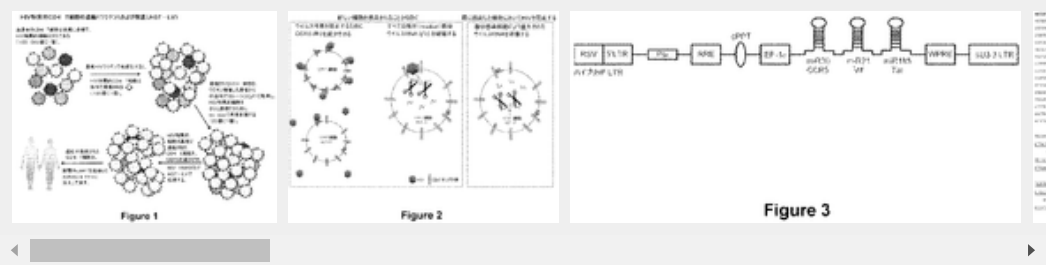
HIV preimmunization and immunotherapy

Images (17)



Classifications

A61K39/21 Retroviridae, eg equine infectious anemia virus

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Inventor: Charles David Pausa,, Charles David Pausa,

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Claims (16)

Hide Dependent

translated from Japanese

A composition comprising transduced CD4 T cells for use in a method of treating HIV infection, wherein said method.
(A) Steps to identify subjects in need of treatment for HIV infection;
(B) Steps to immunize the subject with a therapeutically effective amount of HIV vaccine;
(C) The step of removing lymphocytes from the subject and purifying CD4 T cells;
(D) In ex vivo, the step of contacting the CD4 T cells with a therapeutically effective amount of HIV vaccine;
(E) In ex vivo, a step of transducing a viral delivery system encoding at least one genetic element into the CD4 T cells , wherein the genetic element is a small RNA capable of targeting an HIV RNA sequence. There is a step;
A composition comprising (f) culturing the transduced CD4 T cells for about 1 to about 35 days; and (g) injecting the transduced CD4 T cells into the subject. The composition according to claim 1, wherein step (b) and step (d) include the same HIV vaccine. The composition of claim 1, comprising an HIV vaccine in which step (b) and step (d) are different. The composition of claim 1, wherein the subject had undergone cART or HAART prior to injecting the transduced CD4 T cells into the subject. The composition of claim 1, wherein the subject receives cyclophosphamide pretreatment prior to injecting the transduced CD4 T cells into the subject. The composition of claim 1, wherein the virus delivery system further comprises a small RNA capable of targeting CCR5. Even without least that can be the HIV RNA sequence targeting one of the small RNA, gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP , CRS, or INS, the composition of claim 6. The composition according to claim 1, wherein the transduced CD4 T cells are cultured for about 1 to about 10 days before the transduced CD4 T cells are injected into the subject.
A composition comprising transduced CD4 T cells for use in a method of achieving functional healing of HIV in an HIV + subject, said method.
(A) Steps to identify objects that are HIV +;
(B) Steps to immunize the subject with a therapeutically effective amount of HIV vaccine;
(C) The step of removing lymphocytes from the subject and purifying CD4 T cells;
(D) In ex vivo, the step of contacting the CD4 T cells with a therapeutically effective amount of HIV vaccine;
(E) In ex vivo, a small step of transducing a CD4 T cell using a viral delivery system encoding at least one genetic element, wherein the genetic element can target an HIV RNA sequence. RNA, step;
(F) The step of culturing the transduced CD4 T cells for about 1 to about 21 days; and (g) the step of injecting the transduced CD4 T cells into the subject, wherein the HIV + subject. A composition that includes steps to achieve functional healing. The composition of claim 9 , wherein step (b) and step (d) comprise the same HIV vaccine. The composition according to claim 9 , wherein step (b) and step (d) contain different HIV vaccines. The composition of claim 9 , wherein the subject had undergone cART or HAART prior to injecting the transduced CD4 T cells into the subject. The composition of claim 9 , wherein the subject receives cyclophosphamide pretreatment prior to injecting the transduced CD4 T cells into the subject. The composition of claim 9 , wherein the virus delivery system further comprises a small RNA capable of targeting CCR5. Even without least that can be the HIV RNA sequence targeting one of the small RNA, gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP , CRS, or INS, the composition of claim 14. The composition according to claim 9 , wherein the transduced CD4 T cells are cultured for about 1 to about 7 days before the transduced CD4 T cells are injected into the subject.

Description

translated from Japanese

Cross-reference to related applications This application claims the priority benefit under US Provisional Patent Application No. 62 / 190,139 filed on July 8, 2015, the disclosure of which is referenced in detail. It is used as.

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

Background of the Invention Although combination antiretroviral therapy (cART) (also known as Highly Active Antiretroviral Therapy or HAART) limits HIV-1 replication and slows the progression of the disease. The emergence of drug toxicity and drug resistant viruses is a challenge for long-term control in HIV-infected individuals. In addition, traditional antiretroviral therapies have been successful in delaying the onset or death of AIDS, but have not yet provided a functional cure. An alternative treatment strategy is clearly needed.

New data showing that the immune system has a major role, although usually inadequate to limit HIV replication, has elicited a keen interest in immunotherapy for HIV infection. Virus-specific T helper cells, which are important for maintaining cytolytic T cell (CTL) function, appear to have a role. Viremia is also affected by neutralizing antibodies, but is generally smaller in HIV infection and cannot keep up with viral variants that evolve in vivo.

Taken together, these data indicate that increased intensity and breadth of HIV-specific cell-mediated immune responses can provide clinical benefits through so-called HIV immunotherapy. There have been several studies testing vaccines against HIV, but to date success has been limited. Moreover, while there has been interest in enhancing HIV immunotherapy by utilizing gene therapy techniques, as with other immunotherapeutic approaches, success is limited. One such method of performing HIV-specific immunotherapy or gene therapy may be with a specially designed viral vector.

Due to the viral mechanism for specific viral envelope-host cell receptor interactions and gene expression, viral vectors can be used to transduce genes into target cells. As a result, viral vectors transfer 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. Has been used as a vehicle for. The ability to introduce and express foreign or modified genes into cells is useful for therapeutic interventions such as gene therapy, somatic reprogramming of induced pluripotent stem cells, and various types of immunotherapy.

Gene therapy is one of the most mature areas of biomedical research that has the potential to create new therapies that may involve the use of viral vectors. Given the wide variety of potential genes available for therapy, efficient delivery of these genes is needed to realize the potential of gene therapy as a means of treating infectious and non-communicable diseases. Means are needed. Several viral systems have been developed as therapeutic gene transfer vectors, including mouse retroviruses, adenoviruses, parvoviruses (adenoviruses), vacciniaviruses, and herpesviruses.

There are many factors to consider when developing a viral vector, including tissue orientation, stability of viral preparations, expression stability and regulation, genomic packaging capability, and construct-dependent vector stability. .. In addition, in vivo applications of viral vectors are often limited by host immune responses to viral structural proteins and / or transduced gene products.

Therefore, toxicity and safety are important hurdles that must be overcome for viral vectors used in vivo for the treatment of subjects. There are numerous historical examples of gene therapy applications in humans with problems associated with host immune responses to gene delivery vehicles or therapeutic gene products. Viral vectors (eg, adenoviruses) that co-transduce several viral genes with one or more therapeutic genes are of particular concern.

Lentiviral vectors generally do not induce cytotoxicity and do not elicit a strong host immune response, but some lentiviral vectors, such as HIV-1, which have several immune stimulatory gene products, cause cytotoxicity. , In vivo, may induce a strong immune response. However, this may not be a problem for lentivirus-derived transduction vectors that do not encode multiple viral genes after transduction. Of course, this may not always be the case, as the purpose of the vector may be to encode a protein that will elicit a clinically useful immune response.

Another important issue with the use of lentiviral vectors is the cytopathogenicity issue that can occur during exposure to some cytotoxic viral proteins. Exposure to certain HIV-1 proteins may induce cell death or functional non-responsiveness in T cells. Similarly, the possibility of recombinantly producing replication-competent virulent viruses is often a problem.

Obviously, there is a need for improved treatment of HIV, and the present invention meets this need.

Abstract of the Invention Therapeutic immunization strategies and methods, as well as highly effective therapeutic lentiviruses, and other vectors capable of inhibiting HIV and reducing or altering the expression of specific targets are disclosed herein. Will be done. The methods and compositions of the disclosed inventions are useful for achieving functional cure of HIV. More specifically, the present invention presents to therapeutic immunization of patients, ex vivo restimulation of patient CD4 T cells, ex vivo lentivirus transfection of enriched T cells, ex vivo culture of cells, and enrichment. Included are methods for functional cure of HIV that are optimally combined with reinjection of modified genetically modified cells. In addition, the invention includes bioassays to measure therapeutic efficacy, continuous changes in therapeutic agent administration, monitoring periods after HAART withdrawal, and methods of diagnosing functional HIV cure.

In one aspect, the disclosed invention is a method of treating an HIV infection, in which (a) a step of identifying a subject in need of treatment of the HIV infection; (b) immunizing the subject with a therapeutically effective amount of an HIV vaccine. Steps to transform; (c) remove lymphocytes from the subject and purify peripheral blood mononuclear cells (PBMC); (d) in ex vivo, treat PBMC with a therapeutically effective amount of HIV vaccine (step). Contact in (b) may be the same as or different from the HIV vaccine used in (b); (e) transfect PBMCs in ex vivo using a viral delivery system encoding at least one genetic element. Steps; (f) Transfected PBMCs for about 1-21 or about 35 days (or, for example, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 9. 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, The step of culturing up to (any time frame between these parameters), such as about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34 or about 35 days), and (g) The present invention relates to a method comprising injecting a transgenic PBMC into a subject.

In some embodiments, steps (b) and (d) utilize the same HIV vaccine, while in other embodiments steps (b) and (d) utilize different HIV vaccines. In some embodiments, the patient may not require step (b) and / or step (d). Thus, in some embodiments, the disclosed method comprises only steps (a), (c), (d), (e), (f) and (g), or some combination thereof. obtain.

In some embodiments, the subject received cART or HAART prior to injecting the transduced PBMC into the subject. In some embodiments, the subject is receiving cyclophosphamide pretreatment prior to injecting the transduced PBMC into the subject.

In some embodiments, at least one genetic element targets a small RNA capable of inhibiting the production of the chemokine receptor CCR5, a small RNA capable of inhibiting the production of the chemokine receptor CXCR4, and an HIV RNA sequence. It is selected from the group consisting of small RNA molecules. In some embodiments, the small RNA molecules that target the HIV RNA sequence are gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS. , Or INS.

In some embodiments, the transduced PBMC is subjected to about 1 to about 7 or about 10 days (or, for example, about 2, about 3, about 4, about 4, before injecting the transduced PBMC into the subject). Incubate to any time frame between these parameters, such as 5, about 6, about 7, about 8, about 9, or about 10 days.

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

In some embodiments, the vector is a lentivirus, but in other embodiments, the vector is a DNA plasmid, adeno-associated virus, or other integrated or non-integrated vector system for gene delivery.

In some embodiments, the small RNA molecules that target the HIV RNA sequence are gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS. , Or INS.

In another aspect, the disclosed invention relates to a bioassay for determining whether an HIV + subject is functionally cured. Such a bioassay includes the step of determining the number of HIV-specific CD4 T cells that have been genetically modified by a therapeutic lentivirus and the number of HIV-specific CD4 T cells that have been genetically modified by a therapeutic lentivirus. However, if the threshold is exceeded after a certain time after treatment according to the disclosed method, the subject is functionally healed.

In some embodiments, the threshold is about $1 \times 10^{8,}$ is a HIV-specific CD4 T cells with a genetic modification by therapy for lentivirus, the threshold may be determined this higher than a value or lower ..

In some embodiments, the specific time after treatment is about 30 to about 60 days (or any time frame between these two values), and in other embodiments, the specific time after treatment is. Approximately 12 to approximately 26 weeks (or any time frame between these two values).

In yet another aspect, the disclosed invention relates to a method of achieving functional cure of HIV in an HIV + subject. The methods are: (a) identifying a subject that is HIV +; (b) immunizing the subject with a therapeutically effective amount of HIV vaccine; (c) removing lymphocytes from the subject and peripheral blood mononuclear cells (PBMC).); (D) In ex-vivo, the step of contacting PBMC with a therapeutically effective amount of HIV vaccine; (e) in ex-vivo, traits to PBMC using a viral delivery system encoding at least one genetic element. Steps to introduce; (f) Incubate transfected PBMCs for about 1 to about 21 or 35 days (or any time frame between these values); and (g) Transfected PBMCs. Includes a step of injecting a virus into a subject, wherein the HIV + subject achieves functional healing.

In some embodiments, steps (b) and (d) include the same HIV vaccine, while in other embodiments steps (b) and (d) contain different HIV vaccines.

In some embodiments, the subject received cART or HAART prior to injecting the transduced PBMC into the subject. In some embodiments, the subject receives cyclophosphamide pretreatment or alternative conditioning therapy to improve T cell engraftment prior to injecting the transduced PBMC into the subject.

In some embodiments, at least one genetic element targets a small RNA capable of inhibiting the production of the chemokine receptor CCR5, a small RNA capable of inhibiting the production of the chemokine receptor CXCR4, and an HIV RNA sequence. It is selected from the group consisting of small RNA molecules. In some embodiments, the small RNA molecules that target the HIV RNA sequence are gag, pol, env, tat, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS. , Or INS.

In some embodiments, the transduced PBMC is injected into the subject for about 1 to about 7 or about 12 days (or any time between these two values) prior to injecting the transduced PBMC into the subject. Incubate (frame, or other period described herein).

The general description described above and the brief description of the drawings below and the embodiments for carrying out the invention are exemplary and descriptive and are intended to provide further description of the claimed invention. Other objectives, advantages, and novel features will be readily apparent to those skilled in the art from the brief description of the drawings below and the embodiments for carrying out the invention.

FIG. 1 shows vaccination, cell collection to obtain PBMC and CD4 T cells, growth cytokines (interleukin-2, interleukin-12, interleukin-15, interleukin-6, interleukin-7 and interleukin- T cell cultures in exvivo after stimulation with vaccine immunogen and / or CD3 / CD28 stimulation and / or mitogen stimulation in the presence of support (including, but not limited to, 23), anti. FIG. 3 shows a flow chart of possible clinical therapies incorporating lentiviral transfection to deliver HIV gene constructs, short-term culture of transfected cells, and infusion back into the original subject.

FIG. 2 shows how gene therapy is used to alter CD4 T cells to prevent other cells from infecting or viral replication.

FIG. 3 outlines an exemplary therapeutic lentivirus construct. Therapeutic lentiviral constructs can replace alternative sequences for promoter regions, regulatory RNA targeting, and regulatory RNA types. In addition, the plasmid used to package the lentivirus particles can be modified according to production requirements.

FIG. 4 shows an exemplary vector sequence. Positive (genome) strand sequences of promoters and miR clusters have been developed to inhibit the spread of CCR5-directed HIV strains. The sequence not shown underline contains the transcriptional EF-1 alpha promoter best selected for this miR cluster. The underlined sequence indicates a miR cluster consisting of miR30 CCR5 (a modification of the native human miR30 that redirects to CCR5 mRNA), miR21 Vif (redirect to the Vif RNA sequence) and miR185 Tat (redirect to the Tat RNA sequence). .. Smaller font sequences, not shown underlined, are restriction endonuclease cleavage sites incorporated into oligonucleotide primers for each miRNA construct.

FIG. 5 shows that knockdown of CCR5 by experimental vectors prevents R5-directed HIV infection in AGTc120 cells. Panel (A) shows CCR5 expression in AGTc120 cells with or without the AGT103 lentiviral vector. Panel (B) shows the susceptibility of transduced AGTc120 cells to infection by HIV BaL virus stock expressing green fluorescent protein (GFP) fused to the HIV Nef gene.

FIG. 6 shows that AGT103 reduces the expression of Tat protein expression in cells transfected with the HIV expression plasmid.

FIG. 7 shows that AGT103 reduces the level of intracellular Vif protein expression transfected with a full-length HIV expression plasmid. Cells are either untreated (left lane and central lane) or transduced with AGT103 (left lane).

FIG. 8 shows the generation of highly enriched CD4 + T cell populations 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. Significant CD4 T cell populations are shown in the upper left quadrant of these analytical flow cytometric dot plots and change from 52% to 57% of all T cells after vaccination series. These are typical data. Panel (B) shows peripheral blood from participants in an HIV therapy vaccine study cultured in +/- interleukin-2 / interleukin-12 or +/- interleukin-7 / interleukin-15 medium for 12 days. The expression of CD4 and CD8 in the CD3 + population of mononuclear cells is shown. Several cultures were stimulated with a duplicate peptide representing the total p55 Gag protein of HIV-1 (JPT PepMix) as a source of epitope peptides for T cell stimulation. Panel (C) shows that the combination of PepMix and interleukin-2 / interleukin-12 results in optimal proliferation of antigen-specific CD4 T cells. The upper panel shows the increase in cytokine (interferon-gamma) secreting cells in the vaccinated specimen exposed to PepMix. Panel (D) introduces AGT103 transfection of antigen-proliferated CD4 T cells capable of producing HIV-specific and HIV-resistant helper CD4 T cells for injection into patients as part of functional cure for HIV. Shown. The upper panel contains the results of an analysis of the CD4 + T cell population in culture. The x-axis is green fluorescent protein (GFP) luminescence indicating that AGT103 has been transduced into individual cells. FIG. 8 shows the generation of highly enriched CD4 + T cell populations 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. Significant CD4 T cell populations are shown in the upper left quadrant of these analytical flow cytometric dot plots and change from 52% to 57% of all T cells after vaccination series. These are typical data. Panel (B) shows peripheral blood from participants in an HIV therapy vaccine study cultured in +/- interleukin-2 / interleukin-12 or +/- interleukin-7 / interleukin-15 medium for 12 days. The expression of CD4 and CD8 in the CD3 + population of mononuclear cells is shown. Several cultures were stimulated with a duplicate peptide representing the total p55 Gag protein of HIV-1 (JPT PepMix) as a source of epitope peptides for T cell stimulation. Panel (C) shows that the combination of PepMix and interleukin-2 / interleukin-12 results in optimal proliferation of antigen-specific CD4 T cells. The upper panel shows the increase in cytokine (interferon-gamma) secreting cells in the vaccinated specimen exposed to PepMix. Panel (D) introduces AGT103 transfection of antigen-proliferated CD4 T cells capable of producing HIV-specific and HIV-resistant helper CD4 T cells for injection into patients as part of functional cure for HIV. Shown. The upper panel contains the results of an analysis of the CD4 + T cell population in culture. The x-axis is green fluorescent protein (GFP) luminescence indicating that AGT103 has been transduced into individual cells.

FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector. FIG. 9 shows the sequences of various exemplary cellular elements known to limit HIV replication that may be incorporated into the disclosed vector.

Detailed Description of Suitable Embodiments Methods and compositions for treating and / or preventing human immunodeficiency virus (HIV) disease to achieve functional cure are disclosed herein. Functional cure is defined as a condition that reduces or eliminates the need for cART and results from the disclosed treatments and methods that may or may not require support for adjuvant therapy. The methods of the invention include gene delivery by incorporating the lentivirus, non-integrated lentivirus, and related viral vector techniques described below.

Methods of their use in therapeutic viral vectors (eg, lentiviral vectors), immunotherapy, and strategies for achieving functional cure for HIV infection are disclosed herein. A common strategy is to generate a strong immune response against HIV in HIV-infected patients with stable suppression of viralemia by daily administration of HAART with the aim of concentrating a fraction of HIV-specific CD4 T cells. Can include initial therapeutic immunity with a vaccine intended for. (1) Isolation of peripheral leukocytes by leukocyte aferesis or purification of PBMC from venous blood, (2) Restimulation of CD4 T cells with HIV vaccine protein in ex vivo, (3) Therapeutic wrench This is followed by viral transfection, ex vivo, T cell culture, and (4) reinjection back into the original donor.

Previous efforts have not been made to achieve cure of HIV, primarily due to the inability to obtain a sufficient number of HIV-specific CD4 T cells with defensive genetic modifications. When this value falls below the critical threshold, withdrawal of antiretroviral therapy leads to HIV recurrence, followed by rapid destruction of HIV-specific CD4 T cells, followed by disease despite previous gene therapy. The progress will be resumed. A new strategy for achieving functional cure of HIV by using the therapeutic immunization in the strategies described herein to provide a highly effective therapeutic lentivirus capable of inhibiting HIV. Was developed.

Novel viral vectors for enhancing HIV-specific CD4 T cells, including lentiviral and non-integrated, episomal replication viral vectors, and methods of using them are also disclosed herein. Episomal replication vectors such as the present invention can be found in the Papovavirus family (eg, bovine papillomavirus or BPV) or the Herpesvirus family (eg, Epstein Barr Virus or EBV) or the Hepadonavirus family (eg, hepatitis B virus or). It can contain viral components derived from viruses such as HBV). Episomal replication vectors derived from these viruses are of replication origin and at least one viral transacting agent, eg, an initiation protein such as E1 for BPV, EBNA-1 or HBV polymerase for EBV, or adenovirus. It can include end-binding proteins. The process of episomal replication typically incorporates both host cell replication mechanisms and viral transacting factors.

I. Human immunodeficiency virus (HIV)

HIV is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS is a condition in which life-threatening opportunistic infections and cancer are rampant due to the progressive failure of the immune system. Without treatment, the average survival time after HIV infection is estimated to be 9-11 years, depending on the HIV subtype. HIV infection is caused by the transfer of body fluids, including but not limited to blood, semen, vaginal fluid, pre-ejaculation, saliva, tears, lymph or cerebrospinal fluid, or breast milk. HIV can be present in infected individuals, both as free viral particles and within infected immune cells.

HIV infects living cells of the human immune system, such as helper T cells, but the orientation can vary within the HIV subtype. 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 includes, but is 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. The mechanism results in reduced levels of CD4 + T cells. When the number of CD4 + T cells drops below critical levels, cell-mediated immunity is lost and the body becomes more progressive and more susceptible to opportunistic infections and cancer.

Structurally, HIV is different from many other retroviruses. The RNA genome contains at least 7 structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS) and at least 9 genes (gag, pol, env, tat, rev) encoding 19 proteins. , Nef, viv, vpr, vpu, and sometimes the tenth tv), which is a fusion of tat, env, and rev. The three gag, pol, and env of these genes contain the information needed to make the structural proteins of new viral particles.

HIV replicates primarily in CD4 T cells, causing cell disruption or dysregulation that reduces host immunity. HIV establishes infection as an integrated provirus and transitions to a latent infection state in which viral expression in a particular cell drops below the level of cytopathology affecting that cell or the level detected by the host immune system. HIV is difficult to treat because of its potential and has not been eradicated even after long-term high-activity antiretroviral therapy (HAART). Although survival can be extended by HAART, in most cases HIV infection causes fatal disease.

The main goal in the fight against HIV is to develop strategies to cure the disease. Researchers are looking to alternative procedures, as extended HAART has not yet achieved this goal. Initial efforts to improve host immunity by therapeutic immunization (using vaccines after infection has occurred) have had little or no impact. Similarly, treatment enhancement had moderate or no impact.

Although some progress has been made with the use of gene therapy, the positive result is sporadic, one of the genes encoding CCR5 (chemokine receptor), which plays an important role in viral penetration of host cells. Or found only among rare humans with deficiencies in both alleles. However, many researchers are optimistic that gene therapy has the greatest potential for ultimately achieving HIV cure.

As disclosed herein, the methods and compositions of the invention can achieve functional healing with or without complete eradication of all HIV from the body. Functional cure is that HIV + individuals who previously required HAART survived with low or undetectable viral replication and are using lower or intermittent doses of HAART, or potentially complete HAART. Defined as a situation or condition that can be discontinued. As used herein, functional cure may still require adjuvant therapy to maintain low levels of viral replication and slow or eliminate disease progression. Absent. A possible prognosis for functional cure is the ultimate eradication of HIV to prevent all possible recurrences.

The main obstacle to achieving functional healing lies in the basic biology of HIV itself. Viral infections delete CD4 T cells, which are important for all immune functions. Most importantly, HIV infection and CD4 T cell depletion require activation of individual cells. Activation is a mechanism specific to individual CD4 T cell clones that recognize pathogens or other molecules using rearranged T cell receptors.

In the case of HIV, infection activates, and eventually depletes, a population of HIV-specific T cells before other less virus-specific T cells, effectively defending the immune system against the virus. Incapacitate. The ability of HIV-specific T-cell response to be reconstituted during long-term HAART; however, when HAART is interrupted, repetitive virus infection repeats the process, again deleting virus-specific cells, Reset the disease progression clock.

Obviously, functional healing is possible only if sufficient HIV-specific CD4 T cells are protected and the host's innate immunity becomes controlled against HIV if HAART is interrupted. In one embodiment, the invention provides methods and compositions for improving the effectiveness of gene therapy to provide a functional cure for HIV disease. In another embodiment, the invention provides methods and compositions for enhancing host immunity to HIV in order to provide functional healing. In yet another embodiment, the invention provides methods and compositions for enriching HIV-specific CD4 T cells in a patient to achieve functional healing.

In one embodiment of the invention, treatment involves about 100%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700%, about 700% of the subject HIV-specific CD4 T cells. 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 40,000%, about 45000%, about 50000%, about 55000% , About 60,000%, about 65,000%, about 70,000%, about 75,000%, about 80,000%, about 85,000%, about 90,000%, about 95,000%, about 100,000% or any value in between, resulting in enrichment.
II. Gene therapy

Viral vectors are used to deliver gene constructs to host cells for the purpose of treating or preventing disease.

A gene construct comprises a functional gene or part of a gene that modifies or complements an existing defect, a DNA sequence encoding a regulatory protein, antisense, short homology RNA, long non-coding RNA, small interfering RNA or others. It can include, but is not limited to, a DNA sequence encoding an RNA molecule and a decoy sequence encoding either an RNA or a protein designed to compete for cell factors important for altering disease status. .. Gene therapy involves delivering these therapeutic gene constructs to target cells to provide treatment or alleviation of a particular disease.

Several efforts have been made to utilize gene therapy in the treatment of HIV disease, but so far the results have been poor. A small number of successful treatments were obtained in rare HIV patients with a spontaneous deletion of the CCR5 gene (an allele known as CCR5 delta32).

Nucleases delivered by lentivirus or other mechanisms for gene deletion / modification can be used to help reduce overall expression of CCR5 and / or reduce HIV replication. There is at least one study reporting successful treatment of the disease when lentivirus was administered to patients with a genetic background of CCR5 delta32. However, this is just one example of success, and many other patients without the CCR5 delta32 genotype have not been successfully treated. As a result, there is a substantial need to improve the performance of viral gene therapy for HIV, both in terms of the performance of individual viral vector constructs and the improvement of vector use by strategies to achieve functional HIV cure. ..

For example, some existing therapies rely on zinc finger nucleases to delete some of CCR5 in an attempt to make cells resistant to HIV infection. However, even after optimal treatment, only 30% of T cells are modified by nucleases, so that only 10% of the modified T cell population prevents HIV infection. It was only modified. In contrast, the disclosed method results in the reduction of CCR5 expression below the levels required to enable HIV infection in virtually all cells carrying the lentivirus transgene.

For the purposes of the disclosed methods, gene therapy includes enhanced affinity T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatives on CD8 T cells), viral proteins. Modification of signaling pathways to avoid induced cell death, reducing HIV replication in TREX, SAMHD1, MxA or MxB proteins, APOBEC complexes, TRIM5-alpha complexes, tetherin (BST2), and mammalian cells. Can include, but is not limited to, increased expression of HIV limiting elements, including similar proteins identified as capable of.

伸長因子－1アルファ(EF1－アルファ)プロモーター(イタリック)

ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAG

GGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA

CAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTT

CCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT

TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATC

TGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGAC

GCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCGATCTGCACACTGGTATTTCGGTTTTTGGGGCC

GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA

GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGC

CCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCA

GGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT

TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAG

CTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGA

GACTGAAGTTAGGCCAGCTTGGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTC

ATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTGGCGAAGCTAATTC

miR30 CCR5 開始

TGCAGATTCGACTGTACAAGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCT

CTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG

miR30 CCR5 終結　　miR21 Vif 開始

ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTT

CTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTA

miR21 Vif 終結　　miR185 Tat 開始

TCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC

CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA

miR185 Tat 終結

CCGCGTCTTCGTCGCGGCCGCTCGAGCATGCAT

Figure 4

III. ImmunotherapyFor example, in some embodiments, the disclosed vectors may include, but are not limited to, the limiting elements found in Table 1 below. The sequences of these exemplary limiting elements are further disclosed in FIG.

伸長因子－1アルファ(EF1－アルファ)プロモーター(イタリック)

ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAG

GGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA

CAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTT

CCACGCCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT

TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATC

TGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGAC

GCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCGATCTGCACACTGGTATTTCGGTTTTTGGGGCC

GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGA

GAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGC

CCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGCCCTGCTGCA

GGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT

TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAG

CTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGA

GACTGAAGTTAGGCCAGCTTGGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTC

ATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAGGAATTGGCGAAGCTAATTC

miR30 CCR5 開始

TGCAGATTCGACTGTACAAGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCT

CTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGG

miR30 CCR5 終結　　miR21 Vif 開始

ACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTT

CTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTA

miR21 Vif 終結　　miR185 Tat 開始

TCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC

CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGA

miR185 Tat 終結

CCGCGTCTTCGTCGCGGCCGCTCGAGCATGCAT

Figure 4

Historically, vaccines have been the go-to weapon for deadly infectious diseases, including smallpox, polio, measles, and yellow fever. Unfortunately, there is currently no approved vaccine for HIV. The HIV virus has a unique means of evading the immune system, and the human body does not appear to be able to implement an effective immune response against it. As a result, scientists do not have a clear idea of what is needed to provide protection against HIV.

However, immunotherapy may provide a solution that was previously unattainable by traditional vaccination approaches. Immunotherapy, also called biological therapy, is a type of treatment designed to strengthen the body's natural defenses to fight infections or cancer. It uses materials made either in the body or in the laboratory to improve, target, or restore the functioning of the immune system.

In some embodiments of the disclosed invention, an immunotherapeutic approach can be used to enrich the population of HIV-specific CD4 T cells with the aim of increasing the host's anti-HIV immunity. In some embodiments of the disclosed invention, integrated or non-integrated lentiviral vectors can be used to transduce host immune cells for the purpose of increasing host anti-HIV immunity. In yet another embodiment of the invention, a dead particle, virus-like particle, HIV peptide or peptide fragment, set, combined with a suitable vehicle and / or biological or chemical adjuvant to increase the immune response of the host. Vaccines containing HIV proteins, including but not limited to replacement viral vectors, recombinant bacterial vectors, purified subunits or plasmid DNA, can be used to concentrate a population of virus-specific T cells or antibodies, as well as These methods can be further enhanced by the use of HIV-targeted gene therapy with lentivirus or other viral vectors.
IV. Method according to the invention

In one aspect, the disclosed invention provides a method of using a viral vector to achieve functional cure of HIV disease. The method further includes immunotherapy to enrich the proportion of HIV-specific CD4 T cells, followed by lentivirus transduction to deliver HIV inhibitors and CCR5 and CXCR4 as needed.

In one embodiment, the method comprises therapeutic immunization as a method for enriching the proportion of HIV-specific CD4 T cells. Therapeutic immunization includes biological or chemicals including purified proteins, inactivated viruses, viral vectorized proteins, bacterial vectorized proteins, peptides or peptide fragments, virus-like particles (VLPs), cytokines and / or chemokines. Can include adjuvants, vehicles, and methods for immunization.

Therapeutic vaccines can include one or more HIV proteins having a protein sequence that represents the dominant viral type in the geographic area being treated. Therapeutic vaccines include purified proteins, inactivated viruses, viral vectorized proteins, bacterial vectorized proteins, peptides or peptide fragments, virus-like particles (VLPs), cytokines and / or chemokines. May include adjuvants, vehicles, and methods for immunization. Vaccination can be administered according to standard methods known in the art, and HIV patients receive antiretroviral therapy during the period of immunization and subsequent ex-vivo lymphocyte culture including lentiviral transfection. You can continue.

In some embodiments, HIV + patients are immunized with the HIV vaccine to reduce the frequency of HIV-specific CD4 T cells to about 2, about 25, about 250, about 500, about 750, about 1000, about 1250, or about 1500. It can be increased by a factor of (or any amount between these values). The vaccine may be a clinically utilized or experimental HIV vaccine that comprises a disclosed lentivirus, other viral vector or other bacterial vector used as a vaccine delivery system. For example, the disclosed vector may include a recombinant bovine attenuated tubercle bacillus vaccine (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 vector can encode virus-like particles (VLPs) to induce higher titers of neutralizing antibodies. In another embodiment, the vectors include, but are limited to, gag, pol, and env, tat, rev, nef, viv, vpr, vpu and tev as well as LTR, TAR, RRE, PE, SLIP, CRS, and INS. It is possible to encode a peptide or peptide fragment associated with HIV that is not HIV. Alternatively, the HIV vaccines used in the disclosed methods include purified proteins, inactivated viruses, viral vectorized proteins, bacterial vectorized proteins, peptides or peptide fragments, virus-like particles (VLPs), or cytokines and / Or may include a biological or chemical adjuvant containing a chemokine.

In one embodiment, the method is for biological or chemical adjuvants, vehicles, and restimulations that include purified proteins, inactivated viruses, viral vectorized proteins, bacterial vectorized proteins, cytokines and / or chemokines. Includes ex-vivo restimulation of CD4 T cells from a person or patient previously immunized by therapeutic vaccination using the method of. Ex vivo restimulation can be performed using the same vaccines or immunostimulatory compounds used for in vivo immunization, or different vaccines or different vaccines used for in vivo immunization. It can be performed using immunostimulatory compounds. Moreover, in some embodiments, the patient does not require prior therapeutic vaccination or restimulation of CD4 T cells if the individual has a sufficiently high antigen-specific CD4 T cell response to the HIV protein. There is. In these embodiments, such patients may only require administration of the disclosed viral vector to achieve functional cure.

For example, peripheral blood mononuclear cells (PBMCs) are obtained by leukocyte apheresis and can be treated with ex vivo $^{\text{to obtain } 1 \times 10^{10}}$ CD4 T cells, of which about 0.1%, about 1%, About 5% or about 10% or about 30% are HIV specific in terms of antigen response and are HIV resistant by having a therapeutically introduced

gene delivered by the disclosed lentivirus vector. Alternatively, restimulate about $1x10^7$, about $1x10^8$, about $1x10^9$, about $1x10^{10}$, about $1x10^{11}$, or about $1x10^{12}$ CD4 T cells. Can be isolated for Any suitable amount of CD4 T cells can be isolated for ex vivo restimulation.

The isolated CD4 T cells can be cultured in a suitable medium through restimulation with HIV vaccine antigen, which may contain antigens present in previous therapeutic vaccinations. Anti-retroviral therapeutic agents, including inhibitors of reverse transcriptase, protease or integrase, can be added to prevent viral reappearance during long-term ex vivo culture. CD4 T cell restimulation is used to concentrate the proportion of HIV-specific CD4 T cells in the culture. The same procedure may also be used for analytical purposes to identify HIV-specific T cells and measure the frequency of this subpopulation using a small amount of blood with peripheral blood mononuclear cells obtained by purification. Can be done.

The PBMC fraction can be enriched for HIV-specific CD4 T cells by contacting the cells with an HIV protein that is consistent or complementary to the components of the vaccine previously used for in vivo immunization. Ex vivo restimulation 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.

The method further comprises combining therapeutic immunization in vivo with lentivirus transduction and culture in ex vivo and restimulation of CD4 T cells in ex vivo.

Thus, in one embodiment, the enriched restimulated PBMC fraction for HIV-specific CD4 T cells is transduced with a therapeutic anti-HIV lentivirus or other vector for about 1 to about 21 days or It was retained in the culture for up to about 35 days. Alternatively, the cells can be cultured for about 1 to about 18 days, about 1 to about 15 days, about 1 to about 12 days, about 1 to about 9 days, or about 3 to about 7 days. Therefore, the transduced cells are 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 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 30 It may be cultured for 31, about 32, about 33, about 34, or about 35 days.

When the transduced cells are fully cultured, the transduced CD4 T cells are injected back into the original patient. Injection can be performed using a variety of machines and methods known in the art. In some embodiments, the infusion may be accompanied by pretreatment with cyclophosphamide or a similar compound to increase the efficiency of reimplantation.

In some embodiments, CCR5 targeted therapy may be added to the subject's antiretroviral therapy regimen continuously throughout the therapeutic process. Examples of CCR5 targeted therapies include, but are not limited to, Maraviroc (CCR5 antagonist) or rapamycin (immunosuppressive agent that lowers CCR5). In some embodiments, antiretroviral therapy is discontinued and subjects can be tested for viral rebound. If rebound does not occur, adjuvant therapy can also be removed and the subject can be retested for viral rebound.

Continued viral suppression with or without reduced or no antiretroviral therapy, including cART or HAART, with or without reduced adjuvant therapy for approximately 26 weeks is a functional cure for HIV. I can think. Other definitions of functional healing are described herein.

The lentiviral vector 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 targeting gene therapy, the viral vectors of the invention are (i) antibodies against toxins produced by antigens associated with infectious diseases or infectious pathogens, (ii) immune cells. Invivo, including cytokines containing interleukin, (iii) CD8 suppressor, which are required for proliferation or function and can be therapies for immunoregulatory deficiencies encountered in HIV and other chronic or acute human viral or bacterial pathogens. Factors that suppress the growth of HIV in, (iv) mutations or deletions in the chemokine receptor CCR5, mutations or deletions in the chemokine receptor CXCR4, or mutations or deletions in the chemokine receptor CXCR5, (v) HIV Antisense DNA or RNA to a specific receptor or peptide related to HIV or a host protein associated with HIV, (vi) Small interfering RNA to a specific receptor or peptide related to HIV or a host protein associated with HIV, or (Vii) It may encode a gene or nucleic acid sequence that includes, but is not limited to, various other therapeutically useful sequences that can be used to treat HIV or AIDS.

A further example of HIV-targeted gene therapy that can be used in the disclosed methods is an affinity-enhanced T cell receptor, a chimeric antigen on CD4 T cells (or alternatives on CD8 T cells). Modification of signaling pathways to avoid cell death caused by receptors, viral proteins, TREX, SAMHD 1, MxA or MxB proteins, APOBEC complex, TRIM5-alpha complex, tetherin (BST2), and HIV in mammalian cells It can include, but is not limited to, increased expression of HIV limiting elements, including similar proteins identified as capable of reducing replication.

In some embodiments, the patient may be receiving cART or HAART at the same time while being treated according to the methods of the invention. In other embodiments, the 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 through treatment according to the methods of the invention and the patient is monitored for HIV viral load in the blood and for the frequency of lentivirus transduced CD4 T cells in the blood. May be good. Preferably, a patient receiving cART or HAART before being treated according to the methods of the invention can discontinue or reduce cART or HAART after treatment according to the methods of the invention.

For efficacy purposes, the frequency of transduced HIV-specific CD4 T cells, a novel substitute marker for gene therapy effects, may be determined to be discussed in more detail in Section VI.

V. Composition according to the present invention

In one aspect, the disclosed invention provides a lentiviral vector capable of delivering a gene construct to inhibit HIV penetration of susceptible cells. For example, one mechanism of action is to reduce mRNA levels of CCR5 and / or CXCR4 chemokine receptors, and thus reduce the rate of viral entry into susceptible cells.

Alternatively, the disclosed lentiviral vector may be able to inhibit the formation of DNA and HIV-infected cells by reducing the stability of incoming HIV genomic RNA. In yet another embodiment, the disclosed lentiviral vector can block HIV production from latently infected cells, the mechanism of action of which comprises short homology, small interfering or other regulatory RNA species. The action of inhibitory RNA is to cause instability of viral RNA sequences.

Therapeutic lentiviruses disclosed in this application generally include at least one of two types of genetic cargo. First, the lentivirus may encode a genetic element that directs the expression of small RNA that can inhibit the production of chemokine receptors CCR5 and / or CXCR4, which are important for HIV penetration of susceptible cells. The second type of gene cargo targets HIV RNA sequences with the aim of blocking reverse transcription, RNA splicing, RNA translation to produce proteins, or packaging of viral genomic RNA for particle production and spread of infection. Contains constructs capable of expressing small RNA molecules. An exemplary structure is illustrated in FIG.

As shown in FIG. 3, an exemplary structure can include a large number of sections or components. For example, in one embodiment, the exemplary LV construct may include the following sections or components:

RSV-Rous Sarcoma virus terminal repetitive sequence;

5'LTR-Part of the HIV end repeat that can be cleaved to block vector replication after chromosomal integration;

Psi-A packaging signal that allows the vector RNA genome to be incorporated into viral particles during packaging;

The RRE-Rev reactive element can be added to improve expression from the transgene by transferring RNA from the nucleus to the cytoplasm of the cell;

C PPT-Poly purine tract that promotes second-strand DNA synthesis prior to integration of the transgene into the host cell chromosome;

Promoters-promoters are those that initiate RNA transcription from an integrated transgene to express a microRNA cluster (or other genetic element of a construct), and in some embodiments the vector is EF. -1 promoter may be used;

Anti-CCR5-A microRNA that targets the messenger RNA of the host cell factor CCR5 and reduces its expression on the cell surface;

-MicroRNAs that target HIV genomic RNA or messenger RNA at the junction between the Anti-Rev / Tat-HIV Rev coding region and the Tat coding region, sometimes referred to as miRNA Tat, or in this application. A similar statement is given;

-MicroRNAs that target HIV genomic RNA or messenger RNA within the Anti-Vif-Vif coding region;

The WPRE-woodchuck hepatitis virus post-transcriptional regulatory element is an additional vector component that can be used to promote nuclear RNA transport; and the delta U3 3'LTR. -A modified version of the HIV 3-prime end repeat sequence, in which part of the U3 region is deleted to improve the safety of the vector.

For those of skill in the art, the above components are merely examples, and such components will be reorganized with other elements as long as the construct can block the expression of the HIV gene and reduce the spread of infection. It can be recognized that it can be modified, replaced, or otherwise modified.

The vectors of the invention can contain one or both of the gene cargo types discussed above (ie, the genetic elements that guide the expression of the gene or small RNAs such as siRNA, shRNA or miRNA that can block translation or transcription). May include, the vectors of the invention may also encode additional useful products for the purpose of treating or diagnosing HIV. For example, in some embodiments, these vectors also encode green fluorescent protein (GFP) for the purpose of tracking the vector or antibiotic resistance gene for the purpose of selectively maintaining genetically modified cells in vivo. You may.

The combination of genetic elements incorporated into the disclosed vector is not particularly limited. For example, the vector is 1 small RNA, 2 small RNA, 3 small RNA, 4 small RNA, 5 small RNA, 6 small RNA, 7 small RNA, 8 small RNA. Small RNA, 9 small RNAs, or 10 small RNAs may be encoded. Such vectors may further encode other genetic elements that function in concert with small RNA to block HIV expression and infection.

Those skilled in the art will appreciate that therapeutic lentiviruses can replace alternative sequences for promoter regions, regulatory RNA targeting, and regulatory RNA types. In addition, the therapeutic lentiviruses of the invention may contain modifications within the plasmid used to package the lentivirus particles; these modifications are required to increase the level of production in vitro. Become.

In some embodiments, the vector used in the disclosed method may be a DNA plasmid, adeno-associated virus, or other integrated or non-integrated vector system for gene delivery.

VI. Bioassay

In one aspect, the invention includes a bioassay to determine the success of HIV treatment to achieve functional cure. These assays may provide a method for measuring the effectiveness of the disclosed immunization and therapeutic methods by measuring the frequency of transduced HIV-specific CD4 T cells in patients. HIV-specific CD4 T cells proliferate, modify the composition of cell surface markers, induce signaling pathways including phosphorylation, or cytokines, chemokines, caspases, phosphorylation signaling molecules or other cytoplasms. It is recognizable because it expresses specific marker proteins that can be and / or nuclear constituents. Specific Response CD4 T cells allow the selection of HIV-specific cells using, for example, flow cytometry sorting, magnetic bead separation or other recognized methods for antigen-specific CD4 T cell isolation. , Labeled monoclonal antibodies or mRNA sequences are recognized using specific in-situ amplification. Isolated CD4 T cells are tested to determine the frequency of cells carrying lentivirus for integrated therapy. Single cell test methods, including mass spectrometry to confirm responsiveness to HIV and the presence of lentivirus for integrated therapy, microfluidic separation of individual cells in combination with PCR, ELISA or immunostaining, are also used. You may.

Thus, in one embodiment, the treatment according to the invention (eg, (a) immunization, (b) ex vivo lymphocyte culture; (c) purified protein, inactivated virus, viral vectorized protein, bacterial vectorized protein. , Biological or chemical adjuvants containing cytokines and / or chemokines, restimulation with vehicles; and (d) injection of concentrated and transfected T cells) to determine the effectiveness of treatment Patients may then be assayed. Threshold of target T cells in the body, in order to measure the functional healing, for example, about $1 \times 10^{8,}$ may be established as a HIV-specific CD4 T cells with a genetic modification by therapy lentivirus. The cell threshold value refers to the contents of the whole body. It cannot be measured directly, but may instead be extrapolated from the blood CD4 T cell count using standard corrections. For example, it is common in the art to assume that 90% of CD4 T cells are present in tissue and only 10% are found in blood.

Alternatively, the threshold value in the patient's body, be approximately between $1 \times 10^{5,}$ about $1 \times 10^{6,}$ about $1 \times 10^{7,}$ about $1 \times 10^{9,}$ or about $1 \times {}^{10}10$ pieces of CD4 T cells, You may.

HIV-specific CD4 T cells that have been genetically modified by a therapeutic lentivirus include, for example, but not limited to, flow cytometry, cell selection, FACS analysis, DNA cloning, PCR, RT-PCR or Q-PCR, It can be determined using any suitable method such as ELISA, FISH, Western blotting, Southern blotting, high throughput sequencing, RNA sequencing, oligonucleotide primer extension, or other methods known in the art. it can.

Methods for defining antigen-specific T cells with genetic modification are known in the art. However, utilizing such methods of combining identified HIV-specific T cells with integrated or non-integrated gene therapy constructs as a standard measure of efficacy is a new concept in the field of HIV therapy.

VII. Dose and dosage form

The disclosed methods and compositions can be used to treat HIV + patients during various stages of the disease. Therefore, the dosing regimen can vary based on the patient's condition and method of administration.

In one embodiment, the first HIV-specific vaccine for in vivo immunization can be administered to the subject in need at various doses. Generally, a virus delivered by intramuscular injection is 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, or inactivation. Includes all viral proteins prepared from virus particles, virus-like particles, or purified viral proteins from recombinant systems, or purified from viral preparations. Recombinant virus or bacterial vectors may be administered by any of the described routes. The intramuscular vaccine contains 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 0. Suspended in 1-5 ml volume of oil, saline, buffer or water, may be soluble or emulsion preparation. Vaccines delivered intraorally, rectal, cheek, genital mucosa or nasal cavity, including some viral or bacterial vectorized vaccines, fusion proteins, liposome preparations or similar preparations, are more viral proteins and May include an adjuvant. Transdermal, subepithelial or subcutaneous vaccines utilize amounts of proteins and adjuvants that are more similar to oral, rectal or intranasal delivery vaccines. Depending on the response to the initial immunization, vaccination may be repeated 1-5 times using the same or alternative route for delivery. The

interval may be 2 to 24 weeks between immunizations. The immune response to vaccination is measured by testing HIV-specific antibodies in serum, plasma, vaginal discharge, rectal discharge, saliva or bronchoalveolar lavage fluid using ELISA or similar methods. Cell-mediated immune responses include in vitro stimulation with vaccine antigens, followed by staining for intracellular cytokine accumulation, followed by flow cytometry or lymphocyte proliferation, expression of phosphorylation signaling proteins or changes in cell surface activation markers. Tested by similar methods including. Dosing limits may be determined on an individual patient basis and may depend on the toxicity / safety profile of an individual product or product lot.

Immunization may be performed once, twice, three times, or repeatedly. For example, drugs for HIV immunization are once a week, once every other week, once every three weeks, once a month, once every other month, once every three months, once every six months, 9 It may be administered to the required subject once a month, once a year, once every 18 months, once every two years, once every 36 months, or once every three years.

Immunization will occur at least once prior to ex vivo proliferation and enrichment of CD4 T cells, and once or twice after culture / restimulation and injection of lymphocytes ex vivo. Immunization may be performed 3 times or more times.

In one embodiment, the HIV vaccine for immunization is administered as a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprising the HIV vaccine can be formulated in a wide variety of nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each dosage form can include various disintegrants, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. Pharmaceutical compositions containing the HIV vaccine can also be formulated for injection.

HIV vaccine compositions for immunization purposes include intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenous, intradermal, intramuscular, subcutaneous, intrathecal, intraperitoneal). Any pharmaceutically acceptable method, such as intrapulmonary, intravaginal, topical, topical, post-absorption topical, mucosal, via aerosol, or buccal or nasal spray formulation. Can be used and administered.

In addition, HIV vaccine compositions include solid dosage forms, tablets, pills, lozenges, capsules, liquid dispersions, gels, aerosols, lung aerosols, nasal aerosols, ointments, creams, semi-solid dosage forms, and suspensions. It can be formulated into any pharmaceutically acceptable dosage form. Further, the composition may be a controlled release preparation, a continuous release preparation, an immediate release preparation, or any combination thereof. In addition, the composition may be a transdermal delivery system.

In another embodiment, the pharmaceutical composition comprising the HIV vaccine can be formulated in a solid dosage form for oral administration, which can be a powder, a granule, a capsule, a tablet or a pill. .. 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, solid dosage forms can include lubricants such as talc or magnesium stearate in addition to excipients. In some embodiments, the oral dosage form can be an immediate release or a modified release form. Modified release dosage forms include controlled or sustained release, intestinal release and the like. Excipients used in modified release dosage forms are generally known to those of skill in the art.

In a further embodiment, the pharmaceutical composition comprising the HIV vaccine can be formulated as a sublingual or buccal dosage form. Such dosage forms include sublingual tablets or solution compositions that are administered under the sublingual and buccal tablets located between the cheek and gums.

In a further embodiment, the pharmaceutical composition containing the HIV vaccine can be formulated as a nasal dosage form. Such dosage forms of the invention include solutions, suspensions and gel compositions for nasal delivery.

In one embodiment, the pharmaceutical composition can be formulated in liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In other embodiments, the liquid dosage form is a variety of excipients such as moisturizers, sweeteners, air fresheners or preservatives, in addition to commonly used simple diluents such as water and liquid paraffin. Can be included. In certain embodiments, the composition comprising the HIV vaccine or a pharmaceutically acceptable salt thereof can be formulated to be suitable for administration to a pediatric patient.

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 solution or suspension can include vegetable oils such as propylene glycol, polyethylene glycol, olive oil, or injectable esters such as ethyl oleate. As the base of the suppository, witepsol, macrogol, tween 61, cocoa oil, lauric oil or glycerinated gelatin can be used.

The dose of the pharmaceutical composition may vary depending on the patient's weight, age, gender, time and form of administration, excretion rate, and severity of the disease.

For restimulation purposes, lymphocytes, PBMCs and / or CD4 T cells are removed from the patient and isolated for restimulation and culture. The isolated cells may be contacted with the same HIV vaccine or activator used for immunization or with a different HIV vaccine or activator. In one embodiment, the isolated cells are contacted with about 10^6 cells (or any other suitable amount) per about 10ng~5μg of HIV vaccine or activator in the culture. More specifically, the isolated cells is about 10 per ^{106} cells in culture, 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 Contact with 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 HIV vaccine or activator. You may.

The activator or vaccine is generally used once for each in vitro cell culture, but may be repeated after an interval of about 15 to about 35 days. For example, repeated dosing is 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. It may be done in 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

For transduction of enriched and restimulated cells, cells may be transduced using a lentiviral vector or other known vector systems disclosed in Section V and FIG. The transduced cells are approximately 1 to 1,000 cells (measured by the RT-PCR assay of the culture medium containing the lentiviral vector) per target cell (or any other suitable amount) in culture. It may be contacted with the viral genome. Lentivirus transduction may be repeated 1-5 times per target cell in culture, using the same range of 1-1,000 viral genomes.

VIII. Definition

As used herein, the term "about" may vary to some extent depending on the context understood and used by those skilled in the art. "About" can mean plus or minus 10% of a particular term if one of ordinary skill in the art has an unclear use of the term, even considering the context in which it is used.

"Treatment" is intended to target and combat the disease state, i.e., to improve or prevent the disease state. Therefore, the particular treatment may depend on the targeted disease condition and the current or future condition of the pharmacotherapy and therapeutic approach. Treatment may have associated toxicity.

The term "administering" or "administering" an activator is such that the activator of the invention can be introduced into the body of an individual in a therapeutically useful form in a therapeutically useful subject. , Should be understood to mean providing.

The term "therapeutically effective amount" is used in compositions suitable for treating or preventing the development of symptoms, progression or complications found in patients suffering from discomfort, injury, illness, or condition suffering from, and. Refers to a sufficient amount of the activator of the invention in the appropriate dosage form. The therapeutically effective amount may vary depending on the condition of the patient or its severity, the age and weight of the subject to be treated, and the like. The therapeutically effective amount can vary depending on any of a number of factors, including, for example, the route of administration, the condition of the subject, and other factors understood by those of skill in the art.

The term "treatment" or "treat" generally refers to interventions in attempts to alter the natural course of the subject being treated, either prophylactically or during the course of clinical pathology. Can be done. Desirable effects include preventing the onset or recurrence of the disease, alleviating the symptoms, suppressing, reducing or inhibiting any direct or indirect pathological consequences of the disease, improving or alleviating the disease state. Including, but not limited to, causing remission or improving prognosis.

The term "functional cure" refers to HIV + individuals who previously required cART or HAART with low or undetectable viral replication using lower doses, intermittent doses or discontinuation of cART or HAART. Refers to a situation or condition in which one can survive in form. Individuals may be said to be "functionally cured" even though they still require adjuvant therapy to maintain low levels of viral replication and slow or eliminate disease progression. A possible consequence of functional cure is the ultimate eradication of HIV to prevent all possible recurrences.

The term "HIV vaccine" includes immunogens, vehicles and adjuvants intended to elicit an HIV-specific immune response. The vaccine is a recombinant viral vector capable of expressing purified inactivated viral particles or whole inactivated viral particles, which may be HIV, or HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides. In addition to the recombinant bacterial vector, it may contain a plasmid DNA or RNA that can induce the cell to produce an HIV protein, a glycoprotein or protein fragment that can induce a particular immunity. Alternatively, anti-CD3 / CD28 beads, T cell receptor, for the purpose of enriching HIV-specific CD4 T cells prior to transfection, or for in vitro assay of lentivirus transfected CD4 T cells. Specific methods for immune stimulation, including body-specific antibodies, mitotic

factors, superantigens and other chemical or biological stimuli, can be used to activate dendritic, T or B cells. .. The activator may be soluble, polymeric aggregate, liposome or endosome-based or linked beads. Adding cytokines, including interleukin-2, 6, 7, 12, 15, 23 or others, to improve the cellular response to stimuli and / or improve the survival of CD4 T cells throughout culture and transduction intervals. Can be done.

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

As used herein, "expressed", "expressed" or "encoded" means the process by which a polynucleotide is transcribed into mRNA and / or the transcribed mRNA followed by a peptide, polypeptide or protein. Refers to the process of being translated into. Expression can include splicing of mRNA in eukaryotic cells, or other forms of post-transcriptional or post-translational modifications.

As used herein, "small RNA" generally refers to uncoded RNA that is less than or less than about 200 nucleotides in length and has silencing or interfering functions. In other embodiments, the small RNA is about 175 nucleotides or less in length, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less. Such RNAs include microRNAs (miRNAs), small interfering RNAs (siRNAs), double-stranded RNAs (dsRNAs), and short-stranded hairpin RNAs (SHRNAs). The "small RNA" of the present disclosure should generally be capable of inhibiting or knocking down gene expression of a target gene through a pathway that results in disruption of the target gene mRNA.

The following examples are given to illustrate the invention. However, it should be understood that the invention is not limited to the particular conditions or details described in these examples. All publications referenced herein are specifically incorporated by reference.

(Example 1)
Clinical research for the treatment of HIV Screening and informed consent. Specific HIV + participants who have received antiretroviral combination therapy (cART) with stable viral load suppression are selected and participated.

Immunization with a therapeutic HIV vaccine. Vaccines that are already in IND status and are used in clinical trials involving HIV + participants are administered to participants. This step can increase the relative frequency of HIV-specific CD4 T cells by approximately 1,000-fold.

Next, blood lymphocytes are taken out by leukocyte apheresis and further purified to obtain a peripheral blood mononuclear cell (PBMC) fraction. Alternatively, cells may be purified from venous blood by column or density gradient method.

Cultured PBMCs are stimulated with HIV proteins or peptides that match or complement the components in the therapeutic vaccine (perhaps use the same vaccine depending on its composition). This step can increase the relative frequency of HIV-specific CD4 T cells approximately 100-fold.

Cultured PBMC cells are infected with a therapeutic lentivirus or other disclosed vector, such as a vector encoding a small RNA to interfere with the translation of CCR5 and viral replication proteins. After transduction, cells are maintained in culture for 3-7 days.

The transduced CD4 T cells are injected back into the original participants. The injection can be carried out according to a method known in the art. This may require pretreatment with cyclophosphamide to increase the efficiency of reimplantation.

For participants undergoing cART, cART can be maintained for the duration of the period. After infusion, the HIV viral load in the blood and the frequency of lentivirus transduced CD4 T cells in the blood are monitored.

Participant eligibility criteria is satisfied (the total amount $^{to>}$ 10 6 of blood and tissue $^{compartments,>}$ 10 7> 10 ^{8,} lentiviral transduction includes having HIV-specific CD4 T cells), they You may move on to studying efficacy. If participants do not meet the eligibility criteria, the same protocol may be used to allow a second dose of therapeutic lentivirus.

Further research and bioassay. Thirty to sixty days after transduced T cell infusion, start testing the efficacy of gene therapy for eligible participants. First, a treatment targeting CCR5 is added to their existing cART regimen. It may be the CCR5 blocker maraviroc or the immunosuppressant rapamycin, which reduces the density of CCR5 receptors on T cells. Therapeutic lentiviruses also target CCR5, and the combined effect of lentivirus with maraviroc or rapamycin should lower CCR5 below the levels required to maintain HIV replication.

Two weeks after the addition of maraviroc or rapamycin, cART therapy is discontinued and participants are closely monitored for HIV virus rebound. In case of rebound, cART is reintroduced and managed by their doctor.

If the HIV virus does not rebound, a step-down of maraviroc or rapamycin is initiated at 2-week intervals until treatment is discontinued.

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

A database of values that correlate the number of HIV-specific, lentivirus transduced CD4 T cells with therapeutic efficacy, including the frequency of cells with latent HIV infection or other markers, can determine other gene therapy protocols. Can establish a gold standard that can be done.
(Example 2)
Development of anti-HIV wrench viral vector

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

Inhibitive RNA Design: Potential siRNA or shRNA candidates that knock down CCR5 levels in human cells using Homo sapiens chemokine CC motif receptor 5 (CCR5) (GC03P046377) mRNA sequences I searched for. Potential RNA interference sequences were selected from candidates selected by siRNA or shRNA design programs such as from the Road Institute or BLOCK-iT RNAi Designer from Thermo Scientific. To regulate shRNA expression, individual selected shRNA sequences were inserted into a lentiviral vector immediately 3'to the RNA polymerase III promoter such as H1, U6, or 7SK. These lentivirus-SHRNA constructs were used to transduce cells and measure changes in specific mRNA levels. The most potent shRNAs for reducing mRNA levels were individually implanted within the microRNA backbone to allow expression by either the CMV or EF-1 alpha RNA polymerase II promoter. MicroRNA backbones were selected from mirbase.org/. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without the use of lentiviral vectors.

The genomic sequence of the 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 that knock down 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, but those skilled in the art have limited use of these regions and other potential targets have been selected. Understand what you get. Highly conserved regions of the Gag or polymerase genes could not be targeted by shRNA because these same sequences were present in the packaging system complementary plasmid required for vector production. Similar to CCR5 (NM 000579.3, NM 001100168.1-specific RNA), potential HIV-specific RNA interference sequences can be obtained from the Gene-E software suite hosted by the Broad Institute (broadinstitute.org/mai/public). Selected from candidates selected by siRNA or shRNA design program such as or BLOCK-iT RNAi Designer (rnadesigner.thermofisher.com/rnaiexpress/setOption.do?designOption=shrna&pid=6712627360706061801) from Thermo Scientific. To regulate shRNA expression, individual selected shRNA sequences were inserted into a lentiviral vector immediately 3'to the RNA polymerase III promoter such as H1, U6, or 7SK. These lentivirus-SHRNA constructs were used to transduce cells and measure changes in specific mRNA levels. The most potent shRNAs for reducing mRNA levels were individually implanted within the microRNA backbone to allow expression by either the CMV or EF-1 alpha RNA polymerase II promoter.

Vector Construction: For CCR5, Tat or shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Open, LLC. The overlapping sense and antisense oligonucleotide sequences were mixed and annealed while cooling from 70 ° C. to room temperature. The lentiviral vector was digested with restriction enzymes BamHI and EcoRI at 37 ° C. for 1 hour. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using Invitrogen's DNA gel extraction kit. DNA concentrations were determined, vector to oligo (3: 1 ratio) were mixed, annealed and ligated. The ligation reaction was performed with T4 DNA ligase at room temperature for 30 minutes. A 2.5 microliter ligation mix was added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat shock at 42 ° C. Bacterial cells were spread on agar plates containing ampicillin, drug-resistant colonies (indicating the presence of ampicillin-resistant plasmids) were collected, purified, and grown on LB broth. To check for oligo sequence insertion, plasmid DNA was extracted from harvested bacterial cultures using the Invitrogen DNA miniprep kit. Insertion of the shRNA sequence into the lentiviral vector was confirmed by DNA sequencing using specific primers for the promoter used to regulate shRNA expression. Illustrative vector sequences and cellular elements known to limit HIV replication can be found in FIGS. 4 and 9, respectively.

For example, the shRNA sequence with the highest activity for CCR5, Tat or Vif gene expression was incorporated into a microRNA (miR) cluster under the control of the EF-1 alpha promoter. The promoter and miR sequences are shown in FIG.

Functional assay: Contains CCR5, Tat or shRNA sequence and expresses green fluorescent protein (GFP) under the control of the CMV Immediate Early Promoter for experimental purposes with AGT103 / CMV-GFP. Each named lentiviral vector was tested for its ability to knock down CCR5, Tat or Vif expression. Lentivirus particles

were transduced into mammalian cells in the presence or absence of polybrene. Cells were harvested after 2-4 days; proteins and RNA were analyzed for CCR5, Tat or Vif expression. Analytical flow sites comparing the fluorescence of modified and unmodified cells using either a CCR5-specific antibody or an isotype control antibody, followed by a Western blot assay or labeling the cells with a specific fluorescent antibody (CCR5 assay). Protein levels were tested by metric.

Initiation of Lentivirus Testing: T cell culture medium was prepared using RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin. Cytokine stocks of IL2 10000 units / ml, IL12 1 µg / ml, IL7 1 µg / ml, IL15 1 µg / ml were also prepared in advance.

Prior to lentivirus transduction, infectious virus titers were determined and used to calculate the amount of virus added for appropriate multiplicity of infection (MOI).

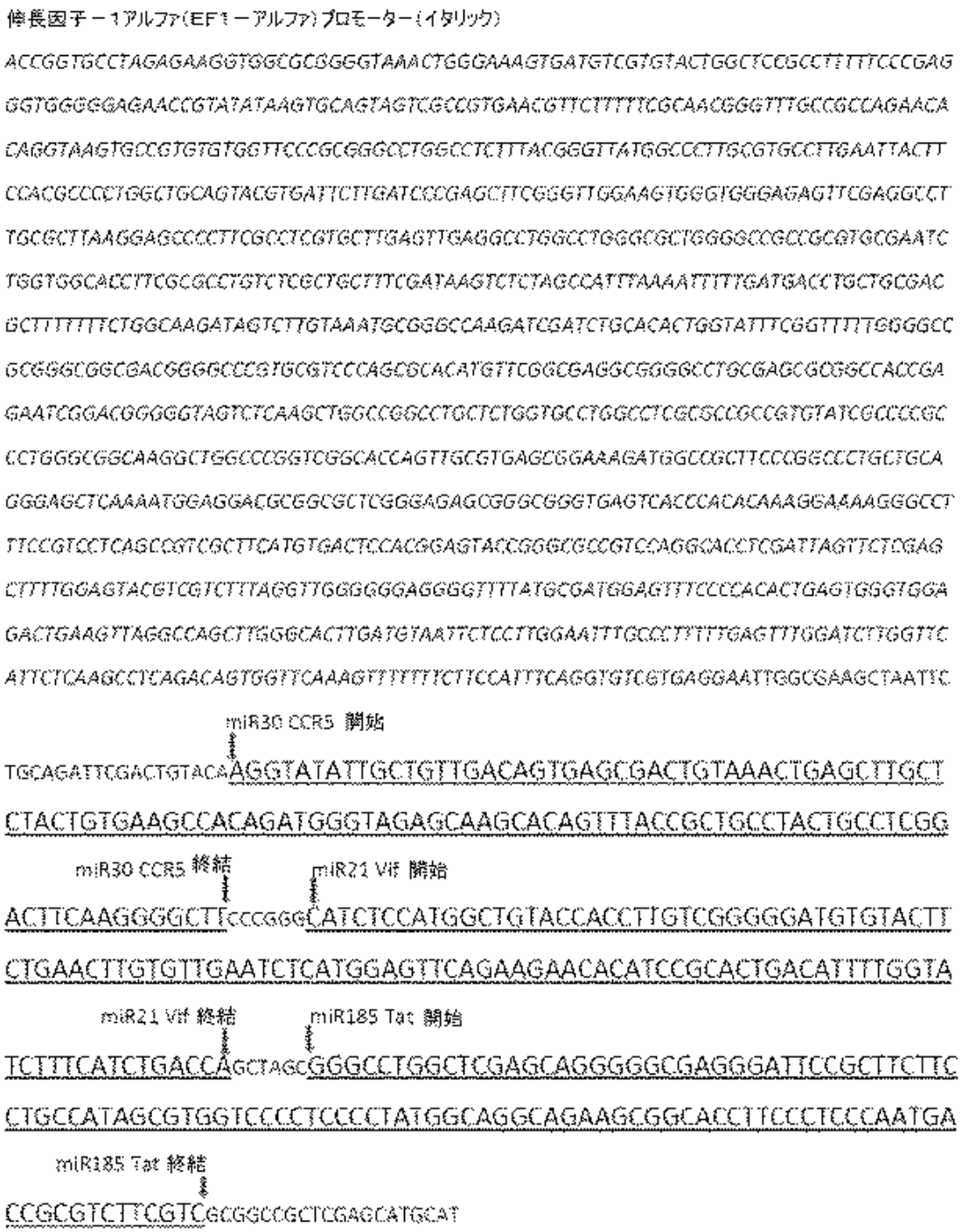

Figure 4

Days 0-12: Antigen-specific concentration: Onday0, cryopreserved PBMCs were thawed, washed in 10 ml of 37 ° C. medium at 1200 rpm for 10 minutes, and 2×10^6 pieces / in 37 ° C. medium. Resuspended at a concentration of ml. Cells were cultured at 0.5 ml / well in a 24-well plate in 5% CO_2 at 37 ° C. To define optimal stimulation

conditions, cells were stimulated with the combination of reagents listed in Table 2 below:

Figure 4

Final concentration: IL2 = 20 units / ml, IL12 = 10 ng / ml, IL7 = 10 ng / ml, IL15 = 10 ng / ml, peptide = 5 μg / ml Individual peptide, MVA MOI = 1.

On days 4 and 8, cytokines were added to stimulated cells in 0.5 ml of fresh medium and listed concentrations (all concentrations indicate final concentrations in culture).

Days 12-24: Non-specific growth and lentivirus transduction: On day 12, stimulated cells were pipetted out of the plate and placed in fresh T-cell medium at a concentration of 1 x 10^6 cells / ml. Resuspended. The resuspended cells were transferred to a T25 culture flask and stimulated with DYNABEADS® Human T-Activator CD3 / CD28 and the cytokines listed above according to the manufacturer's instructions; the flask was incubated in a vertical position.

On day 14, AGT 103 / CMV-GFP was added at MOI 20 and the cultures were returned to the incubator in 2 days. At this point, cells were harvested by pipetting, collected by centrifugation at 1300 rpm for 10 minutes, resuspended in the same volume of fresh medium and centrifuged again to form loose cell pellets. The cell pellet in fresh medium containing the same cytokines as used in the previous step, and resuspended in 0.5 × 10^6 pieces of viable cells per 1 ml.

From 14th to 23rd day, the number of cells was evaluated every 2 days, and the cells were diluted with fresh medium to 0.5 × 10^6 cells / m1. Cytokines were added each time.

Cells were collected on day 24 and beads were removed from the cells. To remove the beads, the cells were transferred to a suitable tube and placed in a sorting magnet for 2 minutes. The supernatant containing the cells was transferred to a new tube. The cells were then cultured in fresh medium for 1 day at 1x10^6 cells / ml. Assays were performed to determine the frequency of antigen-specific T cells and lentivirus transduced cells.

Amprenavir (0.5 ng / ml) was added to the culture on the first day of stimulation and every other day during culture to prevent possible viral growth.

IFN- [gamma] determine the antigen-specific T cells by intracellular cytokine staining for: after peptide stimulation or 1x10^6 cells / ml medium cultured cells after lentiviral transduction alone (negative control), Gag peptide (5 [mu] g / Stimulation with ml of individual peptide) or PHA (5 µg / ml, positive control). After 4 hours, BD GOLGIPLUG ™ (1: 1000, BD Biosciences) was added to block Golgi transport. After 8 hours, the cells are washed and extracellular (CD3, CD4 or CD8; BD Biosciences) antibodies and intracellular (IFN-gamma; BD) using the BD CYTOFIX / CYTOPERM ™ kit according to the manufacturer's instructions. Biosciences) antibody was stained. Samples were analyzed on a BD FACSCALIBUR ™ flow cytometer. Control samples labeled with the appropriate isotype-matched antibody were included in each experiment. Data were analyzed using Flowjo software.

The lentivirus transduction rate was determined by the frequency of GFP + cells. Transduced antigen-specific T cells are determined by the frequency of CD3 + CD4 + GFP + IFN gamma + cells; testing of CD3 + CD8 + GFP + IFN gamma + cells is included as a control.

These results can be transduced into the target T cell population, CD4 T cells, using a lentivirus designed to specifically knock down the expression of HIV-specific proteins, and thus into the virus. In contrast, it is shown to produce a proliferative population of immune T cells. This example provides proof of the concept that the disclosed lentiviral constructs can be used in combination with vaccination to produce functional cure in HIV patients.
(Example 3)
CCR5 knockdown with experimental vector

AGTc120 is a HeLa cell line that stably expresses large amounts of CD4 and CCR5. Transduction was performed using AGTc120 with or without LV-CMV-mCherry (red fluorescent protein mCherry expressed under the control of the CMV immediate early promoter) or AGT103 / CMV-mCherry. Gene expression of mCherry fluorescent protein was regulated by a CMV (cytomegalovirus immediate early promoter) expression cassette. The AGT103 / CMV-mCherry expressed therapeutic miRNAs for CCR5, Vif and Tat, while the LV-CMV-mCherry vector lacked microRNA clusters.

As shown in FIG. 5A, the transduction efficiency was> 90%. After 7 days, cells were collected, stained with a fluorescent monoclonal antibody against CCR5 and subjected to analytical flow cytometry. Isotype controls are shown in gray in these histograms plotting the number of cells normalized for mode (y-axis) against the average fluorescence intensity (x-axis) of CCR5 APC. After staining for cell surface CCR5, cells treated without lentivirus or with control lentivirus (expressing only mCherry markers) showed no change in CCR5 density, while AGT103 (right section) showed CCR5 staining intensity. It was reduced to near isotype control levels. After 7 days, cells were infected with or without the R5 directional HIV reporter virus Bal-GFP. After 3 days, cells were collected and analyzed by flow cytometry. Over 90% of cells were transduced. AGT103-CMV / CMVmCherry reduced CCR5 expression in transduced AGTc120 cells and blocked R5-directed HIV infection compared to cells treated with a control vector.

FIG. 5B shows the relative insensitivity of transfected AGTc120 cells to HIV infection. As shown above, the lentiviral vector expresses the mCherry protein, and HIV-infected transduced cells (expressing GFP) appear as double-positive cells in the upper right quadrant of the pseudocolor flow cytometry dot plot. .. In the absence of HIV (upper panel), GFP + cells were not present under any conditions. After HIV infection (lower panel), 56% of cells were infected in the absence of lentivirus transduction, and 53.6% of AGTc120 cells transduced with LV-CMV-mCherry were infected. When transduced into cells using the Therapeutic AGT103 / CMV-mCherry vector, only 0.83% of the cells appeared in a double positive quadrant, indicating that they were transduced and infected. There is.

Dividing 53.62 (ratio of double-positive cells to control vector) by 0.83 (ratio of double-positive cells to therapeutic vector), AGT103 is more than 65-fold relative to HIV in this experimental system. It is shown that it provided protection.

(Example 4)

AGT103 reduces Tat and Vif expression

Figure 4

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

Figure 4

Transduced into a T lymphoblastoid cell line (CEM; CCRF-CEM; United States Cultured Cell Line Conservation Agency Catalog No. CCL119) using AGT103 / CMV-GFP. After 48 hours, cells were transfected with an HIV expression plasmid encoding the entire viral sequence. After 24 hours, RNA was extracted from the cells and tested for

the level of intact Tat sequences using the reverse transcriptase polymerase chain reaction. Relative expression levels of intact Tat RNA decreased from approximately 850 in the presence of the control lentiviral vector to approximately 200 in the presence of AGT103 / CMV-GFP,> 4-fold reduction, as shown in FIG. did.

In a similar experiment, HEK 293T cells (human fetal kidney 293T; United States Cell Culture Preservation Agency Catalog No. CRL-3216) were transduced using AGT103 / CMV-GFP, followed by HIV 7 days after transfection. Cells were transfected with the expression plasmid (controls were not HIV-transfected). Twenty-four hours after transfection, cells were lysed and analyzed by Western blot using an antibody specific for actin (cell loading control) or HIV Vif protein. As shown in FIG. 7, the presence of AGT103 / CMV-GFP (right lane) caused a dramatic decrease in Vif protein expression levels.
(Example 5)
Generation of populations of CD4 + T cells enriched for HIV specificity and transduced with AGT103 / CMV-GFP

Therapeutic vaccination against HIV had minimal effect on the distribution of CD4 +, CD8 + and CD4 + / CD8 + T cells. As shown in FIG. 8A, the CD4 T cell population is shown in the upper left quadrant of the analytical flow cytometry dot plot and changes from 52% to 57% of all T cells after the vaccination sequence. These are typical data.

Peripheral blood mononuclear cells from participants in the HIV therapy vaccine study were cultured in +/- interleukin-2 / interleukin-12 or +/- interleukin-7 / interleukin-15 medium for 12 days. As a source of epitope peptides for T cell stimulation, several cultures were stimulated with overlapping peptides representing the total p55 Gag protein (JPT PepMix) of HIV-1. These peptides are 10 to 20 amino acids in length and overlap 20 to 50% of their length, representing the entire Gag precursor protein (p55) from the HIV-1 BaL strain. The composition and sequence of individual peptides may be adjusted to compensate for regional variation in the major circulating HIV sequence, or if detailed sequence information is available to individual patients receiving this therapy. it can. At the end of culture, cells were harvested, stained with anti-CD4 or anti-CD8 monoclonal antibody, and the CD3 + population was gated and labeled here. PepMix stimulation for either pre-vaccination or post-vaccination samples was similar to medium control, indicating that PepMix was not toxic to cells and did not act as a polyclonal mitogen. ing. The results of this analysis can be found in FIG. 8B.

PepMix and interleukin-2 / interleukin-12 were provided for optimal proliferation of antigen-specific CD4 T cells. As shown in the upper panel of FIG. 8C, there was an increase in cytokine (interferon-gamma) secreting cells in post-vaccinated specimens exposed to PepMix. In pre-vaccinated samples, cytokine-secreting cells increased from 0.43 to 0.69% as a result of exposure to antigenic peptides. In contrast, post-vaccinated samples showed an increase in cytokine-secreting cells from 0.62 to 1.76% of all CD4 T cells as a result of peptide stimulation. These data demonstrate the strong effect of vaccination on the CD4 T cell response to HIV antigen.

Finally, AGT103 / CMV-GFP transfection of antigen-proliferated CD4 T cells produces HIV-specific and HIV-resistant helper CD4 T cells that are required to be injected into patients as part of functional cure for HIV. (Depending on various other aspects and embodiments, AGT103 may be used alone or without additional elements; for example, clinical embodiments may not include the CMV-GFP segment). The upper panel in FIG. 8D shows the results of analysis of the CD4 + T cell population in culture. The x-axis in FIG. 8D shows the release of green fluorescent protein (GFP), which indicates that AGT103 / CMV-GFP was transduced into individual cells. In the post-vaccinated sample, 1.11% of all CD4 T cells that secreted both cytokines were recovered, which means that the cells responded specifically to the HIV antigen and AGT103 / CMV- It shows that GFP is transfected. It is a target cell population and clinical product intended for HIV injection and functional healing. The efficiency of cell proliferation during ex vivo culture antigen stimulation and subsequent polyclonal proliferation $^{\text{can produce } 4 \times 10\ 8}$ antigen-specific, lentivirus transduced CD4 T cells. This is four-fold above the target of cell production and achieves an antigen-specific and HIV-resistant CD4 T cell count of approximately 40 cells / microliter of blood or approximately 5.7% of all-circulating CD4 T cells. Will be able to.

Figure 4

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

Figure 4

Although some preferred embodiments of the present invention have been described and exemplified above, the present invention is not intended to be limited to such embodiments. Various modifications may be made therein without departing from the scope and spirit of the invention.

In certain embodiments, for example, the following items are provided:
(Item 1)
A way to treat HIV infection
(A) Steps to identify subjects in need of treatment for HIV infection;
(B) Steps to immunize the subject with a therapeutically effective amount of HIV vaccine;
(C) The step of removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC);
(D) In ex vivo, the step of contacting the PBMC with a therapeutically effective amount of HIV vaccine;
(E) In ex vivo, the step of transducing a viral delivery system encoding at least one genetic element into the PBMC;
(F) The step of culturing the transduced PBMC for about 1 to about 35 days; and
(G) Step of injecting the transduced PBMC into the subject
How to include.
(Item 2)
The method of item 1, wherein step (b) and step (d) include the same HIV vaccine.
(Item 3)
The method of item 1, comprising HIV vaccines in which step (b) and step (d) are different.
(Item 4)
The method of item 1, wherein the subject had undergone cART or HAART prior to injecting the transduced PBMC into the subject.
(Item 5)
The method of item 1, wherein the subject receives cyclophosphamide pretreatment prior to injecting the transduced PBMC into the subject.
(Item 6)
From a small RNA capable of inhibiting the production of the chemokine receptor CCR5, a small RNA capable of inhibiting the production of the chemokine receptor CXCR4, and a small RNA molecule targeting the HIV RNA sequence. Item 1. The method according to item 1, which is selected from the group consisting of.
(Item 7)
The small RNA molecule that targets the HIV RNA sequence targets gag, pol, env, tar, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS. The method according to item 6.
(Item 8)
The method of item 1, wherein the transduced PBMC is cultured for about 1 to about 10 days before the transduced PBMC is injected into the subject.
(Item 9)
Selected from the group consisting of small RNAs capable of inhibiting the production of the chemokine receptor CCR5, small RNAs capable of inhibiting the production of the chemokine receptor CXCR4, and small RNA molecules targeting the HIV RNA sequence, at least. A viral vector for transfecting HIV-specific CD4 T cells, which encodes a single genetic element.
(Item 10)
The viral vector according to item 9, which is a lentivirus.
(Item 11)
The virus vector according to item 9, which is a vector-in-vector system.
(Item 12)
The small RNA molecule that targets the HIV RNA sequence targets gag, pol, env, tar, rev, nef, vif, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS. The virus vector according to item 9.
(Item 13)
A bioassay for determining whether an HIV + subject is functionally cured, comprising the step of determining the number of HIV-specific CD4 T cells that have been genetically modified by a therapeutic lentivirus, said therapeutic. A bioassay in which the subject is functionally cured when the number of HIV-specific CD4 T cells carrying a lentivirus genetic modification exceeds the threshold after a particular time after the treatment according to item 1.
(Item 14)
The threshold is about 1×10^{8}. an HIV-specific CD4 T cells with a genetic modification by therapy for lentivirus The method of claim 13.
(Item 15)
13. The method of item 13, wherein the particular time after treatment is about 30 to about 60 days.

(Item 16)
13. The method of item 13, wherein the particular time after treatment is about 12 to about 26 weeks.
(Item 17)
A method of achieving functional cure of HIV in HIV + subjects,
(A) Steps to identify objects that are HIV +;
(B) Steps to immunize the subject with a therapeutically effective amount of HIV vaccine;
(C) The step of removing lymphocytes from the subject and purifying peripheral blood mononuclear cells (PBMC);
(D) In ex vivo, the step of contacting the PBMC with a therapeutically effective amount of HIV vaccine;
(E) In ex vivo, the step of transducing the PBMC using a viral delivery system encoding at least one genetic element;
(F) The step of culturing the transduced PBMC for about 1 to about 21 days; and
(G) A step of injecting the transduced PBMC into the subject, wherein the HIV + subject achieves functional healing.
How to include.
(Item 18)
The method of item 18, wherein steps (b) and (d) include the same HIV vaccine.
(Item 19)
The method of item 18, wherein steps (b) and (d) include different HIV vaccines.
(Item 20)
18. The method of item 18, wherein the subject had undergone cART or HAART prior to injecting the transduced PBMC into the subject.
(Item 21)
18. The method of item 18, wherein the subject receives cyclophosphamide pretreatment prior to injecting the transduced PBMC into the subject.
(Item 22)
From a small RNA capable of inhibiting the production of the chemokine receptor CCR5, a small RNA capable of inhibiting the production of the chemokine receptor CXCR4, and a small RNA molecule targeting the HIV RNA sequence. Item 18. The method of item 18, which is selected from the group of
(Item 23)
The small RNA molecule that targets the HIV RNA sequence targets gag, pol, env, tar, rev, nef, viv, vpr, vpu, tev, LTR, TAR, RRE, PE, SLIP, CRS, or INS. The method according to item 22.
(Item 24)
18. The method of item 18, wherein the transduced PBMC is cultured for about 1 to about 7 days before the transduced PBMC is injected into the subject.

Patent Citations (7)

Publication number	Priority date	Publication date	Assignee	Title
Family To Family Citations				
US6410013B1 *	1999-01-25	2002-06-25	Musc Foundation For Research Development	Viral vectors for use in monitoring HIV drug resistance
ES2612538T3 *	2007-05-29	2017-05-17	Christopher B. Reid	Methods for production and uses of multipotent cell populations, pluripotent cell populations, differentiated cell populations, and HIV resistant cell populations
EP2090659A1 *	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung eV	Infectious particle, process for its preparation and use thereof
WO2010051521A1 *	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection

WO2014016817A2 *	2012-07-17	2014-01-30	University of Geneve	Nucleic acids for down-regulation of gene expression
AU2014296059B2 *	2013-08-02	2020-12-10	The Regents Of The University Of California	Engineering antiviral T cell immunity through stem cells and chimeric antigen receptors
WO2016186708A1 *	2015-05-18	2016-11-24	Calimmune, Inc.	Gene therapeutic for the treatment of hiv and uses thereof

* Cited by examiner, † Cited by third party

Cited By (11)

Publication number	Priority date	Publication date	Assignee	Title
Family To Family Citations				
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
EP4310500A3	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositons for the activation of gamma-delta t-cells
EP3413926A4	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
WO2017156311A2	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
AU2017292582C1	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
EP3565564A4 *	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
WO2019191314A1 *	2018-03-27	2019-10-03	American Gene Technologies International Inc.	Methods of manufacturing genetically-modified lymphocytes
CN113234676B *	2021-04-13	2022-12-13	华南农业大学	Method for promoting duck T cell proliferation and application thereof

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
JP6890831B2	2021-06-18	HIV preimmunization and immunotherapy
JP7162895B2	2022-10-31	HIV pre-immunization and immunotherapy
JP7153332B2	2022-10-14	HIV vaccination and immunotherapy
JP7260170B2	2023-04-18	HIV immunotherapy without prior immunization step
US20240115604A1	2024-04-11	Methods of manufacturing genetically-modified lymphocytes
US20210015868A1	2021-01-21	Methods of manufacturing genetically-modified lymphocytes

Priority And Related Applications

Child Applications (1)

Application	Priority date	Filing date	Relation	Title
JP2021084813A	2015-07-08	2021-05-19	Division	Hiv pre-immunization and immunotherapy

Priority Applications (1)

Application	Priority date	Filing date	Title
JP2021084813A	2015-07-08	2021-05-19	Hiv pre-immunization and immunotherapy

Applications Claiming Priority (3)

Application	Filing date	Title
US201562190139P	2015-07-08	
US62/190,139	2015-07-08	
PCT/US2016/041456	2016-07-08	Hiv pre-immunization and immunotherapy

Legal Events

Date	Code	Title	Description

2018-04-24	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20180326
2019-07-05	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20190705
2020-01-10	A521	Request for written amendment filed	**Free format text** : JAPANESE INTERMEDIATE CODE: A523 **Effective date** : 20200110
2020-06-19	A131	Notification of reasons for refusal	**Free format text** : JAPANESE INTERMEDIATE CODE: A131 **Effective date** : 20200619
2020-09-18	A601	Written request for extension of time	**Free format text** : JAPANESE INTERMEDIATE CODE: A601 **Effective date** : 20200918
2020-11-18	A521	Request for written amendment filed	**Free format text** : JAPANESE INTERMEDIATE CODE: A523 **Effective date** : 20201118
2021-04-15	TRDD	Decision of grant or rejection written	
2021-04-21	A01	Written decision to grant a patent or to grant a registration (utility model)	**Free format text** : JAPANESE INTERMEDIATE CODE: A01 **Effective date** : 20210421
2021-05-21	A61	First payment of annual fees (during grant procedure)	**Free format text** : JAPANESE INTERMEDIATE CODE: A61 **Effective date** : 20210519
2021-05-28	R150	Certificate of patent or registration of utility model	**Ref document number** : 6890831 **Country of reference document** : JP **Free format text** : JAPANESE INTERMEDIATE CODE: R150

Concepts

machine-extracted

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Name	Image	Sections	Count	Query match

immunotherapy	title, description	12	0.000
T-lymphocyte	claims, description	153	0.000
method	claims, description	89	0.000
Bacterial small RNA	claims, description	47	0.000
HIV vaccine	claims, description	45	0.000
virological effect	claims, description	41	0.000
mixture	claims, description	40	0.000
treatment	claims, description	33	0.000
(ribonucleotides)n+m	claims, description	32	0.000
Viruses	claims, description	29	0.000
HIV Infections	claims, description	26	0.000
HIV infectious disease	claims, description	twenty three	0.000
human immunodeficiency virus infectious disease	claims, description	twenty three	0.000
Nucleic acid sequence	claims, description	twenty one	0.000
genetic effect	claims, description	19	0.000
lymphocyte	claims, description	13	0.000
targeting	claims, description	13	0.000
healing	claims, description	12	0.000
Cyclophosphamide	claims, description	8	0.000
cyclophosphamide	claims, description	8	0.000
culturing	claims, description	Five	0.000
transducing effect	claims, description	Four	0.000
CC chemokine receptor type 5	claims	2	0.000
CC chemokine receptor type 5	claims	2	0.000

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