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 21 276038/2 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 22 276038/2 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) 23 276038/2 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 24 276038/2 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 UBI HIV-1 Peptide

Immunogen, Multivalent study 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- MRKAd5 HIV-1 gag/pol/nef 1 Trigene Vaccines 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 276038/2 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. 26 276038/2 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, , 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, 27 276038/2 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 28 276038/2 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 29 276038/2 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (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 TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC 276038/2 CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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. 31 276038/2 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (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 32 276038/2 TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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 33 276038/2 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (SEQ ID NO: 1). In a preferred embodiment, the at least one genetic element comprises: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTT (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 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (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 TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTC GTC (SEQ ID NO: 3). In a preferred embodiment, the at least one genetic element includes CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCA ATGACCGCGTCTTCGTC (SEQ ID NO: 3). 34 276038/2 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 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAG CCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAA GGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCT GAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGG TATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCC GCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTT CCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31). In a preferred embodiment, the microRNA cluster includes: AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTC GGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATG TGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGC GGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (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. 276038/2 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. 36 276038/2 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. 37 276038/2 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. 38 276038/2 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. 39 276038/2 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 40 276038/2 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 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 7 8 9 10 lentivirus vector. Alternatively, about 1x10 , about 1x10 , about 1x10 , about 1x10 , about 11 12 1x10 , or about 1x10 CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells are isolated for ex vivo re-stimulation. 41 276038/2 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 42 276038/2 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. 43 276038/2 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. 44 276038/2 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 45 276038/2 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 46 276038/2 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 47 276038/2 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), 48 276038/2 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 49 276038/2 (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 8 established to measure a functional cure at a determined value, for example, at about 1x10 HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus. Alternatively, the 6 7 8 9 threshold value may be about 1x10 , about 1x10 , about 1x10 , about 1x10 , about 1x10 , or about 1x10 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 50 276038/2 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. 51 276038/2 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 52 276038/2 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 6 with about 10 ng to 5 µg of an HIV vaccine or activating agent per about 10 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 6 per about 10 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 gag conditioned media ("CM"). The levels of supernatant p24 ("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 53 276038/2 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 54 276038/2 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 55 276038/2 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 56 276038/2 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: GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGA GGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATA AAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA TCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGT ACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTG ACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAA 57 276038/2 GGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTTGCCAT AAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAAT AAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGT TAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGT TCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACA ATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGG ATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAA CAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCTGA CTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTG AGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTT GGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCC AGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAAT TGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTA GGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTAG AACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATGA CCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGAC ATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGA ATGAAGGGTGCCCACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAA ATAGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCA TACAAAAGGAAACATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGA TTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTA GAGAAAGAACCCATAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATA GGGAAACTAAATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAG TTGTCCCCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCT AGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATATGCA TTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAA TAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACA CAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTGGTCAGTGCTGGAATCAG GAAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATAT CACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCACCTGTAGTAGCAA AAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGG ACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGA AAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAA 58 276038/2 TTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAG ATGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAGTACTACA GTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACA ATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAATTAAAGAAAATTAT AGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGT ATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAA AGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAA ATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTG GAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGA TAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTAT GGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (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: TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTC AGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCC GACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATC CATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCTGT GCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTG GAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTAC AATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGG AGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAG ACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAG GCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAA GAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCC TCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTA ATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACT CGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTT TAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAA AGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAA AGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTA 59 276038/2 ACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGA CTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCC AAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCA CAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTA ATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGG GAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGC CCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCC ATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAG CTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCT AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTT CACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA TGTATCTTATCAGCGGCCGCCCCGGG (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: ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATA
TGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG
GACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAG TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAA
TGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA GTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTC
TGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCG
GGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCA GCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGC
GGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTC GCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCG
CGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAG CGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGG
GCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTG 60 276038/2
TGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCT GCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCC
GGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGC GGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGT
AACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGC GGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCG
GCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGG GGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGC
AGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCA AATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCG
GGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGC GTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGA
CGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACC GGCGGGAATTC (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: GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAAG
TTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAA TTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAGGCA
CAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGGTTG GATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGGACCGA
AGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGAA AGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAA
GTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCT CACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCACAGTTCATCA
ACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTACAACCTGGCAT TCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCCATGGACATCAC
CTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAGGGCACAGGGTTC AGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAATGCAATACT 61
276038/2 GCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATAA
GGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTG CTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTG
GATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTC TCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCCTGCTTTCA
CCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGAGTCGATATT GCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACTACCACAGAAA
GGGAACTGTGGGATGACTGGGCACCATATGAAGACGTGGAAATTGGACCCAATGG AGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACATGGTA
TGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCGAACATCCTCAC ATTCAAGACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACT
GGGCTATCCAAAAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAA GCTCTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCT
CCGAGTTGGTATCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGACAGATTT ATACAGACATAGAGATGAGAATTC (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: 62 276038/2 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:
TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGC GCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGC
AGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACT CACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATAC
CTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTG
CAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCA AATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCAT
ATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACA GCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTT
TTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACAT GTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCT
CTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCAT AGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC
GGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAA TTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATC
CGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCC CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATT
TATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAG GCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATA
ATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCA CTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCACCCGGG (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 63 276038/2 which the CMV promoter is replaced with the RSV promoter. The DNA sequence was as follows: CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTT
AGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAGT AGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTAGT
CTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCA CCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGG
CAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCAGAGA TAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGACCATTCACCAC
ATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCC
CCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGAC GAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCAC
CTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGA GAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTAGCACTTATCT
GGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTC TTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCA
AATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA (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 64 276038/2 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 (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 65 276038/2 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=67126273 60706061801). 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 66 276038/2 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 th Cloning: A Laboratory Manual, 4 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); 67 276038/2 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 68 276038/2 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. 69 276038/2 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. 70 276038/2 Table 2: Development of HIV Vectors Internal Material Description Remarks Decision Code 1 SIH-H1- Lentiviral shRNA Wrong target, lab Abandon shRT-1,3 vector construct for virus, no virus test RT of LAI strain 2 SIH-H1- Lentiviral H1 promoter Tat protein knock- Lead shRT43 vector shRNA down >90% (Tat/Rev Tat/Rev NL4-3) overlap 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'- GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTT TT-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- Lentiviral shRNA Inhibits Gag Abandon shGag-1 vector construct for expression but will LAI Gag inhibit packaging 71 276038/2 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- Lentiviral shRNA Inhibits Pol Abandon shPol-1 vector construct for expression but will Pol inhibit packaging 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 72 276038/2 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.

SIH-H1- Lentiviral shRNA Best of 5 Lead shCCR5-1 vector construct for candidates, CCR5 Extracellular CCR5 protein reduction >90% 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'- 73 276038/2 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'-GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTAAACTGAGCTTGCTCTTTTT- 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- Lentiviral U6 promoter- Toxic to cells Abandon TAR vector TAR 7 SIH-U6- Lentiviral U6 promoter- Toxic to cells Abandon TAR-H1- vector TAR-H1-shCCR5 shCCR5 8 U6-TAR- Lentiviral U6 promoter- Suppress HIV, Abandon H1-shRT vector TAR-H1-RT toxic to cells, poor packaging 9 U6-TAR- Lentiviral Change Toxic, poor Abandon 7SK-shRT vector shRNA packaging promoter to 7SK U6-TAR- Lentiviral U6 promoter- Toxic, poor Abandon H1-shRT- vector TAR-H1-RT- packaging, H1 H1-shCCR5 H1-shCCR5 repeats 11 U6-TAR- Lentiviral Change Toxic, poor Abandon 7SK-shRT- vector shRNA packaging H1-CCR5 promoter to 7SK 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'-CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTCTCGACAGCGACCAGATCT 74 276038/2 GAGCCTGGGAGCTCTCTGGCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAAT TCTTACTGAGCAAGAAA-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'- GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGG CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAG GGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCA TAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACT TGGATCCGCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCT CCGCTTTTT-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'- GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAGG CGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAG GGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACCA TAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTTCTGTATGAGACCACT TGGATCCGTGTCAAGTCCAATCTATGTTCAAGAGACATAGATTGGACTTGACAC TTTTT-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 microRNA Extracellular CCR5 Lead vector sequence protein reduction >90% 75 276038/2 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'- CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTAC TGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTC GCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCC GTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTT GAATTACTTCCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGG TTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTC GTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGT GGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTT TTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGC CAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCC CGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTC GCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCA GTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAA TGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAA AAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGC GCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGT TGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACT GAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTT CCATTTCAGGTGTCGTGA-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 76 276038/2 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'- AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTT-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.

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 microRNA Vif protein Lead vector sequence reduction>80% 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'-

CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATC TGACCA-3') (SEQ ID NO: 2). The miR Vif target sequence is (5'- GGGATGTGTACTTCTGAACTT-3') (SEQ ID NO: 6).

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. 77 276038/2 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 microRNA Tat RNA Lead vector sequence reduction>80% 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'- GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTT CGTC-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. shCCR5- Lentiviral microRNA CCR5 Candidate shVif-shTat vector cluster reduction>90%, sequence Vif protein reduction>80%, Tat RNA reduction>80%, >95% inhibition of HIV replication 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'- 78 276038/2 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGA AGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTT CAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTA CTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGAC ATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGA GGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAG CGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC-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 79 276038/2 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 6 of 2x10 /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+ Peptides+ MVA+ IL- MVA+ IL- IL-2+IL-12 IL-7+IL-15 2+IL-12 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 6 cytokines used in previous steps, with cells at 0.5x10 viable cells per ml. 80 276038/2 From days 14 to 23, the number of the cells was evaluated every 2 days and the cells 6 were diluted to 0.5 x 10 /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 6 cultured for 1 day in fresh medium at 1x10 /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. 6 Cultured cells after peptide stimulation or after lentivirus transduction at 1x10 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 81 276038/2 (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 82 276038/2 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'- 83 276038/2 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'GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCTTCGTCGCTGTCTCCGCTTT TT-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- 84 276038/2 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- CMV-mCherry-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE- mCherry Control-GFP CMV-mCherry AGT103/CMV-GFP CMV-GFP-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE- Abbreviations: EF-1: elongation factor 1 transcriptional promoter miR30CCR5 – synthetic microRNA capable of reducing CCR5 protein on cell surfaces miR21Vif – synthetic microRNA capable of reducing levels of HIV RNA and Vif protein expression 85 276038/2 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'-GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGT CCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCG TC-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'- 86 276038/2 CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGA ATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTG ACCA-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. 87 276038/2 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'AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATG TTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTG CTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTA TGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTG ACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACT TTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGC TGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGA AATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGA CGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCC TGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGG ATCTCCCTTTGGGCCGCCTCCCCGCCT-3') (SEQ ID NO: 32) 88 276038/2 with a modified AGT103 vector containing a shortened WPRE element (5'AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTAT GTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTAATGCCTCTGTATCATGCTATT GCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTT ATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCT GACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGGGAC TTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCC GCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTC-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 89 276038/2 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'TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCCTCTGCAAAGAAAAAAAAA
AAAAAAAAAGAACAAAGGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAGATGAA GCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGGAGGCGGATCCTGTCAGCTTTGCT 90 276038/2 CTCTCTGTGGCTGGCAGTTTCTCCAAAGGGTAACAGGTGTCAGCTGGCTGAGCCTA
GGCTGAACCCTGAGACATGCTACCTCTGTCTTCTCATGGCTGGAGGCAGCCTTTGT AAGTCACAGAAAGTAGCTGAGGGGCTCTGGAAAAAAGACAGCCAGGGTGGAGGT
AGATTGGTCTTTGACTCCTGATTTAAGCCTGATTCTGCTTAACTTTTTCCCTTGACT TTGGCATTTTCACTTTGACATGTTCCCTGAGAGCCTGGGGGGTGGGGAACCCAGCT
CCAGCTGGTGACGTTTGGGGCCGGCCCAGGCCTAGGGTGTGGAGGAGCCTTGCCA TCGGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGCAGA-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: 91 276038/2 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 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 92 276038/2 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 93 276038/2 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 1×10 cells per dose that will contain 8 approximately 1×10 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. 94 276038/2 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 95 276038/2 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. 96 276038/2 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 97 276038/2 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. 98 276038/2 + 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 99 276038/2 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 -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 100 276038/2 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 8 stimulation and subsequent polyclonal expansion phases of ex vivo culture, 4x10 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 Percentage HIV- Percentage HIV- Material/manipulation Total CD4 T cells specific and specific HIV-resistant Leukapheresis pack 8 ~7x10 ~0.12 N/A from HIV+ patient Peptide expansion ex 8 ~8x10 ~2.4 N/A vivo Mitogen expansion ~1.5x10 ~2.4 N/A Lentivirus transduction ~1.5x10 ~2.4 ~1.6 Example 20: Clinical Study for Treatment of HIV 7 AGT103T is a genetically modified autologous PBMC containing > 5 x 10 HIV-specific CD4 T cells that are also transduced with AGT103 lentivirus vector. 101 276038/2 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, 3 CD4+ T-cell counts >600 cells per mm 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 > 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 < 1x10 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 7 the product must include > 0.5 x 10 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 < 1 x10 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 102 276038/2 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 103 276038/2 • 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 4 weekly draws not exceeding 5x10 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 genetically modified, autologous CD4+ T cells 7 containing > 5 x 10 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 104 276038/2 autologous CD4 T cells (AGT103T) in adult research participants with confirmed HIV infection, 3 CD4+ T-cell counts >600 cells per mm 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 < 1x10 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 7 include > 0.5 x 10 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 < 1 x10 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 105 276038/2 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 Description Sequence NO: 1 miR30 CCR5 AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACT GAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGA GCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT TCAAGGGGCTT 2 miR21 Vif CATCTCCATGGC TG TACCACCTTGTCGGGGGATGTG TACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAG AAGAACACATCCGCACTGACATTTTGGTATCTTTCA TCTGACCA 3 miR185 Tat GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT TCTTCCTGCCATAGCGTGG TCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCC CTCCCAATGACCGCGTCTTCGTC 4 Elongation CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG Factor-1 alpha GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC 106 276038/2 (EF1-alpha) GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT promoter CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCC TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACAC TGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTC GAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGA GGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA AGTTTTTTTCTTCCATTTCAGGTGTCGTGA CCR5 target GAGCAAGCTCAGTTTACA sequence 6 Vif target GGGATGTGTACTTCTGAACTT sequence 107 276038/2 7 Tat target TCCGCTTCTTCCTGCCATAG sequence 8 TAR decoy CTTGCAATGATGTCGTAATTTGCGTCTTACCTCGTTC sequence TCGACAGCGACCAGATCTGAGCCTGGGAGCTCTCTG GCTGTCAGTAAGCTGGTACAGAAGGTTGACGAAAA TTCTTACTGAGCAAGAAA 9 Rev/Tat target GCGGAGACAGCGACGAAGAGC sequence Rev/Tat shRNA GCGGAGACAGCGACGAAGAGCTTCAAGAGAGCTCT sequence TCGTCGCTGTCTCCGCTTTTT 11 Gag target GAAGAAATGATGACAGCAT sequence 12 Gag shRNA GAAGAAATGATGACAGCATTTCAAGAGAATGCTGT sequence

CATCATTTCTTCTTTTT 13 Pol target CAGGAGCAGATGATACAG sequence 14 Pol shRNA CAGGAGATGATACAGTTCAAGAGACTGTATCATCTG sequence CTCCTGTTTTT
CCR5 target GTGTCAAGTCCAATCTATG sequence #1 16 CCR5 shRNA GTGTCAAGTCCAATCTATGTTCAAGAGACATAGATT sequence #1 GGACTTGACACTTTTT 17 CCR5
target GAGCATGACTGACATCTAC sequence #2 18 CCR5 shRNA GAGCATGACTGACATCTACTTCAAGAGAGTAGATGT sequence #2 CAGTCATGCTCTTTTT 19 CCR5 target
GTAGCTCTAACAGGTTGGA sequence #3 CCR5 shRNA GTAGCTCTAACAGGTTGGATTCAAGAGATCCAACCT sequence #3 GTTAGAGCTACTTTTT 21 CCR5 target
GTTCAGAAACTACCTCTTA sequence #4 108 276038/2 22 CCR5 shRNA GTTCAGAAACTACCTCTTATTCAAGAGATAAGAGGT sequence #4 AGTTTCTGAACTTTTT 23
CCR5 target GAGCAAGCTCAGTTTACACC sequence #5 24 CCR5 shRNA GAGCAAGCTCAGTTTACACCTTCAAGAGAGGTGTA sequence #5 AACTGAGCTTGCTCTTTTT
Homo sapiens ATGGATTATCAAGTGTCAAGTCCAATCTATGACATC CCR5 gene, AATTATTATACATCGGAGCCCTGCCAAAAAATCAAT sequence 1
GTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTC TACTCACTGGTGTTCATCTTTGGTTTTGTGGGC 26 Homo sapiens
AACATGCTGGTCATCCTCATCCTGATAAACTGCAAA CCR5 gene, AGGCTGAAGAGCATGACTGACATCTACCTGCTCAAC sequence 2
CTGGCCATCTCTGACCTGTTTTTCCTTCTTACTGTCC CCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACT TTGGAAATACAATGTGTCAACTCTTGACAGGGCTCT
ATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCAT CCTCCTGACAATCGATAGGTACCTGGCTGTCGTCCA TGCTGTGTTTGCTTTAAAAGCCAGGACGGTCACCTT
TGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGC TGTGTTTGCGTCTCTCCCAGGAATCATCTTTACCAG ATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTC
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AATCCTAAAAACTCTGCTTCGGTGTCGAAATGAGAA GAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCAT CATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAAC
ATTGTCCTTCTCCTGAAC 27 Homo sapiens ACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGT CCR5 gene, AGCTCTAACAGGTTGGACCAAGCTATGCAGGTGA sequence
3 109 276038/2 28 Homo sapiens CAGAGACTCTTGGGATGACGCACTGCTGCATCAACC CCR5 gene, CCATCATCTATGCCTTTGTCGGGGAGAAGTTCAGAA sequence 4
ACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCA AACGCTTCTGCAAATGCTGTTCTATTTTCCAG 29 Homo sapiens CAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACAC
CCR5 gene, CCGATCCACTGGGGAGCAGGAAATATCTGTGGGCTT sequence 5 GTGA CD4 promoter TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCC sequence
TCTGCAAAGAAAAAAAAAAAAAAAAAAAGAACAAA GGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAGAT GAAGCCTCTTCTTTCAAGGGAGTGGGGTTGGGGTGG
AGGCGGATCCTGTCAGCTTTGCTCTCTCTGTGGCTG GCAGTTTCTCCAAAGGGTAACAGGTGTCAGCTGGCT GAGCCTAGGCTGAACCCTGAGACATGCTACCTCTGT
CTTCTCATGGCTGGAGGCAGCCTTTGTAAGTCACAG AAAGTAGCTGAGGGGCTCTGGAAAAAAGACAGCCA GGGTGGAGGTAGATTGGTCTTTGACTCCTGATTTAA
GCCTGATTCTGCTTAACTTTTTCCCTTGACTTTGGCA TTTTCACTTTGACATGTTCCCTGAGAGCCTGGGGGG TGGGGAACCCAGCTCCAGCTGGTGACGTTTGGGGCC
GGCCCAGGCCTAGGGTGTGGAGGAGCCTTGCCATC GGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGC AGA 31 miR30-
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACT CCR5/miR21- GAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGA Vif/miR185 Tat
GCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACT microRNA TCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCA cluster sequence
CCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTG AATCTCATGGAGTTCAGAAGAACACATCCGCACTG ACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGC
CTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAG 110 276038/2
AAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT C 32 Long WPRE AATCAACCTCTGATTACAAAATTTGTGAAAGATTGA sequence
CTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATG TGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATA
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GCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTG CTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTC GCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCT
CCCCGCCT 33 Elongation CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG Factor -1 alpha GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC
GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT (EF1 -alpha) CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC promoter; miR30CCR5;
CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC miR21Vif; CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG
miR185 Tat ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG
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CTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAG AAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGT C 34 Rous
Sarcoma GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACAT virus (RSV) GGTAACGATGAGTTAGCAACATGCCTTACAAGGAG promoter
AGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTA AGGTGGTACGATCGTGCCTTATTAGGAAGGCAACA GACGGGTCTGACATGGATTGGACGAACCACTGAAT 112 276038/2
TGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAG CTCGATACAATAAACG 5’ Long terminal GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC repeat (LTR)
TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTG CCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCC
TCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA 36 Psi Packaging TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGG signal AGAGAG 37 Rev response
AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG element (RRE) AAGCACTATGGGCGCAGCCTCAATGACGCTGACGG
TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG
CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA CCTAAAGGATCAACAGCTCC 38 Central TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTG polypurine tract
CAGGGGAAAGAATAGTAGACATAATAGCAACAGAC (cPPT) ATACAAACTAAAGAATTACAAAAACAAATTACAAA ATTCAAAATTTTA 39 3’ delta LTR
TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACC AGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGA
ACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAG TGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT CTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAG
TGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA 40 Helper/Rev; TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT CMV early
TCATAGCCCATATATGGAGTTCCGCGTTACATAACT (CAG) enhancer; TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG Enhance
ACCCCCGCCCATTGACGTCAATAATGACGTATGTTC Transcription CCATAGTAACGCCAATAGGGACTTTCCATTGACGTC 113 276038/2
AATGGGTGGACTATTTACGGTAAACTGCCCACTTGG CAGTACATCAAGTGTATCATATGCCAAGTACGCCCC CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC
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beta CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCA actin (CAG) ATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGC promoter;
GATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGG Transcription CGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG
AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC GGGCG 42
Helper/Rev; GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC Chicken beta CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG actin intron;
ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG Enhance gene CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAA expression
TGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGC CTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGG GGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC
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276038/2 CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGC GGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT
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ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA Gag; Viral ATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG capsid
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CTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCA GCGACCCCTCGTCACAATAA 44 Helper/Rev; HIV ATGAATTTGCCAGGAAGATGGAAACCAAAAATGAT Pol; Protease

and AGGGGGAATTGGAGGTTTTATCAAAGTAGGACAGT reverse ATGATCAGATACTCATAGAAATCTGCGGACATAAA transcriptase
GCTATAGGTACAGTATTAGTAGGACCTACACCTGTC AACATAATTGGAAGAAATCTGTTGACTCAGATTGGC TGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTG
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ACACAAAGGAATTGGAGGAAATGAACAAGTAGATG GGTTGGTCAGTGCTGGAATCAGGAAAGTACTA 45 Helper Rev; HIV TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA
Integrase; TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA Integration of GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAA viral RNA
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GTAGACAGGATGAGGATTAA 118 276038/2 46 Helper/Rev; HIV AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG RRE; Binds Rev
AAGCACTATGGGCGCAGCGTCAATGACGCTGACGG element TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA
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ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC Rev; Nuclear TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC export and
AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA stabilize viral CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG mRNA
AGAGACAGAGACAGATCCATTCGATTAGTGAACGG ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT
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AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT Rabbit beta CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA globin poly A;
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TCATAGCCCATATATGGAGTTCCGCGTTACATAACT 119 276038/2 enhancer; TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG Enhance
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CTTGGCAGTACATCTACGTATTAGTCATC 50 Helper; Chicken GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTG beta actin (CAG)
CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCA promoter; ATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGC Transcription
GATGGGGGCGGGGGGGGGGGGGGCGCGCCAGG CGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG
CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC GGGCG 51 Helper; Chicken
GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC beta actin intron; CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG Enhance gene

ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG expression CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAA TGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGC
CTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGG GGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC
GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTG TGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGG GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG
GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGG GGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTG TAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGA
GCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGG GGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG 120 276038/2
TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCC GCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCG GCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGG
CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGC GGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT
CTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCA GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCG CCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGG
GGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGAC CGGCGG 52 Helper; HIV
ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA Gag; Viral ATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG capsid
GGGGAAAGAAAAAATATAAATTAAAACATATAGTA TGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACA
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AGGGGGAATTGGAGGTTTTATCAAAGTAGGACAGT reverse ATGATCAGATACTCATAGAAATCTGCGGACATAAA transcriptase
GCTATAGGTACAGTATTAGTAGGACCTACACCTGTC AACATAATTGGAAGAAATCTGTTGACTCAGATTGGC TGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTG
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Integrase; TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA Integration of GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAA viral RNA
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GTAGACAGGATGAGGATTAA 55 Helper; HIV AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG RRE; Binds Rev AAGCACTATGGGCGCAGCGTCAATGACGCTGACGG
element TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG
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TCTTATGAAGATC 57 Rev; RSV ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC promoter; TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC Transcription
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CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG 125 276038/2 58 Rev; HIV Rev;
ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC Nuclear export TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC and stabilize
AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA viral mRNA CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG AGAGACAGAGACAGATCCATTCGATTAGTGAACGG
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CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG 59 Rev; Rabbit beta
AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT globin poly A; CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA RNA stability
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TACGGGGTCATTAGTTCATAGCCCATATATGGAGTT Transcription CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG
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GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCG globin intron; CTATTGTAAAATTCATGTTATATGGAGGGGGCAAAG Enhance gene
TTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCT expression TGTATCACCATGGACCCTCATGATAATTTTGTTTCTT TCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTT
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TCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTT CATGCCTTCTTCTCTTTCCTACAG 62 Envelope; VSV- ATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTG G;
Glycoprotein GGGTGAATTGCAAGTTCACCATAGTTTTTCCACACA envelope-cell ACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAATT entry
ACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGC ATAATGACTTAATAGGCACAGCCTTACAAGTCAAA ATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGG
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poly CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA A; RNA stability AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA
TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGC
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TCTCTTATGGAGATC 64 Promoter; EF-1 CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC
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276038/2 GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT
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276038/2 CGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTG AACGCCGATGATTATATAAGGACGCGCCGGGTGTG GCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCG
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CCTTTTGAAATGTAATCATTTGGGTCAATATGTAAT TTTCAGTGTTAGACTAGTAAA 67 Poly A; SV40 GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA
TAGCATCACAAATTTCACAAATAAAGCATTTTTTTC ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA TGTATCTTATCA 68 Poly A; bGH
GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC ACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATT 131
276038/2 GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGG ATTGGGAAGACAATAGCAGGCATGCTGGGGATGCG
GTGGGCTCTATGG 69 HIV Gag; Bal ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA ATTAGATAGGTGGGAAAAAATTCGGTTAAGGCCAG
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276038/2 CACTAGAAGAAATGATGACAGCATGTCAGGGAGTG GGAGGACCCAGCCATAAAGCAAGAATTTTGGCAGA AGCAATGAGCCAAGTAACAAATTCAGCTACCATAA
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AGGAATATGGCAGCTAGATTGTACACATTTAGAAG GAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG GATATATAGAAGCAGAAGTAATTCCAGCAGAGACA
GGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCA GGAAGATGGCCAGTAAAAACAGTACATACAGACAA TGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGC
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GGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAA GGGGCAGTAGTAATACAAGATAATAGTGACATAAA AGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGG
ATTATGGAAAACAGATGGCAGGTGATGATTGTGTG GCAAGTAGACAGGATGAGGATTAA 84 DNA Fragment TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGA containing
Rev, AGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCT RRE and rabbit CTATCAAAGCAACCCACCTCCCAATCCCGAGGGGA beta globin poly
CCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGG A AGAGAGAGACAGAGACAGATCCATTCGATTAGTGA ACGGATCCTTGGCACTTATCTGGGACGATCTGCGGA 149
276038/2 GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACT TACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGG
AATCTCCTACAATATTGGAGTCAGGAGCTAAAGAAT AGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCA GGAAGCACTATGGGCGCAGCGTCAATGACGCTGAC
GGTACAGGCCAGACAATTATTGTCTGGTATAGTGCA GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC AACAGCATCTGTTGCAACTCACAGTCTGGGGCATCA
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276038/2 GAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTT TTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAAT GGTTACAAATAAAGCAATAGCATCACAAATTTCAC
AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT TTGTCCAAACTCATCAATGTATCTTATCAGCGGCCG CCCCGGG 85 DNA fragment
ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTC containing the ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTAC CAG
ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC enhancer/promot CAACGACCCCCGCCCATTGACGTCAATAATGACGTA er/intron
TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTG sequence ACGTCAATGGGTGGACTATTTACGGTAAACTGCCCA CTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC
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CGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTC GGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGG GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG
ACCGGCGGGAATTC 86 DNA fragment GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTAT containing VSV- TCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTC G
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276038/2 AACGGAAAATGCAGCAATTACATATGCCCCACTGTC CATAACTCTACAACCTGGCATTCTGACTATAAGGTC AAAGGGCTATGTGATTCTAACCTCATTTCCATGGAC
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containing RRE AGCAGGAAGCACTATGGGCGCAGCGTCAATGACGC and rabbit beta TGACGGTACAGGCCAGACAATTATTGTCTGGTATAG globin poly A
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ATGGCAGGAAGAAGCGGAGTTCAAGAGACTCCGCT TCTTCCTGCCATTTTTT 91 H1 promoter and GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG shRT sequence
CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA
TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC 155 276038/2 ACTTGGATCCGCGGAGACAGCGACGAAGAGCTTCA
AGAGAGCTCTTCGTCGCTGTCTCCGCTTTTT 92 H1 CCR5 GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG sequence
CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA
TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC ACTTGGATCCGTGTCAAGTCCAATCTATGTTCAAGA
GACATAGATTGGACTTGACACTTTTT 93 Primer AGGAATTGATGGCGAGAAGG 94 Primer CCCCAAAGAAGGTCAAGGTAATCA 95 Primer AGCGCGGCTACAGCTTCA 96
Primer GGCGACGTAGCACAGCTTCP 97 AGT103 CCR5 TGTAAACTGAGCTTGCTCTA miR30 98 AGT103-R5-1 TGTAAACTGAGCTTGCTCGC 99 AGT103-R5-2
CATAGATTGGACTTGACAC 100 CAG promoter TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT TCATAGCCCATATATGGAGTTCCGCGTTACATAACT
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CTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGT ATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGG GGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGC 156
276038/2 GGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGG AGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCT CCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCG
GCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG 101 H1 element GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG
CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA
TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC ACTT 102 3′ LTR
TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACC AGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGA
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TGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA 103 7SK promoter CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGC
ATTCTGGATAGTGTCAAAACAGCCGGAAATCAAGT CCGTTTATCTCAAACTTTAGCATTTTGGGAATAAAT GATATTTGCTATGCTGGTTAAATTAGATTTTAGTTA

AATTTCCTGCTGAAGCTCTAGTACGATAAGCAACTT GACCTAAGTGTAAAGTTGAGATTTCCTTCAGGTTTA TATAGCTTGTGCGCCGCCTGGCTACCTC 104 miR155 Tat CTGGAGGCTTGCTGAAGGCTGTATGCTGTCCGCTTC TTCCTGCCATAGGGTTTTGGCCACTGACTGACCCTA TGGGGAAGAAGCGGACAGGACACAAGGCCTGTTAC 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 157 276038/2 embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present inventio 158

Patent Citations (153)

Publication number	Priority date	Publication date	Assignee	Title
Family To Family Citations				
US5668255A	1984-06-07	1997-09-16	Seragen, Inc.	Hybrid molecules having translocation region and cell-binding region
AU6014094A	1992-12-02	1994-06-22	Baylor College Of Medicine	Episomal vectors for gene therapy
WO1995002697A1	1993-07-13	1995-01-26	Rhone-Poulenc Rorer S.A.	Defective adenovirus vectors and use thereof in gene therapy
CA2265460A1	1996-09-11	1998-03-19	The Government Of The United States Of America, Represented By The Secre Tary, Department Of Health And Human Services	Aav4 vector and uses thereof
WO1999009139A1	1997-08-15	1999-02-25	Rubicon Laboratory, Inc.	Retrovirus and viral vectors
WO1999021979A1	1997-10-28	1999-05-06	Maxygen, Inc.	Human papillomavirus vectors
JP2002506652A	1998-03-20	2002-03-05	トラステイーズ・オブ・ザ・ユニバーシテイ・オブ・ペンシルベニア	Compositions and methods for helper-free production of recombinant adeno-associated virus
DK1115290T3	1998-10-01	2009-06-22	Univ Southern California	Retroviral gene delivery system and methods for its use
US6156514A	1998-12-03	2000-12-05	Sunol Molecular Corporation	Methods for making recombinant cells
US6410013B1	1999-01-25	2002-06-25	Musc Foundation For Research Development	Viral vectors for use in monitoring HIV drug resistance
WO2000072886A1	1999-05-26	2000-12-07	Dana-Farber Cancer Institute, Inc.	Episomally replicating lentiviral vectors
AU2001257611A1	2000-04-28	2001-11-12	Avigen, Inc.	Polynucleotides for use in recombinant adeno-associated virus virion production
AU2001261515A1	2000-05-12	2001-11-26	The Regents Of The University Of California	Treatment of human papillomavirus (hpv)-infected cells
WO2001091802A1	2000-05-30	2001-12-06	Baylor College Of Medicine	Chimeric viral vectors for gene therapy
NO314588B1 *	2000-09-04	2003-04-14	Bionor Immuno As	HIV peptides, antigens, vaccine composition, immunoassay test kits and a method for detecting

				antibodies induced by HIV
US7122181B2	2000-12-19	2006-10-17	Research Development Foundation	Lentiviral vector-mediated gene transfer and uses thereof
US20030119770A1	2001-08-02	2003-06-26	Zhennan Lai	Intercellular delivery of a herpes simplex virus VP22 fusion protein from cells infected with lentiviral vectors
WO2003015708A2	2001-08-18	2003-02-27	Myriad Genetics, Inc	Composition and method for treating hiv infection
US7737124B2	2001-09-13	2010-06-15	California Institute Of Technology	Method for expression of small antiviral RNA molecules with reduced cytotoxicity within a cell
WO2003040311A2	2001-10-25	2003-05-15	The Government Of The United States Of America As Represented By The Secretary Of Health And Human Services	Efficient inhibition of hiv-1 viral entry through a novel fusion protein including of cd4
US20070203333A1	2001-11-30	2007-08-30	Mcswiggen James	RNA interference mediated inhibition of vascular endothelial growth factor and vascular endothelial growth factor receptor gene expression using short interfering nucleic acid (siNA)
CA2479530A1	2002-03-20	2003-10-02	Massachusetts Institute Of Technology	Hiv therapeutic
US20040142416A1	2002-04-30	2004-07-22	Laipis Philip J.	Treatment for phenylketonuria
WO2004037847A2	2002-05-07	2004-05-06	Chiron Corporation	Hiv envelope-cd4 complexes and hybrids
US20040161412A1	2002-08-22	2004-08-19	The Cleveland Clinic Foundation	Cell-based VEGF delivery
DK1545204T3	2002-09-06	2016-11-14	The Government Of The Us Secretary Dept Of Health And Human Services	Immunotherapy with in vitro selected antigen-specific lymphocytes following non-myeloablative lymphodepletive chemotherapy
JP2006505288A	2002-11-04	2006-02-16	ユニバーシティー オブ マサチューセッツ	Allele-specific RNA interference
AU2003283174A1	2002-12-11	2004-06-30	Cytos Biotechnology Ag	Method for protein production
TW200502391A	2003-05-08	2005-01-16	Xcyte Therapies Inc	Generation and isolation of antigen-specific t cells
WO2004104591A2	2003-05-23	2004-12-02	Institut National De La Sante Et De La Recherche Medicale	Improvements to gamma delta t cell-mediated therapy
EP1644508A1	2003-07-11	2006-04-12	Cytos Biotechnology AG	Gene expression system
US20050019927A1	2003-07-13	2005-01-27	Markus Hildinger	DECREASING GENE EXPRESSION IN A MAMMALIAN SUBJECT IN VIVO VIA AAV-MEDIATED RNAi EXPRESSION CASSETTE TRANSFER
US20050138677A1	2003-09-16	2005-06-23	Pfister Herbert J.	Transgenic animal model for the treatment of skin tumors

WO2005028634A2	2003-09-18	2005-03-31	Emory University	Improved mva vaccines
WO2005033282A2	2003-10-01	2005-04-14	Pharmacia & Upjohn Company Llc	Polyamide compositions and therapeutic methods for treatment of human papilloma virus
US20080039413A1	2003-10-21	2008-02-14	Morris David W	Novel compositions and methods in cancer
JPWO2005051927A1	2003-11-26	2007-12-06	株式会社クレハ	Method for culturing CD4-positive T cells by stimulation culture of HIV-1-infected peripheral blood mononuclear cells, and HIV-1 growth inhibitor
EP1753777B1	2004-02-25	2014-05-07	Dana-Farber Cancer Institute, Inc.	METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HIV INFECTION USING TRIM5a
EP1737956A2	2004-03-01	2007-01-03	Massachusetts Institute of Technology	Rnai-based therapeutics for allergic rhinitis and asthma
TWI439284B	2004-04-09	2014-06-01	Abbvie Biotechnology Ltd	Multiple-variable dose regimen for treating tnfα-related disorders
US20080227736A1	2004-06-03	2008-09-18	Regents Of The University Of California,	Targeting Pseudotyped Retroviral Vectors
WO2006012221A2	2004-06-25	2006-02-02	The Regents Of The University Of California	Target cell-specific short interfering rna and methods of use thereof
WO2006023491A2	2004-08-16	2006-03-02	The Cbr Institute For Biomedical Research, Inc.	Method of delivering rna interference and uses thereof
WO2006039721A2	2004-10-08	2006-04-13	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods for bone resorption diseases, cancer, bone pain, immune disorders, and infectious diseases
EP1647595A1 *	2004-10-15	2006-04-19	Academisch Medisch Centrum bij de Universiteit van Amsterdam	Nucleic acids against viruses, in particular HIV
WO2006048215A1	2004-11-02	2006-05-11	Istituto Di Ricerche Di Biologia Molecolare P Angeletti Spa	Adenoviral amplicon and producer cells for the production of replication-defective adenoviral vectors, methods of preparation and use thereof
US7790446B2	2005-02-11	2010-09-07	Kosagen Cell Factory Oü	Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products
CN101160055A	2005-02-16	2008-04-09	莱蒂恩公司	Lentiviral vectors and their use
EP2573185A3	2005-02-16	2013-06-05	Lentigen Corporation	Lentiviral vectors and their use
DK2002003T3	2005-05-27	2016-03-21	Ospedale San Raffaele Srl	Gene vector comprising miRNA
WO2007015122A1	2005-08-02	2007-02-08	Genexel, Inc.	Therapy for alzheimer’s disease
US20070032443A1	2005-08-02	2007-02-08	Jaeseob Kim	Therapy for Alzheimer's disease

WO2007056388A2	2005-11-07	2007-05-18	The General Hospital Corporation	Compositions and methods for modulating poly (adp-ribose) polymerase activity
WO2007133674A2	2006-05-12	2007-11-22	Lentigen Corporation	Lentiviral vector compositions, methods and applications
US8535897B2	2006-06-19	2013-09-17	The Trustees Of Columbia University In The City Of New York	Assays for non-apoptotic cell death and uses thereof
US20080003225A1	2006-06-29	2008-01-03	Henri Vie	Method for enhancing the antibody-dependent cellular cytotoxicity (ADCC) and uses of T cells expressing CD16 receptors
WO2008008719A2	2006-07-10	2008-01-17	Alnylam Pharmaceuticals, Inc.	Compositions and methods for inhibiting expression of the myc gene
EP1878440A1	2006-07-13	2008-01-16	INSERM (Institut National de la Santé et de la Recherche Médicale)	Methods and compositions for increasing the efficiency of therapeutic antibodies using gamma delta cell activator compounds
CN101516365A	2006-07-26	2009-08-26	诺瓦提斯公司	Inhibitors of undecaprenyl pyrophosphate synthase
US20080199961A1	2006-08-25	2008-08-21	Avi Biopharma, Inc.	ANTISENSE COMPOSITION AND METHOD FOR INHIBITION OF miRNA BIOGENESIS
WO2008100292A2	2006-10-16	2008-08-21	Genelux Corporation	Modified vaccinia virus strains for use in diagnostic and therapeutic methods
ES2639568T3 *	2007-01-23	2017-10-27	Janssen Pharmaceutica Nv	Method to design a drug regimen for HIV-infected patients
CA2682694A1	2007-04-12	2008-10-23	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods with enhanced potency for multiple targets including fpps, ggpps, and dpps
US20080293142A1	2007-04-19	2008-11-27	The Board Of Regents For Oklahoma State University	Multiple shRNA Expression Vectors and Methods of Construction
EP2008656A1	2007-06-28	2008-12-31	Bergen Teknologioverforing AS	Compositions for the treatment of hyperphenylalaninemia
US8673477B2	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
WO2009026328A2 *	2007-08-21	2009-02-26	Immune Disease Institute, Inc.	Methods of delivery of agents to leukocytes and endothelial cells
BRPI0821998A2	2008-01-16	2019-08-27	Opal Therapeutics Pty Ltd	immunomodulation compositions and uses thereof.
GB0802754D0	2008-02-14	2008-03-26	Inst Superiore Di Sanito	Antisense RNA targetting CXCR4
EP2090659A1	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.	Infectious particle, process for its preparation and use thereof

WO2009120947A1	2008-03-28	2009-10-01	Virxsys Corporation	Lentivirus-based immunogenic vectors
GB0810209D0	2008-06-04	2008-07-09	Cambridge Entpr Ltd	Pluripotency associated epigenetic factor
US8629334B2	2008-07-16	2014-01-14	University Of Florida Research Foundation, Inc.	Viral-based transient-expression vector system for trees
WO2010022195A2	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system
EP2342321B1	2008-09-17	2018-04-11	Isogenis, Inc.	Construction of fully-deleted adenovirus-based gene delivery vectors and uses thereof
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
WO2010051521A1	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection
US8734795B2	2008-10-31	2014-05-27	Biogen Idec Ma Inc.	Light targeting molecules and uses thereof
WO2011071476A2	2008-11-14	2011-06-16	Life Technologies Corporation	Compositions and methods for engineering cells
EP2191834A1	2008-11-26	2010-06-02	Centre National De La Recherche Scientifique (Cnrs)	Compositions and methods for treating retrovirus infections
WO2010117974A2	2009-04-09	2010-10-14	Stemcyte Inc.	Hiv-resistant stem cells and uses thereof
EP2419113B1	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
EP2425001A4	2009-04-30	2012-11-14	Univ California	Combination anti-hiv vectors, targeting vectors, and methods of use
EP3329772B1	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
SG178909A1	2009-10-08	2012-04-27	Bavarian Nordic As	Generation of a broad t-cell response in humans against hiv
US20120027725A1	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules to treat liver cancer
CN101805750B	2009-12-29	2011-11-30	浙江大学	Construction and application of farnesyl pyrophosphoric acid synthetase RNA (Ribonucleic Acid) interference recombinant lentivirus vector
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WO2011119942A1	2010-03-25	2011-09-29	Vistagen Therapeutics, Inc.	Induction of ips cells using transient episomal vectors
WO2011133687A2	2010-04-20	2011-10-27	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-

				adrenergic receptor degradation
LT2561078T	2010-04-23	2019-01-10	Cold Spring Harbor Laboratory	NOVEL STRUCTURALLY DESIGNED shRNAs
US20110293571A1	2010-05-28	2011-12-01	Oxford Biomedica (Uk) Ltd.	Method for vector delivery
WO2012020757A1	2010-08-10	2012-02-16	タカラバイオ株式会社	Production method for cell populations
US20130281493A1	2010-10-07	2013-10-24	The Trustees Of The University Of Columbia In The City Of New York	Method for Treating Cancer Harboring a p53 Mutation
WO2012061075A2	2010-10-25	2012-05-10	The Regents Of The University Of California	Hiv resistant and functional hematopoietic stem/progenitor cells and macrophages from induced pluripotent stem cells
WO2012115980A1	2011-02-22	2012-08-30	California Institute Of Technology	Delivery of proteins using adeno-associated virus (aav) vectors
JP2014511704A	2011-04-13	2014-05-19	イミュニカム・エイビイ	Method for priming T cells
US9226976B2	2011-04-21	2016-01-05	University Of Massachusetts	RAAV-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
EP2782596A4	2011-11-22	2015-07-29	Philadelphia Children Hospital	Virus vectors for highly efficient transgene delivery
US9745631B2	2011-12-20	2017-08-29	Dana-Farber Cancer Institute, Inc.	Methods for diagnosing and treating oncogenic kras-associated cancer
BR112014019431A8	2012-02-07	2017-07-11	Global Bio Therapeutics Usa Inc	COMPARTMENTALIZED METHOD OF DELIVERY OF NUCLEIC ACID AND COMPOSITIONS AND USES THEREOF
WO2013174404A1	2012-05-23	2013-11-28	Ganymed Pharmaceuticals Ag	Combination therapy involving antibodies against claudin 18.2 for treatment of cancer
AU2013273483A1	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
WO2014016817A2	2012-07-17	2014-01-30	Universite De Geneve	Nucleic acids for down-regulation of gene expression
CA2922005A1	2012-09-27	2014-04-03	Population Diagnostics, Inc.	Methods and compositions for screening and treating developmental disorders
JP6391582B2	2012-11-13	2018-09-19	コディアック バイオサイエンシズ インコーポレイテッド	Methods for delivering therapeutic agents
CA2892448A1	2012-12-05	2014-06-12	Sangamo Biosciences, Inc.	Methods and compositions for regulation of metabolic disorders
US9642921B2	2012-12-20	2017-05-09	Tocagen Inc.	Cancer combination therapy and recombinant vectors
WO2014117050A2	2013-01-26	2014-07-31	Mirimus, Inc.	Modified mirna as a scaffold for shrna

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WO2015042308A2	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors
AU2014340083B2	2013-10-22	2019-08-15	Translate Bio, Inc.	mRNA therapy for phenylketonuria
CN106459995B	2013-11-07	2020-02-21	爱迪塔斯医药有限公司	CRISPR-associated methods and compositions using dominant grnas
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GB201322091D0	2013-12-13	2014-01-29	Cambridge Entpr Ltd	Modified serpins for the treatment of bleeding disorders
CA2946312A1	2014-04-23	2015-10-29	Juno Therapeutics, Inc.	Methods for isolating, culturing, and genetically engineering immune cell populations for adoptive therapy
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PL3689899T3	2014-04-25	2022-01-31	2Seventy Bio, Inc.	Mnd promoter chimeric antigen receptors
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SI3197472T1	2014-09-22	2022-01-31	Tanea Medical Ab	Recombinant phe-free proteins for use in the treatment of phenylketonuria
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CN107405357B	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
JP2017534284A *	2014-10-27	2017-11-24	インターナショナル エイズ バクシーン イニシアティブ	Genetically stable replicable Sendai virus vector containing and expressing an optimized HIV gene
WO2016069716A1	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds

WO2016186708A1	2015-05-18	2016-11-24	Calimmune, Inc.	Gene therapeutic for the treatment of hiv and uses thereof
GB201509202D0	2015-05-28	2015-07-15	Ge Healthcare Bio Sciences Ab	Semi-static cell culture
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WO2017007994A1 *	2015-07-08	2017-01-12	American Gene Technologies International Inc.	Hiv pre-immunization and immunotherapy
JP6780870B2	2015-08-13	2020-11-04	北昊干細胞与再生医学研究院有限公司ＢｅｉｈａｏＳｔｅｍＣｅｌｌＡｎｄＲｅｇｅｎｅｒａｔｉｖｅＭｅｄｉｃｉｎｅＲｅｓｅａｒｃｈＩｎｓｔｉｔｕｔｅＣｏ．，Ｌｔｄ．	Induced expanded pluripotent stem cells, how to make and use
CN105112370B	2015-08-25	2019-02-05	杭州优善生物科技有限公司	A kind of method and its application of stimulated in vitro peripheral blood gamma delta T cells high efficiently multiplying
JP7059179B2	2015-10-20	2022-04-25	アンスティチュ ナショナル ドゥ ラ サンテ エ ドゥ ラ ルシェルシュ メディカル	Methods and products for genetic engineering
US11389546B2	2015-12-09	2022-07-19	Modernatx, Inc.	Heterologous UTR sequences for enhanced mRNA expression
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JP7173548B2	2016-06-08	2022-11-16	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
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WO2018025923A1	2016-08-03	2018-02-08	国立大学法人鹿児島大学	Anti-htlv-1 drug and therapeutic agent for htlv-1-associated myelopathy/tropical spastic paraparesis (ham/tsp)
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EP3565564A4	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
CN110621322A	2017-02-08	2019-12-27	达纳-法伯癌症研究所有限公司	Modulatable endogenous protein degradation with heterobifunctional compounds
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
US20200181645A1	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
CN111433368A	2017-10-02	2020-07-17	美国基因技术国际有限公司	Vector with promoter and enhancer combination for treating phenylketonuria
WO2020011247A1	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
KR20220068954A	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression

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EP3565564A4 *	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
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CN112105731A	2018-03-30	2020-12-18	日内瓦大学	micro-RNA expression constructs and uses thereof
JPWO2020027094A1 *	2018-07-31	2021-09-16	サイアス株式会社	A method for producing a regenerated T cell population via iPS cells
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US20220211845A1 *	2019-05-08	2022-07-07	The Wistar Institute Of Anatomy And Biology	Dna encoded il-36 gamma as an adjuvant
CN112048523A *	2019-06-05	2020-12-08	南京艾德免疫治疗研究院有限公司	Method for preparing high-titer lentiviral vector by conventional centrifugation
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* Cited by examiner, † Cited by third party, ‡ Family to family citation

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Publication	Publication Date	Title
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US20210121561A1	2021-04-29	Methods of producing cells resistant to hiv infection
US20200384021A1	2020-12-10	Hiv immunotherapy with no pre-immunization step
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Priority And Related Applications

Applications Claiming Priority (4)

Application	Filing date	Title
US201662360185P	2016-07-08	
US201662385864P	2016-09-09	
US201662409270P	2016-10-17	
PCT/US2017/013019	2017-01-11	Hiv pre-immunization and immunotherapy

Concepts

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Name	Image	Sections	Count	Query match
immunization		title,claims,description	34	0.000
immunization		title,claims,description	33	0.000
immunotherapy		title,claims,description	18	0.000
cell		claims,description	338	0.000
vector		claims,description	302	0.000
T-lymphocyte		claims,description	257	0.000
plasmid		claims,description	141	0.000
peripheral blood mononuclear cell		claims,description	113	0.000
method		claims,description	112	0.000
proteins and genes		claims,description	111	0.000
microRNA		claims,description	109	0.000
MicroRNAs		claims,description	104	0.000
genetic effect		claims,description	102	0.000
processed proteins & peptides		claims,description	99	0.000
vaccine		claims,description	69	0.000
particle		claims,description	64	0.000

chemical substances by application	claims,description	57	0.000
infectious disease	claims,description	57	0.000
packaging method and process	claims,description	53	0.000
stimulating effect	claims,description	52	0.000
virological effect	claims,description	49	0.000
Bacterial small RNA	claims,description	45	0.000
Nucleic acid sequence	claims,description	43	0.000
HIV vaccine	claims,description	42	0.000
manufacturing process	claims,description	42	0.000
inhibitory effect	claims,description	38	0.000
antigen	claims,description	29	0.000
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targeting	claims,description	28	0.000
4-amino-1-[(2r)-6-amino-2-[[(2r)-2-[[(2r)-2-[[(2r)-2-amino-3-phenylpropanoyl]amino]-3-phenylpropanoyl]amino]-4-methylpentanoyl]amino]hexanoyl]piperidine-4-carboxylic acid	claims,description	27	0.000
Homo sapiens	claims,description	26	0.000
CCR5	claims,description	21	0.000
Envelope protein	claims,description	19	0.000
Protein X	claims,description	19	0.000
transducing effect	claims,description	19	0.000
pol Genes	claims,description	15	0.000
gag Genes	claims,description	13	0.000
rev Genes	claims,description	12	0.000
Gag polyprotein	claims,description	11	0.000
culturing	claims,description	10	0.000

leukocyte	claims,description	9	0.000
transfection	claims,description	6	0.000
rev gene	claims,description	5	0.000
decreasing effect	claims,description	4	0.000
engineering process	claims,description	4	0.000
Syncytin-1	claims	4	0.000
C-C chemokine receptor type 5	claims	1	0.000
C-C chemokine receptor type 5	claims	1	0.000

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