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(71) Applicant: **AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.** [US/US]; 15010 Broschart Road, Suite 110, Rockville, Maryland 20850 (US).

(72) Inventor: **PAUZA, Charles David**; c/o American Gene Technologies International Inc., 15010 Broschart Road, Suite 110, Rockville, Maryland 20850 (US).

(74) Agents: **SIMKIN, Michele M.** et al.; Foley & Lardner LLP, 3000 K Street NW, Suite 600, Washington, District of Columbia 20007 (US).

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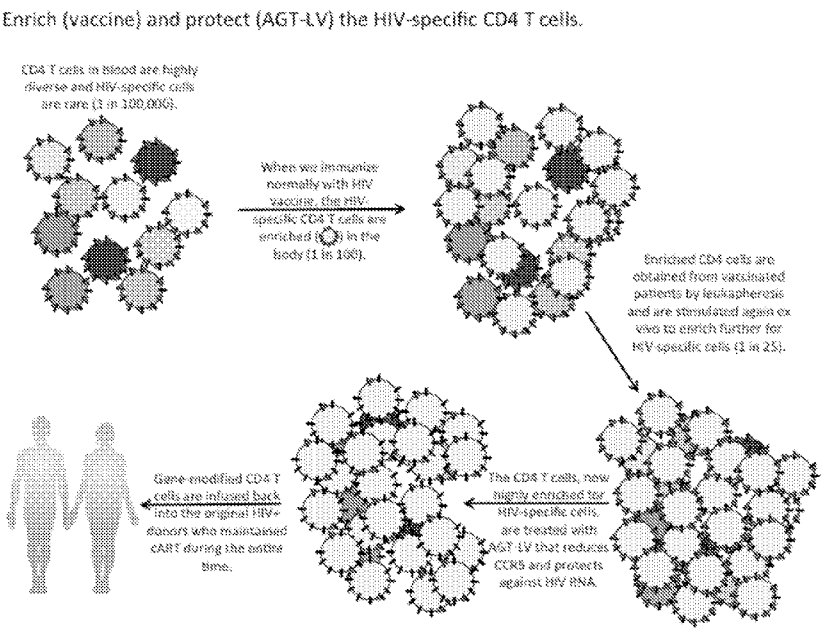

Figure 1

(57) Abstract: The present invention relates generally to immunization and immunotherapy for the treatment or prevention of HIV. In particular, the methods relate to in vivo and ex vivo enrichment of HIV-specific CD4 T cells. In certain embodiments, the disclosed compositions and methods can incorporate therapy in order to further enhance the HIV-specific CD4 T cells.

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HIV PRE-IMMUNIZATION AND IMMUNOTHERAPY

CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority to US Provisional Application No. 62/190,139, filed on July 8, 2015, the disclosure of which is specifically incorporated by reference.

FIELD OF THE INVENTION

[0002] 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 system for the delivery of genes and other therapeutic or diagnostic compositions.

BACKGROUND OF THE INVENTION

[0003] 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 clearly needed.

[0004] Intense interest in immunotherapy for HIV infection has been precipitated by emerging data indicating that the immune system has a major, albeit usually insufficient, role in limiting HIV replication. Virus-specific T-helper cells, which are critical to maintenance of cytolytic T cell (CTL) function, likely have 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*.

[0005] Together these data indicate that increasing the strength and breadth of HIV-specific cellular immune responses might have a clinical benefit through so-called HIV immunotherapy. Some studies have tested vaccines against HIV, but success has been limited to date.

Additionally, there has been interest in augmenting HIV immunotherapy by utilizing gene therapy techniques, but as with other immunotherapy approaches, success has been limited. One such method of implementing an HIV-specific immunotherapy or gene therapy could be via specially designed viral vectors.

[0006] 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.

[0007] 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 developed as therapeutic gene transfer vectors.

[0008] 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.

[0009] 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.

[0010] 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.

[0011] 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.

[0012] Clearly, there is a need for improved treatment of HIV, and the present invention satisfies this need.

SUMMARY OF THE INVENTION

[0013] Disclosed herein are therapeutic immunization strategies and methods as well as highly effective therapeutic lentiviruses and other vectors capable of inhibiting HIV and reducing or altering expression of specific targets. The methods and compositions of the disclosed invention are useful for achieving a functional cure of HIV. More specifically, the invention includes methods for a functional cure of HIV that optimally combine therapeutic immunization of a patient, *ex vivo* re-stimulation of the patient's CD4 T cells, *ex vivo* lentivirus transduction of the enriched T cells, *ex vivo* culture of the cells, and reinfusion of the enriched, genetically modified cells. Additionally, the invention includes bioassays to measure treatment efficacy, sequential changes in therapeutic drug administration, monitoring intervals following withdrawal of HAART, and methods of diagnosis of a functional HIV cure.

[0014] In one aspect, the disclosed invention relates to a method of treating an HIV infection, comprising: (a) identifying a subject in need of treatment of HIV infection; (b) immunizing the

subject with a therapeutically effective amount of an HIV vaccine; (c) removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC); (d) contacting the PBMC *ex vivo* with a therapeutically effective amount of an HIV vaccine (which can be the same as or different from the HIV vaccine used in step (b)); (e) transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; (f) culturing the transduced PBMC for about 1 to about 21 or up to about 35 days (or any time frame in between these parameters, such as 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); and (g) infusing the transduced PBMC into the subject.

[0015] In some embodiments, step (b) and step (d) utilize the same HIV vaccine, while in other embodiments, step (b) and step (d) utilize different HIV vaccines. In some embodiments, a patient may not require step (b) and/or step (d). Accordingly, in some embodiments, the disclosed methods may only comprise steps (a), (c), (d), (e), (f), and (g), or some combination thereof.

[0016] In some embodiments, the subject received cART or HAART prior to infusing the transduced PBMC into the subject. In some embodiments, the subject receives a cyclophosphamide pre-treatment prior to infusing the transduced PBMC into the subject.

[0017] In some embodiments, at least one genetic element is selected from the group consisting of a small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences. In some embodiments, the small RNA molecules targeting HIV RNA sequences are directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

[0018] In some embodiments, the transduced PBMC are cultured for about 1 to about 7 or up to about 10 days (or for any time frame in between these parameters, such as about 2, about 3,

about 4, about 5, about 6, about 7, about 8, about 9, or about 10 days) prior to infusing the transduced PBMC into the subject.

[0019] In another aspect, the disclosed invention relates to a viral vector for transducing HIV-specific CD4 T cells, wherein the viral vector encodes at least one genetic element selected from the group consisting of small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences.

[0020] In some embodiments, the vector is a lentivirus, but in other embodiments the vector is a DNA plasmid, adeno-associated virus, or other integrating or non-integrating vector systems for gene delivery.

[0021] In some embodiments, the small RNA molecules targeting HIV RNA sequences are directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

[0022] In another aspect, the disclosed invention relates to a bioassay for determining whether a HIV+ subject is functionally cured. Such a bioassay comprises determining the number of HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus, wherein the subject is functionally cured if the number of HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus is above a threshold value after a specified time following treatment according to disclosed methods.

[0023] In some embodiments, the threshold value is about $1x10^8$ HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus, but the threshold value may be determined to be higher or lower than this value.

[0024] In some embodiments, the specified time following treatment is about 30 to about 60 days (or any time frame in between these two values), while in other embodiments the specified time following treatment is about 12 to about 26 weeks (or any time frame in between these two values).

[0025] In yet another aspect, the disclosed invention relates to a method of achieving a functional cure for HIV in a HIV+ subject. The method comprises: (a) identifying a subject that is HIV+; (b) immunizing the subject with a therapeutically effective amount of an HIV vaccine; (c) removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC); (d) contacting the PBMC *ex vivo* with a therapeutically effective amount of an HIV vaccine; (e) transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element; (f) culturing the transduced PBMC for about 1 to about 21 or up to 35 days (or any timeframe in between these values); and (g) infusing the transduced PBMC into the subject, wherein the HIV+ subject achieves a functional cure.

[0026] In some embodiments step (b) and step (d) comprise the same HIV vaccine, while in other embodiments, step (b) and step (d) comprise different HIV vaccines.

[0027] In some embodiments, the subject received cART or HAART prior to infusing the transduced PBMC into the subject. In some embodiments, the subject receives a cyclophosphamide pre-treatment or alternative conditioning therapies to improve T cell engraftment prior to infusing the transduced PBMC into the subject.

[0028] In some embodiments, at least one genetic element is selected from the group consisting of a small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences. In some embodiments, the small RNA molecules targeting HIV RNA sequences are directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

[0029] In some embodiments, the transduced PBMC are cultured for about 1 to about 7 or up to about 12 days (or any timeframe in between these two values, or other time periods described herein) prior to infusing the transduced PBMC into the subject.

[0030] The foregoing general description and following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be

readily apparent to those skilled in the art from the following brief description of the drawings and detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] Figure 1 shows a flow chart diagram of possible clinical therapy incorporating vaccination, cell collection to obtain PBMC and CD4 T cells, *ex vivo* T cell culture after stimulating with vaccine immunogens and/or CD3/CD28 stimulation and/or mitogen stimulation in the presence of supporting growth cytokines (including, but not limited to, interleukin-2, interleukin-12, interleukin-15, interleukin-6, interleukin-7, and interleukin-23), lentivirus transduction to deliver anti-HIV genetic constructs, brief culture of transduced cells, and infusion back into the original subject.

[0032] Figure 2 shows how CD4 T cells may be altered using gene therapy to prevent other cells from becoming infected or to prevent viral replication.

[0033] Figure 3 shows a schematic of an exemplary therapeutic lentiviral construct. Therapeutic lentivirus constructs may substitute alternate sequences for the promoter region, targeting of regulatory RNA, and types of regulatory RNA. Further, the plasmids used for packaging the lentivirus particles can be altered according to production needs.

[0034] Figure 4 shows exemplary vector sequences. Positive (genomic) strand sequence 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 best for this miR cluster. Sequences that are underlined show the miR cluster consisting of miR30 CCR5 (a modification of the natural human miR30 that redirects to CCR5 mRNA), miR21 Vif (redirects to Vif RNA sequence) and miR185 Tat (redirects to Tat RNA sequence). The sequences that are not underlined and in smaller font are restriction endonuclease cleavage sites that were incorporated into the oligonucleotide primers for each of the miRNA constructs.

[0035] Figure 5 shows that knockdown of CCR5 by an experimental vector prevents R5-tropic HIV infection in AGTc120 cells. Panel (**A**) shows CCR5 expression in AGTc120 cells with or without AGT103 lentivirus vector. Panel (**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.

[0036] Figure 6 shows AGT103 reduces expression of Tat protein expression in cells transfected with HIV expression plasmid.

[0037] Figure 7 shows AGT103 reduces levels of Vif protein expression in cells transfected with a full-length HIV expression plasmid. Cells were untreated (left lane and center lane) or transduced with AGT103 (left lane).

[0038] Figure 8 shows generating a CD4+ T cell population that is highly enriched for HIV-specific, AGT103-transduced CD4 T cells. Panel (A) shows that therapeutic vaccination against HIV has minimal effect on the distribution of CD4+, CD8+ and CD4+/CD8+ T cells. An important CD4 T cell population is shown in the upper left quadrant of these analytical flow cytometry dot plots, and changes from 52% to 57% of total T cells after the vaccination series. These are representative data. Panel (B) shows the expression of CD4 and CD8 in a CD3+ population of peripheral blood mononuclear cells from a participant in an HIV therapeutic vaccine trial that 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 (JPT PepMix) as a source of epitope peptides for T cell stimulation. Panel (C) shows that a combination of PepMix and interleukin-2/interleukin-12 provides for optimal expansion of antigen-specific CD4 T cells. The upper panels show the increase in cytokine (interferon-gamma) secreting cells in post-vaccination specimens exposed to PepMix. Panel (D) shows AGT103 transduction of antigen-expanded CD4 T cells can produce HIV-specific and HIV-resistant helper CD4 T cells for infusion into patients as part of a functional cure for HIV. The upper panels contain the results of analyzing the CD4+ T cell population in culture. The x axis is Green Fluorescent Protein (GFP) emission indicating that individual cells were transduced with the AGT103.

[0039] Figure 9 shows the sequences of various exemplary cellular elements known to restrict HIV replication that may be incorporated into the disclosed vectors.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0040] Disclosed herein are methods and compositions for treating and/or preventing human immunodeficiency virus (HIV) disease to achieve a functional cure. A functional cure is defined as a condition resulting from the disclosed treatments and methods that reduces or eliminates the need for cART and may or may not require supporting adjuvant therapy. The methods of the invention include gene delivery by integrating lentivirus, non-integrating lentivirus, and related viral vector technology as described below.

[0041] Disclosed herein are therapeutic viral vectors (e.g., lentivirus vectors), immunotherapies, and methods for their use in a strategy to achieve a functional cure for HIV infection. The general strategy can include first therapeutic immunization with vaccines intended to produce strong immune responses against HIV in HIV-infected patients with stable suppression of viremia due to daily administration of HAART, for the purpose of enriching 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) re-stimulating CD4 T cells *ex vivo* with HIV vaccine proteins, (3) performing therapeutic lentivirus transduction, *ex vivo* T cell culture, and (4) re-infusion back into the original donor.

[0042] Previous efforts to achieve a cure for HIV have fallen short principally due to the failure to obtain sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications. When this value is below a critical threshold, removing antiretroviral therapy allows HIV re-emergence, followed by 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 the strategy described herein and providing highly effective therapeutic lentiviruses capable of inhibiting HIV, a new strategy for achieving a functional cure of HIV has been developed.

[0043] Also disclosed herein are novel viral vectors for enhancing HIV-specific CD4 T cells including lentiviral vectors and non-integrating, episomally replicating viral vectors and methods of using the same. Episomally replicating vectors like the present invention can comprise viral components from viruses like Papovaviridae (e.g. bovine papillomavirus or BPV) or Herpesviridae (e.g. Epstein Barr Virus or EBV) or Hepadnaviridae (e.g. Hepatitis B Virus or HBV). Episomal replicating vectors derived from these viruses may comprise a replication origin and at least one viral trans-acting factor, e.g., an initiator protein, such as E1 for BPV and EBNA-1 for EBV or HBV polymerase or terminus binding protein of Adenovirus. The process of episomal replication typically incorporates both host cell replication machinery and viral trans-acting factors.

I. Human Immunodeficiency Virus (HIV)

[0044] 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.

[0045] HIV infects vital cells in the human immune system such as helper T cells, although tropism can vary among HIV subtypes. Immune cells that may be specifically susceptible to HIV infection include but are not limited to CD4+ T cells, macrophages, and dendritic cells. HIV infection leads to low levels of CD4+ T cells through a number of mechanisms, including but not limited to apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected CD4+ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections and cancer.

[0046] 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.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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. 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.

[0051] 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 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.

[0052] 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, effectively crippling 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.

[0053] 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.

[0054] 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%, about 1000%, about 1500%, about 2000%, about 2500%, about 3000%, about 3500%, about 4000%, about 4500%, about 5000%, about 5500%, about 6000%, about 6500%, about 7000%, about 7500%, about 8000%, about 8500%, about 9000%, about 9500%, about 10000%, about 11000%, about 12000%, about 13000%, about 14000%, about 15000%, about 16000%, about 17000%, about 18000%, about 19000%, about 20000%, about 25000%, about 30000%, about 35000%, about 40000%, about 45000%, about 50000%, about 55000%, about 60000%, about 65000%, about 70000%, about 75000%, about

80000%, about 85000%, about 90000%, about 95000%, about 100000%, or any value in between.

II. Gene Therapy

[0055] Viral vectors are used to deliver genetic constructs to host cells for the purposes of disease therapy or prevention.

[0056] 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.

[0057] 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).

[0058] 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.

[0059] 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.

[0060] 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.

[0061] For example, in some embodiments the disclosed vectors may include, but are not limited to, the restriction elements found in Table 1 below. The sequences of these exemplary restriction elements are further disclosed in Figure 9.

Table 1

Gene	**Accession Number**
TREX1	NM_016381 (human) XM_015128506.1 (Macaca mulatta)
TREX2	NM_080701/NM_007205 (human XM_015128506.1 (Macaca mulatta)
SAMHD1	NM_015474 (human) JN936895.1 (Macaca mulatta)
MxA	NM_001144925 (human) JX297237.1 (Macaca mulatta)
MxB	NM_002463 (human)
APOBEC3G	NM_021822 (human) XM_015150306 (Macaca mulatta)

TRIM5-alpha	NM_033034 (human) NM_001032910.1 (Macaca mulatta)
Tetherin	NM_004335 (human) FJ943432.1 (Macaca mulatta)

III. Immunotherapy

[0062] 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.

[0063] 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.

[0064] 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.

IV. Methods According to the Invention

[0065] In one aspect, the disclosed invention provides methods for using viral vectors to achieve a functional cure for HIV disease. The methods further 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.

[0066] In one embodiment, the methods include therapeutic immunization as a method for enriching the proportion of HIV-specific CD4 T cells. Therapeutic immunization can 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.

[0067] Therapeutic vaccines can include one or more HIV protein with protein sequences representing the predominant viral types of the geographic region where treatment is occurring. Therapeutic vaccines will include purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, peptides or peptide fragments, virus-like particles (VLPs), biological or chemical adjuvants including cytokines and/or chemokines, vehicles, and methods for immunization. Vaccinations may be administered according to standard methods known in the art and HIV patients may continue antiretroviral therapy during the interval of immunization and subsequent *ex vivo* lymphocyte culture including lentivirus transduction.

[0068] In some embodiments, HIV+ patients can be 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. For instance, the disclosed vector may comprise a recombinant Bacille Calmette Guerin (BCG) strain expressing HIV VLP. BCG is mycobacterium bovis attenuated for use as a human vaccine against tuberculosis. In another embodiment, the vectors can encode virus-like particles (VLPs) to induce higher titers of neutralizing antibodies. In another embodiment, the vectors can 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.

[0069] 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 may 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.

[0070] For example, peripheral blood mononuclear cells (PBMCs) can be obtained by leukapheresis and treated *ex vivo* to obtain about $1x10^{10}$ CD4 T cells of which about 0.1%, about 1%, about 5% or about 10% or about 30% are both HIV-specific in terms of antigen responses, and HIV-resistant by virtue of carrying the therapeutic transgene delivered by the disclosed lentivirus vector. Alternatively, about $1x10^{7}$, about $1x10^{8}$, about $1x10^{9}$, about $1 x10^{10}$, about $1x10^{11}$, or about $1x10^{12}$ CD4 T cells may be isolated for re-stimulation. Any suitable amount of CD4 T cells can be isolated for *ex vivo* re-stimulation.

[0071] 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.

[0072] 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 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold.

[0073] 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.

[0074] 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 about 1 to about 21 days or 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.

[0075] Once the transduced cells have been sufficiently cultured, transduced CD4 T cells are infused back into the original patient. Infusion can be performed using various machines 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.

[0076] In some embodiments, a CCR5-targeted therapy may be added to a subject's antiretroviral therapy regimen, which was continued throughout the treatment process. Examples

of CCR5-targeted therapies include but are not limited to Maraviroc (a CCR5 antagonist) or Rapamycin (immunosuppressive agent that lowers CCR5). In some embodiments, the antiretroviral therapy may be ceased and the subject can be tested for virus rebound. If no rebound occurs, adjuvant therapy can also be removed and the subject can be tested again for virus rebound.

[0077] Continued virus suppression with reduced or no antiretroviral therapy include 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.

[0078] 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 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.

[0079] 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.

[0080] In some embodiment, 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.

[0081] 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 in Section VI.

V. Compositions According to the Invention

[0082] In one aspect, the disclosed invention provides lentiviral vectors capable of delivering genetic constructs to inhibit HIV penetration of susceptible cells. For instance, one mechanism of action is to reduce mRNA levels for CCR5 and/or CXCR4 chemokine receptors and thus reduce the rates for viral entry into susceptible cells.

[0083] Alternatively, the disclosed lentiviral vectors may be capable of inhibiting the formation of DNA and 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.

[0084] The therapeutic lentiviruses disclosed in this application 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.

[0085] As shown in Figure 3, 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;
- c PPT - 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 a\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-prime long terminal repeat where a portion of the U3 region has been deleted to improve safety of the vector.

One of 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.

[0086] Vectors of the invention may include either or both of the types of genetic cargo discussed above (i.e. genetic elements that direct expression of a gene or small RNAs, such as siRNA, shRNA, or miRNA that can prevent translation or transcription), and the vectors of the invention may also encode additionally useful products for the purpose of treatment or diagnosis of HIV. For instance, in some embodiments, these vectors may also encode green fluorescent protein (GFP) for the purpose of tracking the vectors or antibiotic resistance genes for the purposes of selectively maintaining genetically-modified cells *in vivo*.

[0087] The combination of genetic elements incorporated into the disclosed vectors is not particularly limited. For example, a vector may encode a single small RNA, two small RNAs, three small RNA, four small RNAs, five small RNAs, sis small RNAs, seven small RNAs, eight small RNAs, nine small RNAs, or ten 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.

[0088] Those of 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 invention may comprise changes in the plasmids

used for packaging the lentivirus particles; these changes are required to increase levels of production in vitro.

[0089] In some embodiments, the vector used in the disclosed methods may be a DNA plasmid, adeno-associated virus, or other integrating or non-integrating vector systems for gene delivery.

VI. Bioassays

[0090] In one aspect, the present invention includes bioassays for determining the success of HIV treatment for achieving a functional cure. These assays will 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 they proliferate, change the composition of cell surface markers, induce signaling pathways including phosphorylation, 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.

[0091] Thus, in one embodiment, following application of a treatment according to the invention (e.g., (a) immunization, (b) *ex vivo* lymphocyte culture; (c) re-stimulation with purified proteins, inactivated viruses, virally vectored proteins, bacterially vectored proteins, biological or chemical adjuvants including cytokines and/or chemokines, vehicles; and (d) infusion of the enriched, transduced T cells), a patient may be subsequently assayed to determine the efficacy of the treatment. A threshold value of target T cells in the body may be established to measure a functional cure at, for instance, about $1x10^8$ HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus. The value threshold value of cells refers to the total

body content. It may not be measured directly, but instead may be extrapolated from blood CD4 T cell counts using a standard correction. For example, it is common in the art to assume that 90% of CD4 T cells are present in tissues and only 10% are found in blood.

[0092] Alternatively, the threshold value may be about $1x10^5$, about $1x10^6$, about $1x10^7$, about $1x10^9$, or about $1x10^{10}$ CD4 T cells in the body of the patient.

[0093] 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.

[0094] Methods for defining antigen specific T cells with genetic modifications are known in the art. However, 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 new concept in the field of HIV treatment.

VII. Doses and Dosage Forms

[0095] 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.

[0096] In one embodiment, HIV-specific vaccines for the initial *in vivo* immunization may be administered to a subject in need in varying doses. In general, vaccines delivered by intramuscular injection include about 10 μg to about 300 μg, about 25 μg to about 275 μg, about 50 μg to about 250 μg, about 75 μg to about 225, or about 100 μg to about 200 μg of HIV protein, either total virus protein prepared from inactivated virus particles, virus-like particles or purified virus protein from recombinant systems or purified from virus preparations. Recombinant viral or bacterial vectors may be administered by any and all of the routes described. Intramuscular vaccines will include about 1 μg to about 100 μg, about 10 μg to about 90 μg, about 20 μg to about 80 μg, about 30 μg to about 70 μg, about 40 μg to about 60 μg, or

about 50 μg of suitable adjuvant molecules and be suspended in oil, saline, buffer or water in volumes of 0.1 to 5 ml per injection dose, and may be soluble or emulsion preparations. Vaccines delivered orally, rectally, bucally, at genital mucosal or intranasally, including some virally-vectored or bacterially-vectored vaccines, fusion proteins, liposome formulations or similar preparations, may contain higher amounts of virus protein and adjuvant. Dermal, sub-dermal or subcutaneous vaccines utilize protein and adjuvant amounts more similar to oral, rectal or intranasal-delivered vaccines. Depending on responses to the initial immunization, vaccination may be repeated 1-5 times using the same or alternate routes for delivery. Intervals may be of 2-24 weeks between immunizations. Immune responses to vaccination are measured by testing HIV-specific antibodies in serum, plasma, vaginal secretions, rectal secretions, saliva or bronchoalveolar lavage fluids, using ELISA or similar methodology. Cellular immune responses are tested by in vitro stimulation with vaccine antigens followed by staining for intracellular cytokine accumulation followed by flow cytometry or similar methods including lymphoproliferation, expression of phosphorylated signaling proteins or changes in cell surface activation markers. Upper limits of dosing may be determined based on the individual patient and will depend on toxicity/safety profiles for each individual product or product lot.

[0097] 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.

[0098] Immunization will 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* lymphocyte culture/re-stimulation and infusion.

[0099] In one embodiment, HIV-vaccines for immunization are administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising an HIV vaccine can be 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.

[0100] 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.

[0101] 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.

[0102] In another embodiment, the pharmaceutical composition comprising an HIV vaccine can be 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 can include 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 can be 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.

[0103] In a further embodiment, the pharmaceutical composition comprising a HIV vaccine can be 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.

[0104] In yet a further embodiment, the pharmaceutical composition comprising an HIV vaccine can be formulated as a nasal dosage form. Such dosage forms of the present invention comprise solution, suspension, and gel compositions for nasal delivery.

[0105] In one embodiment, the pharmaceutical composition can be 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 can be formulated to be suitable for administration to a pediatric patient.

[0106] In one embodiment, the pharmaceutical composition can be 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 can include propyleneglycol, polyethyleneglycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate. As a base for suppositories, witepsol, macrogol, tween 61, cacao oil, laurin oil or glycerinated gelatin can be used.

[0107] 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.

[0108] For the purposes of re-stimulation, lymphocytes, PBMCs, and/or CD4 T cells are removed from a patient and isolated for re-stimulation and culturing. The isolated cells may be contacted with the same HIV vaccine or activating agent used for immunization or a different HIV vaccine or activating agent. In one embodiment, the isolated cells are contacted with about 10 ng to 5 μg of an HIV vaccine or activating agent per about 10^6 cells in culture (or any other suitable amount). More specifically, the isolated cells may be contacted with about 50 ng, about 100 ng, about 200 ng, about 300 ng, about 400 ng, about 500 ng, about 600 ng, about 700 ng, about 800 ng, about 900 ng, about 1 μg, about 1.5 μg, about 2 μg, about 2.5 μg, about 3 μg, about 3.5 μg, about 4 μg, about 4.5 μg, or about 5 μg of an HIV vaccine or activating agent per about 10^6 cells in culture.

[0109] 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.

[0110] For transduction of the enriched, re-stimulated cells, the cells may be transduced with lentiviral vectors or with other known vector systems as disclosed in Section V and Figure 3. 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.

VIII. Definitions

[0111] 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.

[0112] "A treatment" is intended to target the disease state and combat it, i.e., ameliorate or prevent the disease state. The particular treatment thus will depend on the disease state to be targeted and the current or future state of medicinal therapies and therapeutic approaches. A treatment may have associated toxicities.

[0113] The terms "administration of" or "administering" an active agent should be understood to mean 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.

[0114] The term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present invention, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering

from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient's condition or its severity, and the age, weight, etc., of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, *e.g.*, the route of administration, the condition of the subject, as well as other factors understood by those in the art.

[0115] 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.

[0116] The term "functional cure" refers to a state or condition wherein HIV+ individuals who previously required cART or HAART, may survive with low or undetectable virus replication using lower doses, intermittent doses, or discontinued dosing of cART or HAART. An individual may be said to have been "functionally cured" while still requiring adjunct therapy to maintain low level virus replication and slow or eliminate disease progression. A possible outcome of a functional cure is the eventual eradication of HIV to prevent all possibility of recurrence.

[0117] The term "HIV vaccine" encompasses immunogens plus vehicle plus adjuvant intended to elicit HIV-specific immune responses. 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.

[0118] The terms "individual," "host," "subject," and "patient" are used interchangeably herein.

[0119] As used herein, "expression," "expressed," or "encodes" refers 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.

[0120] 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, generally through pathways that result in the destruction of the target gene mRNA.

[0121] The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

Examples

Example 1 – Clinical Study for Treatment of HIV

[0122] *Screening and informed consent.* Select HIV+ participants receiving combination antiretroviral therapy (cART) with stable suppression of virus burden are chosen to participate.

[0123] *Immunization with a therapeutic HIV vaccine.* A vaccine that already has IND status and has been used in a clinical trial involving HIV+ participants is administered to participants. This

step will increase the relative frequency of HIV-specific CD4 T cells by approximately 1,000-fold.

[0124] Next, blood lymphocytes are removed by leukapheresis and further purified to the peripheral blood mononuclear cell (PBMC) fraction. Alternately, cells may be purified from venous blood by column or density gradient methods.

[0125] Cultured PBMC are stimulated with HIV proteins or peptides matching or complementing the components in the therapeutic vaccine (perhaps using the same vaccine depending on its composition). This step will increase the relative frequency of HIV-specific CD4 T cells by approximately 100-fold.

[0126] Cultured PBMC cells are infected with therapeutic lentivirus or other disclosed vectors, for instance, one that encodes small RNA to interfere with the translation of CCR5 and viral replication proteins. After transduction, the cells are maintained in culture 3-7 days.

[0127] Transduced CD4 T cells are infused back into the original participant. Infusion can be performed according to methods known in the art. This may require pre-treatment with cyclophosphamide to increase the efficiency of re-engraftment.

[0128] For participants receiving cART, cART will be maintained throughout. Following infusion, monitor HIV viral burden in blood and frequency of lentivirus-transduced CD4 T cells in blood.

[0129] If participants meet qualifying criteria (including having $>10^6$, $>10^7$, $> 10^8$ Lentivirus-transduced, HIV-specific CD4 T cells in the total of blood and tissue compartments), they may move to an efficacy study. If participants miss the qualifying criterion they may be eligible to receive a second dose of therapeutic lentivirus using the same protocol.

[0130] *Further Study and Bioassay*. For qualified participants at 30-60 days after transduced T cell infusion, begin testing the efficacy of gene therapy. First, add a CCR5-targeted treatment to their existing cART regimen. This may be the CCR5-blocking drug Maraviroc or the immune suppressing drug Rapamycin that lowers CCR5 receptor density on T cells. The therapeutic

lentivirus also targets CCR5 and the combined effects of lentivirus plus Maraviroc or Rapamycin should decrease CCR5 below levels needed to sustain HIV replication.

[0131] Two weeks after adding Maraviroc or Rapamycin, the cART therapy is stopped and participants are monitored closely for HIV virus rebound. If they rebound, cART is reintroduced and managed by their regular physician.

[0132] If HIV virus does not rebound, step-down of the Maraviroc or Rapamycin is started in 2 week intervals until treatment is stopped.

[0133] If viremia does not return within 12-26 weeks, the participant has achieved a functional cure of HIV.

[0134] A database of values relating the number of HIV-specific, lentivirus-transduced CD4 T cells to treatment efficacy (including the frequency of cells with latent HIV infection or other markers) will establish a Gold Standard by which other gene therapy protocols could be judged.

Example 2 – Developing an Anti-HIV Lentivirus Vector

[0135] The purpose of this example was to develop an anti-HIV lentivirus vector.

[0136] *Inhibitory RNA Designs:* The sequence of *Homo sapiens* chemokine C-C motif receptor 5 (CCR5) (GC03P046377) mRNA was used to search for potential siRNA or shRNA candidates to knockdown CCR5 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT RNAi Designer from Thermo Scientific. Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org/. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

[0137] 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. Highly conserved regions of Gag or Polymerase genes could not be targeted by shRNA because these same sequences were present in the packaging system complementation plasmids needed for vector manufacturing. As with the CCR5 (NM 000579.3, NM 001100168.1-specific RNAs, potential HIV-specific RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Gene-E Software Suite hosted by the Broad Institute (broadinstitute.org/mai/public) or the BLOCK-iT RNAi Designer from Thermo Scientific (rnadesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=shrna&pid=6712627360706061801). Individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. These lentivirus-shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the CMV or EF-1alpha RNA polymerase II promoters

[0138] *Vector Constructions:* For CCR5, Tat or Vif shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Invitrogen. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after

heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered, purified and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Exemplary vector sequences and cellular elements known to restrict HIV replication can be found in Figures 4 and 9, respectively.

[0139] 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 4.

[0140] *Functional Assays:* Individual lentivirus vectors containing CCR5, Tat or Vif shRNA sequences and, for experimental purposes, expressing green fluorescent protein (GFP) under control of the CMV Immediate Early Promoter, and designated AGT103/CMV-GFP were tested for their ability to knockdown CCR5, Tat or Vif expression. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days; protein and RNA were analyzed for CCR5, Tat or Vif expression. Protein levels were tested by Western blot assay or by labeling cells with specific fluorescent antibodies (CCR5 assay), followed by analytical flow cytometry comparing modified and unmodified cell fluorescence using either the CCR5-specific or isotype control antibodies.

[0141] *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 10000units/ml, IL12 1µg/ml, IL7 1µg/ml, IL15 1µg/ml were also prepared in advance.

[0142] 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).

[0143] *Day 0-12: antigen-specific enrichment*: On day 0, cryopreserved PBMC were thawed, washed with 10 ml 37˚C medium at 1200 rpm for 10 minutes and resuspended at a concentration

of $2x10^6$/ml in 37°C medium. The cells were cultured at 0.5 ml/well in a 24-well plate at 37°C in 5% CO_2. To define the optimal stimulation conditions, cells were stimulated with combinations of reagents as listed in Table 2 below:

Table 2

1	2	3	4	5	6
IL2+IL12	IL7+IL15	Peptides+ IL2+IL12	Peptides+ IL7+IL15	MVA+ IL2+IL12	MVA+ IL7+IL15

[0144] Final concentrations: IL2=20 units/ml, IL12=10 ng/ml, IL7=10 ng/ml, IL15=10 ng/ml, peptides=5 μg/ml individual peptide, MVA MOI=1.

[0145] 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.

[0146] *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 $1x10^6$/ml. The resuspended cells were transferred to T25 culture flasks and stimulated with DYNABEADS® Human T-Activator CD3/CD28 following the manufacturer's instruction plus cytokine as listed above; flasks were incubated in the vertical position.

[0147] On day 14, AGT103/CMV-GFP was added at MOI 20 and cultures were returned to the incubator for 2 days. At this time, cells were recovered by pipetting, collected by centrifugation at 1300 rpm for 10 minutes, resuspended in the same volume of fresh medium, and centrifuged again to form a loose cell pellet. That cell pellet was resuspended in fresh medium with the same cytokines used in previous steps, with cells at $0.5x10^6$ viable cells per ml.

[0148] From days 14 to 23, the number of the cells was evaluated every 2 days and the cells were diluted to $0.5x10^6$/ml with fresh media. Cytokines were added every time.

[0149] On day 24, the cells were collected and the beads were removed from the cells. To remove the beads, cells were transferred to a suitable tube that was placed in the sorting magnet for 2 minutes. Supernatant containing the cells was transferred to a new tube. Cells were then

cultured for 1 day in fresh medium at $1x10^6$/ml. Assays were performed to determine the frequencies of antigen-specific T cells and lentivirus transduced cells.

[0150] 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.

[0151] *Examine antigen-specific T cells by intracellular cytokine staining for IFN-gamma:* Cultured cells after peptide stimulation or after lentivirus transduction at $1x10^6$ cells/ml were stimulated with medium alone (negative control), Gag peptides (5µg/ml individual peptide), or PHA (5µg/ml, positive control). After 4 hours, BD GOLGIPLUG™ (1:1000, BD Biosciences) was added to block Golgi transport. After 8 hours, cells were washed and stained with extracellular (CD3, CD4 or CD8; BD Biosciences) and intracellular (IFN-_gamma; BD Biosciences) antibodies with BD CYTOFIX/CYTOPERM™ kit following the manufacturer's instruction. Samples were analyzed on a *BD FACSCALIBUR*™ Flow Cytometer. Control samples labeled with appropriate isotype-matched antibodies were included in each experiment. Data were analyzed using Flowjo software.

[0152] 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.

[0153] These results indicate that CD4 T cells, the target T cell population, can be transduced with lentiviruses that are designed to specifically knock down the expression of HIV-specific proteins, thus producing an expandable population of T cells that are immune to the virus. This example serves as a proof of concept indicating that the disclosed lentiviral constructs can be used in combination with vaccination to produce a functional cure in HIV patients.

Example 3 – CCR5 Knockdown with experimental vectors

[0154] AGTc120 is a Hela cell line that stably expresses large amounts of CD4 and CCR5. AGTc120 was transduced with or without LV-CMV-mCherry (the red fluorescent protein mCherry expressed under control of the CMV Immediate Early Promoter) or AGT103/CMV-mCherry. Gene expression of the mCherry fluorescent protein was controlled by a CMV

(cytomegalovirus immediate early promoter) expression cassette. The LV-CMV-mCherry vector lacked a microRNA cluster, while AGT103/CMV-mCherry expressed therapeutic miRNA against CCR5, Vif, and Tat.

[0155] As shown in Figure 5A, 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.

[0156] Figure 5B 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.

[0157] 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 4 - AGT103 decreases expression of Tat and Vif

[0158] Cells were transfected with exemplary vector AGT103/CMV-GFP. AGT103 and other exemplary vectors are defined in Table 3 below.

Table 3

Vector Designation	Composition
AGT103	EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE
Control-mCherry	CMV-mCherry
AGT103/CMV-mCherry	CMV-mCherry-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE-
Control-GFP	CMV-mCherry
AGT103/CMV-GFP	CMV-GFP-EF1-miR30CCR5-miR21Vif-miR185-Tat-WPRE-
Abbreviations: 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 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	

[0159] 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 6.

[0160] In a similar experiment, HEK 293T cells (Human Embryonic Kidney 293T; American Type Culture Collection Catalogue number CRL-3216) cells were transduced with AGT103/CMV-GFP and then an HIV expression plasmid was transfected into cells 7 days after

transduction (control was not transfected with HIV). 24 hours after transfection cells were lysed and analyzed by Western blot using antibodies specific for Actin (cellular loading control) or HIV Vif protein. The presence of AGT103/CMV-GFP (right lane) caused a dramatic reduction in Vif protein expression levels as shown in Figure 7.

Example 5 – Generating a population of CD4+ T cells enriched for HIV-specificity and transduced with AGT103/CMV-GFP

[0161] Therapeutic vaccination against HIV had minimal effect on the distribution of CD4+, CD8+ and CD4+/CD8+ T cells. As shown in Figure 8A, 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.

[0162] 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 (JPT PepMix) as a source of epitope peptides for T cell stimulation. These peptides are 10-20 amino acids in length and overlap by 20-50% of their length to represent the entire Gag precursor protein (p55) from HIV-1 BaL strain. The composition and sequence of individual peptides can be adjusted to compensate for regional variations in the predominant circulating HIV sequences or when detailed sequence information is available for an individual patient receiving this therapy. At culture end, cells were recovered and stained with anti-CD4 or anti-CD8 monoclonal antibodies and the CD3+ population was gated and displayed here. The PepMix stimulation for either pre- or post-vaccination samples was similar to the medium control indicating that PepMix was not toxic to cells and was not acting as a polyclonal mitogen. The results of this analysis can be found in Figure 8B.

[0163] PepMix and interleukin-2/interleukin-12 provided for optimal expansion of antigen-specific CD4 T cells. As shown in the upper panels of Figure 8C, there was an increase in cytokine (interferon-gamma) secreting cells in post-vaccination specimens exposed to PepMix. In the pre-vaccination sample, cytokine secreting cells increased from 0.43 to 0.69% as a result of exposure to antigenic peptides. In contrast, the post-vaccination samples showed an increase

of cytokine secreting cells from 0.62 to 1.76% of total CD4 T cells as a result of peptide stimulation. These data demonstrate the strong impact of vaccination on the CD4 T cell responses to HIV antigen.

[0164] 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 may be used alone or without further additional elements; for example, clinical embodiments may not include the CMV-GFP segment). The upper panels of Figure 8D show the results of analyzing the CD4+ T cell population in culture. The x axis of Figure 8D shows Green Fluorescent Protein (GFP) emission indicating that individual cells were transduced with the AGT103/CMV-GFP. In the post-vaccination samples 1.11% of total CD4 T cells that were both cytokine secreting was recovered, indicating that the cells are responding specifically to HIV antigen, and transduced with AGT103/CMV-GFP. This is the target cell population and the clinical product intended for infusion and functional cure of HIV. With the efficiency of cell expansion during the antigen stimulation and subsequent polyclonal expansion phases of *ex vivo* culture, $4x10^8$ antigen-specific, lentivirus transduced CD4 T cells can be produced. This exceeds the target for cell production by 4-fold and will allow achievement of a count of antigen-specific and HIV-resistant CD4 T cells of approximately 40 cells/microliter of blood or around 5.7% of total circulating CD4 T cells.

[0165] Table 4 below shows the results of the *ex vivo* production of HIV-specific and HIV-resistant CD4 T cells using the disclosed vectors and methods.

Table 4

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

* * *

[0166] While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention.

WHAT IS CLAIMED IS:

1. A method of treating HIV infection, comprising:

(a) identifying a subject in need of treatment of HIV infection;

(b) immunizing the subject with a therapeutically effective amount of an HIV vaccine;

(c) removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC);

(d) contacting the PBMC *ex vivo* with a therapeutically effective amount of an HIV vaccine;

(e) transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element;

(f) culturing the transduced PBMC for about 1 to about 35 days; and

(g) infusing the transduced PBMC into the subject.

2. The method of claim 1, wherein step (b) and step (d) comprise the same HIV vaccine.

3. The method of claim 1, wherein step (b) and step (d) comprise different HIV vaccines.

4. The method of claim 1, wherein the subject was receiving cART or HAART prior to infusing the transduced PBMC into the subject.

5. The method of claim 1, wherein the subject receives a cyclophosphamide pre-treatment prior to infusing the transduced PBMC into the subject.

6. The method of claim 1, wherein the at least one genetic element is selected from the group consisting of small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences.

7. The method of claim 6, wherein the small RNA molecules targeting HIV RNA sequences is directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

8. The method of claim 1, wherein the transduced PBMC are cultured for about 1 to about 10 days prior to infusing the transduced PBMC into the subject.

9. A viral vector for transducing HIV-specific CD4 T cells, wherein the viral vector encodes at least one genetic element selected from the group consisting of small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences.

10. The viral vector of claim 9, wherein the vector is a lentivirus.

11. The viral vector of claim 9, wherein the vector is a vector-in-vector system.

12. The viral vector of claim 9, wherein the small RNA molecules targeting HIV RNA sequences is directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

13. A bioassay for determining whether a HIV+ subject is functionally cured, comprising determining the number of HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus, wherein the subject is functionally cured if the number of HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus is above a threshold value after a specified time following treatment according to claim 1.

14. The method of claim 13, wherein the threshold value is about $1x10^8$ HIV-specific CD4 T cells bearing genetic modification from therapeutic lentivirus.

15. The method of claim 13, wherein the specified time following treatment is about 30-about 60 days.

16. The method of claim 13, wherein the specified time following treatment is about 12-about 26 weeks.

17. A method of achieving a functional cure for HIV in a HIV+ subject, comprising:

(a) identifying a subject that is HIV+;

(b) immunizing the subject with a therapeutically effective amount of an HIV vaccine;

(c) removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC);

(d) contacting the PBMC *ex vivo* with a therapeutically effective amount of an HIV vaccine;

(e) transducing the PBMC *ex vivo* with a viral delivery system encoding at least one genetic element;

(f) culturing the transduced PBMC for about 1- about 21 days; and

(g) infusing the transduced PBMC into the subject, wherein the HIV+ subject achieves a functional cure.

18. The method of claim 18, wherein step (b) and step (d) comprise the same HIV vaccine.

19. The method of claim 18, wherein step (b) and step (d) comprise different HIV vaccines.

20. The method of claim 18, wherein the subject was receiving cART or HAART prior to infusing the transduced PBMC into the subject.

21. The method of claim 18, wherein the subject receives a cyclophosphamide pre-treatment prior to infusing the transduced PBMC into the subject.

22. The method of claim 18, wherein the at least one genetic element is selected from the group consisting of small RNA capable of inhibiting the production of chemokine receptor CCR5, small RNA capable of inhibiting the production of chemokine receptor CXCR4, and small RNA molecules targeting HIV RNA sequences.

23. The method of claim 22, wherein the small RNA molecules targeting HIV RNA sequences is directed to gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS.

24. The method of claim 18, wherein the transduced PBMC are cultured for about 1 to about 7 days prior to infusing the transduced PBMC into the subject.

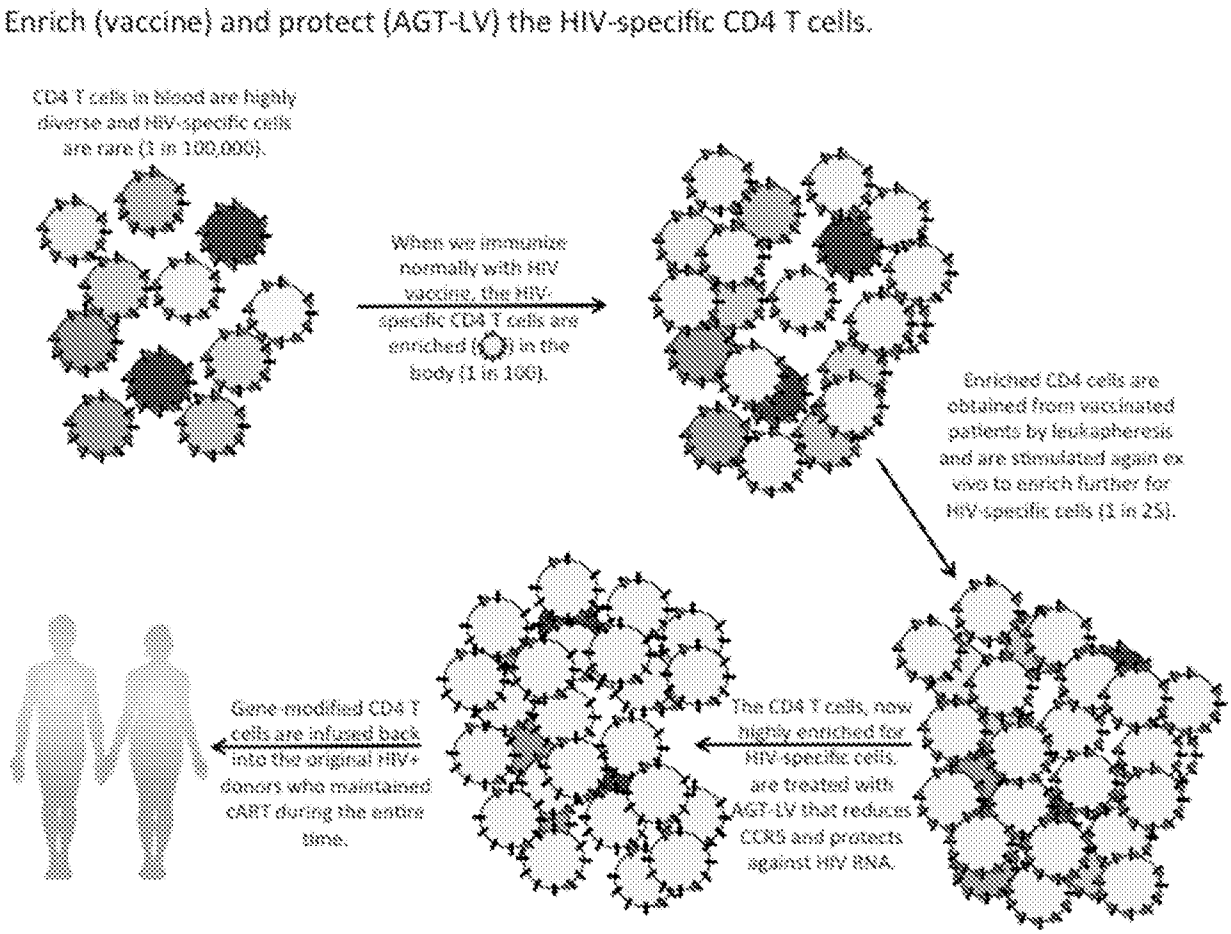
Enrich (vaccine) and protect (AGT-LV) the HIV-specific CD4 T cells.
CD4 T cells in blood are highly diverse and HIV-specific cells are rare (1 in 100,000).
When we immunize normally with HIV vaccine, the HIV-specific CD4 T cells are enriched (○) in the body (1 in 100).
Enriched CD4 cells are obtained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

Figure 1

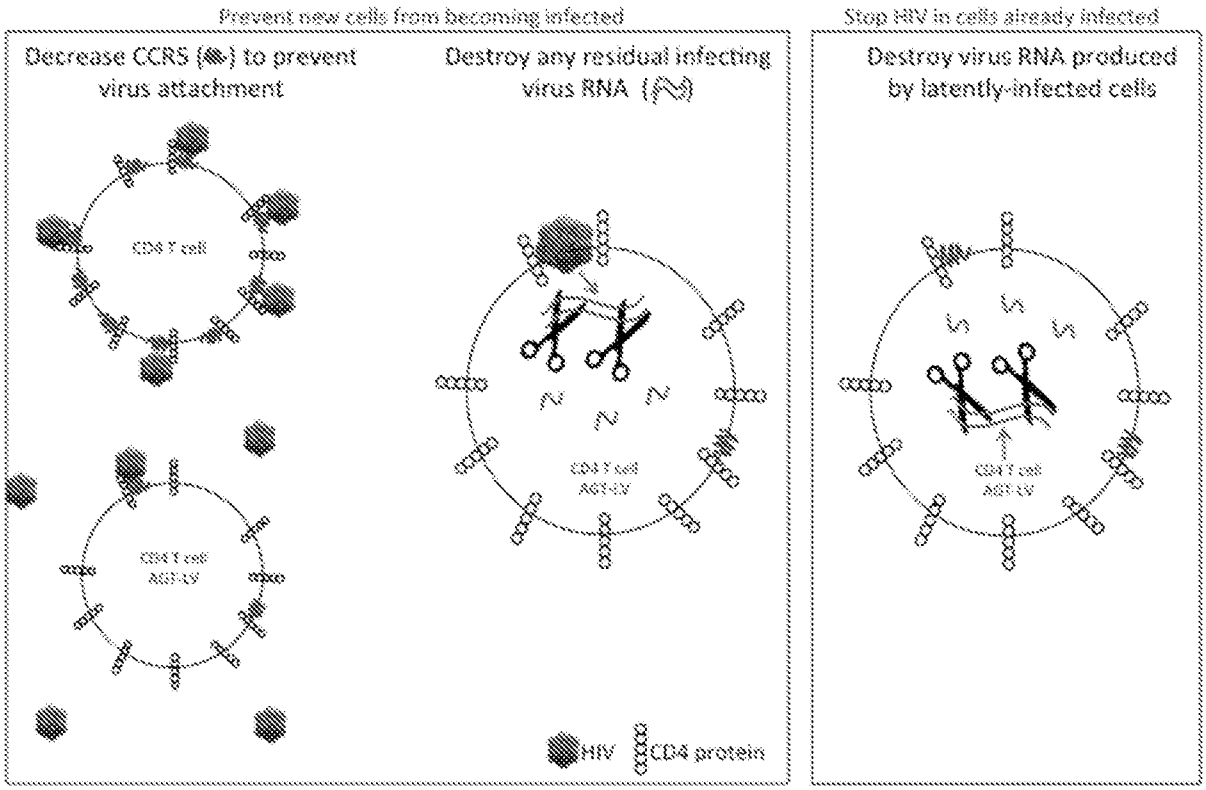
Prevent new cells from becoming infected
Stop HIV in cells already infected
Decrease CCR5 to prevent virus attachment
Destroy any residual infecting virus RNA
Destroy virus RNA produced by latently-infected cells
CD4 T cell
HIV
CD4 protein

Figure 2

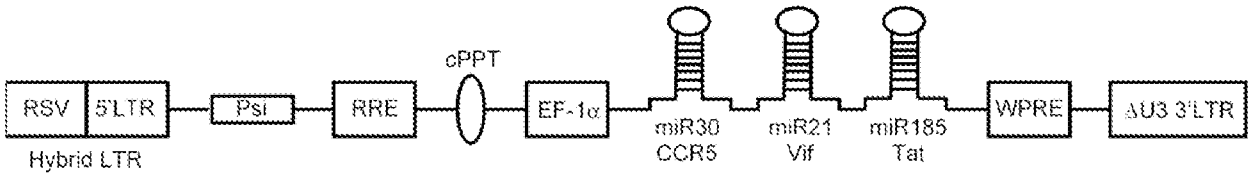
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
EF-1α
miR30
CCR5
miR21
Vif
miR185
Tat
WPRE
ΔU3 3'LTR

Figure 3

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

ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAG
GGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA
CAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTT
CCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT
TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATC
TGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGAC
GCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCGATCTGCACACTGGTATTTCGGTTTTTGGGGCC
GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA
GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGC
CCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCA
GGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT
TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAG
CTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGA
GACTGAAGTTAGGCCAGCTTGGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTC
ATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTGGCGAAGCTAATTC

miR30 CCR5 start

TGCAGATTCGACTGTACAAGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCT
CTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG

miR30 CCR5 end miR21 Vif start

ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTT
CTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTA

miR21 Vif end miR185 Tat start

TCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA

miR185 Tat end

CCGCGTCTTCGTCGCGGCCGCTCGAGCATGCAT

Figure 4

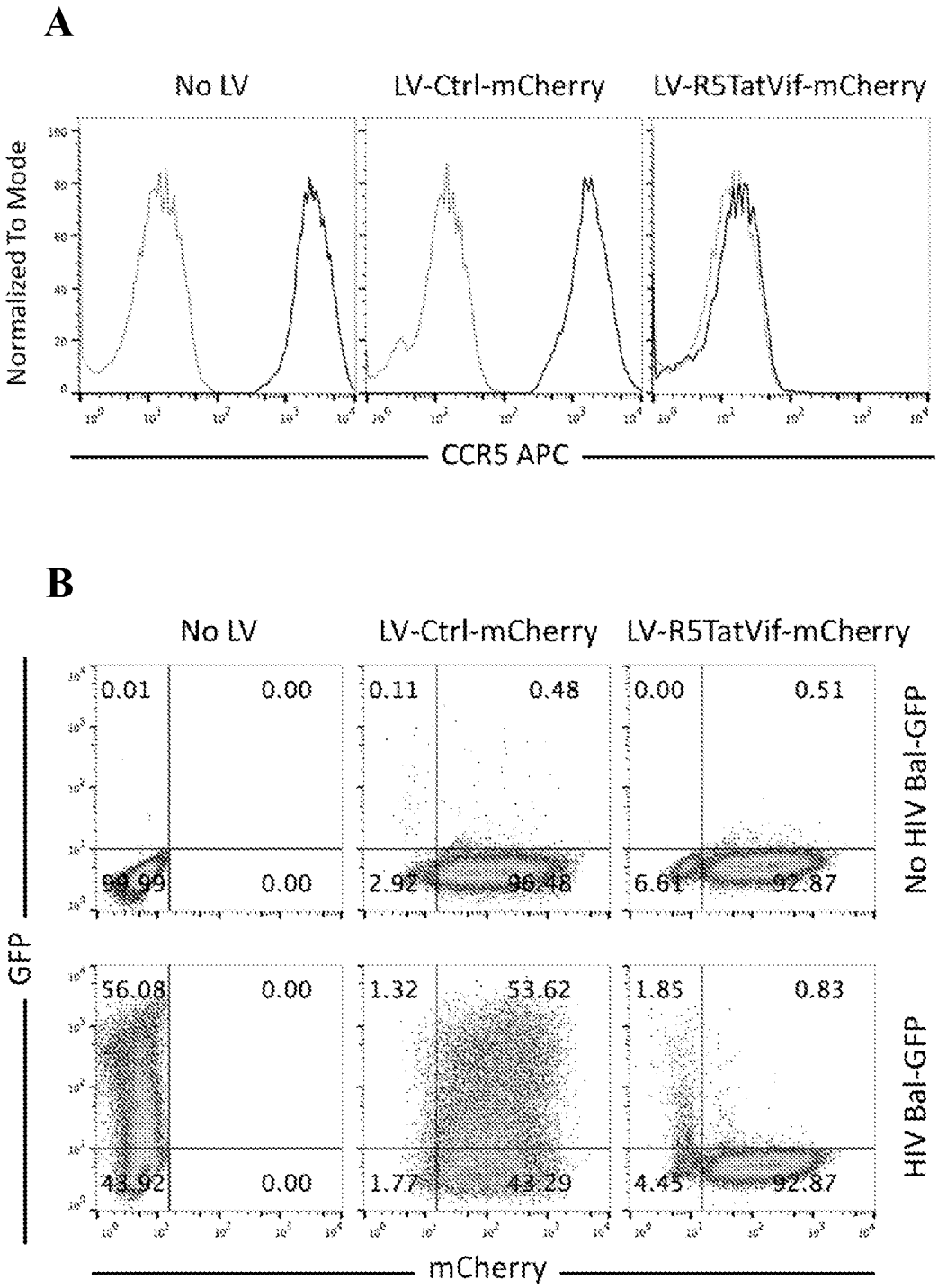
A
No LV
LV-Ctrl-mCherry
LV-R5TatVif-mCherry
Normalized To Mode
CCR5 APC
B
No LV
LV-Ctrl-mCherry
LV-R5TatVif-mCherry
0.01
0.00
0.11
0.48
0.00
0.51
99.99
0.00
2.92
96.48
6.61
92.87
No HIV Bal-GFP
56.08
0.00
1.32
53.62
1.85
0.83
43.92
0.00
1.77
43.29
4.45
92.87
HIV Bal-GFP
GFP
mCherry

Figure 5

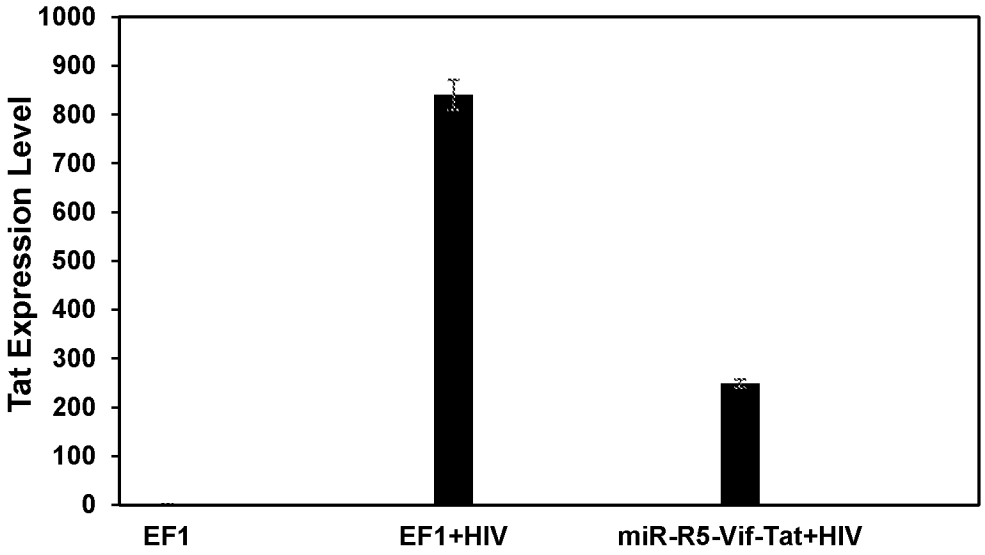
Tat Expression Level
1000
900
800
700
600
500
400
300
200
100
0
EF1
EF1+HIV
miR-R5-Vif-Tat+HIV

Figure 6

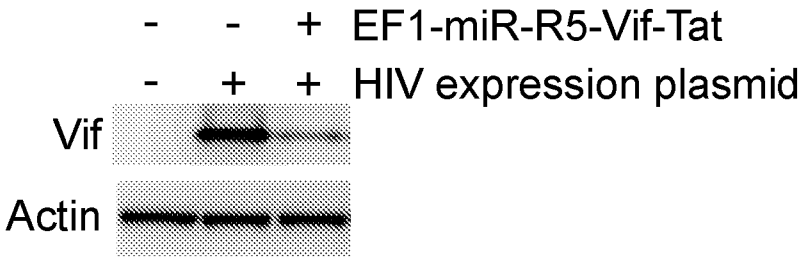

Figure 7

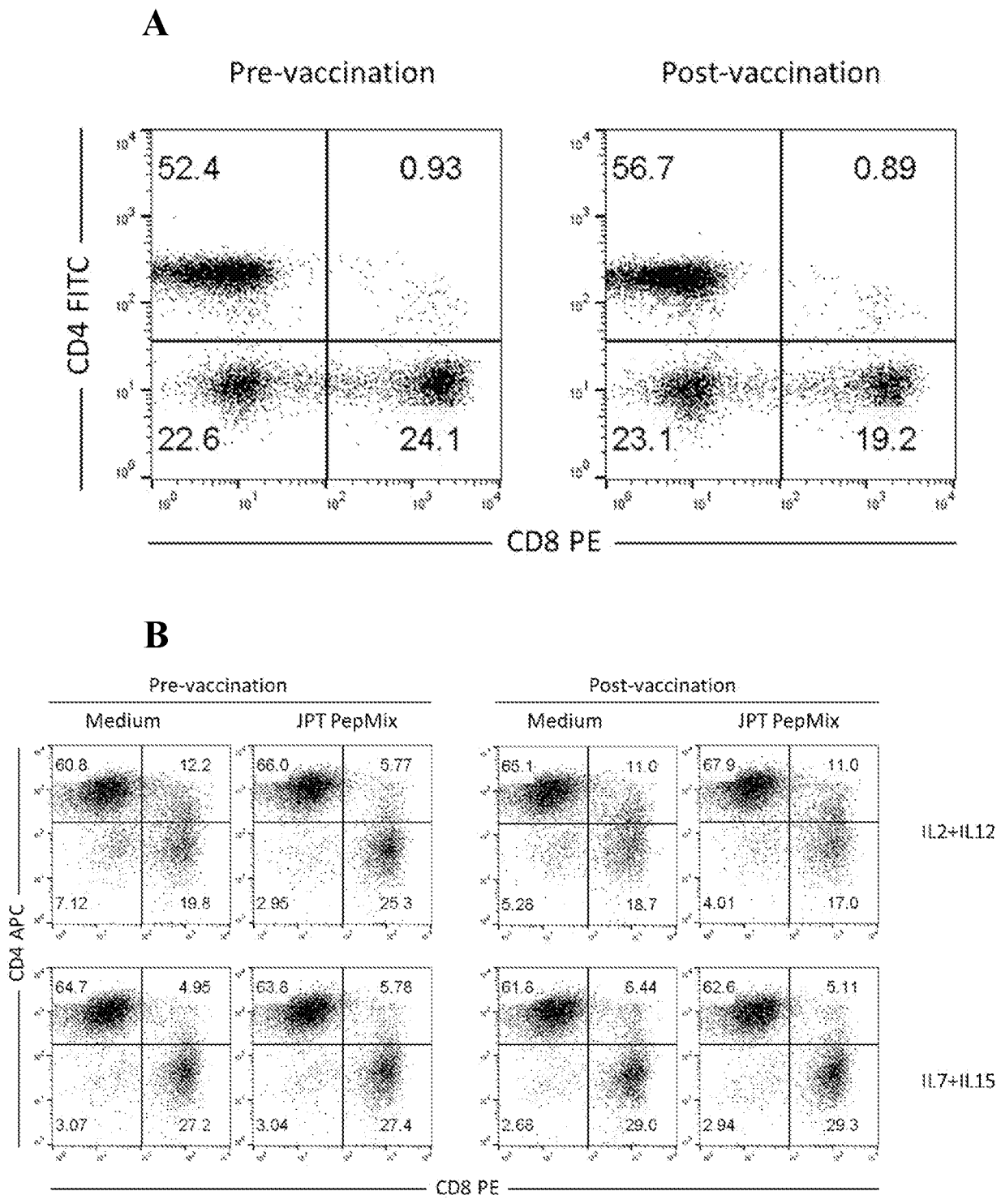
A
Pre-vaccination
Post-vaccination
52.4
0.93
22.6
24.1
56.7
0.89
23.1
19.2
CD4 FITC
CD8 PE
B
Pre-vaccination
Post-vaccination
Medium
JPT PepMix
Medium
JPT PepMix
60.8
12.2
7.12
19.8
66.0
5.77
2.95
25.3
65.1
11.0
5.28
18.7
67.9
11.0
4.01
17.0
IL2+IL12
64.7
4.95
3.07
27.2
63.6
5.78
3.04
27.4
61.6
8.44
2.68
29.0
62.6
5.11
2.94
29.3
IL7+IL15
CD4 APC
CD8 PE

Figure 8

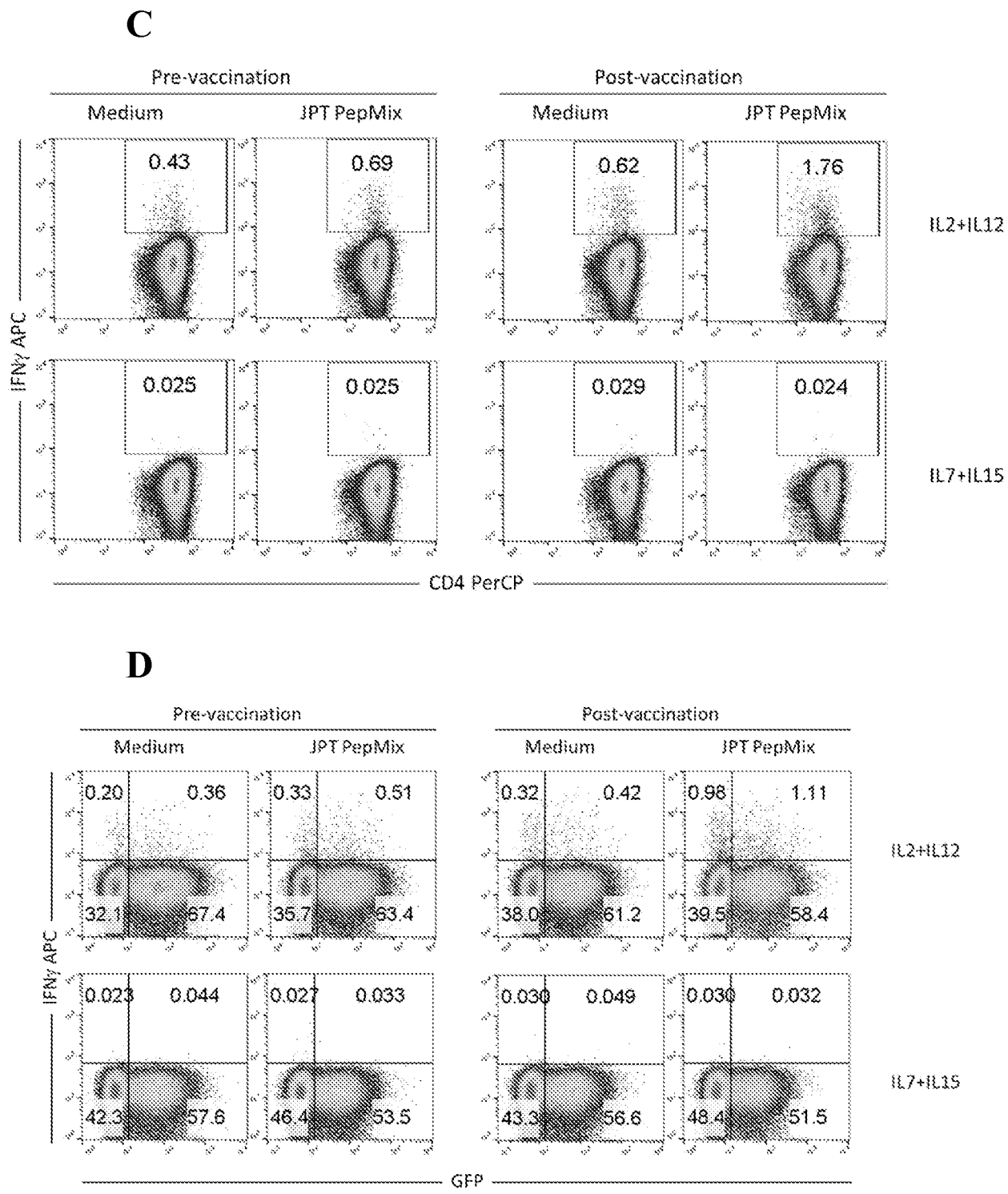

Figure 8 Con't

TREX1 - human; open reading frame; three prime repair exonuclease 1

```
   1 atgcagaccc tcatcttttt cgacatggag gccactggct tgccctttct ccagcccaag
  61 gtcacggagc tgtgcctgct ggctgtccac agatgtgccc tggagagccc ccccacctct
 121 caggggccac ctcccacagt tcctccacca ccgcgtgtgg tagacaagct ctccctgtgt
 181 gtggctccgg ggaaggcctg cagccctgca gccagcgaga tcacaggtct gagcacagct
 241 gtgctggcag cgcatgggcg tcaatgtttt gatgacaacc tggccaaacct gctcctagcc
 301 ttcctgcggc gccagccaca gccctggtgc ctggtggcac acaatggtga ccgctacgac
 361 ttccccctgc tccaagcaga gctggctatg ctgggcctca ccagtgctct ggatggtgcc
 421 ttctgtgtgg atagcatcac tgcgctgaag gccctggagc gagcaagcag ccccctcagaa
 481 cacggcccaa ggaagagcta cagcctaggc agcatctaca ctgccctgta tgggcagtcc
 541 cctccagact cgcacacggc tgagggtgat gtcctggccc tgctcagcat ctgtcagtgg
 601 agaccacagg ccctgctgcg gtgggtggat gctcacgcca ggccttttcgg caccatcagg
 661 cccatgtatg gggtcacagc ctctgctagg accaagccaa gaccatctgc tgtcacaacc
 721 actgcacacc tggccacaac caggaacact agtcccagcc ttcgagagag cagggtacc
 781 aaggatcttc ctccagtgaa ggaccctgga gccctatcca gggaggggct gctggcccca
 841 ctgggtctgc tggccatcct gaccttggca gtagccacac tgtatggact atccctggcc
 901 acacctggcg attaa
```

Figure 9

TREX2 - human; open reading frame; three prime repair exonuclease 2

```
   1 cacctttggtc acactccaga gctcgcttca tccccacgag cggcacctga tctccagtga
  61 cggggagact gaggcctggc aaagaggacc tgttgccttt gtgtaactgg ccccagcatg
 121 ggggaggcat tggccccagc atgggggagg cattgacccc aggcctgcca ggcctggcag
 181 ccacagcctg gctaggtgga ggttactgcc ttccgcacca ctctggcttc ctccccgtgc
 241 ctgtgaagag ctcggggcct gcttcctaat ttgtaaacac ggggcgtgtg tctcagtggc
 301 tgtgagctag cgaggggtg gcgagcgagc cggctgcgca ggtcctgagg ccccaggcct
 361 cattgttggc caacaggcag ctgggggcgg gctgcggccg ctgattaaag gccgcctaga
 421 gcagcctgtg tggcgacagg tgcccagaag cccaggaagc cggtcagtgc ccgccccagt
 481 cctcagggtt tgtgcctctc gctcggacag tttgaggact tgctatcccc gtgggaacat
 541 caccatgtcc gaggcacccc gggccgagac ctttgtcttc ctggacctgg aagccactgg
 601 gctccccagt gtggagcccg agattgccga gctgtcccta tttgctgtcc accgctccta
 661 cctggagaac ccggagcacg acgagtctgg tgccctagta ttgccccggg tcctggacaa
 721 gctcacgctg tgcatgtgcc cggagcgccc cttcactgcc aaggccagcg agatcaccgg
 781 cctgagcagt gagggcctgg cgcgatgccg gaaggctggc tttgatggcg ccgtggtgcg
 841 gacgctgcag gccttcctga gccgccaggc agggcccatc tgccttgtgg cccacaatgg
 901 ctttgattat gatttccccc tgctgtgtgc cgagctgcgg cgcctgggtg cccgcctgcc
 961 ccgggacact gtctgcctgg acacgctgcc ggccctgcgg ggcctggacc gcgcccacag
1021 ccacggcacc cgggccccgg gccgccaggg ttacagcctc ggcagcctct tccaccgcta
1081 cttccgggca gagccaagcg cagcccactc agccgagggc gacgtgcaca ccctgctcct
1141 gatcttcctg cacccgcgcg cagagctgct cgcctgggcc gatgagcagg ccccgtgggtg
1201 ggcccacatc gagcccatgt acttgccgcc tgatgacccc agcctggagg cctga
```

Figure 9 Con't

SAMHD1 - human; open reading frame; SAM domain and HD domain-containing protein

```
   1 attgcgcctg cgcagggagc ccaaggcaag agccgctagg ctgccctgcc cgaagggctc
  61 aactgtcagt gagcctgcgc aggaggccaa taggctgcca atactccttg gactccccgc
 121 cagggccctg ctgtcagtgc gcctgcgcgc gggtccggcg ccgaggttct tgactgctgt
 181 gccggacgcc aggtgtagcc atgcagcgag ccgattccga gcagccctcc aagcgtcccc
 241 gttgcgatga cagcccgaga acccccctcaa acacccctc cgcagaggca gactggtccc
 301 cgggcctgga actccatccc gactacaaga catggggtcc ggagcaggtg tgctcctcc
 361 tcaggcgcgg tggctttgaa gagccggtgc tgctgaagaa catccgagaa aatgaaatca
 421 caggcgcatt actgccttgt cttgatgagt ctcgttttga aaatcttgga gtaagttcct
 481 tgggggagag gaagaagctg cttagttata tccagcgatt ggttcaaatc cacgttgata
 541 caatgaaggt aattaatgat cctatccatg gccacattga gctccaccct ctcctcgtcc
 601 gaatcattga tacacctcaa tttcaacgtc ttcgatacat caaacagctg ggaggtggtt
 661 actatgtttt tccaggagct tcacacaatc gatttgagca tagtctaggg gtggggtatc
 721 tagcaggatg tctagttcac gcactgggtg aaaaacaacc agagctgcag ataagtgaac
 781 gagatgttct ctgtgttcag attgctggac tttgtcatga tctcggtcat gggccatttt
 841 ctcacatgtt tgatggacga tttattccac ttgctcgccc ggaggtgaaa tggacgcatg
 901 aacaaggctc agttatgatg tttgagcacc ttattaattc taatggaatt aagcctgtca
 961 tggaacaata tggtctcatc cctgaagaag atatttgctt tataaaggaa caaattgtag
1021 gaccacttga atcacctgtc gaagattcat tgtggccata taaagggcgt cctgaaaaca
1081 aaagcttcct ttatgagata gtatctaata aaagaaatgg cattgatgtg gacaaatggg
1141 attattttgc cagggactgc catcatcttg gaatccaaaa taatttttgat tacaagcgct
1201 ttattaagtt tgcccgtgtc tgtgaagtag acaatgagtt gcgtatttgt gctagagata
1261 aggaagttgg aaatctgtat gacatgttcc acactcgcaa ctctttacac cgtagagctt
1321 atcaacacaa agttggcaac attattgata caatgattac agatgctttc ctcaaagcag
1381 atgactacat agagattaca ggtgctggag gaaaaaagta tcgcatttct acagcaattg
1441 acgacatgga agcctatact aagctgacag ataacatttt tctggagatt ttatactcta
1501 ctgatcccaa attgaaagac gcacgagaga tttttaaaaca aattgaatac cgtaatctat
1561 tcaagtatgt gggtgagacg cagccaacag gacaaataaa gattaaaagg gaggactatg
1621 aatctcttcc aaaagaggtt gccagtgcta aacccaaagt attgctagac gtgaaactga
1681 aggctgaaga ttttatagtg gatgttatca acatggatta tggaatgcaa gaaaagaatc
1741 caattgatca tgttagcttc tattgtaaga ctgccccccaa cagagcaatc aggattacta
1801 aaaaccaggt ttcacaactt ctgccagaga aatttgcaga gcagctgatt cgagtatatt
1861 gtaagaaggt ggacagaaag agtttgtatg ccgcaagaca atattttgtt cagtggtgtg
1921 cagacagaaa tttcaccaag ccgcaggatg gcgatgttat agccccactc ataacacctc
1981 aaaaaaagga atggaacgac agtacttcag tccaaaatcc aactcgcctc cgagaagcat
2041 ccaaaagcag agtccagctt tttaaagatg acccaatgtg aatgtctgta gtcagttgtt
2101 tacaaactcc ctctcctgca caattcattt agaggcttca atcatagaat tctgcaaatt
2161 aatgacaact catgctttaa ttttgtattt tgaatgtaca cgcatgctga agctaagtaa
2221 cttttaatca aagaaataag atggtattag gcaaatctta ctatactatg aaaagcatta
2281 ccttgcctat ttttaatatt attaaagcct ttctccttca gtagtctatt ttcttagaat
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2401 ctctgttgcc cagcttggac tcgaactttc ctgggctcaa gcgaccctcc tgcctcagcc
2461 ccccaagtag ctgggactaa agtcatgtgc caccacaccc agcttactct gaacttttat
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2581 gaaattgaca ttgtcattga caaacatggc taaaaacaaa acatcaaatc ctgccccccgt
2641 gaagagttcc ctgtcacctc aagtttgagg atagtcctct aagagtgacc taagcataag
2701 tgaaagacac ctcccctcac ccttctagcc ccctacaagg tgccaggttg gggtaaagag
2761 ttggagatga tggccaggag tggcctccaa cacgctggtg agaggcctga ttaggttttg
2821 gggaagatct gagagctctg gcctcttcgt gagtggaaca taaagccgcc tcttgttggg
2881 agatcctacc ccagtgacag aggaatcccc caaactaggc tgtgccctgg ctccgtggcg
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3001 ttcccactgc ccagcctgga gaaggcctcc cctgacctgc tctctcagta tcctggagag
3061 ctggccagag gccatcacag gcatcatcct cagagctcct cagacctggg actttgtttt
3121 tgctggttca gtgcattttg tgtatttaag agcaaacact agccaggcgt ggcggcgtgt
```

Figure 9 Con't

MxA - human; open reading frame; influenza virus resistance 1

```
   1 cgagcagaaa tgaaaccgaa actgaattgt ccgggaaatt cgcggtgggg gcggagagcg
  61 cagggagaag taagcccagt gcaggatcct gaggcccgtg tttgcaggac cagggccggc
 121 cttccgattc cccattcatt ccagaagcac cgaaccacgc tgtgccccgga tcccaagtgc
 181 agcggcaccc agcgtgggcc tggggttgcc ggttgacccg gtcctcagcc tggtagcaga
 241 ggccaggcca gtgccacaag gcacctaagt ccacctgggc ctggagcagg acaggttgca
 301 aaagaaaata tctcgggacc cccaaactcc ttatgctaag ggaaacatcg agcctgggaa
 361 ctgagccatc aacgctgcca ttcttttcc caaacagaac cctgttgtca gaggtacacc
 421 cagagcaact ccacaccggg tgcatgccac agcaactcca tcttaaatag gagctggtaa
 481 aacgaggctg atacctactg ggctgcattc ccagacggca tagcgaggag gtgctgaaga
 541 gcgcaggttt ggagaatgat cacctggatt ggaaccatag ctctaccaat atggaacccа
 601 gctccttagg cctcggtctt ctcatggaga acatggtgtg ataatcctac tcctctggga
 661 gggtggctgt taagccttgg accgcagttg ccggccagga atcccagtgt cacggtggac
 721 acgcctccct cgcgcccttg ccgcccacct gctcacccag ctcaggggct ttggaatttct
 781 gtggccacac tgcgaggaga tcggttctgg gtcggaggct acaggaagac tcccactccc
 841 tgaaatctgg agtgaagaac gccgccatcc agccaccatt ccaaggaggt gcaggagaac
 901 agctctgtga taccatttaa cttgttgaca ttacttttat ttgaaggaac gtatattaga
 961 gcttactttg caaagaagga agatggttgt ttccgaagtg gacatcgcaa aagctgatcc
1021 agctgctgca tcccacccтc tattactgaa tggagatgct actgtggccc agaaaaatcc
1081 aggctcggtg gctgagaaca acctgtgcag ccagtatgag gagaaggtgc gcccctgcat
1141 cgacctcatt gactccctgc gggctctagg tgtggagcag gacctggccc tgccagccat
1201 cgccgtcatc ggggaccaga gctcgggcaa gagctccgtg ttggaggcac tgtcaggagt
1261 tgcccttccc agaggcagcg ggatcgtgac cagatgcccg ctggtgctga aactgaagaa
1321 acttgtgaac gaagataagt ggagaggcaa ggtcagttac caggactacg agattgagat
1381 ttcggatgct tcagaggtag aaaaaggaaat taataaagcc cagaatgcca tcgccgggga
1441 aggaatggga atcagtcatg agctaatcac cctggagatc agctcccgag atgtcccgga
1501 tctgactcta atagaccttc ctggcataac cagagtggct gtgggcaatc agcctgctga
1561 cattgggtat aagatcaaga cactcatcaa gaagtacatc cagaggcagg agacaatcag
1621 cctggtggtg gtccccagta atgtggacat cgccaccaca gaggctctca gcatggccca
1681 ggaggtggac cccgagggag acaggaccat cggaatcttg acgaagcctg atctggtgga
1741 caaaggaact gaagacaagg ttgtggacgt ggtgcggaac ctcgtgttcc acctgaagaa
1801 gggttacatg attgtcaagt gccgggggcca gcaggagatc caggaccagc tgagcctgtc
1861 cgaagccctg cagagagaga agatcttctt tgagaaccac ccatatttca gggatctgct
1921 ggaggaagga aaggccacgg ttccctgcct ggcagaaaaa cttaccagcg agctcatcac
1981 acatatctgt aaatctctgc ccctgttaga aaatcaaatc aaggagactc accagagaat
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2101 cttcctgata gataaagtta atgcctttaa tcaggacatc actgctctca tgcaaggaga
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2281 ccagaaattt gaaaatcagt atcgtggtag agagctgcca ggctttgtga attacaggac
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3121 gtgagagcag tttggtttct agcatgaaga cagagcccca ccctcagatg cacatgagct
3181 ggcgggattg aaggatgctg tcttcgtact gggaaaggga ttttcagccc tcagaatcgc
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```

Figure 9 Con't

MxB - human; open reading frame

```
   1 aagagatgat ttctccatcc tgaacgtgca gcgagcttgt caggaagatc ggaggtgcca
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 121 agccttggcc ctaccgggagg agaagtcaat tttcttctcg aaaatacctg aaaaaagaaa
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 241 ctccaaactg gcagggggca gagaaggacg ctgctttcct cgccaaggac ttcaactttc
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 481 accagagctc gggcaagagc tctgtgctgg aggcactgtc aggagtcgcg cttcccagag
 541 gcagcggaat cgtaaccagg tgtccgctgg tgctgaaact gaaaaagcag cctgtgagg
 601 catgggccgg aaggatcagc taccggaaca ccgagctaga gcttcaggac cctggccagg
 661 tggagaaaga gatacacaaa gcccagaacg tcatggccgg gaatggccgg ggcatcagcc
 721 atgagctcat cagcctggag atcaacctcc ctgaggttcc agacctgacc atcattgacc
 781 ttcccggcat caccagggtg gctgtggaca accagccccg agacatcgga ctgcagatca
 841 aggctctcat caagaagtac atccagaggc agcagacgat caacttggtg gtggttccct
 901 gtaacgtgga cattgccacc acggaggcgc tgagcatggc ccatgaggtg gacccggaag
 961 gggacaggac catcggtatc ctgaccaaac cagatctaat ggacaggggc actgagaaaa
1021 gcgtcatgaa tgtggtgcgg aacctcacgt accccctcaa gaagggctac atgattgtga
1081 agtgccgggg ccagcaggag atcacaaaca ggctgagctt ggcagaggca accaagaaag
1141 aaattacatt ctttcaaaca catccatatt tcagagtttc cctggaggag gggtcagcca
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1441 atgagacccg tttatacaac aaaatcagag aggatttaa aaactgggta ggcatacttg
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1561 agtatcgagg caaggagctt ctgggatttg tcaactacaa gacatttgag atcatcgtgc
1621 atcagtacat ccagcagctg gtggagcccg cccttagcat gctccagaaa gccatggaaa
1681 ttatccagca agctttcatt aacgtggcca aaaaacattt tggcgaattt ttcaacctta
1741 accaaactgt tcagagcacg attgaagaca taaaagtgaa acacacagca aaggcagaaa
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1861 gtgttgttct gaagaaagtc cgagaagaga ttttttaacc tctggggacg ccttcacaga
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1981 taggcatcca cctgaatgcc tacttcttgg aaaccagcaa acgtctgcc aaccagatcc
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2281 tgtgcgtact cattcattct aaggggagtc ggtgcaggat gccgcttctg ctttgggccc
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2761 ggtcccttta agatccagtg gccatgcccc ctgcttccca tggttcactg tcattgtgtt
2821 tcccagcctc tccactcccc cgccagaaag gagcctgagt gattctcttt tcttcttgtt
2881 tccctgatta tgatgagctt ccattgttct gttaagtctt gaagaggaat ttaataaagc
2941 aaagaaactt tttaaaaacg t
```

Figure 9 Con't

APOBEC3G - human; open reading frame; apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G)

```
   1 gtgctctgct ggctcagcct ggtgtggacc cacctccccgg gcgctggctg caatgacttt
  61 ctctttccct ttgcaattgc cttgggtcct gccgcacaga gcgggcctgtc tttatcagag
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 181 gataaagcgt cccagggcct cctacaccag cgcctgagca ggaagcggga ggggccatga
 241 ctacgaggcc ctgggaggtc actttaggga gggctgtcct aaaaccagaa gcttggagca
 301 gaaagtgaaa ccctggtgct ccagacaaag atcttagtcg ggactagccg gccaaggatg
 361 aagcctcact tcagaaacac agtggagcga atgtatcgag acacattctc ctacaacttt
 421 tataatagac ccatcctttc tcgtcggaat accgtctggc tgtgctacga agtgaaaaca
 481 aagggtccct caaggccccc tttggacgca aagatctttc gaggccaggt gtattccgaa
 541 cttaagtacc acccagagat gagattcttc cactggttca gcaagtggag gaagctgcat
 601 cgtgaccagg agtatgaggt cacctggtac atatcctgga gccccctgcac aaagtgtaca
 661 agggatatgg ccacgttcct ggccgaggac ccgaaggtta ccctgaccat ctttgttgcc
 721 cgcctctact acttctggga cccagattac caggaggcgc ttcgcagcct gtgtcagaaa
 781 agagacggtc cgcgtgccac catgaagatc atgaattatg acgaatttca gcactgttgg
 841 agcaagttcg tgtacagcca aagagagcta tttgagcctt ggaataatct gcctaaatat
 901 tatatattac tgcacatcat gctgggggag attctcagac actcgatgga tccacccaca
 961 ttcactttca actttaacaa tgaaccttgg gtcagaggac ggcatgagac ttacctgtgt
1021 tatgaggtgg agcgcatgca caatgacacc tgggtcctgc tgaaccagcg cagggggctt
1081 ctatgcaacc aggctcccca taaacacggt ttccttgaag gccgccatgc agagctgtgc
1141 ttcctggacg tgattccctt ttggaagctg gacctggacc aggactacag ggttacctgc
1201 ttcacctcct ggagcccctg cttcagctgt gcccaggaaa tggctaaatt catttcaaaa
1261 aacaaaacacg tgagcctgtg catcttcact gcccgcatct atgatgatca aggaagatgt
1321 caggaggggc tgcgcaccct ggccgaggct ggggccaaaa tttcaataat gacatacagt
1381 gaatttaagc actgctggga caccttttgtg gaccaccagg gatgtccctt ccagccctgg
1441 gatggactag atgagcacag ccaagacctg agtgggaggc tgcgggccat tctccagaat
1501 caggaaaact gaaggatggg cctcagtctc taaggaaggc agagacctgg gttgagcctc
1561 agaataaaag atcttcttcc aagaaatgca aacaggctgt tcaccaccat ctccagctga
1621 tcacagacac cagcaaagca atgcactcct gaccaagtag attcttttaa aaattagagt
1681 gcattacttt gaatcaaaaa tttatttata tttcaagaat aaagtactaa gattgtgctc
1741 aatacacaga aaagtttcaa acctactaat ccagcgacaa tttgaatcgg ttttgtaggt
1801 agaggaataa aatgaaatac taaatctttc tgtaaaaaaa aaaaaaaa
```

Figure 9 Con't

TRIM5 alpha - human; open reading frame; tripartite motif containing 5 (TRIM5)

```
   1 agtttatctt tcactttcct gccctgagtg tgagcaagaa tttcctgcgg ttcctctagg
  61 aaaattcctt tgtgcagatc aggcccgtgg attggtgagt gaatcctaac cacgtctttc
 121 ctggcctgtc ttcactcttc tccccagaat caccactttct gcactggtgt ctgaaggtgt
 181 attgagtgat tttgtggagg gcagaagtag gaagtctttg ggacaaaact gtatttacct
 241 tgggatctgt gaacaagagg aacctcagca gccaggacag gcaggagcag tggaatagct
 301 actatggctt ctggaatcct ggttaatgta aaggaggagg tgacctgccc catctgcctg
 361 gaactcctga cacaaccctt gagcctggac tgcggccaca gcttctgcca agcatgcctc
 421 actgcaaacc acaagaagtc catgctagac aaaggagaga gtagctgccc tgtgtgccgg
 481 atcagttacc agcctgagaa catacggcct aatcggcatg tagccaacat agtggagaag
 541 ctcagggagg tcaagttgag cccagagggg cagaaagttg atcattgtgc acgccatgga
 601 gagaaacttc tactcttctg tcaggaggac gggaaggtca tttgctggct ttgtgagcgg
 661 tctcaggagc accgtggtca ccacacgttc ctcacagagg aggttgcccg ggagtaccaa
 721 gtgaagctcc aggcagctct ggagatgctg aggcagaagc agcaggaagc tgaagagtta
 781 gaagctgaca tcagagaaga gaaagcttcc tggaagactc aaatacagta tgacaaaacc
 841 aacgtcttgg cagattttga gcaactgaga gacatcctgg actgggagga gagcaatgag
 901 ctgcaaaacc tggagaagga ggaggaagac attctgaaaa gccttacgaa ctctgaaact
 961 gagatggtgc agcagaccca gtccctgaga gagctcatct cagatctgga gcatcggctg
1021 cagggqtcag tgatggagct gcttcagggt gtggatggcg tcataaaaag gacggagaac
1081 gtgaccttga agaagccaga aacttttcca aaaaatcaaa ggagagtgtt tcgagctcct
1141 gatctgaaag gaatgctaga agtgtttaga gagctgacag atgtccgacg ctactgggtt
1201 gatgtgacag tggctccaaa caacatttca tgtgctgtca tttctgaaga taagagacaa
1261 gtgagctctc cgaaaccaca gataatatat ggggcacgag ggacaagata ccagacattt
1321 gtgaatttca attattgtac tggcatcctg ggctctcaaa gtatcacatc agggaaacat
1381 tactgggagg tagacgtgtc caagaaaact gcttggatcc tggggtatg tgctggcttc
1441 caacctgatg caatgtgtaa tattgaaaaa aatgaaaatt atcaacctaa atacggctac
1501 tgggttatag ggttagagga aggagttaaa tgtagtgctt tccaggatag ttccttccat
1561 actccttctg ttcctttcat tgtgcccctc tctgtgatta tttgtcctga tcgtgttgga
1621 gtttttcctag actatgaggc ttgcactgtc tcattcttca atatcacaaa ccatggattt
1681 ctcatctata agttttctca ctgttctttt tctcagcctg tatttccata tttaaatcct
1741 agaaaatgtg gagtccccat gactctgtgc tcaccaagct cttgaacctt cttacacact
1801 cagccccttc tgtacagcac ctcttgtcca ggtgcatctc atacacctga actcatttgc
1861 atcattttaa ccatcttttc cttgctgtct ccctttcttc tatttgaacg tccttcactc
1921 atcagtaaaa tgtaataatt gccttgtgcc atattgtccc caatatttta ttgacatttg
1981 atagcaattt ttttcatcat tttccgtact cctaaggaaa actgacctat acctcataaa
2041 atgagaccgc tatttaggta ttacttctgc cagatattta tcacccaatt gcctctgaca
2101 ctgactaaga agatgaagaa aagcttttca acagcctttc tatatcatcg tgtgataatt
2161 gttcaccaat gaatgagtcc ttagccctgt gtcagtttac cctcgatgcc cttatttgtg
2221 agttaaagag aaaatatcat aaatggtata ctcttaagta tagaggtttt gtatctagag
2281 gatctcagtt caactcctgt ctctccatat accagcagtg taactgtgaa taacatactt
2341 aaatggctgt gcttatttcc ttttcttttc ttttttcttt ttttttttt ttgagatgaa
2401 gttttgctct tgttccccag gctggagtgc aatggcacga tctcggttca ctgcaacctc
2461 cacctctcag attcaagcaa ttctcctgcc tcagcctccc aagtagctgg gattacaggt
2521 gcccaccacc acccctggct aaatttgtat tttcagtaga gacggggttt ccccatgttg
2581 gttaggctcg tctagaacct ctgacctcag gtgatccacc cgcctcggcc tcccaaagtg
2641 ctgggattac aggcgtgagc cacggcgccc agcctgtgct tatttttctta aaataatttt
2701 tgtattaaaa acttcacatt aaataagtgc taatgtttta ttgcatagta gggtgactag
2761 agttaacaat aacctattgc atatattttg aaatagctag aagagaggat tttgaaagtt
2821 ctcaacacaa agaaatgaca catatttgag gtgatggata tgctaattac cctggttcgg
2881 ttattacgca atgtatacat gtatcaaaac atcacactgt accacataaa tatgtatatt
2941 tattatttgt caattaaaag caaaataaaa caaaaaacct tcatctaata ctttggatca
3001 ttgtgaaaaa ataaattcct gaagtataaa gcatctatct aagtgtcttg atctaataag
3061 tacttgttct acaaatttatt gaaaaacata aactctgtta atgtctcatg gaacaggttg
3121 tgccttcagg gaaactagga ttggatttac taaattctca ttttttagat ctcagatact
3181 actgtcaaaa tgacttcaat tctgccttct atatataata cacacatata tttaggattt
```

Figure 9 Con't

Tetherin (BST2) - human; open reading frame

```
   1 ataaaggggt ggcccgtaga agattccagc accctccccc aactccaggc cagactcctt
  61 tcagctaaag gggagatctg gatggcatct acttcgtatg actattgcag agtgcccatg
 121 gaagacgggg ataagcgctg taagcttctg ctggggatag gaattctggt gctcctgatc
 181 atcgtgattc tgggggtgcc cttgattatc ttcaccatca aggccaacag cgaggcctgc
 241 cgggacggcc ttcgggcagt gatggagtgt cgcaatgtca cccatctcct gcaacaagag
 301 ctgaccgagg cccagaaggg ctttcaggat gtggaggccc aggccgccac ctgcaaccac
 361 actgtgatgg ccctaatggc ttccctggat gcagagaagg cccaaggaca aaagaaagtg
 421 gaggagcttg agggagagat cactacatta aaccataagc ttcaggacgc gtctgcagag
 481 gtggagcgac tgagaagaga aaaccaggtc ttaagcgtga gaatcgcgga caagaagtac
 541 taccccagct cccaggactc cagctccgct gcggcgcccc agctgctgat tgtgctgctg
 601 ggcctcagcg ctctgctgca gtgagatccc aggaagctgg cacatcttgg aaggtccgtc
 661 ctgctcggct tttcgcttga acattcccct gatctcatca gttctgagcg ggtcatgggg
 721 caacacggtt agcgggggaga gcacggggta gccggagaag ggcctctgga gcaggtctgg
 781 aggggccatg gggaagtcct gggtgtgggg acacagtcgg gttgacccag ggctgtctcc
 841 ctccagagcc tccctccgga caatgagtcc cccctcttgt ctcccaccct gagattgggc
 901 atggggtgcg gtgtgggggg catgtgctgc ctgttgttat gggtttttct tgcgggggcg
 961 gttgcttttt totggggtct ttgagctcca aaaaataaac acttcctttg agggagagca
1021 cacctgaaaa aaaaaaaaaa aaaaaaaa
```

Figure 9 Con't

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041456

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/12(2006.01)i, A61K 35/12(2006.01)i, A61K 35/17(2014.01)i, A61K 39/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 39/12; C12N 15/867; A61P 31/18; C12N 15/10; C12N 15/74; C12Q 1/70; A61K 31/711; A61K 35/12; A61K 35/17; A61K 39/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: viral vector, HIV, CD4 T cell, small RNA, CXCR4, CCR5, lentivirus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010-051521 A1 (LENTIGEN CORPORATION) 06 May 2010 See abstract; and claims 1-143.	9-16
X	TEBAS, P. et al., "Antiviral effects of autologous CD4 T cells genetically modified with a conditionally replicating lentiviral vector expressing long antisense to HIV", Blood, 2013, Vol. 121, No. 9, pp. 1524-1533 See abstract; and pages 1526, 1527.	9-16
A	WO 2015-017755 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 05 February 2015 See the whole document.	9-16
A	US 2002-0168345 A1 (DONG, J. Y.) 14 November 2002 See the whole document.	9-16
A	WO 2009-100928 A1 (FRAUNHOFER GESELLSCHAFT ZUR FORDERUNG DER ANGEWANDTEN FOR SCHUNG E.V.) 20 August 2009 See the whole document.	9-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2016 (19.10.2016)	**19 October 2016 (19.10.2016)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
PARK, Jung Min
Telephone No. +82-42-481-3516

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/041456

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-8, 17-24
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-8, 17-24 pertain to a method for treating a human body, as well as diagnostic methods, and thus relate to a subject matter not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2016/041456

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010-051521 A1	06/05/2010	None	
WO 2015-017755 A1	05/02/2015	AU 2014-296059 A1	03/03/2016
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		US 2014-0057354 A1	27/02/2014