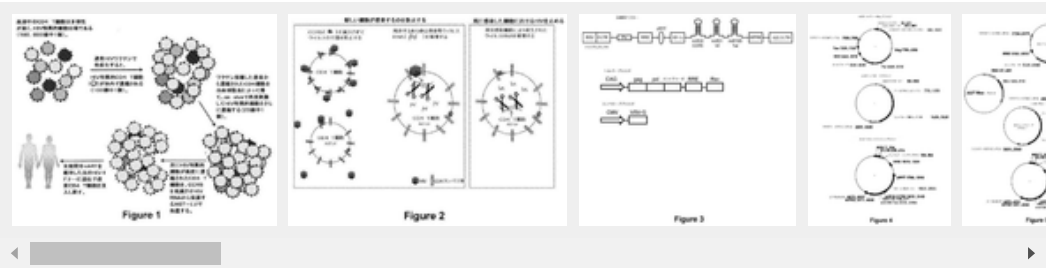
HIV preimmunization and immunotherapy

Images (25)



Classifications

A61K31/7105 Natural ribonucleic acids, i.e. containing only riboses attached to adenine, guanine, cytosine or uracil and having 3'-5' phosphodiester links

View 37 more classifications

Landscapes

Health & Medical Sciences

Life Sciences & Earth Sciences

Show more

JP6971492B2
Japan

Download PDF | Find Prior Art | Similar

Other languages: Japanese

Inventor: チャールズ デイビッド パウザ, , ハイシャン リ, , テイラー ラフーゼン,

Worldwide applications

2017 • AU EP *IL* CA JP *WO* EP US JP *WO* US 2018 • US US 2019 • IL US 2020 • *IL* 2021 • AU US *IL* JP 2022 • JP 2023 • US US US

Application JP2019500475A events

2017-01-11 • Application filed by アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド

2019-09-26 • Publication of JP2019527055A

2020-05-07 • Publication of JP2019527055A5

2021-10-26 • Priority to JP2021174409A

2021-11-24 • Application granted

2021-11-24 • Publication of JP6971492B2

Status • Active

2037-01-11 • Anticipated expiration

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

External links: Espacenet, Global Dossier, Discuss

Claims (27)

Hide Dependent

translated from Japanese

A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster comprises a sequence having at least 90% sequence identity with SEQ ID NO: 31, and the encoded microRNA cluster is: A lentiviral vector capable of inhibiting the production of the chemokine receptor CCR5 and targeting the HIV Vif and HIV Tat sequences. The lentiviral vector according to claim 1, wherein the encoded microRNA cluster comprises a sequence having at least 95% sequence identity with SEQ ID NO: 31. Lentiviral particles that are produced by packaging cells and can infect target cells.

a. Envelope proteins that can infect the target cells; and b. It comprises a encoded microRNA cluster comprising a sequence having at least 90% sequence identity with SEQ ID NO: 31, said encoded microRNA cluster being capable of inhibiting the production of the chemokine receptor CCR5 and HIV Virus. Lentivirus particles capable of targeting sequences and HIV Tat sequences. The lentivirus particle of claim 3, wherein the encoded microRNA cluster comprises a sequence having at least 95% sequence identity with SEQ ID NO: 31. The lentivirus particle according to claim 3, wherein the target cell is a CD4 + T cell. Modified cells containing primary T cells infected with lentivirus particles, wherein the lentivirus particles are

a. Envelope proteins that can infect the target cells; and b. Containing a encoded microRNA cluster comprising a sequence having at least 90% sequence identity with SEQ ID NO: 31, the encoded microRNA cluster is capable of inhibiting the production of the chemokine receptor CCR5 and HIV Vif. Modified cells capable of targeting sequences and HIV Tat sequences. The modified cell of claim 6, wherein the encoded microRNA cluster comprises a sequence having at least 95% sequence identity with SEQ ID NO: 31. The modified cell according to claim 6, wherein the primary T cell is a primary CD4 + T cell. A method for producing transduced peripheral blood mononuclear cells (PBMC) ex vivo.

a. carried out in ex vivo, and away step the P BM C isolated from a subject infected with HIV, in contact with the ex vivo stimulants increase the fraction of HIV-specific CD4T cells,

b. The step of transducing the PBMC with lentiviral particles ex vivo, wherein the lentiviral particles are:

i. Envelope proteins that can infect the PBMC; and ii. Containing a encoded microRNA cluster comprising a sequence having at least 90% sequence identity with SEQ ID NO: 31, the encoded microRNA cluster is capable of inhibiting the production of the chemokine receptor CCR5 and HIV Vif. Steps that can target sequences and HIV Tat sequences, and

c. A method comprising culturing the transduced PBMC for at least about 1 day. 9. The method of claim 9, wherein the encoded microRNA cluster comprises a sequence having at least 95% sequence identity with SEQ ID NO: 31. A composition comprising transduced PBMCs produced by the method of claim 9, wherein the composition is injected into a subject. The method of claim 9, further comprising the step of positively selecting HIV-specific CD4 + T cells from the PBMC. A lentiviral vector comprising an encoded microRNA cluster, wherein the encoded microRNA cluster has (i) at least 90% sequence identity with SEQ ID NO: 1 and (ii) at least 90% with SEQ ID NO: 2. The encoded microRNA cluster comprising sequence identity, and (iii) a sequence containing at least 90% sequence identity with SEQ ID NO: 108 , can inhibit the production of the chemokine receptor CCR5 and is HIV Virus. A lentiviral vector capable of targeting sequences and HIV Tat sequences. The encoded microRNA cluster has (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 108. It comprises a sequence comprising a sequence identity, lentiviral vector of claim 1 3. The encoded micro RNA clusters, SEQ ID NO: 1, comprising the sequence comprising SEQ ID NO: 2 or SEQ ID NO: 108, lentiviral vector of claim 1 3. Lentiviral particles that can infect target cells

a. Envelope proteins that can infect the target cells; and b. (I) Contains a sequence comprising at least 90% sequence identity with SEQ ID NO: 1, (ii) sequence identity with at least 90% with SEQ ID NO: 2, and (iii) sequence identity with at least 90% with SEQ ID NO: 108. A lentivirus particle comprising an encoded microRNA cluster, wherein the encoded microRNA cluster is capable of inhibiting the production of the chemokine receptor CCR5 and targeting the HIV Vif and HIV Tat sequences. The encoded microRNA cluster has (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 108. The lentivirus particle according to claim 16 , which comprises a sequence comprising a sequence identity of. The lentivirus particle of claim 16 , wherein the encoded microRNA cluster comprises a sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 108. The lentivirus particle according to claim 16 , wherein the target cell is a CD4 + T cell. Modified cells containing primary T cells infected with lentivirus particles, wherein the lentivirus particles are

a. Envelope proteins that can infect the target cells; and b. (I) Contains a sequence comprising at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with SEQ ID NO: 2, and (iii) SEQ ID NO: 108 with at least 90% sequence identity. Modified cells comprising the encoded microRNA cluster, wherein the encoded microRNA cluster is capable of inhibiting the production of the chemokine receptor CCR5 and targeting the HIV Vif sequence and the HIV Tat sequence. The encoded microRNA cluster has (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 108. It comprises a sequence comprising the sequence identity, the modified cell of claim 2 0. The encoded micro RNA clusters, SEQ ID NO: 1, comprising the sequence comprising SEQ ID NO: 2 or SEQ ID NO: 108, modified cell of claim 2 0. A method for producing peripheral blood mononuclear cells (PBMC) ex vivo.

a. carried out in ex vivo, and away step the P BM C isolated from a subject infected with HIV, in contact with the ex vivo stimulants increase the fraction of HIV-specific CD4T cells,

b. The step of transducing the PBMC with lentiviral particles ex vivo, wherein the lentiviral particles are:

i. Envelope proteins that can infect the PBMC; and ii. (I) Contains a sequence comprising at least 90% sequence identity with SEQ ID NO: 1, (ii) at least 90% sequence identity with

SEQ ID NO: 2, and (iii) SEQ ID NO: 108 with at least 90% sequence identity. Containing a encoded microRNA cluster, wherein the encoded microRNA cluster is capable of inhibiting the production of the chemokine receptor CCR5 and targeting the HIV Vif sequence and the HIV Tat sequence. ,Method. The encoded microRNA cluster has (i) at least 95% sequence identity with SEQ ID NO: 1, (ii) at least 95% sequence identity with SEQ ID NO: 2, or (iii) at least 95% sequence identity with SEQ ID NO: 108. It comprises a sequence comprising a sequence identity of a method according to claim 2 3. The encoded micro RNA clusters, SEQ ID NO: 1, comprising the sequence comprising SEQ ID NO: 2 or SEQ ID NO: 108, The method of claim 2 3. A composition comprising a PBMC transduced produced by the method of claim 2 3, wherein the composition is characterized in that it is injected into a subject, the composition. Further comprising the step of positive selection of HIV-specific CD4 + T cells from the PBMC, The method according to claim 2 3.

Description

translated from Japanese

Mutual reference to related applications This application is filed on July 8, 2016, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTERAPY", US Provisional Patent Application Nos. 62 / 360,185, "HIV PRE-IMMUNIZATION AND". US Provisional Patent Application No. 62 / 385,864 filed September 9, 2016, entitled "IMMUNOTERAPY", and filed October 17, 2016, entitled "HIV PRE-IMMUNIZATION AND IMMUNOTHERAPPY". Claims priority to US Provisional Patent Application No. 62 / 409,270 (these disclosures are incorporated herein by reference).

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

Combination anti-retroviral therapy (cART) (also known as Highly Active Active Anti-Retroviral Therapy or HAART) limits HIV-1 replication and promotes disease progression. , Drug toxicity and the emergence of drug-resistant viruses are challenges in long-term control of HIV-infected persons. Moreover, traditional antiretroviral drug therapies have been successful in delaying the onset or death of AIDS, but have not resulted in functional cure. Alternative treatment strategies are needed.

With the advent of data showing that the immune system plays a major role, albeit usually inadequate, in limiting HIV replication, there is a great deal of interest in immunotherapy for HIV infection. Virus-specific T helper cells, which are important for maintaining the function of cytotoxic T cells (CTLs), are likely to play a role. Viremia is also affected by neutralizing antibodies, but they are generally less important in HIV infection and lag behind in vivo evolving viral variants.

Taken together, this data shows that increased intensity and breadth of HIV-specific cell-mediated immune responses may have clinical utility with so-called HIV immunotherapy. Vaccines against HIV have been tested in several studies, but have not been sufficiently successful to date. In addition, interest has been shown in enhancing HIV immunotherapy by utilizing gene therapy technology, but, like other immunotherapeutic approaches, it has not been sufficiently successful.

Viral vectors can be used to transduce genes into target cells due to the viral mechanism for specific viral envelope and host cell receptor interactions and gene expression. 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. The ability to introduce and express foreign or altered genes in 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 therapeutic agents that may involve the use of viral vectors. Given the wide variety of potentially genes available for therapy, efficient means of delivering these genes to meet the expectations of gene therapy as a means of treating infectious and non-infectious diseases. is required. Several viral systems have been proposed as therapeutic gene transfer vectors, including mouse retroviruses, adenoviruses, parvoviruses (adenoviruses), vacciniaviruses, and herpesviruses.

Many must be considered when developing viral vectors, including tissue tropism, viral preparation stability, expression stability and regulation, genomic packaging capacity, and construct-dependent vector stability. There is a factor. In addition, in vivo application of viral vectors is often limited by the host immune response to viral structural proteins and / or transduced gene products.

Thus, toxicity and safety are the main hurdles that must be overcome for the use of viral vectors in vivo for the treatment of subjects. There are many historical examples of the application of gene therapy in humans facing problems related to host immune responses to gene delivery vehicles or therapeutic gene products. Viral vectors (eg, adenoviruses) that co-transduce several viral genes in combination with one or more therapeutic genes are particularly problematic.

Although lentiviral vectors generally do not induce cell damage and do not elicit a strong host immune response, some lentiviral vectors, such as HIV-1, which have several immunostimulatory gene products, are in vivo cell damage. And has the potential to induce a strong immune response. However, this may not be a concern for lentiviral-derived transduction vectors that do not encode multiple viral genes after transduction. Of course, this is not always the case. This is because, in some cases, the purpose of the vector is to encode a protein that elicits a clinically useful immune response.

Another important issue with the use of lentiviral vectors is the potential for cell degeneration when exposed to some cytotoxic viral proteins. Exposure to certain HIV-1 proteins can induce cell death or functional non-reactivity of T cells. Similarly, in many cases, there is concern that recombination may result in replicable virulent viruses. Therefore, improved HIV treatment is still in need.

In one aspect, a method of treating HIV-infected cells is provided. The method encodes at least one genetic element with a step of contacting peripheral blood mononuclear cells (PBMC) isolated from an HIV-infected subject with a therapeutically effective amount of stimulant, performed in ex vivo. It comprises various steps of exvivo transducing the virus delivery system into the PBMC and culturing the transduced PBMC for a period sufficient to ensure adequate transduction. In embodiments, transduced PBMCs can be cultured for about 1 to about 35 days. The method may further include injecting the transduced PBMC into the subject. The subject can be human. The stimulant may include any agent suitable for stimulating the T cell response in the subject. In embodiments, the stimulant is a peptide or a mixture of peptides, and in embodiments, it comprises a gag peptide. Stimulants may also include vaccines. The vaccine may be an HIV vaccine, and in embodiments, the HIV vaccine is an MVA / HIV62B vaccine or a variant thereof. In embodiments, the virus delivery system comprises lentiviral particles. In embodiments, at least one genetic element comprises a small RNA that can inhibit the production of the chemokine receptor CCR5. In a further embodiment, the at least one genetic element comprises at least one small RNA capable of targeting the HIV RNA sequence. In a further embodiment, the at least one genetic element may include a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. The HIV RNA sequence contains any HIV sequence suitable for targeting by a viral delivery system. In embodiments, the HIV RNA sequence comprises one or more of the HIV Vif sequence, the HIV Tat sequence, or variants thereof. The at least one genetic element comprises any genetic element that can be expressed by the viral delivery system. In embodiments, the at least one genetic element comprises microRNA or shRNA. In a further embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC A (SEQ ID NO: 2)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG. TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititijitijititijieieitishitishieitijijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG

TCTTCGTC (SEQ ID NO: 31)
Includes sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT
TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT
TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, a method of treating an HIV infection in a subject is disclosed. The method comprises various steps of immunizing the subject with an effective amount of the first stimulant and removing leukocytes from the subject to obtain peripheral blood mononuclear cells (PBMC). The method comprises ex vivo contacting the PBMC with a therapeutically effective amount of a second stimulant, ex vivo transducing a viral delivery system encoding at least one genetic element into the PBMC, and the appropriate traits. It further comprises the step of culturing transduced PBMCs for a period sufficient to ensure introduction. In embodiments, transduced PBMCs can be cultured for about 1 to about 35 days. In embodiments, the method further comprises the step of injecting the transduced PBMC into the subject. The subject can be human. The first and second stimulants may be the same or different. The first and second stimulants may comprise one or more of the peptides or mixtures of peptides. In embodiments, at least one of the first and second stimulants comprises a gag peptide. At least one of the first and second stimulants may include a vaccine. The vaccine may be an HIV vaccine and in a preferred embodiment the HIV vaccine is the MVA / HIV62B vaccine or a variant thereof. In a preferred embodiment, the virus delivery system comprises lentiviral particles. In embodiments, at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5. In embodiments, the at least one genetic element comprises at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. The HIV RNA sequence may include an HIV Vif sequence, an HIV Tat sequence, or a variant thereof. At least one genetic element may include microRNA or shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT
(SEQ ID NO: 1)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT
(SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC
A (SEQ ID NO: 2)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG.
TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititijitijititijieieitishitishieitijijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG
GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG

TCTTCGTC (SEQ ID NO: 31)
Includes sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT
TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT
TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, the lentiviral vector is disclosed. The lentiviral vector comprises at least one encoded genetic element, the at least one encoded genetic element comprising a small RNA capable of inhibiting the production of the chemokine receptor CCR5. The at least one encoded genetic element may include at least one small RNA capable of targeting the HIV RNA sequence. In another embodiment, the at least one encoded genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. .. The HIV RNA sequence may include an HIV Vif sequence, an HIV Tat sequence, or a variant thereof. At least one encoded genetic element can include microRNA or shRNA. At least one encoded genetic element can include microRNA clusters.

In another aspect, at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT
(SEQ ID NO: 1)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT
(SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC
A (SEQ ID NO: 2)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG.
TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
Includes microRNAs having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the at least one genetic element is
ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititijitijititijieieitishitishieitijijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG
GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG
TCTTCGTC (SEQ ID NO: 31)
Includes sequences having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with. In a preferred embodiment, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT
TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT
TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, a lentiviral vector system for expressing lentiviral particles is disclosed. This system comprises the lentiviral vector described herein, preferably an enveloped plasmid for expressing an enveloped protein optimized to infect cells, and at least one for expressing the gene of interest. Includes helper plasmids. In embodiments, the gene of interest comprises one or more of the gag, pol, and rev genes. In embodiments, the lentiviral vector, envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line. In a further embodiment, the lentiviral particles are produced by a packaging cell line. In embodiments, lentiviral particles can regulate the production of the target of interest. In embodiments, the target of interest is either the chemokine receptor CCR5 or the HIV RNA sequence. The system may further include a first helper plasmid and a second helper plasmid. In embodiments, the first helper plasmid expresses the gag and pol genes and the second helper plasmid expresses the rev gene.

In another aspect, lentiviral particles capable of infecting cells are provided. Lentiviral particles preferably include enveloped proteins optimized to infect cells and the lentiviral vectors described herein. In embodiments, the enveloped protein may be optimized to infect T cells. In a preferred embodiment, the enveloped protein is optimized to infect CD4 + T cells.

In another aspect, modified cells are provided. Modified cells include any cells capable of infecting the lentiviral vector system for use in accordance with this embodiment and embodiments. In embodiments, the cells are CD4 + T cells infected with lentivirus particles. In embodiments, CD4 + T cells are also selected to recognize the HIV antigen. In embodiments, the HIV antigen comprises a gag antigen. In embodiments, CD4 + T cells express reduced levels of CCR5 after infection with lentiviral particles.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is provided. The method is a step of immunizing a subject with an effective amount of a first stimulant; removing leukocytes from the subject, purifying peripheral blood mononuclear cells (PBMC) and making it into at least one factor associated with PBMC. With the step of determining the first quantifiable measure associated with; the second measurement associated with at least one factor associated with PBMC by contacting the PBMC with a therapeutically effective amount of the second stimulant exvivo. It includes various steps to determine the value, where the subject is selected for the treatment regimen if the second quantifiable measurement is higher than the first quantifiable measurement. The at least one factor may include either T cell proliferation or IFN gamma production.

The overall description above, as well as the brief and detailed description of the drawings below, 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 of skill in the art from the brief description of the drawings below and the detailed description of the invention.

FIG. 1 shows a flow chart of the ex vivo treatment method of the present disclosure.

FIG. 2 shows alteration of CD4 + T cells and prevention of new infections according to the present disclosure.

FIG. 3 shows an exemplary lentiviral vector system consisting of therapeutic vectors, helper plasmids, and enveloped plasmids. The therapeutic vector shown herein is a preferred therapeutic vector, also referred to herein as AGT103, and contains miR30CCR5-miR21Vif-miR185-Tat.

FIG. 4 shows an exemplary three-vector lentiviral vector system in a circularized form.

FIG. 5 shows an exemplary 4-vector lentiviral vector system in a circularized form.

FIG. 6 shows an exemplary vector sequence. We have developed a positive (ie, genomic) chain sequence of promoters and miR clusters to inhibit the transmission of CCR5-tropic HIV strains. The unlined sequence comprises the transcriptional EF-1alpha promoter (SEQ ID NO: 105) selected as the preferred promoter for this miR cluster. The underlined sequence indicates a miR cluster consisting of miR30 CCR5 (SEQ ID NO: 1) , miR21 Vif (SEQ ID NO: 2) , and miR185 Tat (SEQ ID NO: 108) (collectively shown in SEQ ID NO: 33).

FIG. 7 shows exemplary lentiviral vector constructs according to various aspects of the present disclosure.

FIG. 8 shows knockdown of CCR5 by experimental vectors in AGTc120 cells and prevention of corresponding R5-tropic HIV infection. (A) Shows the expression of CCR5 in AGTc120 cells with or without the AGT103 lentiviral vector. (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. 9 shows data demonstrating the regulation of CCR5 expression by shRNA inhibitor sequences in the disclosed lentiviral vectors. (A) Screening data for potential candidates are shown. (B) CCR5 knockdown data after transduction of CCR5 shRNA-1 (SEQ ID NO: 16) is shown.

FIG. 10 shows data demonstrating the regulation of HIV components by shRNA inhibitor sequences in the disclosed lentiviral vectors. (A) Knockdown data of the rev / tat target gene are shown. (B) Knockdown data of the gag target gene are shown.

FIG. 11 shows data demonstrating that AGT103 reduces Tat protein expression in cells transfected with an HIV expression plasmid, as described herein.

FIG. 12 shows data demonstrating the regulation of HIV components by synthetic microRNA sequences in the disclosed lentiviral vectors. (A) Tat knockdown data is shown. (B) Vif knockdown data is shown.

FIG. 13 shows data demonstrating the regulation of CCR5 expression by synthetic microRNA sequences in the Lentiviral vector of the present disclosure.

FIG. 14 shows data demonstrating the regulation of CCR5 expression by synthetic microRNA sequences in the lentiviral vectors of the present disclosure containing either long or short WPRE sequences.

FIG. 15 shows data demonstrating the regulation of CCR5 expression by synthetic microRNA sequences in the Lentiviral vectors of the present disclosure with or without WPRE sequences.

FIG. 16 shows data demonstrating the regulation of CCR5 expression by the CD4 promoter that regulates synthetic microRNA sequences in the lentiviral vectors of the present disclosure.

FIG. 17 shows data demonstrating the detection of HIV Gag-specific CD4 T cells.

FIG. 18 shows data demonstrating the expansion and proliferation of HIV-specific CD4 T cells and lentivirus transduction. (A) An exemplary schedule of treatment is shown. (B) IFN gamma production in CD4 gated T cells as described herein is shown. (C) IFN gamma production and GFP expression in CD4 gated T cells as described herein are shown. (D) The frequencies of HIV-specific CD4 + T cells described herein are shown. (E) IFN gamma production from PBMCs after vaccination as described herein is shown.

FIG. 19 shows data demonstrating a functional assay for increasing dose response of AGT103-GFP and inhibition of CCR5 expression. (A) Dose response data for increasing doses of AGT103-GFP are shown. (B) A normally distributed population in terms of CCR5 expression is shown. (C) The percentage inhibition of CCR5 expression by increasing doses of AGT103-GFP is shown.

FIG. 20 shows data demonstrating the transduction efficiency of AGT103 into primary human CD4 + T cells. (A) As described herein, the frequency of transduced cells (GFP positive) is indicated by FACS. (B) The number of vector copies per cell described herein is shown.

FIG. 21 shows data demonstrating AGT103 inhibition of HIV replication in primary CD4 + T cells as described herein.

FIG. 22 shows data demonstrating AGT103 protection of primary human CD4 + T cells from HIV-induced depletion.

FIG. 23 shows data demonstrating the generation of highly enriched CD4 + T cell populations of HIV-specific, AGT103-transduced CD4 T cells. (A) Shows the expression profiles of CD4 and CD8 for the cell populations described herein. (B) Shows the expression profiles of CD4 and CD8 for the cell populations described herein. (C) The expression profiles of IFN gamma and CD4 for the cell populations described herein are shown. (D) The expression profiles of IFN gamma and GFP for the cell populations described herein are shown. FIG. 23 shows data demonstrating the generation of highly enriched CD4 + T cell populations of HIV-specific, AGT103-transduced CD4 T cells. (A) Shows the expression profiles of CD4 and CD8 for the cell populations described herein. (B) Shows the expression profiles of CD4 and CD8 for the cell populations described herein. (C) The expression profiles of IFN gamma and CD4 for the cell populations described herein are shown. (D) The expression profiles of IFN gamma and GFP for the cell populations described herein are shown.

Overview herein discloses methods and compositions for treating and / or preventing human immunodeficiency virus (HIV) disease to achieve functional cure. The methods and compositions include integrated lentiviruses, non-incorporated lentiviruses, and related viral vector techniques, as described below.

Disclosed herein are therapeutic viral vectors (eg, lentiviral vectors), immunotherapy, and methods of using them to treat HIV infection. In embodiments, methods and compositions for achieving functional cure of HIV infection are provided. As shown in FIG. 1 herein, various aspects and embodiments are HIV-infected, eg, in HIV-infected patients, with stable suppression of viralemia, eg, by daily administration of HAART. Includes a first therapeutic immunization with a vaccine intended to provide a strong immune response to HIV. In embodiments, the first stimulus event enriches the fraction of HIV-specific CD4 T cells. This is followed by (1) isolation of peripheral leukocytes by leukocyte depletion or purification of PBMCs from venous blood, (2) a second stimulating event, such as any vaccine or protein, such as HIV or HIV-related peptides. Ex vivo restimulation of CD4 T cells with a suitable stimulant, (3) therapeutic lentivirus characterization, ex vivo T cell culture, and (4) reinjection into the original patient follow.

Various methods and compositions can be used to prevent new cells, such as CD4 + T cells, from becoming infected with HIV. For example, as illustrated in FIG. 2, in order to prevent infection of new cells, CCR5 expression can be targeted to prevent viral attachment. In addition, it is possible to target the destruction of any remaining infectious viral RNA. With respect to the above and with reference to FIG. 2 herein, there are provided compositions and methods for stopping the cycle of the HIV virus in cells already infected with HIV. To stop the HIV virus cycle, target viral RNA produced by latently infected cells such as latently infected CD4 + T cells.

Previous attempts to achieve cure for HIV have been unsuccessful, especially due to the lack of sufficient numbers of HIV-specific CD4 T cells with protective genetic modifications. If this number is below the critical threshold, the functional cure described herein will not be achieved. For example, when antiretroviral drug therapy ends, the reappearance of HIV usually follows. Patients then often experience rapid destruction of HIV-specific CD4 T cells, and despite previous gene therapy, resumption of disease progression continues. By using therapeutic immunization according to the compositions and methods described herein, new HIV treatment regimens have been developed that include functional cure in various embodiments.

Definitions and Interpretations Unless otherwise defined herein, scientific and technical terms used in the context of this disclosure shall have meaning generally understood by one of ordinary skill in the art. Further, unless otherwise required by the context, the singular term shall include the plural and the plural term shall include the singular. In general, the nomenclature used herein in connection with cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry, and hybridization, as well as these. The technology is well known and commonly used in the art. Unless otherwise stated, the methods and techniques of this disclosure are generally well known in the art and are described in various general and more specific references cited and discussed throughout this specification. , Performed according to conventional methods. For example, Sambrook J. et al. And Russel D. Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. et al. Y. (2000), Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Clinical Biology, Wiley, Wiley, John. (2002), Harlow and Lane Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. et al. Y. (1998), and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. See (2003). Any enzymatic reaction or purification technique is performed according to the manufacturer's specifications, as is commonly performed in the art or as described herein. The nomenclatures used in the context of analytical chemistry, synthetic organic chemistry, and pharmaceuticals and pharmaceutical chemistry, as described herein, as well as these testing techniques and techniques are well known and commonly used in the art. It is what has been done.

As used herein, the term "about" will be understood by those of skill in the art and will vary somewhat depending on the context in which the term is used. Given the context in which the term "about" is used, if the use of the term is not clear to one of ordinary skill in the art, "about" means up to plus 10% or minus 10% of a particular term.

As used herein, the term "administering" or "administering" the activator is used in a form and therapeutically effective amount that is therapeutically useful to the body of this individual to a subject in need of treatment. It is meant to provide the activator of the invention in a form that can be introduced.

As used herein, the term "AGT103" is a specific practice of a lentiviral vector containing a miR30-CCR5 / miR21-Vif / miR185-Tat microRNA cluster sequence detailed herein. Refers to the form.

As used herein, the term "AGT103T" refers to cells transduced with lentivirus containing the AGT103 lentiviral vector.

Throughout the specification and claims, the word "comprise" or variants such as "comprises" or "comprising" include the stated integers or groups of integers. It is understood to imply, but not to imply the exclusion of any other integer or group of integers. Further, as used herein, the term "includes" is meant to include without limitation.

As used herein, the term "engraftment" is capable of determining the quantitative level of sustained engraftment in a subject after injection of a cell source. (For example, Rosenberg et al., N. Engl. J. Med., Vol. 323: 570-578 (1990), Dudley et al., J. Immunother., Vol. 24: 363-373 (2001), Yee. Et al., Curr. Opin. Immunol., Vol. 13, pp. 141-146 (2001), Rooney et al., Blood, Vol. 92: pp. 1549-1555 (1998)).

The terms "expressed," "expressed," or "encoded" refer to the process by which a polynucleotide is transcribed into mRNA and / or the process by which the transcribed mRNA is subsequently translated into a peptide, polypeptide, or protein. Point to. Expression may include splicing of mRNA in eukaryotic cells, or other forms of post-transcriptional or post-translational modification.

As mentioned above, and further defined herein, the term "functional cure" refers to HIV + individuals who have previously required continuous HIV therapy, such as cART or HAART, for such HIV therapy. A situation or condition in which virus replication is low or undetectable if a lower dose is used, an intermittent dose is used, or the dose is discontinued. Individuals may be referred to as "functionally cured" even if adjuvant therapy is still required to maintain low levels of viral replication and slow or terminate disease progression. Possible outcomes of functional cure relapse within a specified time frame, such as 1 month, 3 months, 6 months, 1 year, 3 years, and 5 years, and all other time frames that can be defined. Is the final eradication of all or virtually all HIV, such that is not detected.

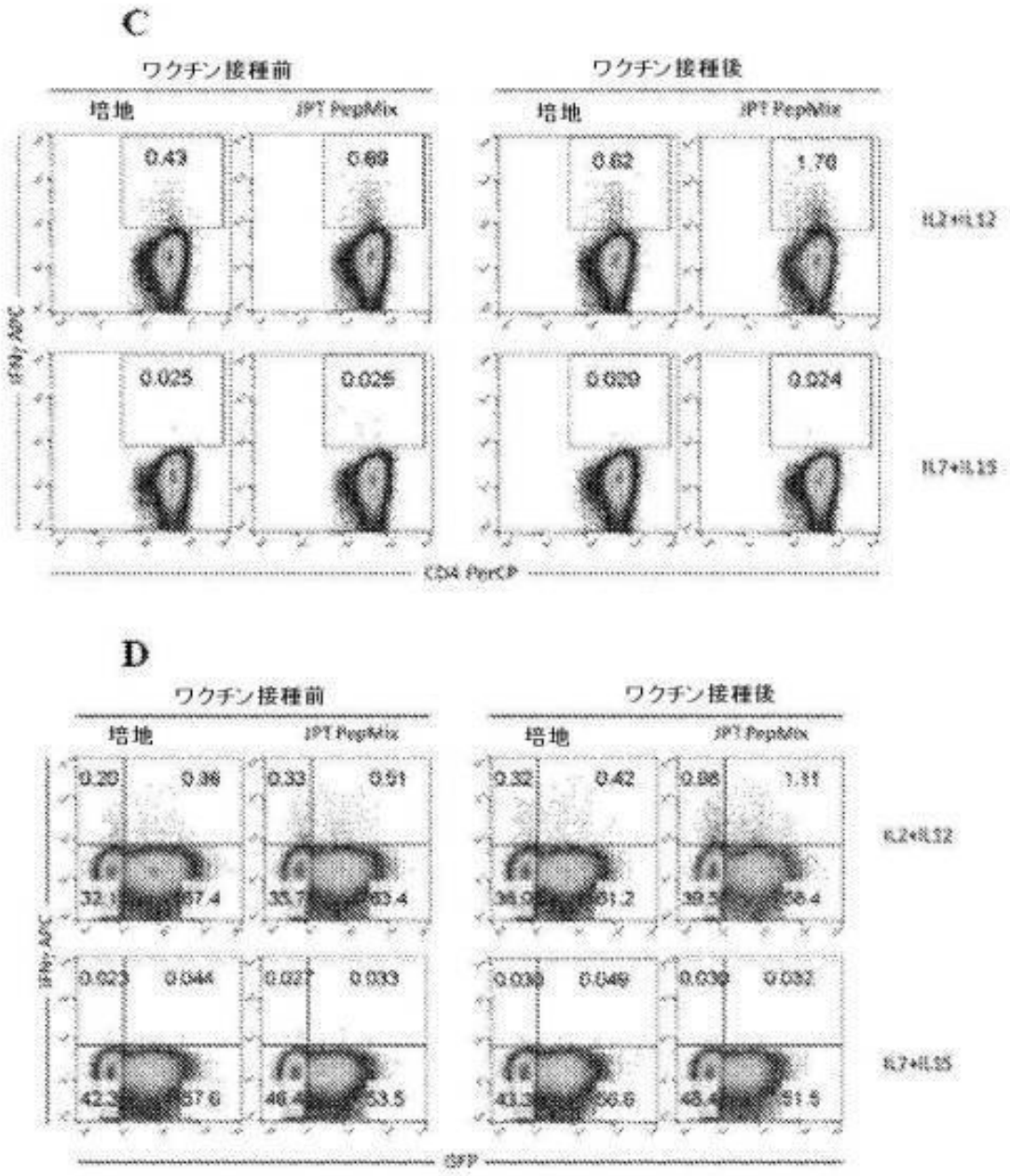
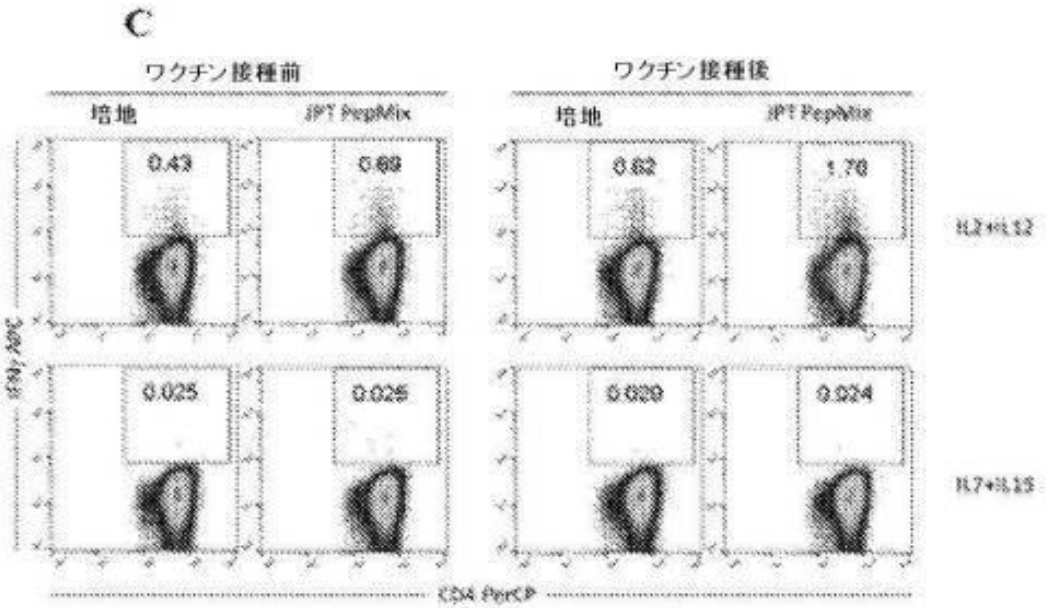

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

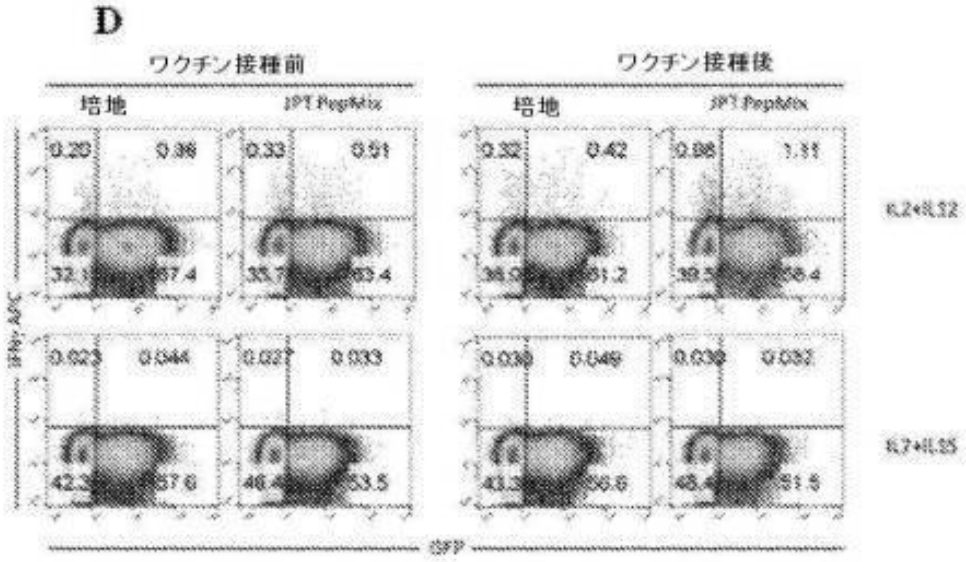
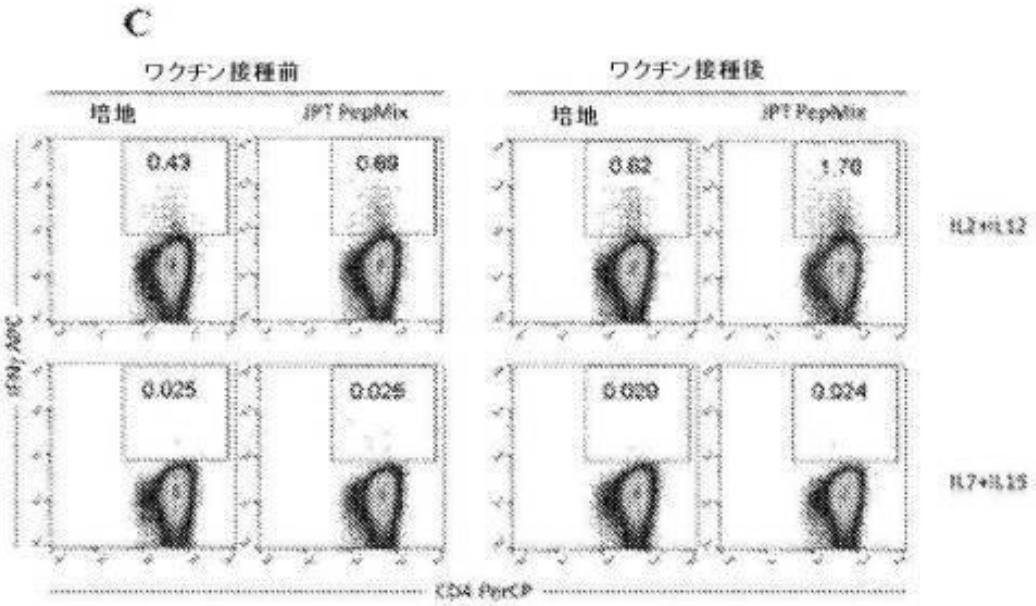

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

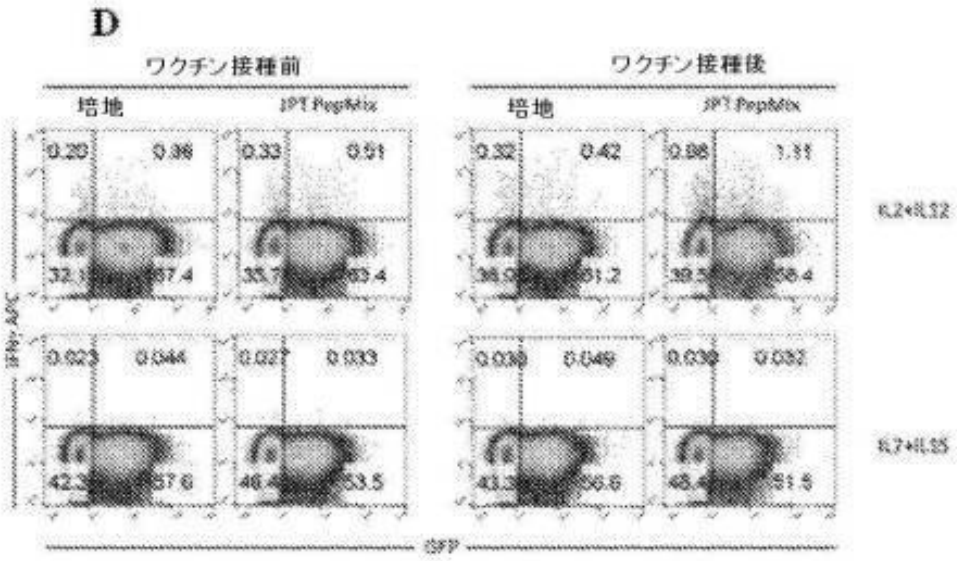
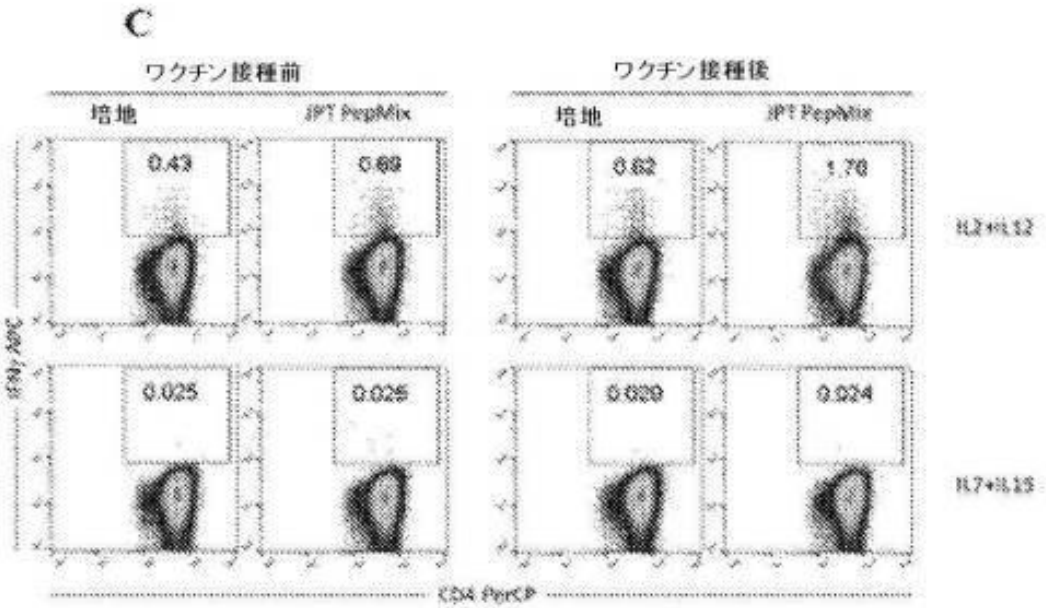

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

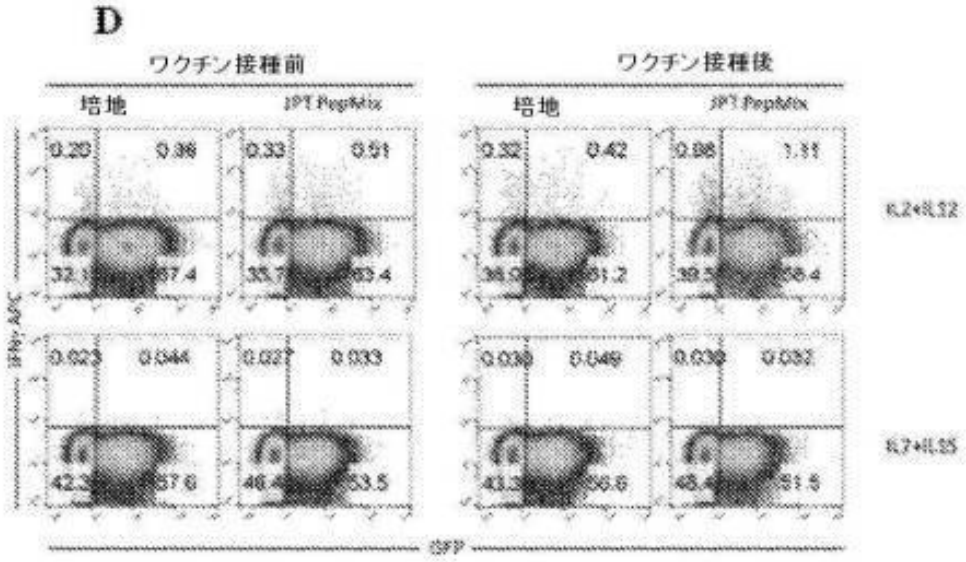
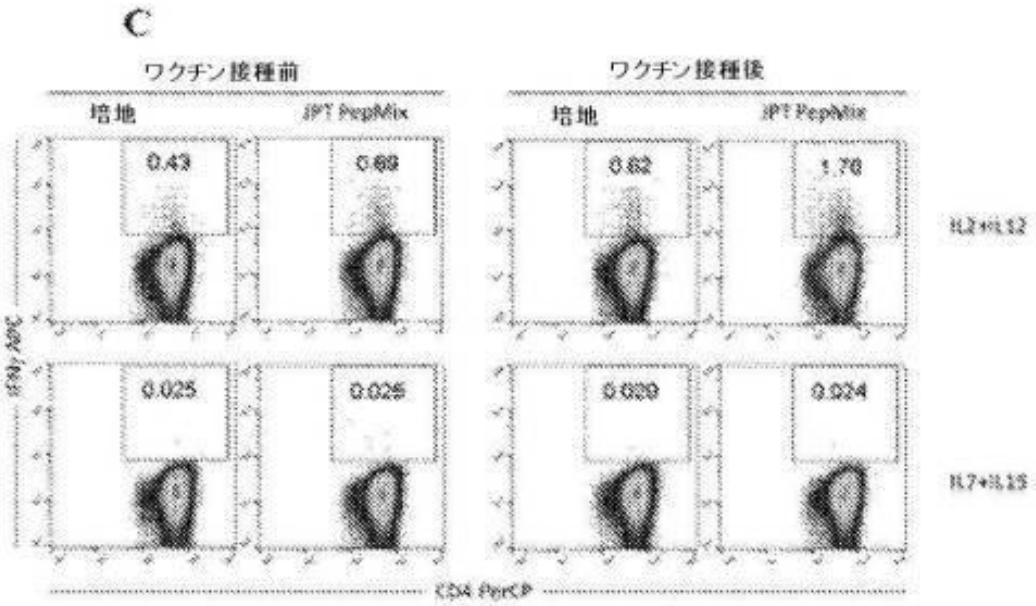

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

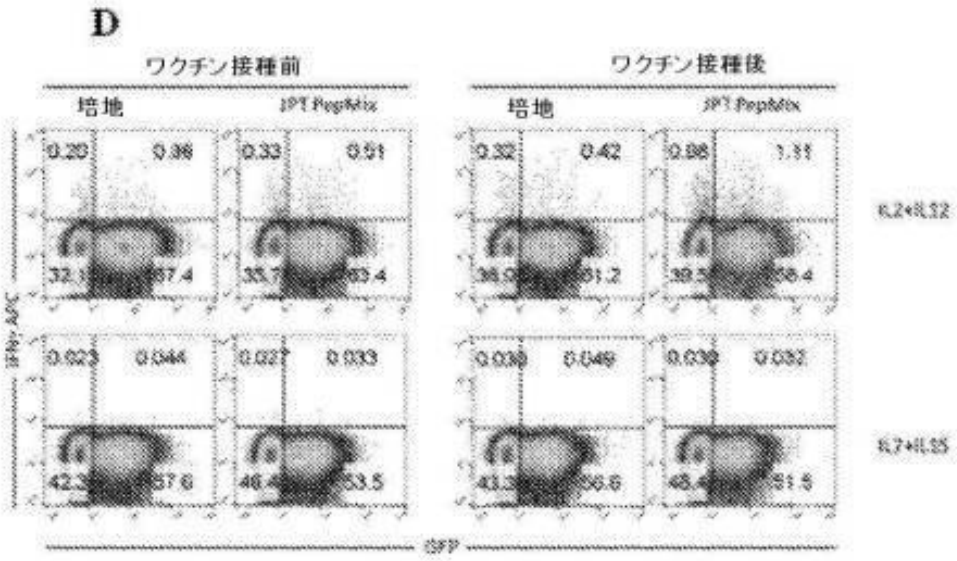
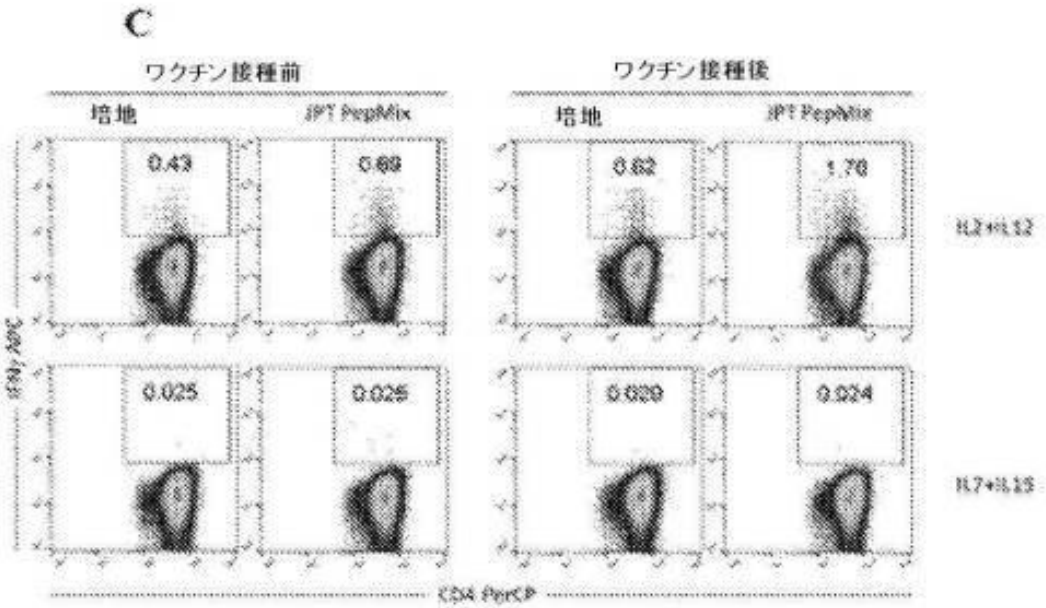

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

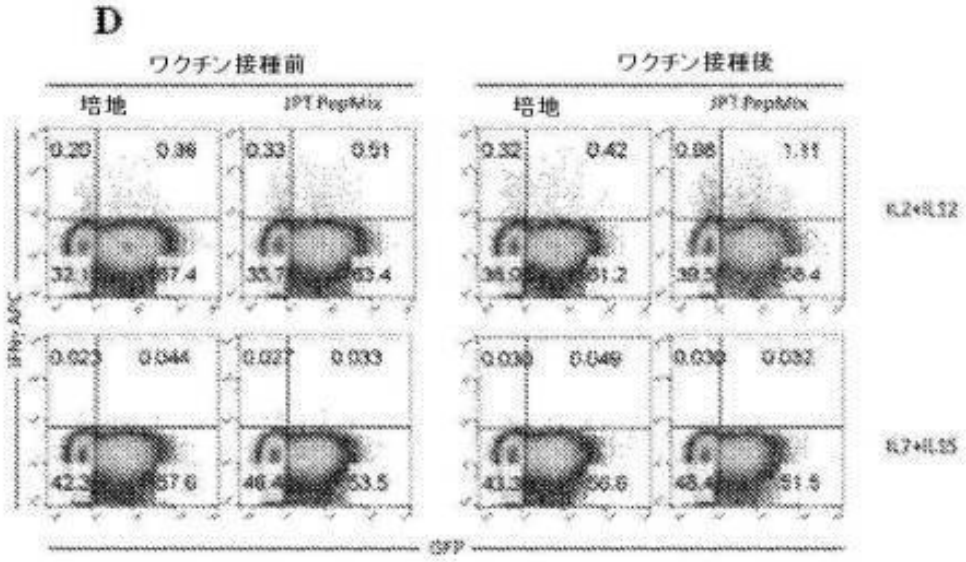
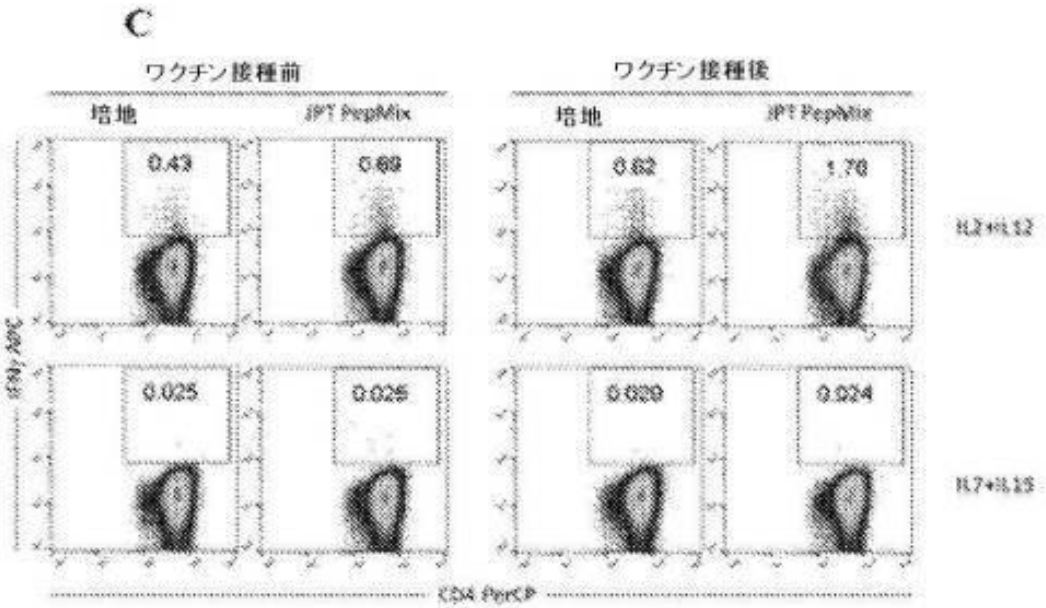

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

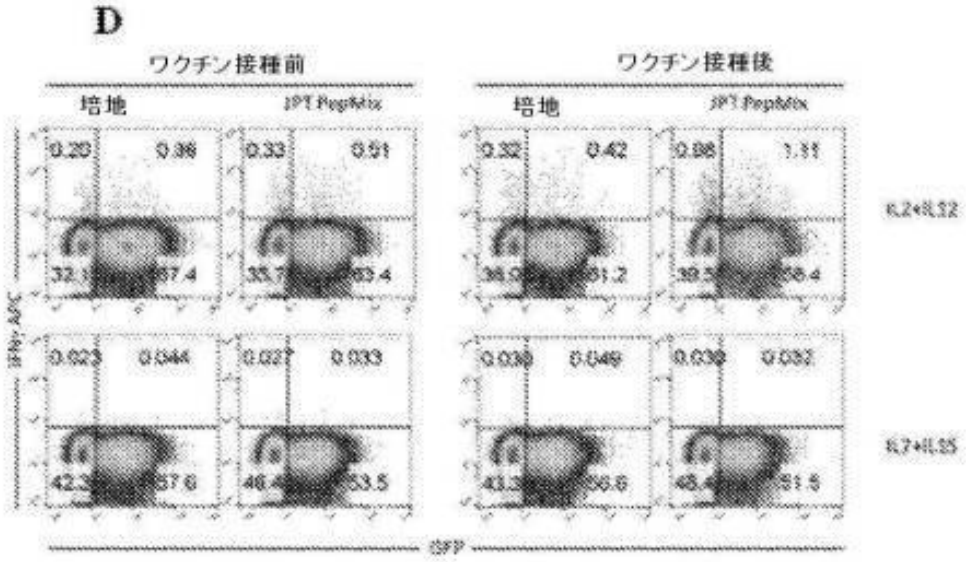
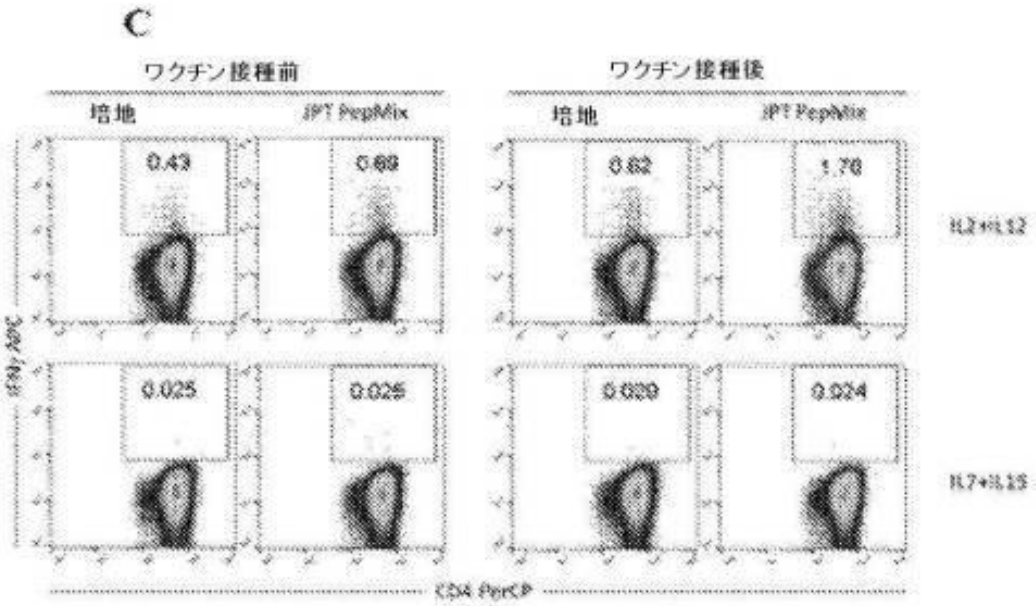

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

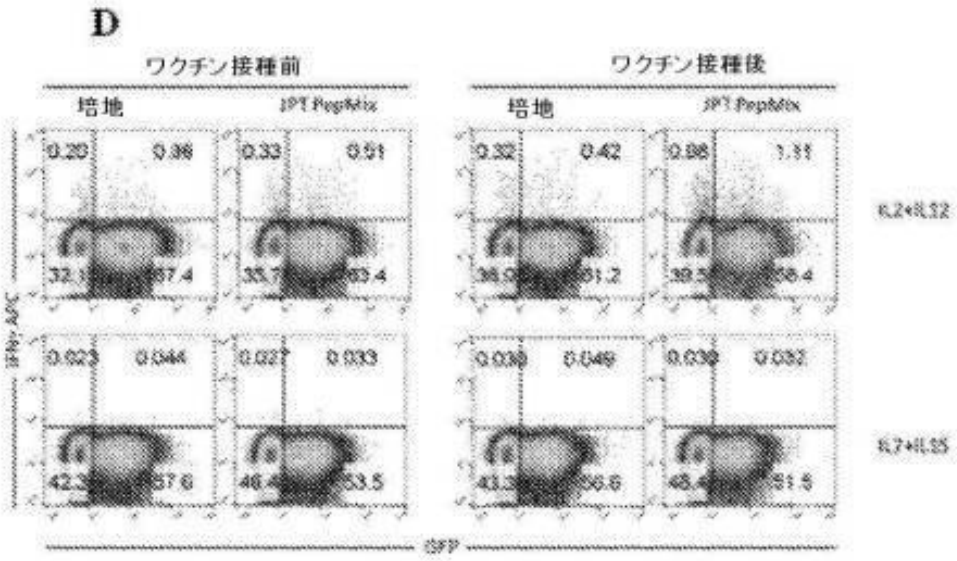

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き

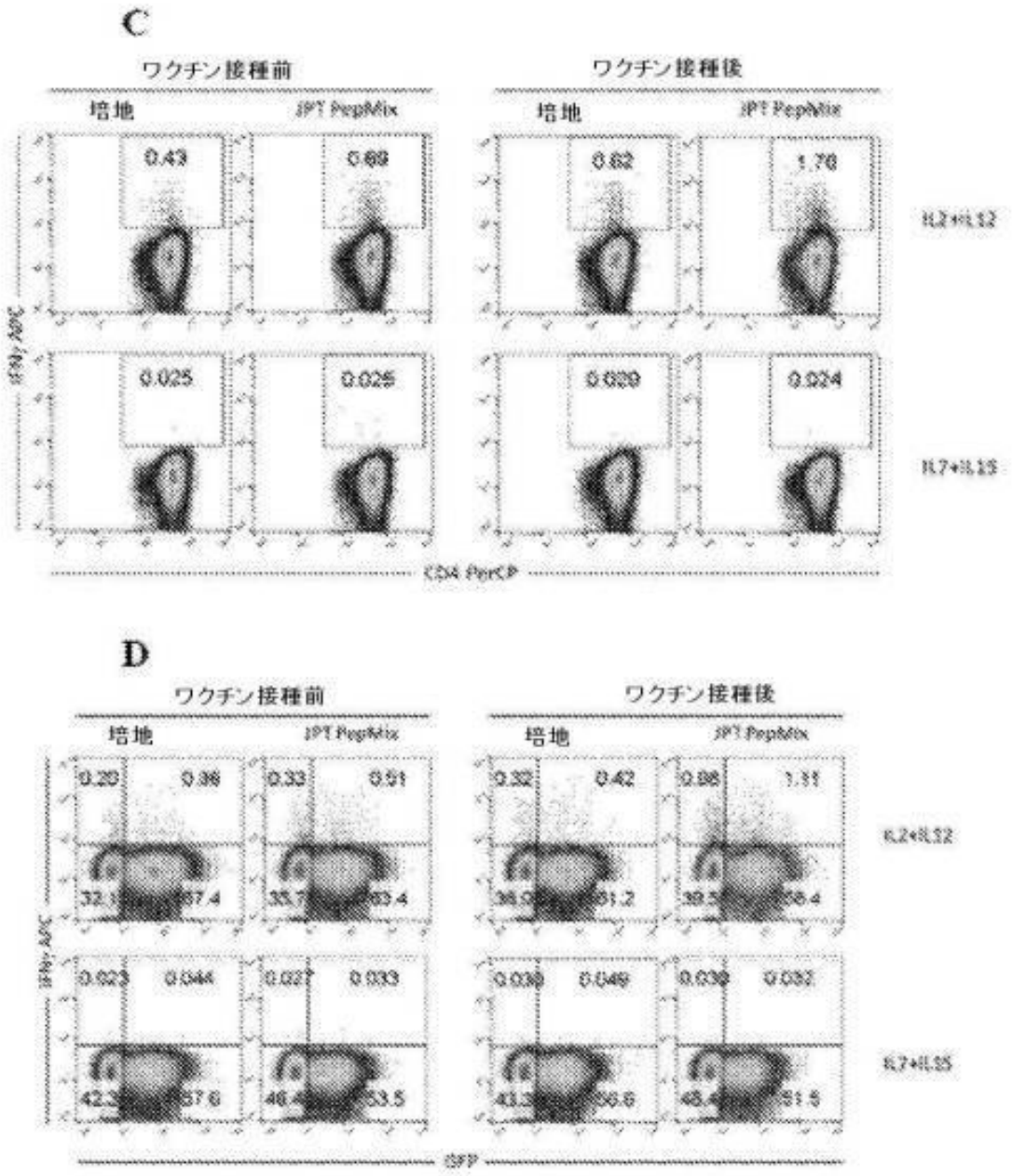


Figure 23 続き

The term "HIV vaccine" includes immunogens, vehicles and adjuvants intended to elicit an HIV-specific immune response. The term "HIV vaccine" is included in the meaning of the term "stimulant" as described herein. An "HIV vaccine" is used to produce cells to produce purified inactivated virus particles or whole inactivated virus particles that can be HIV, or HIV proteins, glycoproteins or protein fragments that can induce specific immunity. In addition to recombinant bacterial vectors, plasmid DNA or RNA that can be directed, recombinant viral vectors capable of expressing HIV proteins, protein fragments or peptides, glycoprotein fragments or glycopeptides can be

included. Instead, anti-CD3 / CD28 beads, T cell receptor-specific, for the purpose of enriching HIV-specific CD4 T cells prior to transfection, or for in vitro assay of lentivirus-transfected CD4 T cells. Dendritic cells, T cells or B cells may be activated using specific methods for immune stimulation, including antibodies, mitogens, superantigens, and other chemical or biological stimuli. The activator may be soluble, polymer aggregate, liposome or endosome-based, or ligated to beads. Cytokines containing interleukins 2, 6, 7, 12, 15, 23, or others to improve the response of cells to stimuli and / or to improve the survival of CD4 T cells during culture and transduction. May be added. Instead, but not limited to any of the above, the term "HIV vaccine" includes the MVA / HIV62B vaccine and its variants. The MVA / HIV62B vaccine is a known highly attenuated double recombinant MVA vaccine. The MVA / HIV62B vaccine was constructed by inserting the HIV-1 gag-pol and env sequences into a known MVA vector (eg, Goepfert et al. (2014) J. Infect. Dis., Vol. 210 (No. 1)). : See pages 99-110; see also WO2006206667; both of which are incorporated herein by reference). The term "HIV vaccine" also includes any one or more vaccines provided in Table 1 below.

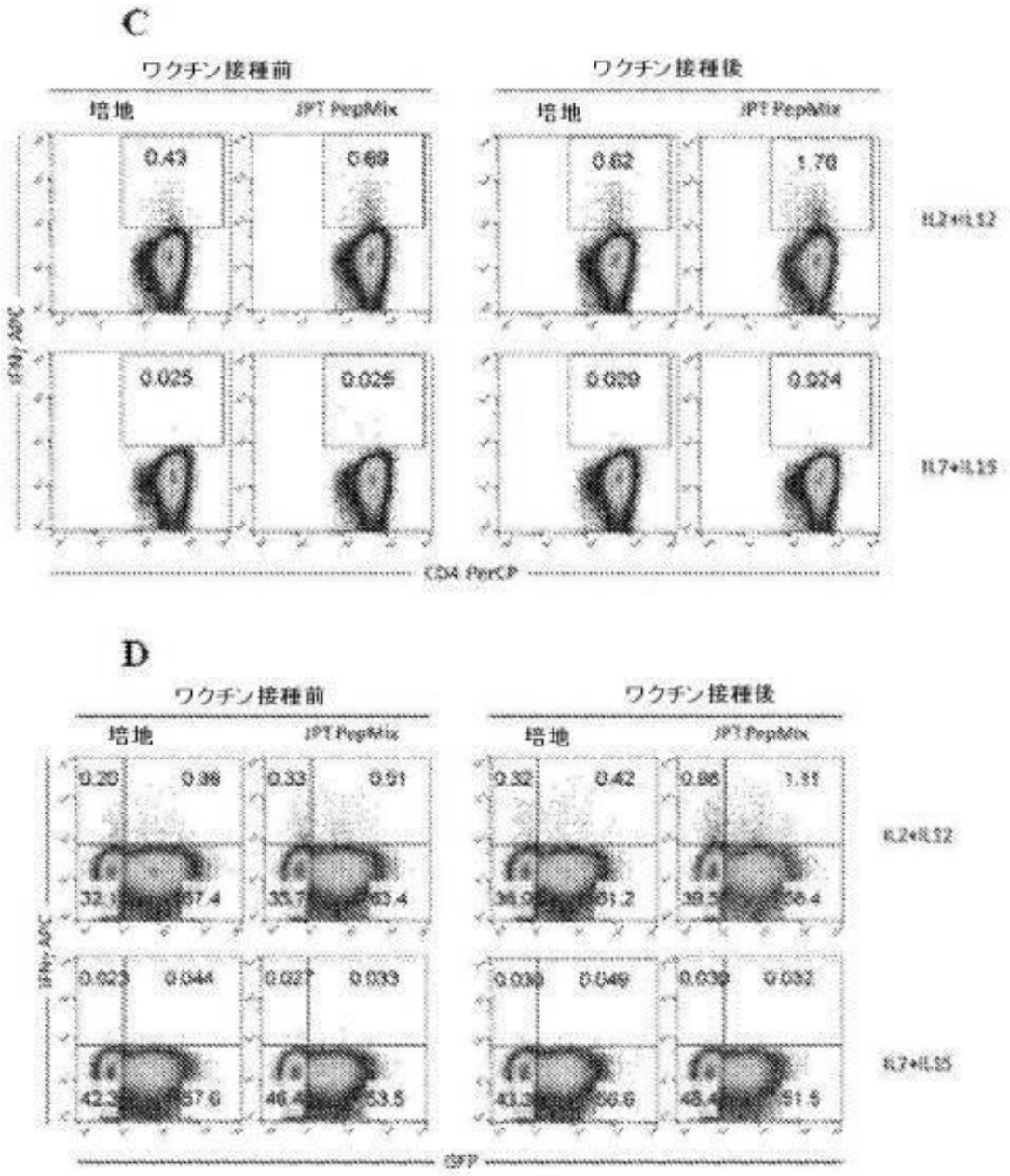
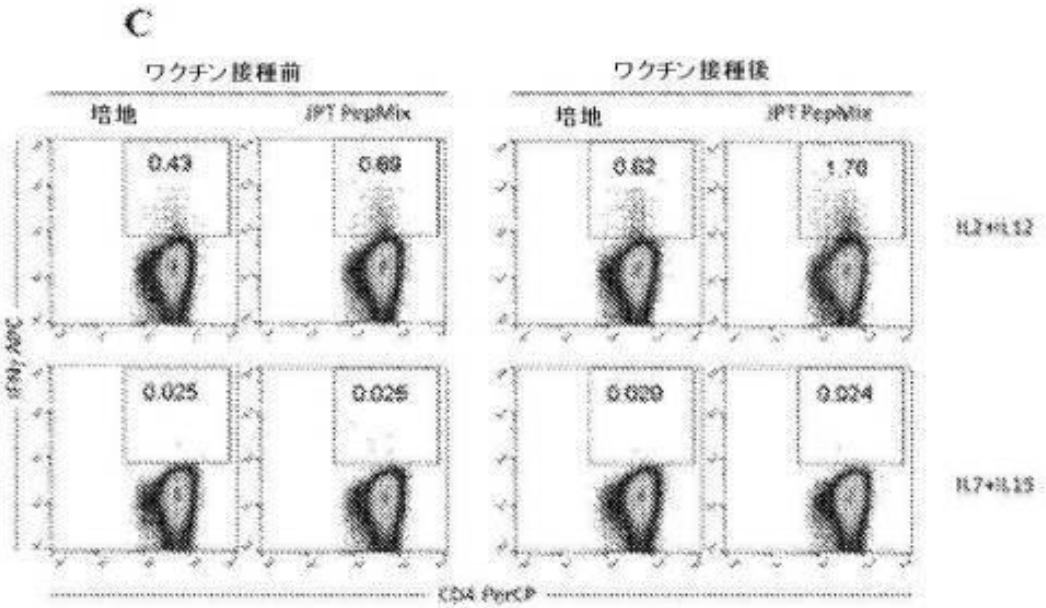

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

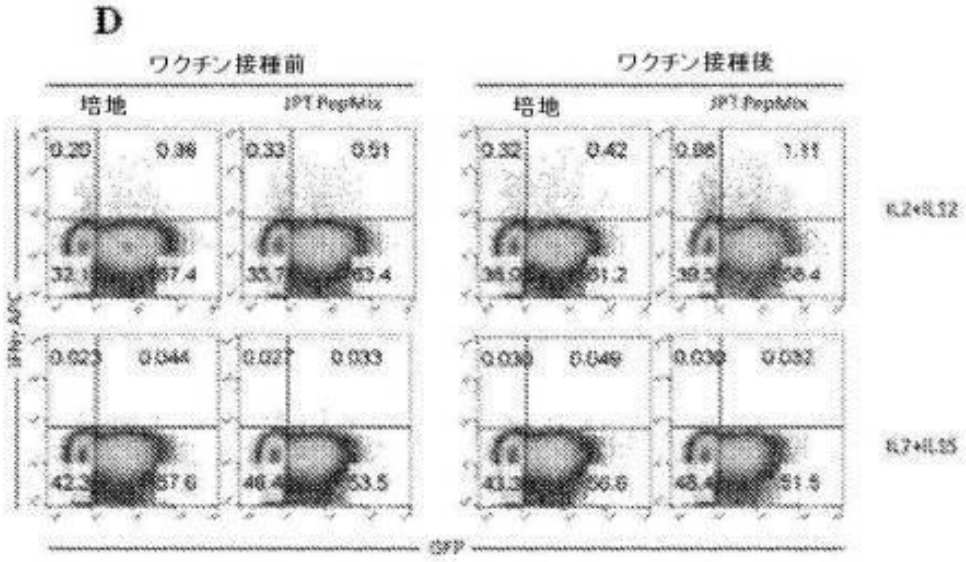
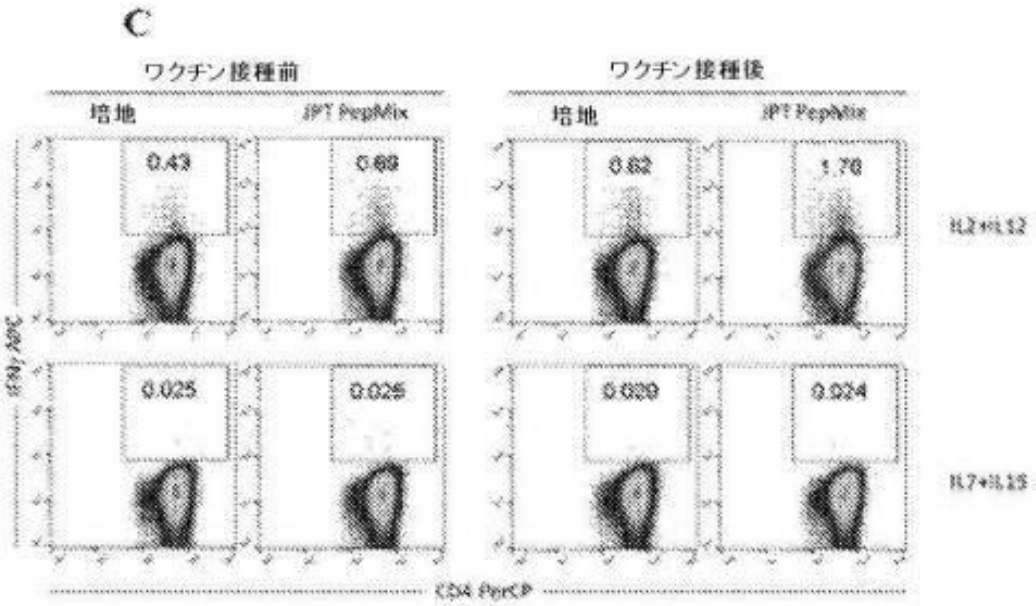

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

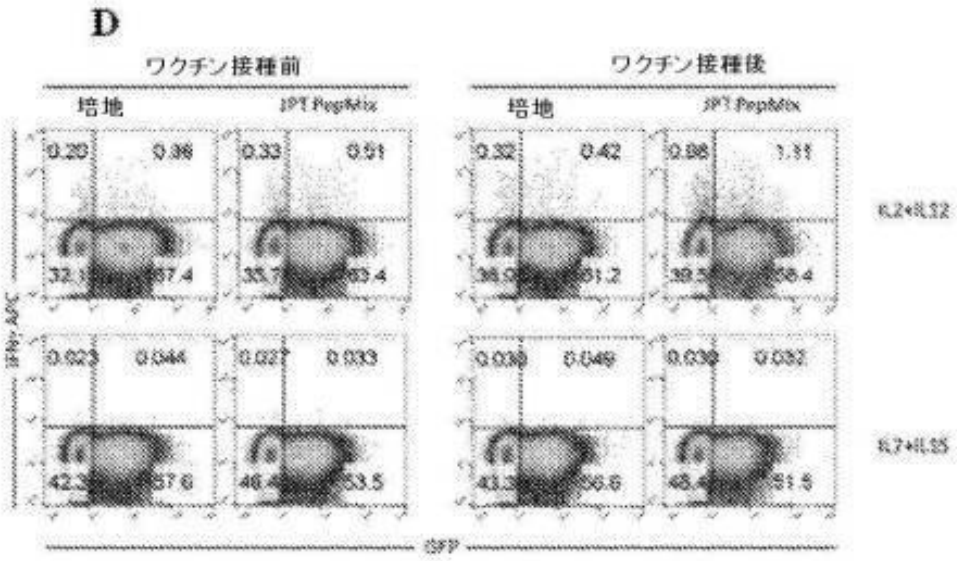

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き

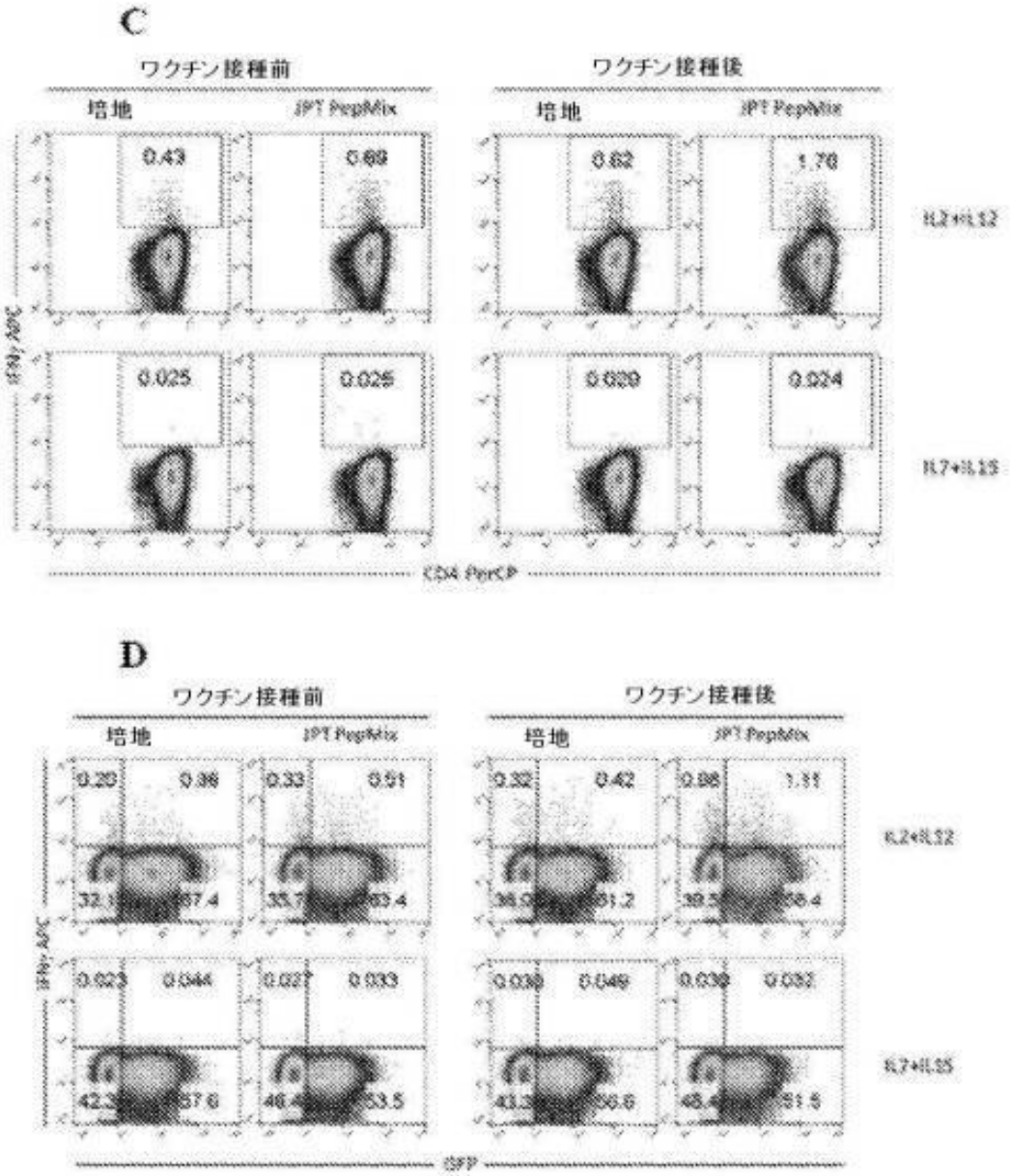
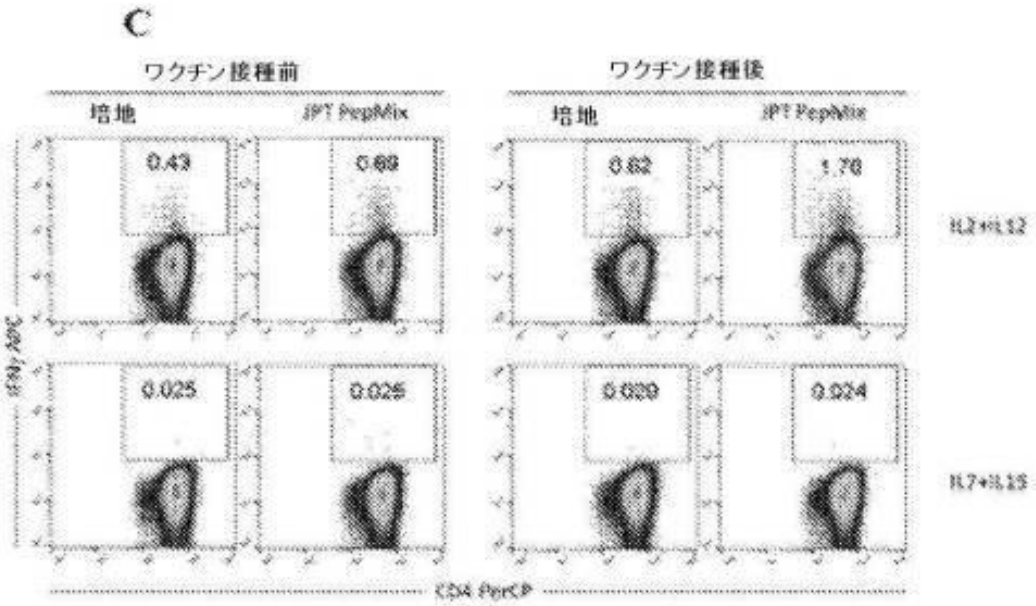

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.020
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

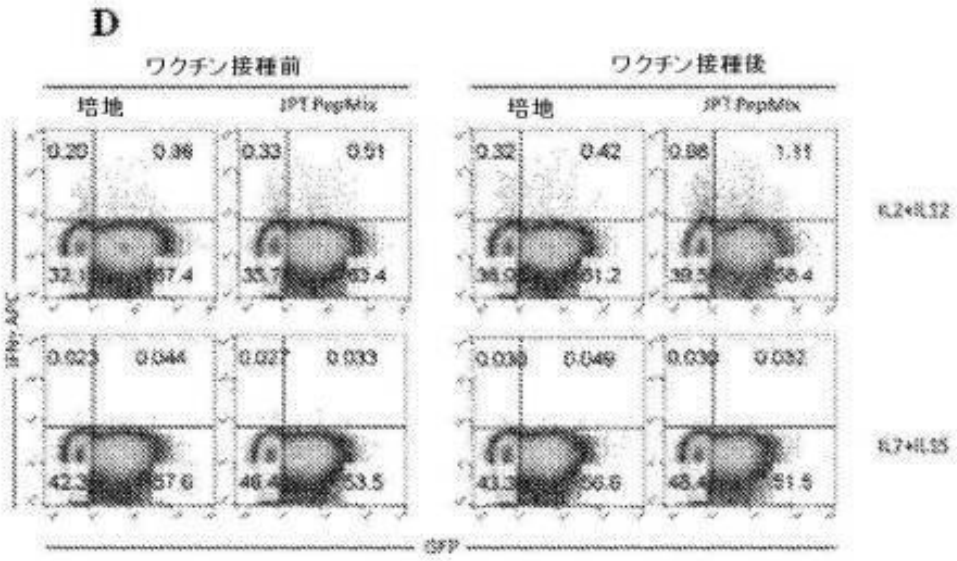
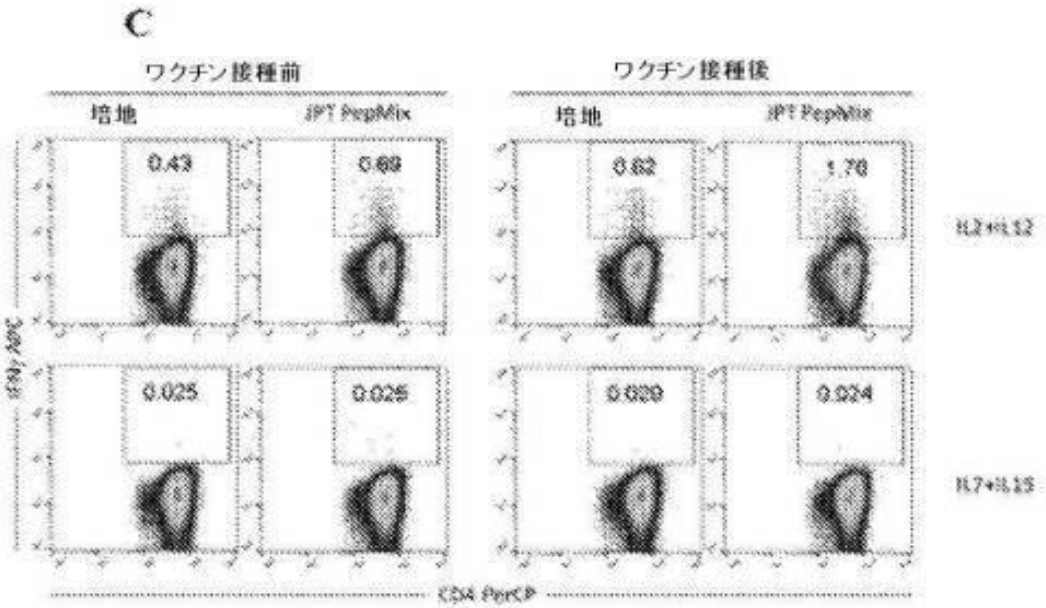

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

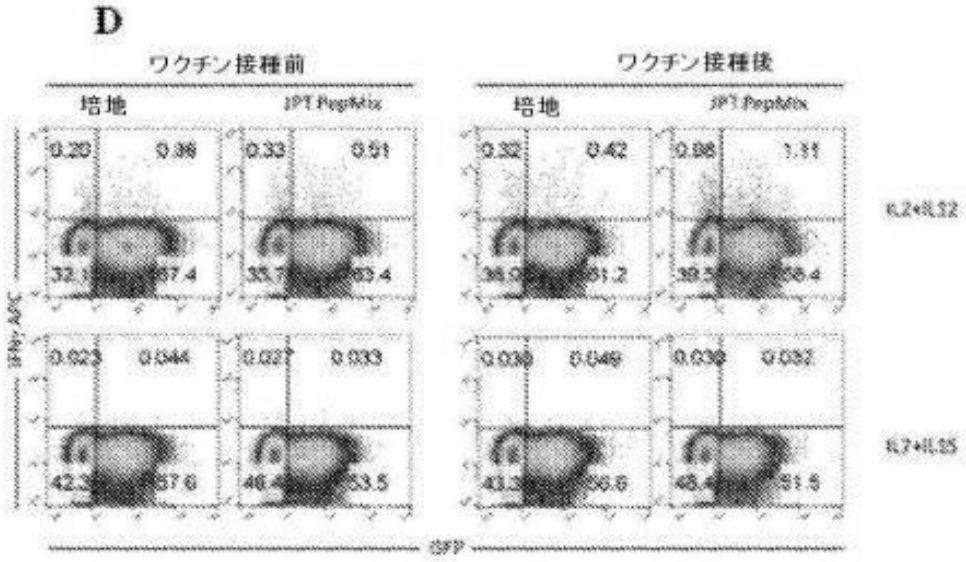
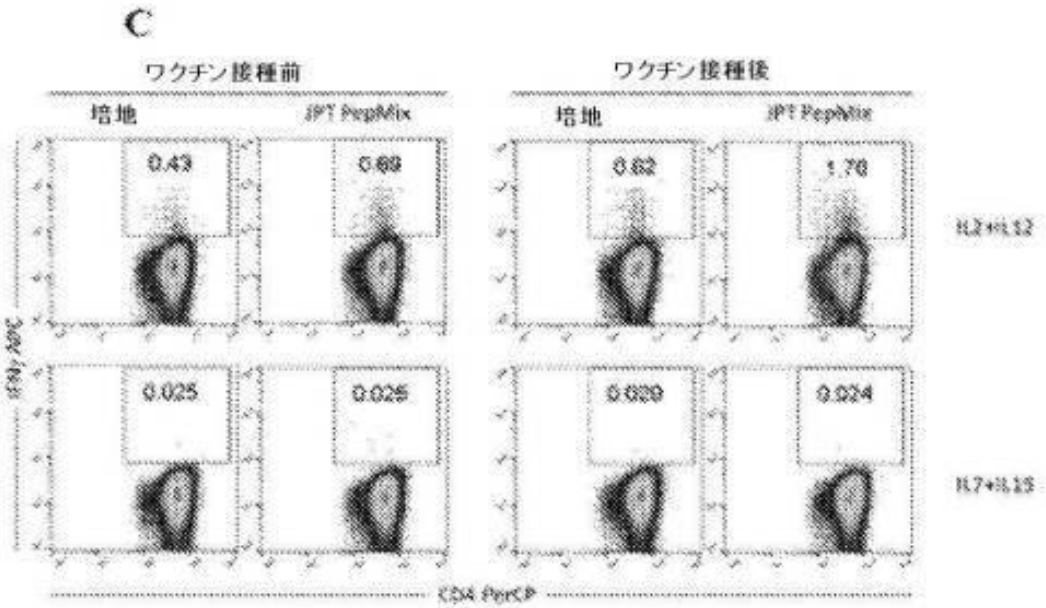

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

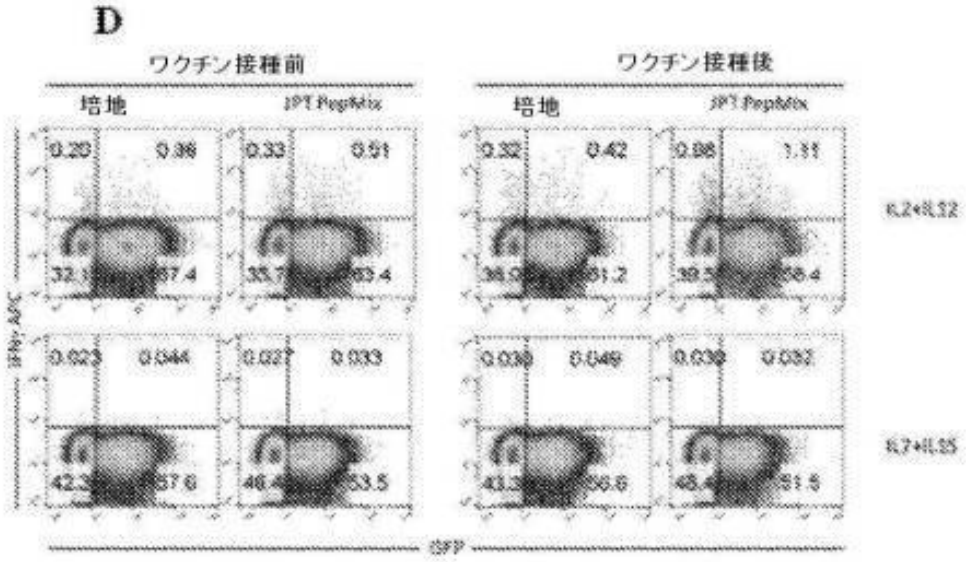
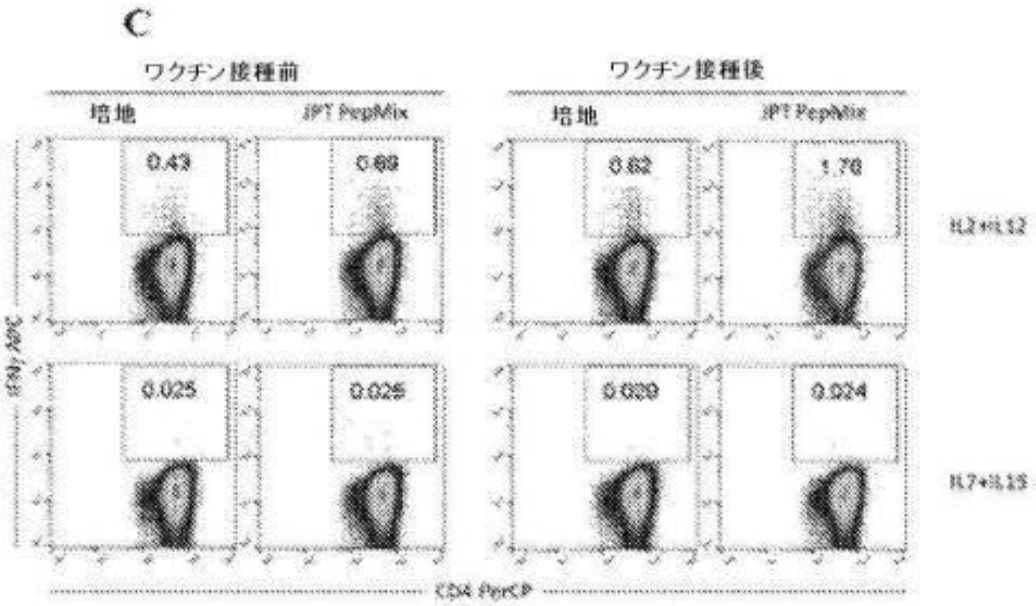

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

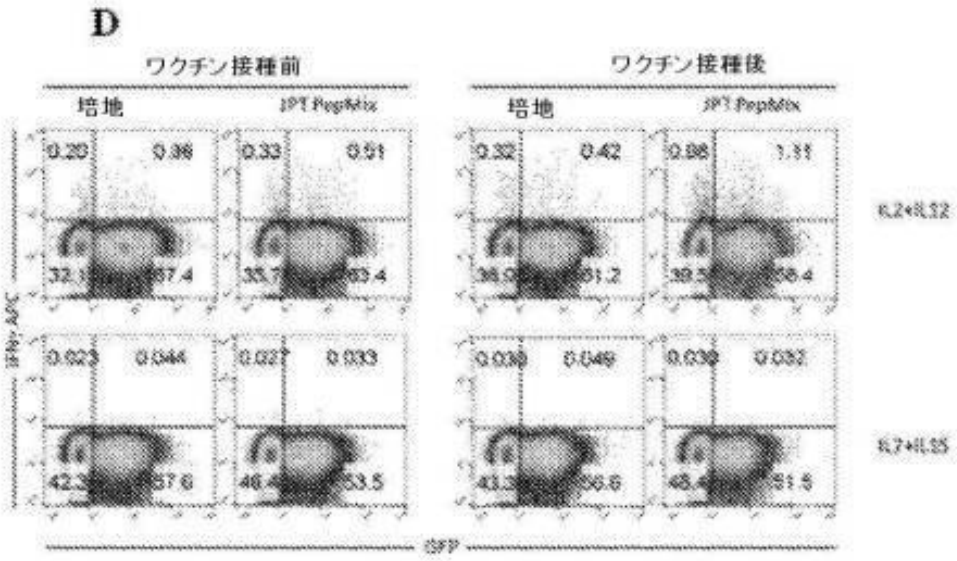

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き

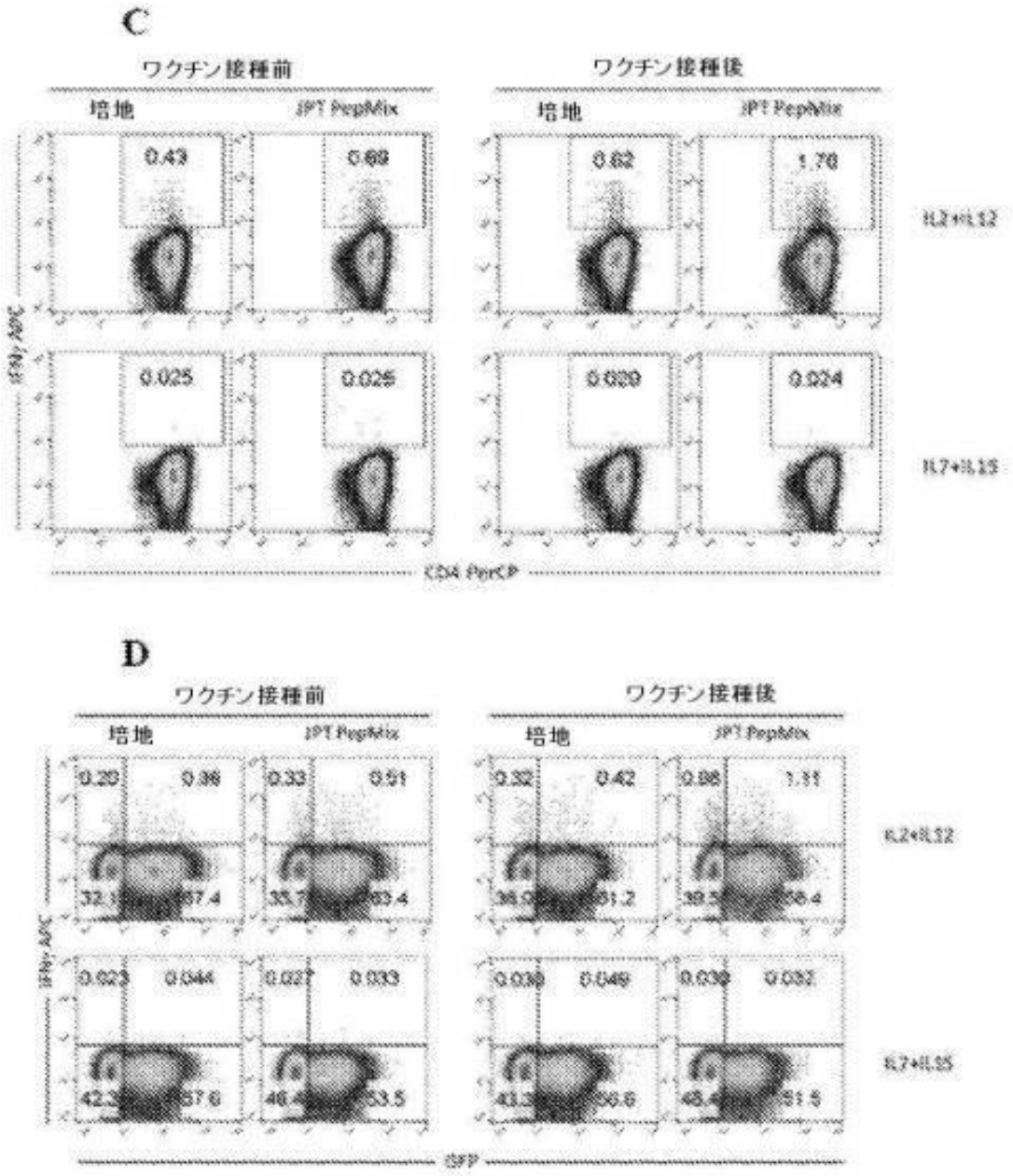


Figure 23 続き

The term "in vivo" refers to the processes that occur in living organisms. The term "ex vivo" refers to a process that occurs outside a living organism. For example, in vivo treatment refers to treatment that occurs within the patient's body, while ex vivo treatment refers to treatment that occurs outside the patient's body but still uses or approaches tissue from that patient. Or a procedure that interacts with the tissue. The ex vivo treatment step may then include a subsequent in vivo treatment step.

The term "miRNA" refers to microRNA and is sometimes referred to herein as "miR". The term "microRNA cluster" refers to at least two microRNAs located close to each other and co-expressed on a vector.

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

The term "percent identity" is used in the context of two or more nucleic acid or polypeptide sequences among the sequence comparison algorithms described below (eg, BLASTP and BLASTN, or other algorithms available to those of skill in the art). Two or more with a specific percentage of nucleotide or amino acid residues that are the same when compared and aligned for maximum match, either using one of them or as measured by visual inspection. Refers to an array or sub-sequence. Depending on the application, "percent identity" may be present over the region of the sequence being compared, eg, across the functional domain, or, alternative, over the full length of the two sequences being compared. There is also. In sequence comparison, one sequence typically serves as a reference sequence, whereas test sequences are compared. When using the sequence comparison algorithm, enter the test and reference sequences into the computer, specify the coordinates of the subarrays as needed, and specify the sequence algorithm program parameters. The sequence comparison algorithm then calculates the percent sequence identity of the test sequence to the reference sequence based on the specified program parameters.

Optimal alignment of sequences for comparison is described, for example, in Smith and Waterman, Adv. Apple. Math. Vol. 2, p. 482 (1981), Local Homology Algorithm, Needleman and Wunch, J. Mol. Mol. Biol. , 48: 443 (1970), Homology Alignment Algorithm, Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA, Vol. 85: pp. 2444 (1988), Similarity Search Methods, These Algorithms (Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis., GAP, BEST, FAS. It can be implemented by computer or by visual inspection (Ausube et al., See General below).

Examples of suitable algorithms for determining percent sequence identity and sequence similarity are described in Altschul et al., J. Mol. Mol. Biol. , 215: 403-410 (1990), the BLAST algorithm. Software for performing BLAST analysis is publicly available from the National Center for Biotechnology Information website.

Percent identity between the two nucleotide sequences was made using the GAP program of the GCG software package (available at http://www.ggcg.com) and NWSgapdna. It can be determined using a CMP matrix, a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. Percent identity between two nucleotide or amino acid sequences is incorporated into the ALIGN program (version 2.0). Meyers and W.M. Determined using Miller's algorithm (CABIOS, Vol. 4, pp. 11-17 (1989)), PAM120 residue weight table, 12 gap length penalties, and 4 gap penalties. You can also do it. In addition, the percent identity between the two amino acid sequences is included in the GAP program of the GCG software package (available at http://www.ggcg.com) Needleman and Wunsch (J. Mol. Biol. (Volume 48): pp. 444-453 (1970)), with either the Blossum62 matrix or the PAM250 matrix, with a gap weight of 16, 14, 12, 10, 8, 6, or 4, and 1, It can be determined using a length weight of 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can be further used as "query sequences" for searching public databases to identify, for example, related sequences. Such a search was performed by Altschul et al. (1990), J. Mol. Mol. Biol. , 215: Can be done using the NBLAST and XBLAST programs (version 2.0) on pages 403-10. By performing a nucleotide search for BLAST using the NBLAST program, score = 100, word length = 12, a nucleotide sequence homologous to the nucleic acid molecule of the present invention can be obtained. By performing a BLAST protein search using the XBLAST program, score = 50, word length = 3, an amino acid sequence homologous to the protein molecule of the present invention can be obtained. To obtain a gapped alignment for comparative purposes, Altschul et al. (1997), Nucleic Acids Res. , Vol. 25 (No. 17): Gapped BLAST described on pages 3389 to 3402 can be used. When using BLAST and Gapped BLAST programs, the default parameters of the respective programs (eg, XBLAST and NBLAST) may be used. http: // www. ncbi. nlm. nih. See gov.

As used herein, "pharmaceutically acceptable" is, within sound medical judgment, an excessive toxicity, irritant effect, allergic response commensurate with a reasonable benefit / risk ratio. , Or a compound, substance, composition, and / or dosage form suitable for use in contact with human and animal tissues, organs, and / or body fluids without any other problems or complications.

As used herein, "pharmaceutically acceptable carrier" is any physiologically compatible solvent, dispersion medium, coating, antibacterial and antifungal agent, isotonic and delayed absorption. Refers to agents and includes these. The composition may comprise a pharmaceutically acceptable salt, such as an acid or base addition salt (see, eg, Berge et al. (1977), J Pharm Sci, Vol. 66: pp. 1-19.).

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

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

As used herein, the term "stimulant" refers to, but is not limited to, any exogenous factor capable of stimulating an immune response, including, but not limited to, vaccines, HIV vaccines, and HIV or HIV-related. Contains peptides. The stimulant is preferably capable of stimulating a T cell response.

As used herein, the term "subject" includes human patients, but also other mammals. The terms "subject," "individual," "host," and "patient" may be used interchangeably herein.

The term "therapeutically effective amount" is a suitable composition and suitable composition sufficient to treat or prevent the symptoms, progression, or onset of complications found in a patient suffering from a given disease, injury, disease, or condition. Refers to the amount of activator of the invention in a suitable dosage form. The therapeutically effective amount depends on the condition of the patient or its severity, and the condition such as the age and weight of the subject to be treated. The therapeutically effective amount may vary depending on any of several factors, including, for example, the route of administration, the condition of the subject, and other factors understood by those of skill in the art.

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

The term "treatment" or "treatment" generally refers to an intervention that attempts to alter the natural course of the subject being treated, may be performed prophylactically, or during a clinical pathological process. May be done in. 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, and ameliorating or alleviating the condition. And, but are not limited to, causing amelioration or an improved prognosis.

The term "vaccine" is used interchangeably with the term "therapeutic vaccine" and refers to extrinsic factors capable of inducing an immune response in an individual, including but not limited to purified proteins, inactivation. Includes viruses, viral vectorized proteins, bacterial vectorized proteins, peptides or peptide fragments, or virus-like particles (VLPs).

Description of Aspects of the Disclosure As detailed herein, one aspect provides a method of treating cells infected with HIV. The method generally involves contacting peripheral blood mononuclear cells (PBMCs) isolated from HIV-infected subjects with a therapeutically effective amount of stimulant and at least one genetic element, performed in ex vivo. It comprises ex-vivo transducing the encoded virus delivery system into a PBMC and culturing the transduced PBMC for a period sufficient to achieve such transduction. In embodiments, transduced PBMCs are cultured for about 1 to about 35 days. The method may further include injecting the transduced PBMC into the subject. The subject can be human. The stimulant may comprise a peptide or a mixture of peptides and, in a preferred embodiment, a gag peptide. Stimulants may include vaccines. The vaccine may be an HIV vaccine and in a preferred embodiment the HIV vaccine is the MVA / HIV62B vaccine or a variant thereof. In a preferred embodiment, the virus delivery system comprises lentiviral particles. In embodiments, at least one genetic element may comprise a small RNA capable of inhibiting the production of the chemokine receptor CCR5. In embodiments, the at least one genetic element comprises at least one small RNA capable of targeting an HIV RNA sequence. In other embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. The HIV RNA sequence may include an HIV Vif sequence, an HIV Tat sequence, or a variant thereof. At least one genetic element can include at least one of microRNA or shRNA. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT

(SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC
A (SEQ ID NO: 2)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or higher percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG
TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is
ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititijitijititijieieitishitishieitijijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG
GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG
TCTTCGTC (SEQ ID NO: 31)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Includes sequences with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT
TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT
TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, a method of treating an HIV infection in a subject is disclosed. The method generally comprises the step of immunizing the subject with an effective amount of the first stimulant and the step of removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC). The method involves ex vivo transduction of PBMCs with a therapeutically effective amount of a second stimulant, ex vivo transduction of a viral delivery system encoding at least one genetic element, and transduction. It further comprises the step of culturing transduced PBMCs for a period sufficient to achieve. The method may further comprise further enrichment of PBMC, for example, preferably by enriching PBMC for CD4 + T cells. In embodiments, transduced PBMCs are cultured for about 1 to about 35 days. The method may further involve injecting the transduced PBMC into the subject. The subject can be human. The first and second stimulants may be the same as or different from each other. At least one of the first and second stimulants may comprise a peptide or a mixture of peptides. In embodiments, at least one of the first and second stimulants comprises a gag peptide. At least one of the first and second stimulants may include a vaccine. The vaccine may be an HIV vaccine and in a preferred embodiment the HIV vaccine is the MVA / HIV62B vaccine or a variant thereof. In embodiments, the first stimulant is an HIV vaccine and the second stimulant is a gag peptide.

In embodiments, the virus delivery system comprises lentiviral particles. In embodiments, at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5. In embodiments, the at least one genetic element comprises at least one small RNA capable of targeting an HIV RNA sequence. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. The HIV RNA sequence may include an HIV Vif sequence, an HIV Tat sequence, or a variant thereof. At least one genetic element can include microRNAs or shRNAs, or clusters thereof. In a preferred embodiment, the at least one genetic element comprises a microRNA cluster.

In another aspect, at least one genetic element is
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT

(SEQ ID NO: 1)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC A (SEQ ID NO: 2)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG. TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititjitijititijieieitishitishieitjijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG TCTTCGTC (SEQ ID NO: 31)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Includes sequences with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the microRNA cluster is AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, the lentiviral vector is disclosed. The lentiviral vector comprises at least one encoded genetic element, which targets a small RNA or HIV RNA sequence capable of inhibiting the production of the chemokine receptor CCR5. Contains at least one small RNA that can be. In another embodiment, the at least one encoded genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting the HIV RNA sequence. , Lentiviral vectors are disclosed. The HIV RNA sequence may include an HIV Vif sequence, an HIV Tat sequence, or a variant thereof. At least one encoded genetic element can include microRNA or shRNA. At least one encoded genetic element can include microRNA clusters.

In another aspect, at least one genetic element is AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
including.

In another aspect, at least one genetic element is
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACATCCCGCACTGACATTTTTTGGTATTTTCATCTGACC
A (SEQ ID NO: 2)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or higher percent identity, or GGGCCTGGCTCGAGCAGGGGGGCGAGGGATTCCGCTTCTTCTGCCATAGACGTGGG
TCCCCTCCCCATGGCAGGCAGAAGCGGCACTTCCTCCCAATGACCGCCGTCTTCGTCG (SEQ ID NO: 3)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Contains microRNAs with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the at least one genetic element is
ShieitishitishishieitijijishitijitieishishieishishititijitishijijijijieitijitijitieishititishitijieieishititijitijititijieieitishitishieitijijieijititishieijieieijieieishieishieitishishijishieishitijieiCATTTTG
GTATCTTTCATCTGACCA (SEQ ID NO: 2); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTC
CTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
including.

In another aspect, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG
TCTTCGTC (SEQ ID NO: 31)
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least Includes sequences with 92%, at least 93%, at least 94%, at least 95%, or higher percent identity. In a preferred embodiment, the microRNA cluster is
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCT
ACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCTTCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACT
TCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCT
TCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTC (SEQ ID NO: 31)
including.

In another aspect, a lentiviral vector system for expressing lentiviral particles is provided. This system comprises the lentiviral vectors described herein; preferably at least one enveloped plasmid for expressing an enveloped protein optimized to infect cells; the gene of interest, eg, gag, pol. , And at least one helper plasmid for expressing any of the rev genes, and when the lentivirus vector, at least one enveloped plasmid, and at least one helper plasmid are transfected into the packaging cells, the lentivirus. The particles are produced by the packaging cells, which can regulate the target sequence of interest, eg, inhibit the production of the chemokine receptor CCR5, or target the HIV RNA sequence.

In another aspect, lentiviral particles capable of infecting cells are disclosed. Lentiviral particles preferably include at least one enveloped protein optimized to infect cells and the lentiviral vector described herein. Envelope proteins may be optimized to infect T cells. In a preferred embodiment, the enveloped protein is optimized to infect CD4 + T cells.

In another aspect, the modified cells are disclosed. In embodiments, the modified cell is a CD4 + T cell. In embodiments, the CD4 + T cells are infected with the lentiviral particles described herein. In embodiments, CD4 + T cells are also selected to recognize HIV antigen based on past immunization with stimulants. In a further preferred embodiment, the HIV antigen recognized by CD4 + T cells comprises a gag antigen. In a more preferred embodiment, CD4 + T cells express reduced levels of CCR5 after infection with lentiviral particles.

In another aspect, a method of selecting a subject for a therapeutic treatment regimen is disclosed. The method generally involves immunizing a subject with an effective amount of a first stimulant; removing leukocytes from the subject, purifying peripheral blood mononuclear cells (PBMC), and at least one associated with PBMC. With the step of determining the first quantifiable measure associated with the factor; the second stimulant contacting the PBMC with a therapeutically effective amount of the second stimulant exvivo and associated with at least one factor associated with the PBMC. If the second quantifiable measurement is different (eg, higher) than the first quantifiable measurement, then the subject is due to a treatment regimen. Is selected for. At least one factor can be T cell proliferation or IFN gamma production.

Human immunodeficiency virus (HIV)

The human immunodeficiency virus, also commonly referred to as "HIV," is a retrovirus that causes acquired immunodeficiency syndrome (AIDS) in humans. AIDS is a condition in which progressive failure of the immune system causes life-threatening opportunistic infections and cancer to spread. If untreated, the average survival time after HIV infection is estimated to be 9-11 years, depending on the HIV subtype. Infection with HIV results from the transfer of body fluids including, but not limited to, blood, semen, vaginal fluid, pre-ejaculate, saliva, tears, lymph or cerebrospinal fluid, or breast milk. HIV may be present as free viral particles in infected individuals or in infected immune cells.

HIV infects cells essential to the human immune system, such as helper T cells, but tropism can vary between HIV subtypes. Immune cells that may be particularly 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 cell-damaging lymphocytes that recognize infected cells. This mechanism results in low levels of CD4 + T cells. When the number of CD4 + T cells drops below critical levels, cell-mediated immunity is lost, making the body increasingly susceptible to opportunistic infections and cancer.

HIV is structurally 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 encoding 19 proteins (gag, pol, env, tat, rev, It consists of nef, viv, vpr, vpu, and in some cases the tenth tev) which is a fusion of tat, env and rev. Three of these genes, gag, pol, and env, contain the information needed to make structural proteins for new viral particles.

HIV replicates primarily in CD4 T cells, causing cell destruction or dysregulation and reducing host immunity. HIV establishes infection as an integrated provirus and may enter a latent state in which viral expression in a particular cell is reduced below the level of cytopathology or detection by the host immune system that affects this cell. Is difficult to treat and has not been eradicated even after long-term high activity antiretroviral drug therapy (HAART). Although HAART can prolong survival in the majority of cases, HIV infection causes fatal disease.

The main goal of the fight against HIV is to develop strategies to cure the disease. Long-term HAART has not achieved this goal, so researchers are looking to alternative procedures. The initial efforts to improve host immunity through therapeutic immunization (use of the vaccine after infection) had little or no effect. Similarly, the effects of enhanced treatment were moderate or none.

Although some progress has been made with the use of gene therapy, the positive results are sporadic and one of the genes encoding CCR5 (chemokine receptor), which plays a decisive role in the invasion of the virus into host cells, or It has only been found in rare humans with defects in both alleles. However, many researchers are optimistic that gene therapy is most likely to eventually achieve cure for HIV.

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. As mentioned above, functional cure is low or undetectable for HIV + individuals who have previously required HAART, when HAART is used at lower or intermittent doses. It is defined as a situation or condition in which it is possible to survive in the condition or to discontinue HAART altogether. As used herein, functional cure may still require adjuvant therapy to maintain low levels of viral replication and slow or terminate disease progression. The possible outcome of functional healing is the ultimate eradication of HIV, which prevents 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 almost all immune functions. Most importantly, HIV infection and CD4 T cell depletion require activation of individual cells. Activation is a specific mechanism of individual CD4 T cell clones that recognize pathogens or other molecules using rearranged T cell receptors.

In the case of HIV, infection activates and infects a population of HIV-specific T cells, resulting in depletion of other T cells that are less specific to the virus, thus protecting the immune system against the virus. Is effectively impaired. The responsiveness of HIV-specific T cells is restored during long-term HAART, but when HAART is interrupted, the rebound of virus infection repeats this process, causing virus-specific cells to be deleted again and time for disease progression. The axis returns.

Obviously, functional healing is possible only if sufficient HIV-specific CD4 T cells are protected so that the host's innate immunity can counteract and control HIV if HAART is interrupted. In one embodiment, the invention provides methods and compositions for improving the efficacy of gene therapy to result in functional cure of HIV disease. In another embodiment, the invention provides methods and compositions for enhancing host immunity to HIV and resulting in 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, the treatment is about 100%, about 200%, about 300%, about 400%, about 500%, about 600%, about 700% of the subject's HIV-specific CD4 T cells. Concentrate about 800%, about 900%, or about 1000%.

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 corrects or complements an existing defect, a DNA sequence encoding a regulatory protein, antisense, short-chain homologous RNA, long non-coding RNA, small interfering RNA, and the like. It may include, but is not limited to, a DNA sequence encoding an RNA molecule and a decoy sequence encoding either RNA or protein designed to compete for cellular factors important for altering the pathology. Gene therapy involves delivering these therapeutic gene constructs to target cells, resulting in treatment or alleviation of a particular disease.

Several efforts are underway to utilize gene therapy in the treatment of HIV disease, but the results so far are inadequate. 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 Delta 32).

Nucleases or other mechanisms of gene deletion / modification delivered by lentivirus can be used to reduce overall expression of CCR5 and / or aid in reducing HIV replication. At least one study reports successful treatment of the disease when lentivirus was administered to patients with a genetic background of CCR5 Delta 32. However, this is the only successful case, and many other patients who do not have the CCR5 Delta 32 genotype have not been successfully treated. As a result, substantial improvements in the performance of viral gene therapy against HIV, both in terms of the performance of individual viral vector constructs and the improvement of vector use through strategies to achieve functional HIV cure. There is a need.

For example, some existing therapies rely on zinc finger nucleases that delete a portion of CCR5 in an attempt to make cells resistant to HIV infection. However, even after optimal treatment, only 30% of T cells were modified by nucleases, of which the modified ones were modified to prevent HIV infection throughout the CD4 T cell population. It was only 10%. In contrast, in the disclosed method, virtually all cells will have a lentivirus transgene with reduced CCR5 expression below the levels required to enable HIV infection.

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

Immunotherapy Historically, vaccines have been the mainstay of countermeasures against deadly infectious diseases such as smallpox, polio, measles, and yellow fever. Unfortunately, there are currently no approved vaccines for HIV. The HIV virus has a unique way of escaping the immune system, and the human body appears to be unable to establish an effective immune response to it. As a result, scientists do not have a clear idea of what is needed to provide protection from HIV.

However, immunotherapy may provide a solution that has not previously been addressed by traditional vaccine approaches. Immunotherapy, also known as biological therapy, is a type of procedure designed to boost the body's natural defenses to combat infection or cancer. Immunotherapy uses materials made either in the body or in the laboratory to improve, target, or restore immune system function.

In some embodiments of the invention disclosed, an immunotherapeutic approach may be used to enrich the HIV-specific CD4 T cell population for the purpose of increasing the host's anti-HIV immunity. In some embodiments of the invention disclosed, integrated or non-integrated lentiviral vectors can be used to transduce a host's immune cells for the purpose of increasing the host's 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 vehicle and / or a biological or chemical adjuvant suitable for increasing the immune response of the host. When vaccines containing HIV proteins, including but not limited to replacement viral vectors, recombinant bacterial vectors, purified subunits or plasmid DNA, are used to concentrate a population of virus-specific T cells or antibodies. These methods can be further enhanced by the use of HIV-targeted gene therapy using lentivirus or other viral vectors.

Methods In one aspect, the present disclosure provides methods for achieving functional cure of HIV disease using viral vectors. The method generally comprises immunotherapy to increase the proportion of HIV-specific CD4 T cells, followed by lentiviral transduction to deliver HIV and, as needed, inhibitors of CCR5 and CXCR4.

In one embodiment, the method comprises a first stimulating event to increase the proportion of HIV-specific CD4 T cells. The first stimulus may include administration of one or more of any suitable agent for concentrating HIV-specific CD4 + T cells in a patient, including but not limited to a vaccine.

Therapeutic vaccines may include one or more HIV proteins having a protein sequence that is representative of the predominant viral type in the geographic area being treated. Therapeutic vaccines are biological or chemical adjuvants, vehicles containing purified proteins, inactivated viruses, viral vectorized proteins, bacterial vectorized proteins, peptides or peptide fragments, virus-like particles (VLPs), cytokines and / or chemokines. , As well as methods of immunization. Vaccination may be given according to standard methods known in the art, and HIV patients are in the interval between immunization and subsequent culture of lymphocytes in exvivo, including lentiviral transfection. Antiretroviral drug therapy may be continued during.

In some embodiments, when an HIV + patient is immunized with the HIV vaccine, the frequency of HIV-specific CD4 T cells is about 2, about 25, about 250, about 500, about 750, about 1000, about 1250, or about. Increase by 1500 times (or any amount between these values). The vaccine may be any clinically utilized or experimental HIV vaccine, including disclosed lentiviral vectors, other viral vectors, or other bacterial vectors used as vaccine delivery systems. .. In another embodiment, the vector encodes a virus-like particle (VLP) to induce a higher titer of neutralizing antibody. In another embodiment, the vector comprises gag, pol, and env, tar, rev, nef, viv, vpr, vpu, and tv, as well as LTR, TAR, RRE, PE, SLIP, CRS, and INS. Encodes a peptide or peptide fragment for HIV, not limited to. Alternatively, 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 /. Alternatively, it may include a biological or chemical adjuvant containing a chemokine.

In one embodiment, the method uses a biological or chemical adjuvant containing purified protein, inactivated virus, viral vectorized protein, bacterial vectorized protein, cytokine and / or chemokine, vehicle, and restimulation method. Includes ex-vivo restimulation of CD4 T cells from individuals or patients previously immunized by therapeutic vaccination. Restimulation in ex vivo may be performed using the same vaccine or immunostimulatory compound used for immunization in vivo, or used for immunization in vivo. It may be done using a different vaccine or immunostimulatory compound. Further in some embodiments, the patient is prior to 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. do not need. In these embodiments, such patients may only require administration of the disclosed viral vectors to achieve functional cure.

In embodiments, peripheral blood mononuclear cells (PBMCs) are obtained by leukocyte depletion and treated with ex vivo to give about 1×10^{10} CD4 T cells, of which about 0.1%, about. Because 1%, about 5%, or about 10%, or about 30% are HIV-specific in terms of antigenic response and have therapeutically introduced genes delivered by the disclosed lentiviral vectors. It is also HIV resistant. Alternatively, about 1×10^{7}, about 1×10^{8}, about 1×10^{9}, about 1×10^{10}, about 1×10^{11}, or about 1×10^{12} CD4 T cells are restimulated. Can be isolated for. Any suitable amount of CD4 T cells is isolated for ex vivo restimulation.

The isolated CD4 T cells can be cultured in the appropriate medium during restimulation with the HIV vaccine antigen, which may contain the antigens present in previous therapeutic vaccinations. Antiretroviral therapeutic agents, including inhibitors of reverse transcriptase, protease, or integrase, may be added to prevent the reappearance of the virus during long-term ex vivo culture. Restimulation of CD4 T cells is used to increase the proportion of HIV-specific CD4 T cells in the culture. The same procedure can also be used for analytical purposes to identify HIV-specific T cells using less blood volume, including peripheral blood mononuclear cells obtained by purification, and to measure the frequency of this subpopulation. ..

The PBMC fraction concentrates HIV-specific CD4 T cells by contacting these cells with HIV proteins that match or complement the components of vaccines previously used for invivo immunization. be able to. Ex vivo restimulation increases the relative frequency of HIV-specific CD4T cells by about 5, about 10, 25, about 50, about 75, about 100, about 125, about 150, about 175, or about 200-fold. I can let you.

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

Thus, in one embodiment, the restimulated PBMC fraction enriched with HIV-specific CD4 T cells is transduced with a therapeutic anti-HIV lentivirus or other vector and is sufficient for such transduction. It can be maintained in culture for a period of time, such as about 1 to about 21 days, up to about 35 days, and so on. Alternatively, these cells may 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 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 can be cultured for 31, about 32, about 33, about 34, or about 35 days.

In a further embodiment, once the transduced cells have been cultured for a sufficient period of time, the transduced CD4 T cells are infused back into the original patient. Injection can be performed using a variety of devices 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 regeneration.

In some embodiments, CCR5 targeted therapy may be added to the subject's antiretroviral therapy regimen that has been continued throughout the treatment process. Examples of therapies targeted to CCR5 include, but are not limited to, maraviroc (CCR5 antagonist) or rapamycin (an immunosuppressant that lowers CCR5). In some embodiments, antiretroviral drug therapy may be discontinued and the subject may be tested for viral rebound. If no rebound occurs, the adjuvant therapy may be removed and the subject can be retested for viral rebound.

In various embodiments, viral suppression persists for approximately 26 weeks with reduced or no antiretroviral drug therapy, including cART or HAART, and with reduced or no adjuvant therapy. If so, it can be regarded as a functional cure for HIV. Other definitions of functional healing are described herein.

The lentivirus vector and other vectors used in the disclosed methods are at least one, at least two, at least three, at least four, or at least five genes, or at least six genes of interest. , Or at least 7 genes, or at least 8 genes, or at least 9 genes, or at least 10 genes, or at least 11 genes, or at least 12 genes. Given the versatility and therapeutic potential of HIV-targeted gene therapy, the viral vectors of the invention are (i) antibodies against toxins produced by antigens associated with infectious diseases or infectious agents, (ii).) Cytokines containing interleukin, (iii) CD8, which are required for the growth or function of immune cells and can be therapeutic agents for immunoregulatory deficiencies encountered in HIV and other chronic or acute human viruses or bacterial pathogens. Factors that suppress HIV growth in vivo, including suppressors, (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) Antisense DNA or RNA against a specific receptor or peptide associated with HIV or a host protein associated with HIV, (vi) Small molecule against a specific receptor or peptide associated with HIV or a host protein associated with HIV It may encode a gene or nucleic acid sequence that includes, but is not limited to, an interfering RNA, or a variety of other therapeutically useful sequences that can be used to treat (vii) HIV or AIDS.

Further examples of HIV-targeted gene therapies that can be used in the disclosed methods are affinity-enhanced T cell receptors, chimeric antigen receptors on CD4 T cells (or alternatives on CD8 T cells). , Modification of signaling pathways to avoid cell death due to viral proteins, HIV replication in TREX, SAMHD1, MxA or MxB proteins, APOBEC complex, TRIM5 alpha complex, tetherin (BST2), and mammalian cells. Increased expression of HIV limiting elements, including, but not limited to, similar proteins identified as being able to be reduced.

In some embodiments, the patient may be receiving cART or HAART at the same time as being treated according to the method of the invention. In other embodiments, the patient may receive cART or HAART before or after being treated according to the methods of the invention. In some embodiments, cART or HAART is maintained throughout the procedure according to the method of the invention and the patient monitors the HIV viral load in the blood and the frequency of CD4 T cells transduced with lentivirus in the blood. Can be. Preferably, a patient who has received cART or HAART before being treated according to the method of the invention can discontinue or reduce cART or HAART after treatment according to the method of the invention.

For efficacy purposes, the frequency of transduced HIV-specific CD4 T cells is a novel alternative marker of the effect of gene therapy and can be determined as discussed in more detail herein.

Composition In various aspects, the present disclosure provides a lentiviral vector capable of delivering a gene construct that inhibits the invasion of HIV into susceptible cells. As an example, one mechanism of action herein is to reduce mRNA levels for CCR5 and / or CXCR4 chemokine receptors in order to reduce the rate of virus entry into susceptible cells.

Alternatively, the disclosed lentiviral vectors can inhibit the formation of HIV-infected cells by reducing the stability of incoming HIV genomic RNA. In yet another embodiment, the disclosed lentiviral vector can prevent HIV production from latently infected cells, the mechanism of action of which is short-chain homologous RNA, small interfering RNA, or other. The action of inhibitory RNA, including regulatory RNA species, causes instability of viral RNA sequences.

The disclosed therapeutic lentiviruses generally contain at least one of two genetic cargoes. First, the lentivirus can encode a genetic element that directs the expression of small RNAs that can inhibit the production of the chemokine receptors CCR5 and / or CXCR4, which are important for the entry of HIV into susceptible cells. The second type of gene cargo targets the RNA sequence of HIV to prevent reverse transcription, RNA splicing, RNA translation that produces proteins, or packaging of viral genomic RNA that produces particles and propagates infection. Contains constructs capable of expressing small RNA molecules. An exemplary structure is illustrated in FIG.

As shown in FIG. 3 (upper panel), an exemplary construct may contain 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 long terminal repeat),

5'LTR (part of HIV-terminal repeat sequence that can be truncated to prevent vector replication after chromosomal integration),
Psi (a packaging signal that allows the integration of the vector RNA genome into viral particles during packaging),
RRE (addition of Rev response element can improve expression from transgene by mobilizing RNA from the nucleus of the cell to the cytoplasm),
CPPT (polypurine tract that promotes second-strand DNA synthesis prior to integration of the transgene into the host cell chromosome),
-Promoters (promoters initiate RNA transcription from integrated transgenes to express microRNA clusters (or other genetic elements of constructs), and in some embodiments the vector uses the EF-1 promoter. Can be),
Anti-CCR5 (microRNA that targets the messenger RNA of the host cell factor CCR5 and reduces its expression on the cell surface),
Anti-Rev / Tat (a microRNA that targets HIV genomic RNA or messenger RNA at the junction between the HIV Rev coding region and the Tat coding region. In this application, it is referred to as miRNA Tat or similar. An explanation may be given),
Anti-Vif (microRNA targeting HIV genomic RNA or messenger RNA within the Vif coding region),
WPRE (Woodchuck hepatitis virus post-transcriptional regulatory element is an additional vector component that can be used to promote nuclear RNA transport), and
Delta U3 3'LTR (U3 to improve vector safety). Modified version of the 3'end repeat sequence of HIV, with a portion of the region deleted).

The above components are merely examples, and as long as the construct can prevent the expression of the HIV gene and reduce the transmission of infection, such components can be reorganized or replaced with other elements. It will be appreciated by those skilled in the art that it may be changed, or otherwise.

The vectors of the invention are gene cargoes of either or both of the above types (ie, gene elements that direct gene expression, or small RNAs such as siRNA, shRNA, or miRNA that can prevent translation or transcription.) May be included, and the vector of the present invention may encode a product that is more useful for the purpose of treating or diagnosing HIV. As an example, in some embodiments, these vectors are 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 code it.

The combination of genetic elements incorporated into the disclosed vector is not particularly limited. For example, the vectors herein are single small RNA, 2 small RNA, 3 small RNA, 4 small RNA, 5 small RNA, 6 small RNA. , 7 small RNAs, 8 small RNAs, 9 small RNAs, or 10 small RNAs, or 11 small RNAs, or 12 small RNAs may be encoded. .. Such vectors may further encode other genetic elements to function in concert with small RNA to prevent HIV expression and infection.

It will be appreciated by those skilled in the art that therapeutic lentiviruses can use alternative sequences in place of promoter regions, regulatory RNA targeting, and regulatory RNA types. In addition, the therapeutic lentiviruses of the present disclosure may contain changes in the plasmid used to package the lentivirus particles, which are required to increase in vitro production levels. NS.

Lentiviral Vector Systems Lentiviral virions (particles) according to the various aspects and embodiments herein are expressed by vector systems that encode the viral proteins required to produce virions (viral particles). In various embodiments, one vector containing a nucleic acid sequence encoding a lentiviral pol protein, operably linked to a promoter, is provided for reverse transcription and integration. In another embodiment, the pol protein is expressed by multiple vectors. In another embodiment, a vector containing a nucleic acid sequence encoding a lentiviral Gag protein, operably linked to a promoter for forming a viral capsid, is provided. In embodiments, the gag nucleic acid sequence is on a vector separate from at least a portion of the pol nucleic acid sequence. In other embodiments, the gag nucleic acid is on a vector separate from all pol nucleic acid sequences encoding the pol protein.

The vectors herein may undergo a number of modifications, which can be used to create particles that further minimize the chances of obtaining wild-type reversion variants. These include, but are not limited to, deletions of the U3 region of the LTR, tat deletions, and matrix (MA) deletions. In embodiments, the gag, pol, and envelope vectors do not contain nucleotides from the lentiviral genome that package the lentiviral RNA, called lentiviral packaging sequences.

The vector forming the particles preferably does not contain a nucleic acid sequence derived from the lentiviral genome expressing the enveloped protein. Preferably, a separate vector containing a nucleic acid sequence encoding an enveloped protein operably linked to the promoter is used. This env vector also does not contain a lentivirus packaging sequence. In one embodiment, the env nucleic acid sequence encodes a lentiviral envelope protein.

In another embodiment, the enveloped protein is not derived from lentivirus, but from a different virus. The resulting particles are called pseudotyped particles. With proper selection of envelopes, virtually any cell can be "infected". For example, influenza virus, VSV-G, alpha virus (Semriki forest virus, Sindbis virus), arenavirus (lymphocytic choroiditis virus), flavivirus (tick-mediated encephalitis virus, dengue virus, hepatitis C virus), Env gene encoding envelope proteins that target intracellular compartments such as those of GB virus), rabdovirus (bulbric stomatitis virus, mad dog disease virus), paramixovirus (mumps or measles), and orthomixovirus (influenza virus). Can be used. Other envelopes that can preferably be used include those derived from Moloney leukemia virus such as MLV-E, MLV-A, and GALV. These latter

envelopes are particularly preferred when the host cell is a primary cell. Other enveloped proteins may be selected depending on the desired host cell. For example, targeting of specific receptors, such as dopamine receptors, can be used for delivery to the brain. Another target can be the vascular endothelium. These cells can be targeted using the filovirus envelope. For example, GP of Ebola become GP by post-transcriptional modification, and GP $_2$ glycoprotein. In another embodiment, different lentiviral capsids with a pseudotyped envelope (eg, FIV or SHIV [US Pat. No. 5,654,195]) can be used. The SHIV pseudotyped vector can be readily used in animal models such as monkeys.

The lentiviral vector system provided herein typically comprises at least one helper plasmid containing at least one of the gag, pol, or rev genes. The gag, pol, and rev genes may each be provided in individual plasmids, or one or more genes may be provided together in the same plasmid. In one embodiment, the gag, pol, and rev genes are provided in the same plasmid (eg, FIG. 4). In another embodiment, the gag and pol genes are provided in the first plasmid and the rev gene is provided in the second plasmid (eg, FIG. 5). Therefore, both 3-vector and 4-vector systems can be used to produce the lentiviruses described herein. In embodiments, the therapeutic vector, at least one enveloped plasmid, and at least one helper plasmid are transfected into a packaging cell, eg, a packaging cell line. A non-limiting example of a packaging cell line is the 293T / 17 HEK cell line. When the therapeutic vector, envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, lentiviral particles are finally produced.

In another aspect, a lentiviral vector system for expressing lentiviral particles is disclosed. This system includes the lentiviral vectors described herein; envelope plasmids for expressing envelope proteins optimized to infect cells; at least for expressing gag, pol, and rev genes. When a lentiviral vector, an enveloped plasmid, and at least one helper plasmid are transfected into a packaging cell line, including one helper plasmid, lentiviral particles are produced by the packaging cell line, and the lentiviral particles are produced. , Inhibition of the production of the chemokine receptor CCR5, or targeting of the HIV RNA sequence is possible.

In another aspect, the lentiviral vector, also referred to herein as a therapeutic vector, is the next element: hybrid 5'end repeat sequence (RSV / 5'LTR) (SEQ ID NOs: 34-35), Psi sequence (RNA packaging). Site) (SEQ ID NO: 36), RRE (Rev response element) (SEQ ID NO: 37), cPPT (polyprint lacto) (SEQ ID NO: 38), EF-1α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2), miR185Tat (SEQ ID NO: 3), woodchuck post-transfer regulatory element (WPRE) (SEQ ID NO: 32 or 80), and ΔU3 3'LTR (SEQ ID NO: 39). In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referenced herein.

In another embodiment, the helper plasmid has the following elements: CAG promoter (SEQ ID NO: 41), HIV component gag (SEQ ID NO: 43), HIV component pol (SEQ ID NO: 44), HIV Int (SEQ ID NO: 45), HIV RRE (SEQ ID NO: 45). SEQ ID NO: 46), and HIV Rev (SEQ ID NO: 47). In another embodiment, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes and a second separate plasmid for expressing the rev gene. In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referenced herein.

In another embodiment, the envelope plasmid comprises the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62). In another aspect, sequence changes due to substitutions, deletions, additions, or mutations can be used to modify the sequences referenced herein.

In various embodiments, the plasmid used for lentivirus packaging is modified by substitution, addition, removal, or mutation of various elements without loss of vector function. For example, but not limited to, the following elements can replace similar elements in the plasmids that make up the packaging system: Elongation Factor 1 (EF-1), Phosphoglycerate Kinase (PGK). , And the ubiquitin C (UbC) promoter can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace rabbit beta globin poly A. The HIV sequence in the helper plasmid may be constructed from different HIV strains or clades. VSV-G glycoproteins include feline endogenous virus (RD114), tenagazal leukemia virus (GALV), mad dog disease (FUG), lymphocytic choriomyelitis virus (LCMV), influenza A poultry virus (fowl plague virus) (It can be replaced with a membrane glycoprotein of FPV), Ross River alpha virus (RRV), mouse leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Various lentivirus packaging systems can be obtained commercially (eg, OriGene Technologies, Inc., Rockville, MD's Lenti-vpak packaging kit) and can also be designed as described herein. can. Moreover, it is within the skill of one of ordinary skill in the art to replace or modify aspects of the lentiviral packaging system to improve any number of relevant factors, including the efficiency of lentiviral particle production.

Bioassay In various aspects, the invention comprises a bioassay for determining the success of HIV treatment to achieve functional cure. These assays provide a method for measuring the effectiveness of the disclosed immunization and treatment methods by measuring the frequency of transduced HIV-specific CD4 T cells in patients. HIV-specific CD4 T cells are recognizable. Because, among others, they proliferate, alter the composition of cell surface markers, induce signaling pathways including

phosphorylation, and / or / or cytokines, chemokines, caspases, phosphorylation signaling molecules, or other cytoplasms and / Or because it expresses a specific marker protein that can be a nuclear component. Certain responding CD4 T cells allow for the sorting of HIV-specific cells using, for example, flow cytometry sorting, magnetic bead separation, or other recognized methods of antigen-specific CD4 T cell isolation. Recognized using specific in-situ amplification of the labeled monoclonal antibody or mRNA sequence. Testing isolated CD4 T cells determines the frequency of cells carrying the integrated therapeutic lentivirus. Single-cell testing methods involving microfluidic separation of individual cells combined with mass spectrometry, PCR, ELISA or antibody staining can also be used to confirm responsiveness to HIV and the presence of integrated therapeutic lentivirus. can.

Thus, in various embodiments, the application of the treatment according to the invention (eg, (a) immunization, (b) culture of leukocytes / lymphocytes in ex vivo, (c) purified protein, inactivated virus, viral vectorization). Assaying patients following (d) injection of enriched transfected T cells) with biological or chemical adjuvants containing proteins, bacterial vectorized proteins, cytokines and / or chemokines, restimulation with vehicles. Allows the effectiveness of the procedure to be determined. Target T cell thresholds in the body have been established to measure functional healing at predetermined values, eg, in about 1×10^{8} HIV-specific CD4 T cells with genetic modification from a therapeutic lentivirus. good. Alternatively, this threshold is about 1×10^{5}, about 1×10^{6}, about 1×10^{7}, about 1×10^{8}, about 1×10^{9}, or about 1×10^{10} in the patient's body. It may be a CD4 T cell.

HIV-specific CD4 T cells with genetic modification by therapeutic lentivirus include flow cytometry, cell sorting, FACS analysis, DNA cloning, PCR, RT-PCR or Q-PCR, ELISA, FISH, Western blotting, Southern blotting, It can be determined using any suitable method, such as, but not limited to, high throughput sequencing, RNA sequencing, oligonucleotide primer extension, or other methods known in the art.

Methods for defining antigen-specific T cells with genetic alterations are known in the art, but such methods are utilized and integrated or non-integrated gene therapy as a standard measure of efficacy. Combining the identification of HIV-specific T cells containing constructs of is a novel concept in the field of HIV treatment, as variously described herein.

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

In various embodiments, the HIV-specific vaccine for initial in vivo immunization is administered to the subject in need at various doses. Generally, vaccines delivered by intramuscular injection are either whole viral proteins prepared from inactivated viral particles, virus-like particles, or viral proteins purified from recombinant systems or purified from viral preparations. It comprises 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. Recombinant viral or bacterial vectors may be administered by any of the described routes. The intramuscular vaccine contains suitable adjuvant molecules of 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 per injection dose. Suspended in 0.1-5 ml volumes of oil, saline, buffer, or water, it may be a soluble or emulsion preparation. Higher doses of vaccines delivered orally, rectal, oral, genital mucosa, or intranasal, including some viral or bacterial vectorized vaccines, fusion proteins, liposome formulations, or similar preparations. It may contain viral proteins and adjuvants. Dermal, sub-dermal, or subcutaneous vaccines utilize amounts of proteins and adjuvants that are more similar to vaccines delivered orally, rectally, or intranasally. Depending on the response to the initial immunization, vaccination may be repeated 1-5 times using the same or alternative delivery routes. The interval can be 2 to 24 weeks between immunizations. 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 methodologies. .. The cell-mediated immune response follows in vitro stimulation with a vaccine antigen, followed by staining for intracellular cytokine accumulation, followed by flow cytometry or lymphocyte proliferation, expression of phosphorylation signaling proteins, or cell surface activation. Tested by a similar method involving changes in markers. Dosing limits may be determined on an individual patient basis and will depend on the toxicity / safety profile for each individual product or product lot.

Immunization may be performed once, twice, three times, or repeatedly. As an example, the drug for HIV immunization is given to the subject in need once a week, once every week, once every three weeks, once a month, every other month. Administered every 3 months, every 6 months, every 9 months, once a year, every 18 months, every 2 years, every 36 months, or every 3 years. It's okay.

Immunization is generally performed at least once prior to the expansion and enrichment and enrichment of CD4 T cells in exvivo, and immunization is performed once or twice after culture / restimulation and infusion of leukocytes / lymphocytes in exvivo. It can be done three times or more.

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

HIV vaccine compositions intended for immunization can be prepared by any pharmaceutically acceptable method, such as intranasal administration, oral administration, sublingual administration, oral administration, rectal administration, ocular administration, parenteral administration (intravenous administration). Administration, intradermal administration, intramuscular administration, subcutaneous administration, intratubal administration, intraperitoneal administration, lung administration, intravaginal administration, local administration, external administration, external administration after random incision, mucosal administration, by aerosol, or It can be administered using oral or nasal spray formulation.

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

In another embodiment, the pharmaceutical composition comprising the HIV vaccine is formulated in a solid dosage form for oral administration, which solid dosage form can be powder, granules, capsules, tablets, or pills. In yet another embodiment, the solid dosage form comprises one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose, or gelatin. In addition, the solid dosage form may include a lubricant such as talc or magnesium stearate in addition to the excipient. In some embodiments, the oral dosage form is an immediate release form or a modified release form. Modified release dosage forms include controlled or extended 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 is formulated as a sublingual or oral dosage form. Such dosage forms include sublingual tablets or solution compositions administered sublingually, and oral tablets placed between the cheek and gingiva.

Still in a further embodiment, the pharmaceutical composition comprising the HIV vaccine is 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 is formulated in liquid dosage form for oral administration, such as suspension, emulsion, or syrup. In other embodiments, the liquid dosage form is a commonly used simple diluent such as water and liquid paraffin, as well as a humectant, sweetener, aromatic compound, or preservative. It may contain a variety of excipients. In certain embodiments, a composition comprising an HIV vaccine or a pharmaceutically acceptable salt thereof is formulated to be suitable for administration to a pediatric patient.

In one embodiment, the pharmaceutical composition is formulated in a dosage form for parenteral administration, such as a sterile aqueous solution, suspension, emulsion, non-aqueous solution, or suppository. In other embodiments, the non-aqueous solution or suspension comprises a vegetable oil such as propylene glycol, polyethylene glycol, olive oil, or an injectable ester such as ethyl oleate. As the base of the suppository, witepsol, macrogol, Tween 61, cocoa butter, lauric oil, or glycerin gelatin can be used.

The dosage of the pharmaceutical composition may vary depending on the patient's weight, age, gender, dosing time and method, excretion rate, and severity of the disease.

For restimulation purposes, generally, 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 HIV vaccine or activators of cells per approximately 10Ng ~ 5myug (or any other suitable amount) in the culture. More specifically, isolated cells, about 10^6 cells per approximately 50ng in culture, about 100 ng, about 200 ng, about 300 ng, about 400 ng, about 500 ng, about 600 ng, about 700 ng, about 800 ng, Can be contacted with 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. good.

The activator or vaccine is generally used once in each in vitro cell culture, but may be repeated after an interval of about 15-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 can be done on 29, about 30, about 31, about 32, about 33, about 34, or about 35 days.

For transduction of enriched and restimulated cells, for example, the lentiviral vector disclosed in FIG. 4 or other known vector system may be transduced into the cell. Transduced cells are measured by RT-PCR assay of about 1-1,000 (or any other suitable amount) of viral genomes (or any other suitable amount) of virus genome per target cell in culture (culture medium containing lentiviral vector).) May be contacted. Lentiviral transduction may be repeated 1-5 times using the same range of viral genomes of 1-1,000 per target cell in culture.

Cell Concentration In various embodiments, cells, such as T cells, are obtained from HIV-infected patients and cultured. Culturing can be performed on a multi-well plate in culture medium containing conditioned medium ("CM"). Superficial p24 ^{gag} levels ("p24") and viral RNA levels can be assessed by standard means. Patients with CM cultured cells having a peak of supernatant p24 level below 1 ng / ml may be suitable patients for large-scale T cell expansion in CM with or without the use of additional antiviral agents. In addition, different drugs or combinations of drugs of interest may be added to different wells and the effect on viral levels in the sample can be assessed by standard means. Combinations of drugs that provide adequate viral suppression are therapeutically useful combinations. It is within the competence of a qualified technician to determine what constitutes moderate viral suppression for a particular subject. To test the efficacy of the drug of interest in limiting the spread of the virus, additional factors such as anti-CD3 antibodies may be added to the culture to stimulate virus production. Unlike the methods of culturing HIV-infected cell samples known in the art, CM allows T cells to be cultivated for a period of more than 2 months, which provides an effective system for assaying long-term drug efficacy. Brought to you.

This approach allows the inhibition of gene expression driven by the HIV LTR promoter region within the cell population by culturing cells in media containing CM. Cultures in CM4 are likely to inhibit HIV LTR-driven gene expression by altering one or more interactions between transcription-mediated proteins and HIV gene expression regulatory elements. Transcription-mediated proteins of interest include host cell-encoded proteins such as AP-1, NF-Kappa B, NF-AT, IRF, LEF-1, and Sp1, as well as the HIV-encoded protein Tat. The HIV gene expression regulatory element of interest comprises a binding site for AP-1, NF Kappa B, NF-AT, IRF, LEF-1, and Sp1 as well as a trans-acting response element ("TAR") that interacts with Tat. ..

In a preferred embodiment, HIV infected cells are obtained from a subject having a sensitive transcription-mediated protein sequence and a sensitive HIV regulatory element sequence. In a more preferred embodiment, HIV infected cells are obtained from a subject having a wild-type transcription-mediated protein sequence and a wild-type HIV regulatory sequence.

Another method of enriching T cells utilizes immune affinity-based selection. The method comprises simultaneous enrichment or selection of first and second cell populations, such as CD4 + and CD8 + cell populations. The cells containing the primary human T cells are, in the incubation composition, a first immunoaffinity reagent that specifically binds to CD4 and a second immunoaffinity reagent that specifically binds to CD8, and their immunity. The affinity reagent is contacted on the cell surface in the sample under conditions that specifically bind to CD4 and CD8 molecules, respectively. Cells bound to the first and / or second immunoaffinity reagent are recovered, resulting in a concentrated composition comprising CD4 + cells and CD8 + cells. This approach may include incubation of the composition with suboptimal yield concentrations of the first and / or second immunoaffinity reagents. Notably, in some embodiments, the transduced cells are a mixed T cell population, and in other embodiments, the transduced cells are not a mixed T cell population. ..

In some embodiments, immunoaffinity-based selection is used in which the solid support is a sphere, eg beads, eg microbeads or nanobeads. In other embodiments, the beads may be magnetic beads. In another embodiment, the antibody comprises one or more binding partners capable of forming a reversible bond with a binding reagent immobilized on a solid surface such as a sphere or a chromatographic matrix, and the antibody is a solid. Reversibly mobilized to the surface. In some embodiments, cells expressing the antibody-bound cell surface marker on the solid surface can be recovered from the matrix by breaking the reversible binding between the binding reagent and the binding partner. In some embodiments, the binding reagent is streptavidin, or a streptavidin analog or variant.

Stable transduction of primary cells of hematopoietic and / or hematopoietic stem cells is obtained by in vitro or ex vivo contact of the cell surface with both the lentiviral vector and at least one molecule that binds to the cell surface. be able to. These cells may be cultured in an aerated container with two or more layers under conditions that promote growth and / or proliferation. In some embodiments, this approach can be used in conjunction with depletion of non-CD4 + T cells and / or wide polyclonal proliferation.

Another approach to enriching T cells is to stimulate PBMCs with peptides to concentrate cells that secrete cytokines such as interferon gamma. This approach generally involves stimulating a mixture of cells containing T cells with the antigen and separating the antigen-stimulated cells according to the extent to which they are labeled with the product. Antigen stimulation is achieved by exposing the cells to at least one antigen under conditions effective in inducing antigen-specific stimulation of at least one T cell. Labeling with the product modifies the surface of the cell to contain at least one capture portion, the product is secreted and released, and specifically binds to the capture portion ("captured" or "captured" or ". Achieved by culturing the cells under "entrapped") conditions and labeling the captured product with a labeled moiety, the labeled cells are part of the labeling procedure as well as part of the isolation procedure. Is not dissolved. The capture moiety may incorporate detection of the cell surface glycoprotein CD3 or CD4 in order to refine the enrichment step and increase the proportion of antigen-specific T cells in general, especially CD4 + T cells.

The following examples are presented to illustrate aspects of 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 referred to herein are expressly incorporated by reference.

(Example 1: Development of lentiviral vector system)
A lentiviral vector system was developed as summarized in FIG. 3 (linear form) and FIG. 4 (cyclic form). First, referring to the upper part of FIG. 3, from left to right, the following elements: hybrid 5'end repeat sequence (RSV / 5'LTR) (SEQ ID NOs: 34 to 35), Psi sequence (RNA packaging site) (SEQ ID NO:). 36), RRE (Rev response element) (SEQ ID NO: 37), cPPT (polyprint lacto) (SEQ ID NO: 38), EF-1α promoter (SEQ ID NO: 4), miR30CCR5 (SEQ ID NO: 1), miR21Vif (SEQ ID NO: 2). , MiR185Tat (SEQ ID NO: 3), Woodchuck post-transfer regulatory element (WPRE) (SEQ ID NO: 32 or 80), and ΔU3 3'LTR (SEQ ID NO: 39). The therapeutic vector detailed in FIG. 3 is also referred to herein as AGT103.

Next, referring to the central part of FIG. 3, from left to right, the following elements: CAG promoter (SEQ ID NO: 41), HIV component gag (SEQ ID NO: 43), HIV component pol (SEQ ID NO: 44), HIV Int (SEQ ID NO: 44). A helper plasmid with No. 45), HIV RRE (SEQ ID NO: 46), and HIV Rev (SEQ ID NO: 47) was designed and produced.

Next, referring to the bottom of FIG. 3, from left to right, the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 60) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 62). The enveloped plasmid to have was designed and produced.

After transfecting therapeutic vectors, envelope plasmids, and helper plasmids (shown in FIG. 3) in 293T / 17 HEK cells (purchased from American Type Culture Collection, Manassas, VA), lentivirus particles were produced. For transfection of 293T / 17 HEK cells that produced functional viral particles, the reagent poly (ethyleneimine) (PEI) was used to increase the efficiency of plasmid DNA uptake. The plasmid and DNA were first added separately to serum-free culture medium in a 3: 1 ratio (mass ratio of PEI to DNA). After 2-3 days, cell culture medium was collected and lentiviral particles were purified by performing anion exchange chromatography after high speed centrifugation and / or filtration. The concentration of lentivirus particles can be expressed in terms of transduction units / ml (TU / ml). The TU determination is made by measuring HIV p24 levels in the culture (p24 protein is incorporated into lentivirus particles) and by quantitative PCR or by infecting cells and using light (vectors are luciferase or (When encoding a fluorescent protein marker), achieved by measuring the number of viral DNA copies per cell.

As mentioned above, a three-vector system (ie, a two-vector lentivirus packaging system) for the production of lentivirus particles was designed. The outline of this three-vector system is shown in FIG. The outline of FIG. 4 is a circularized version of the linear system previously described in FIG. Briefly with reference to FIG. 4, the top vector is, in this case, a helper plasmid containing Rev. The vector in the center of FIG. 4 is an enveloped plasmid. The bottom vector is the therapeutic vector described so far.

More specifically, referring to FIG. 4, the helper + Rev plasmids are CAG enhancer (SEQ ID NO: 40), CAG promoter (SEQ ID NO: 41), chicken beta actinintron (SEQ ID NO: 42), HIV gag (SEQ ID NO: 43), HIV. Includes Pol (SEQ ID NO: 44), HIV Int (SEQ ID NO: 45), HIV RRE (SEQ ID NO: 46), HIV Rev (SEQ ID NO: 47), and Rabbit Betaglobin Poly A (SEQ ID NO: 48).

Envelope plasmids include the CMV promoter (SEQ ID NO: 60), betaglobin intron (SEQ ID NO: 61), VSV-G (SEQ ID NO: 62), and rabbit betaglobin poly A (SEQ ID NO: 63).

Synthesis of a two-vector lentivirus packaging system containing helper (+ Rev) and enveloped plasmid.
material and method:
Construction of helper plasmids: Helper plasmids were constructed by initial PCR amplification of DNA fragments from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing the Gag, Pol, and integrase genes. Primers were designed to amplify fragments with EcoRI and NotI restriction sites that could be used for insertion into the same site of the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTC ATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 81) and the reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 82). The sequences of Gag, Pol and integrase fragments were as follows.
GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAG

GTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAG
CCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT
GAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAAT
TAGGAATACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTAC
CATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCT
TTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGT
TGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAA
AGGACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAA
GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACA
GGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCAT
TTAAAAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAA
AGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGA
AGTTATGGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGTAACTGACAGAGGAA
GACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATATG
CATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAAG
GAATTGGAGGAAATGAACAAGTAGATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGA
GAGCAATGGCTAGTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA
TAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGC
CAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGCAG
CAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAATT
AAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG
GGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAA
AGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGA
TGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (SEQ ID NO: 83)

Next, DNA fragments containing Rev, RRE, and rabbit beta-globin poly A sequences with XbaI and XmaI flanking restriction sites were synthesized by the MWG Operon. This DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites. The DNA sequence was as follows.
TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGC
CCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTAC
CACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTA
AAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCA
GCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATC
AACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT
TTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGG
TGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCT
TTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACT
ACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCA
CACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGC
CAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTT
TATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATG
GTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG (SEQ ID NO: 84)

Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG enhancer / promoter + chicken beta actin intron sequence. DNA fragments containing CAG enhancer / promoter / intron sequences with MluI and EcoRI flanking sites were synthesized by MWG Operon. This DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows.
ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC

CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT
ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
ATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGG
GGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGT
TTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCG
CCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCT
GCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGG
CGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC
CGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACC
CCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGG
GGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTT
TATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCG
CCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACG
GGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC (SEQ ID NO: 85)

Construction of VSV-G envelope plasmid:
Bullous stomatitis Indiana viral glycoprotein (VSV-G) sequences with adjacent EcoRI restriction sites were synthesized by the MWG Operon. This DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and sequenced with CMV-specific primers to determine the correct orientation. The DNA sequence was as follows.
GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAATTACC
ATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGC
TTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAACGAAA
CAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATAC
ACAGGAGAATGGGTTGATTCACAGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTACAACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATT
CTAACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGG
CCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGT
CAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCC
AATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCCTGCTT
TCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACTACCACAGAAAGGGA
ACTGTGGGATGACTGGGCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACATGGTATGTTGGACTCC
GATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATC
CAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGC
ATTAAATTAAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC (SEQ ID NO: 86)

A 4-vector system (ie, a 3-vector lentivirus packaging system) was also designed and produced using the methods and materials described herein. The outline of this 4-vector system is shown in FIG. Briefly with reference to FIG. 5, the topmost vector is, in this case, a Rev-free helper plasmid. The second vector from the top is a separate Rev plasmid. The second vector from the bottom is the envelope plasmid. The bottom vector is the therapeutic vector described so far.

With reference to FIG. 5 in part, helper plasmids include CAG enhancer (SEQ ID NO: 49), CAG promoter (SEQ ID NO: 50), chicken beta actinintron (SEQ ID NO: 51), HIV gag (SEQ ID NO: 52), HIV Pol (SEQ ID NO: 52). SEQ ID NO: 53), HIV Int (SEQ ID NO: 54), HIV RRE (SEQ ID NO: 55), and Rabbit Betaglobinpoly A (SEQ ID NO: 56).

The Rev plasmid contains the RSV promoter (SEQ ID NO: 57), HIV Rev (SEQ ID NO: 58), and rabbit beta globinpoly A (SEQ ID NO: 59).

Envelope plasmids include the CMV promoter (SEQ ID NO: 60), betaglobin intron (SEQ ID NO: 61), VSV-G (SEQ ID NO: 62), and rabbit betaglobin poly A (SEQ ID NO: 63).

Synthesis of a 3-vector lentivirus packaging system containing helpers, Rev, and enveloped plasmids.
material and method:
Construction of Rev-free helper plasmid:

A Rev-free helper plasmid was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequences. This sequence with adjacent XbaI and XmaI restriction sites was synthesized by MWG Operon. The RRE / rabbit polyA beta globin sequence was then inserted into a helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows.
TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGC
AGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA
CAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTT
TTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTG
GCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTT
AACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGC
TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCT
AACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCAT
CCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT
TTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATA
ATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCACCCGGG (SEQ ID NO: 87)

Construction of Rev plasmid:
The RSV promoter and HIV Rev sequences with flanking MfeI and XbaI restriction sites were synthesized by the MWG Operon as a single DNA fragment. This DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites where the CMV promoter has been replaced by the RSV promoter. The DNA sequence was as follows.
CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCG
CTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGA
TTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTA
GCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTC
CATAGAAGACACCGGGACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATC
AAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG
GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGT
GGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA (SEQ ID NO: 88)

The plasmids for the two-vector and three-vector packaging systems could be modified with similar elements and the intron sequence could be removed without loss of vector function. For example, the following elements could replace similar elements in 2-vector and 3-vector packaging systems.

Promoters: Elongation Factor 1 (EF-1) (SEQ ID NO: 64), Phosphoglycerate Kinase (PGK) (SEQ ID NO: 65), and Ubiquitin C (UbC) (SEQ ID NO: 66) are CMV (SEQ ID NO: 60) or CAG. It can replace the promoter (SEQ ID NO: 100). These sequences can also be further altered by additions, substitutions, deletions, or mutations.

Poly A sequence: SV40 poly A (SEQ ID NO: 67) and bGH poly A (SEQ ID NO: 68) can replace rabbit beta globin poly A (SEQ ID NO: 48). These sequences can also be further altered by additions, substitutions, deletions, or mutations.

HIV Gag, Pol, and Integrase Sequences: The HIV sequences in the helper plasmid may be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 69), HIV Pol (SEQ ID NO: 70), and HIV Int (SEQ ID NO: 71) from the Bal strain are included in the helper / helper + Rev plasmids outlined herein. It can be exchanged for plasmids and int sequences. These sequences can also be further altered by additions, substitutions, deletions, or mutations.

Envelope: VSV-G glycoproteins include feline endogenous virus (RD114) (SEQ ID NO: 72), tenagazal leukemia virus (GALV) (SEQ ID NO: 73), mad dog disease (FUG) (SEQ ID NO: 74), lymphocytic choroidal medulla. Flame virus (LCMV) (SEQ ID NO: 75), Influenza A poultry pestovirus (FPV) (SEQ ID NO: 76), Ross River alpha virus (RRV) (SEQ ID NO: 77), Murine leukemia virus 10A1 (MLV) (SEQ ID NO: 78) , Or can be replaced with a membrane sugar protein from Ebola virus (EboV) (SEQ ID NO: 79). The arrangement of these envelopes is specified in parts of the arrangement herein. In addition, these sequences can be further altered by additions, substitutions, deletions, or mutations.

In summary, the 3-vector vs. 4-vector systems can be partially compared and contrasted as follows. The three-vector lentiviral vector system is 1. Helper plasmids: HIV Gag, Pol, integrase, and Rev / Tat; 2. Envelope plasmid: VSV-G / FUG envelope; as well as 3. Therapeutic Vectors: Contains RSV 5'LTR, Psi Packaging Signal, Gag Fragment, RRE, Env Fragment, cPPT, WPRE, and 3'Delta LTR. The four-vector lentiviral vector system is 1. Helper plasmids: HIV Gag, Pol, and integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G / FUG envelope; and 4. Therapeutic Vectors: Contains RSV 5'LTR, Psi Packaging Signal, Gag Fragment, RRE, Env Fragment, cPPT, WPRE, and 3'Delta LTR. The sequences corresponding to the above elements are specified in parts of the sequence listing herein.

(Example 2: Development of anti-HIV lentiviral vector)
The purpose of this example was to develop an anti-HIV lentiviral vector.

Inhibitory RNA design. The mRNA sequence of Homo sapiens chemokine C-C motif receptor 5 (CCR5) (GC03P046377) was used to search for potential siRNA or shRNA candidates that knock down CCR5 levels in human cells. Potential RNA interference sequences were selected from candidates selected by the Broad Institute program or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-iT RNAi Designer. The individual selected shRNA sequences were inserted immediately 3'to the RNA polymerase III promoter of the lentiviral vector, eg, H1, U6, or 7SK, to regulate shRNA expression. These lentivirus-SHRNA constructs were used for cell transduction and specific changes in mRNA levels were measured. The most potent shRNAs were individually embedded within the microRNA backbone to reduce mRNA levels, allowing expression by either the CMV or the EF-1 alpha RNA polymerase II promoter. The microRNA backbone is mirbase. Selected from 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 human immunodeficiency virus type 1 Bal strain (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, this search focused on regions of the Tat and Vif genes of HIV, but to those of skill in the art, the use of these regions is non-limiting and other potential targets. It will be understood that can be selected. Importantly, highly conserved regions of the gag or pol gene could not be targeted by shRNA because the same sequences were present on the packaging complementary plasmids required for vector production. Similar to CCR5 (NM 000579.3, NM 001100168.1) RNA, Gene-E Software Suite (broadinstation.org/mai/Public) or Thermo Scientific (Thermo Design) sponsored by the Broad Institute. Potential HIV-specific RNA interference sequences selected from siRNA or candidates selected by the shRNA design program, such as rnadesigner.thermovisher.com/rnaiexpress.do?designOption=shrna&pid=6712627360670618001). The individual selected shRNA sequences were inserted immediately 3'to the RNA polymerase III promoter of the lentiviral vector, eg, H1, U6, or 7SK, to regulate shRNA expression. These lentivirus-SHRNA constructs were used for cell transduction and specific changes in mRNA levels were measured. The most potent shRNAs were individually embedded within the microRNA backbone to reduce mRNA levels, allowing expression by either the CMV or the EF-1 alpha RNA polymerase II promoter.

Construction of vector. Oligonucleotide sequences containing BamHI and EcoRI restriction sites for CCR5, Tat, or Vif shRNA were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI at 37 degrees Celsius 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 concentration was determined, vector and oligo (3: 1 ratio) were mixed, annealed and ligated. The ligation reaction was carried out at room temperature for 30 minutes using T4 DNA ligase. A 2.5 microliter ligation mix was added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin, drug-resistant colonies (indicating the presence of ampicillin-resistant plasmids) were harvested, purified and expanded in LB broth. To examine the insertion of oligo sequences, plasmid DNA was extracted from the collected cell cultures using the Invitrogen DNA miniprep kit. DNA sequencing using promoter-specific primers used to regulate shRNA expression validated insertion of the shRNA sequence into the lentiviral vector. An exemplary vector sequence determined to limit HIV replication can be found in FIG. For example, the shRNA sequence most active against CCR5, Tat, or Vif gene expression was then assembled into microRNA (miR) clusters under the control of the EF-1alpha promoter. The promoter and miR sequence are shown in FIG.

In addition, using standard molecular biology techniques (eg, Sambrook; Molecular Cloning: A Laboratory Manual, 4th Edition) and the techniques described herein, as shown in FIG. 7 herein. , Developed a series of lentiviral vectors.

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

Vector 2 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); H1 element (SEQ ID NO: 101); shRev / Tat (SEQ ID NO: 10); H1 element (SEQ ID NO: 101).); ShCCR5 (SEQ ID NO: 16, 18, 20, 22, or 24); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NO: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102). do.

Vector 3 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); H1 element (SEQ ID NO: 101); shGag (SEQ ID NO: 12); H1 element (SEQ ID NO: 101); It contains shCCR5 (SEQ ID NO: 16, 18, 20, 22, or 24); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NO: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102).

Vector 4 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); 7SK element (SEQ ID NO: 103); shRev / Tat (SEQ ID NO: 10); H1 element (SEQ ID NO: 101).); ShCCR5 (SEQ ID NO: 16, 18, 20, 22, or 24); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NO: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102). do.

Vector 5 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat. (SEQ ID NO: 3); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NOs: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102).

Vector 6 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR155Tat. (SEQ ID NO: 104); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NOs: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102).

Vector 7 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); EF1 element (SEQ ID NO: 4); miR30CCR5 (SEQ ID NO: 1); MiR21Vif (SEQ ID NO: 2); miR185Tat. (SEQ ID NO: 3); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NOs: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102).

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

Vector 9 was developed, from left to right, the terminal repeat sequence (LTR) portion (SEQ ID NO: 35); CD4 element (SEQ ID NO: 30); miR30CCR5 (SEQ ID NO: 1); miR21Vif (SEQ ID NO: 2); miR185Tat. (SEQ ID NO: 3); post-transcriptional regulatory element of Woodchuck hepatitis virus (WPRE) (SEQ ID NOs: 32, 80); and terminal repeat sequence portion (SEQ ID NO: 102).

Vector Development It should be noted that not all of the vectors developed for these experiments worked as expected. More specifically, lentiviral vectors against HIV are: 1) inhibitory RNA that lowers the level of HIV-binding protein (receptor) on the surface of the target cell to block early viral attachment and invasion, and 2) virus. Three major key factors are overexpression of the HIV TAR sequence, which sequesters the Tat protein and reduces its ability to transactivate viral gene expression, and 3) inhibitory RNA that attacks important conserved sequences within the HIV genome. May contain ingredients.

With respect to the first point above, an important cell surface HIV binding protein is the chemokine receptor CCR5. HIV particles attach to susceptible T cells by binding to CD4 and CCR5 cell surface proteins. Since CD4 is an essential glycoprotein on the cell surface that is important for the immunological function of T cells, it was not selected as a target for manipulating its expression level. However, people born as homozygotes for null mutations in the CCR5 gene and who are completely deficient in receptor expression, except for increased susceptibility to some infectious diseases and the possibility of rare autoimmunity. Live a normal life. Therefore, conditioning CCR5 was determined to be a relatively safe approach and was a major target in the development of anti-HIV lentiviral vectors.

With respect to the second point above, the viral TAR sequence is a highly structured region of HIV genomic RNA that binds tightly to the viral Tat protein. The Tat: TAR complex is important for the efficient production of viral RNA. Overexpression of the TAR region was envisioned as a decoy molecule that sequesters the Tat protein and reduces the level of viral RNA. However, TAR has proven toxic to most mammalian cells, including the cells used to produce lentiviral particles. In addition, TAR was inefficient in inhibiting viral gene expression in other laboratories and was rejected as a viable component in HIV gene therapy.

In various embodiments, i) sequences reasonably conserved across a series of HIV isolates represent epidemics in the region of interest; ii) due to the activity of inhibitory RNA in the viral vector. Reducing RNA levels reduces the corresponding protein levels by an amount sufficient to significantly reduce HIV replication; and iii) viral gene sequences targeted by inhibitory RNA are viral vectors during production. A viral gene sequence was identified that met three criteria: absent from the gene required

for particle packaging and assembly. In various embodiments, the sequences at the junction of the HIV Tat and Rev genes, as well as the second sequence within the HIV Vif gene, were targeted by the inhibitory RNA. Tat / Rev targeting has the additional benefit of reducing the expression of HIV enveloped glycoproteins because this region overlaps with enveloped genes within the HIV genome.

Various methods for vector development and testing first identify suitable targets (described herein) and then express individual or multiple inhibitory RNA species for testing in a cell model. It relies on constructing plasmid DNA and finally constructing a lentiviral vector containing inhibitory RNA with proven anti-HIV function. Lentivirus vectors are tested for efficacy against HIV in terms of toxicity, yield during in vitro production, and inhibition of viral replication by reducing CCR5 expression levels or reducing viral gene products.

Table 2 below demonstrates the progression to reach clinical candidates through multiple versions of the inhibitory construct. First, a shRNA (short-chain homologous RNA) molecule was designed and expressed from a plasmid DNA construct.

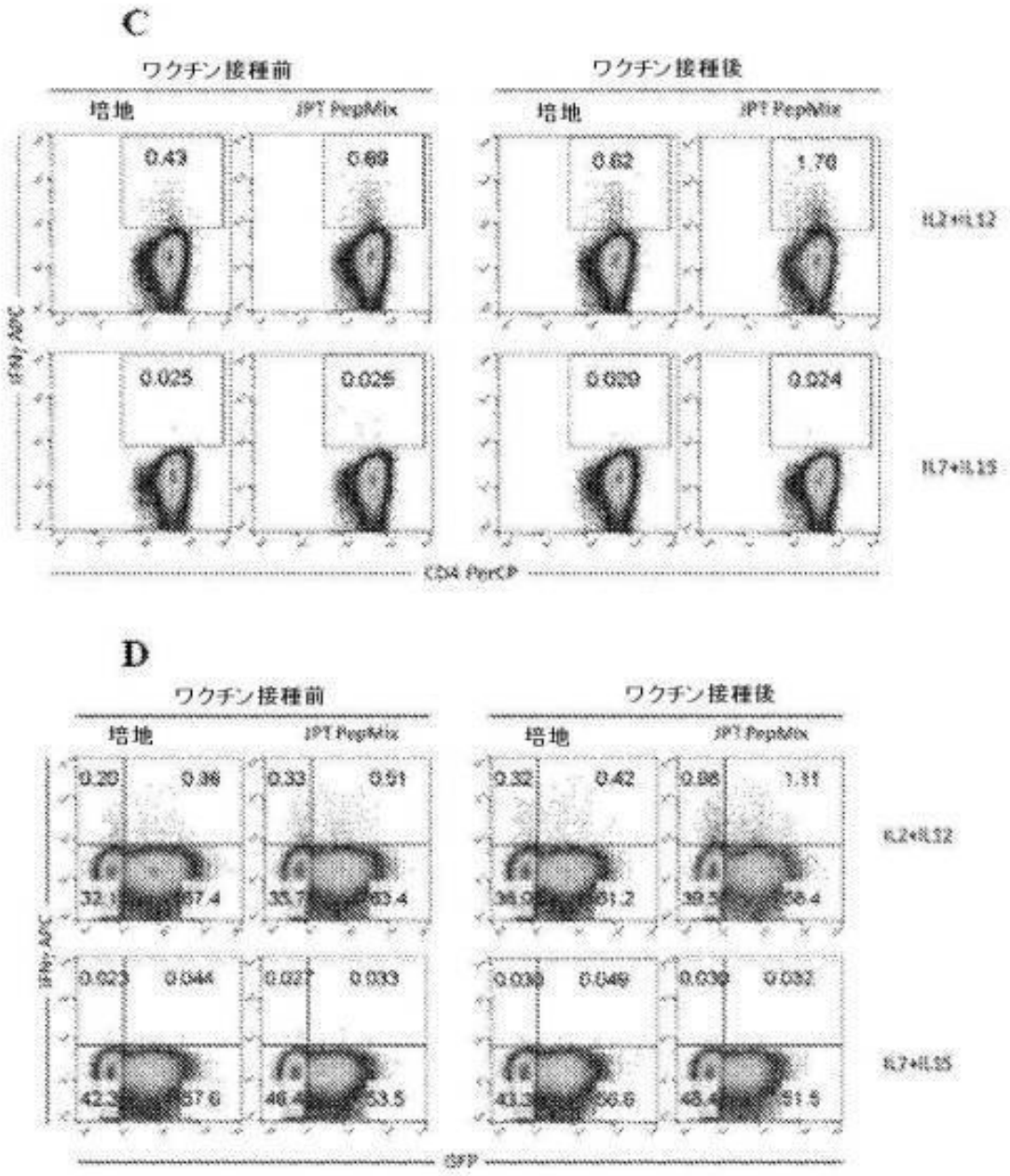

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き

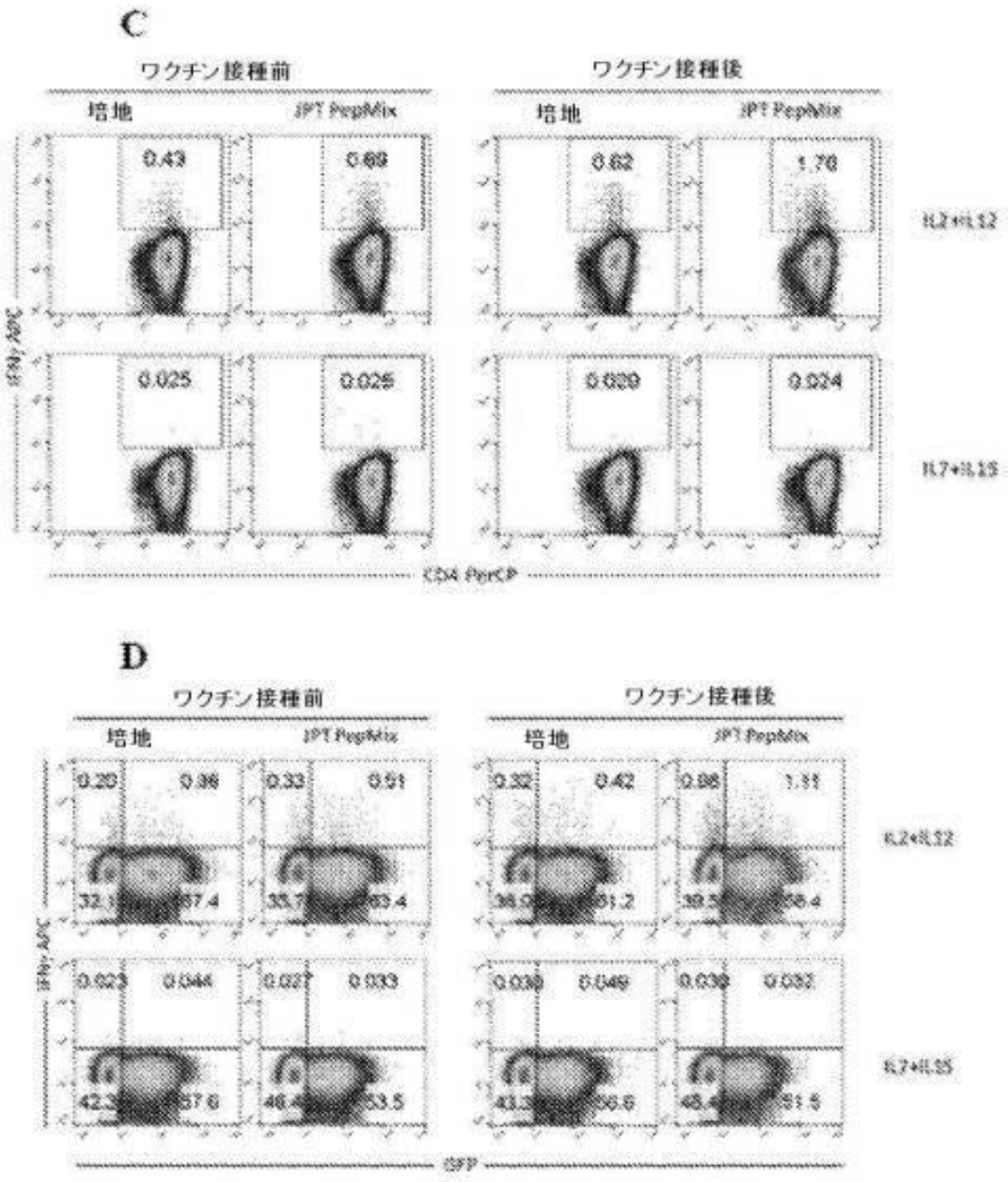
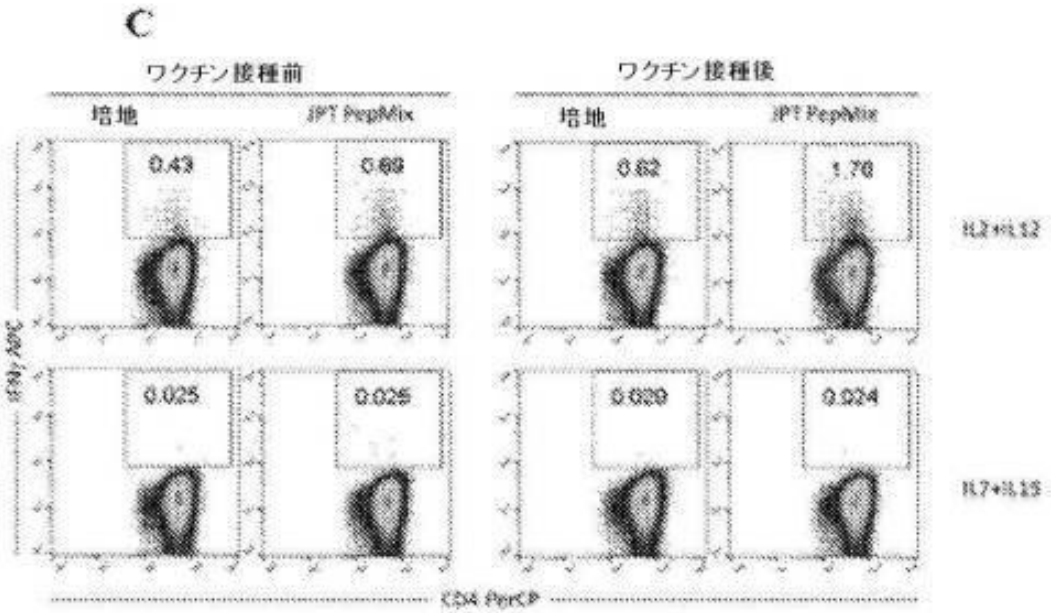

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.020
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL13
0.025
0.025
0.020
0.024
IL7+IL15
CD4 PerCP

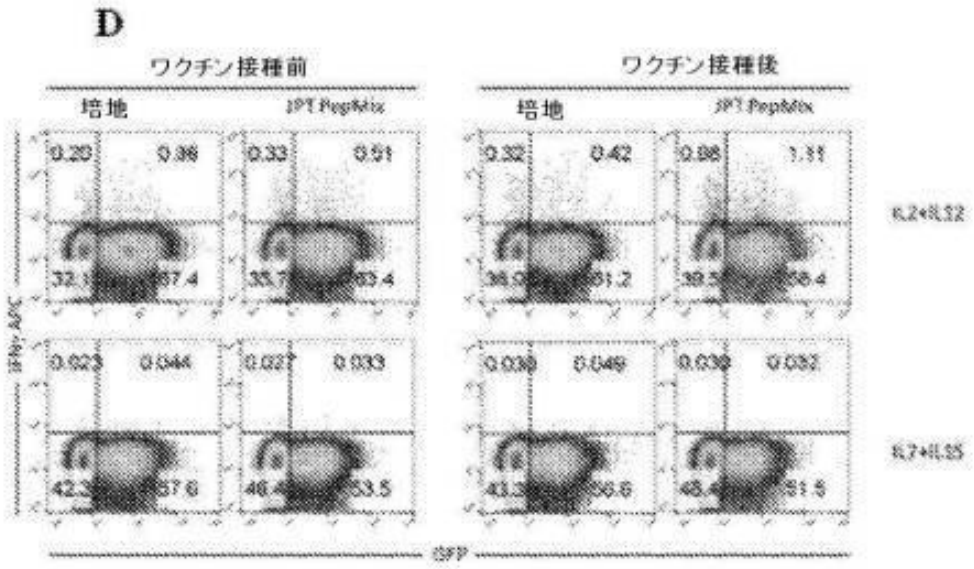
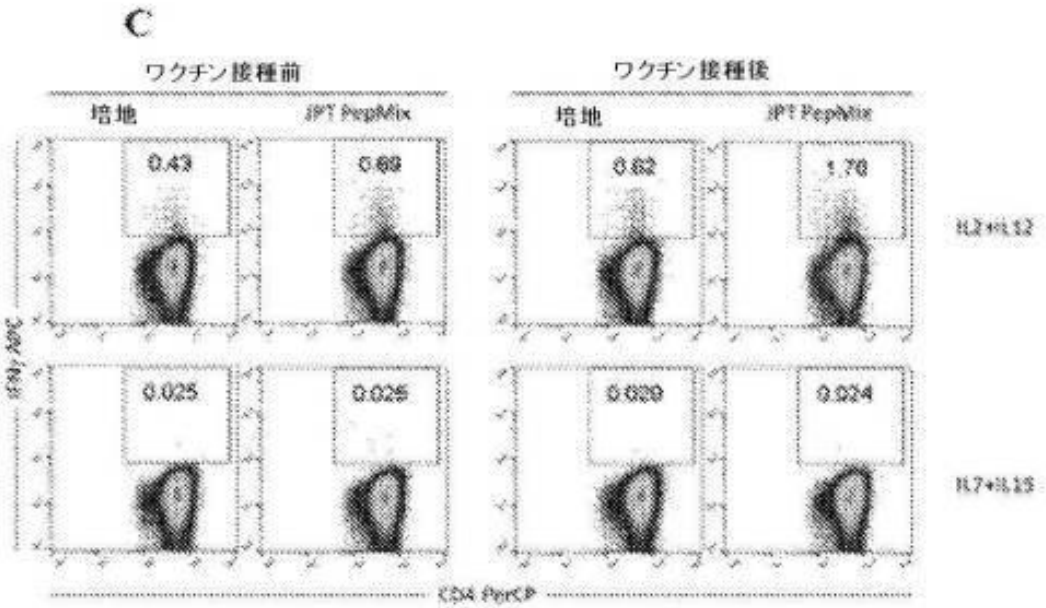

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.91
0.32
0.42
0.86
1.11
IL2+IL13
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

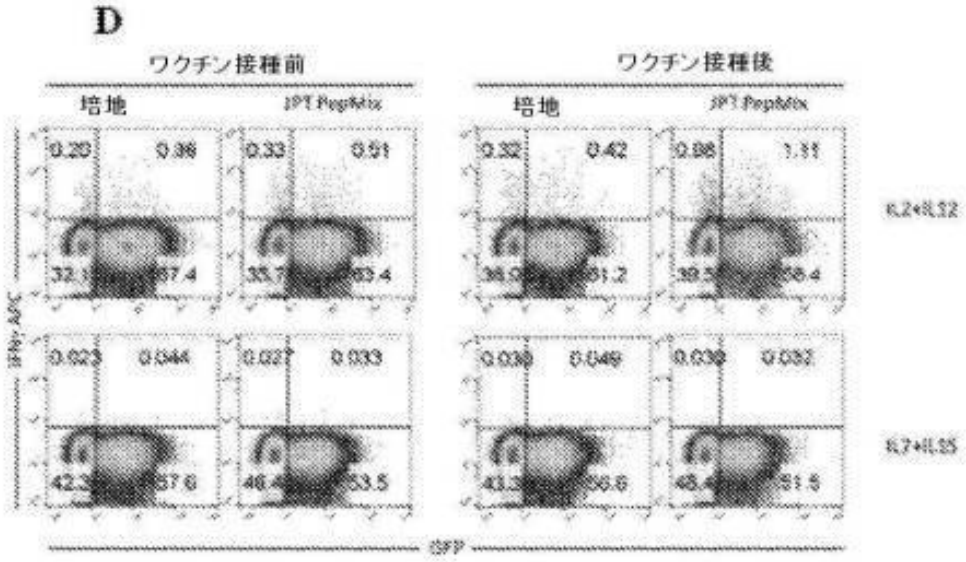
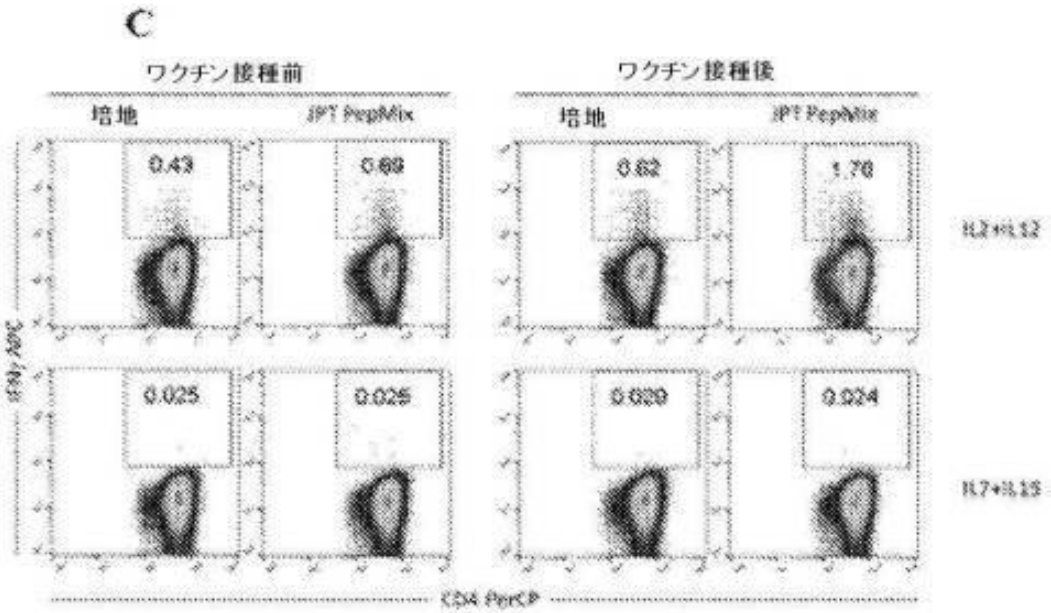

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL13
0.025
0.025
0.020
0.024
IL7+IL15
CD4 PerCP

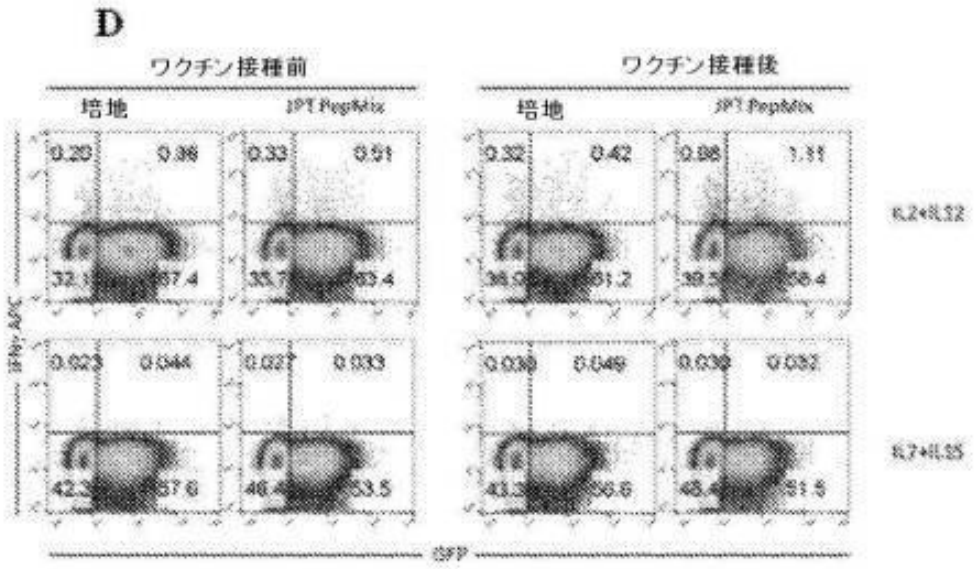

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.91
0.32
0.42
0.86
1.11
IL2+IL13
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
CFP

Figure 23 続き

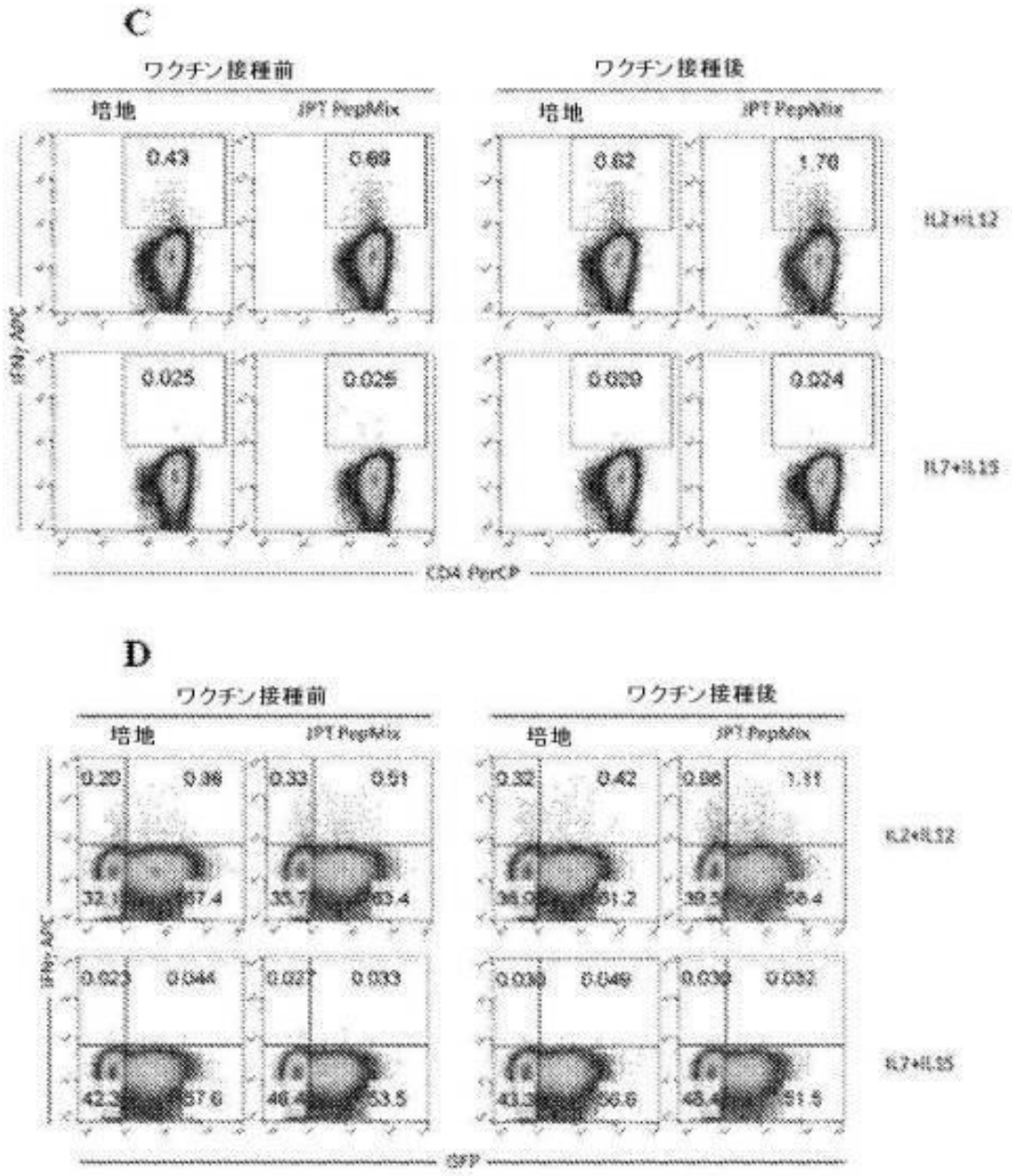


Figure 23 続き

The plasmids 1-4, detailed in Table 2 below, were tested for shRNA sequences for the Gag, Pol, and RT genes of HIV. Although each shRNA was active in suppressing viral protein expression in the cell model, there were two important problems that hindered further development. First, these sequences were targeted to laboratory isolates of HIV that are not representative of the Clade B HIV strain currently circulating in North America and Europe. Second, these shRNAs will target important components in the lentiviral vector packaging system and will significantly reduce the vector yield during production. The plasmid 5 detailed in Table 2 was selected for targeting CCR5

and yielded read candidate sequences. The plasmids 6, 7, 8, 9, 10, and 11 detailed in Table 2 incorporate the TAR sequence and are unacceptable toxicity to mammalian cells, including the cells used to produce the lentiviral vector. It turned out that it brought. The plasmid 2 detailed in Table 2 identified a shRNA sequence capable of reducing Tat RNA expression. The plasmid 12, detailed in Table 2, demonstrated the efficacy of shCCR5 expressed as microRNA (miR) in the lentiviral vector and confirmed that it should be present in the end product. The plasmid 13 detailed in Table 2 demonstrated the efficacy of shVif expressed as microRNA (miR) in the lentiviral vector and confirmed that it should be present in the end product. The plasmid 14 detailed in Table 2 demonstrated the efficacy of shTat expressed as microRNA (miR) in the lentiviral vector and confirmed that it should be present in the end product. The plasmid 15, detailed in Table 2, contained miR CCR5, miR Tat and miR Vif in the form of miR clusters expressed from a single promoter. These miRs did not target important components in the lentiviral vector packaging system and were demonstrated to have negligible toxicity to mammalian cells. The miRs within the cluster were equally effective against the previously tested individual miRs, and the overall effect was a substantial reduction in replication of the CCR5-tropic HIV BaL strain.

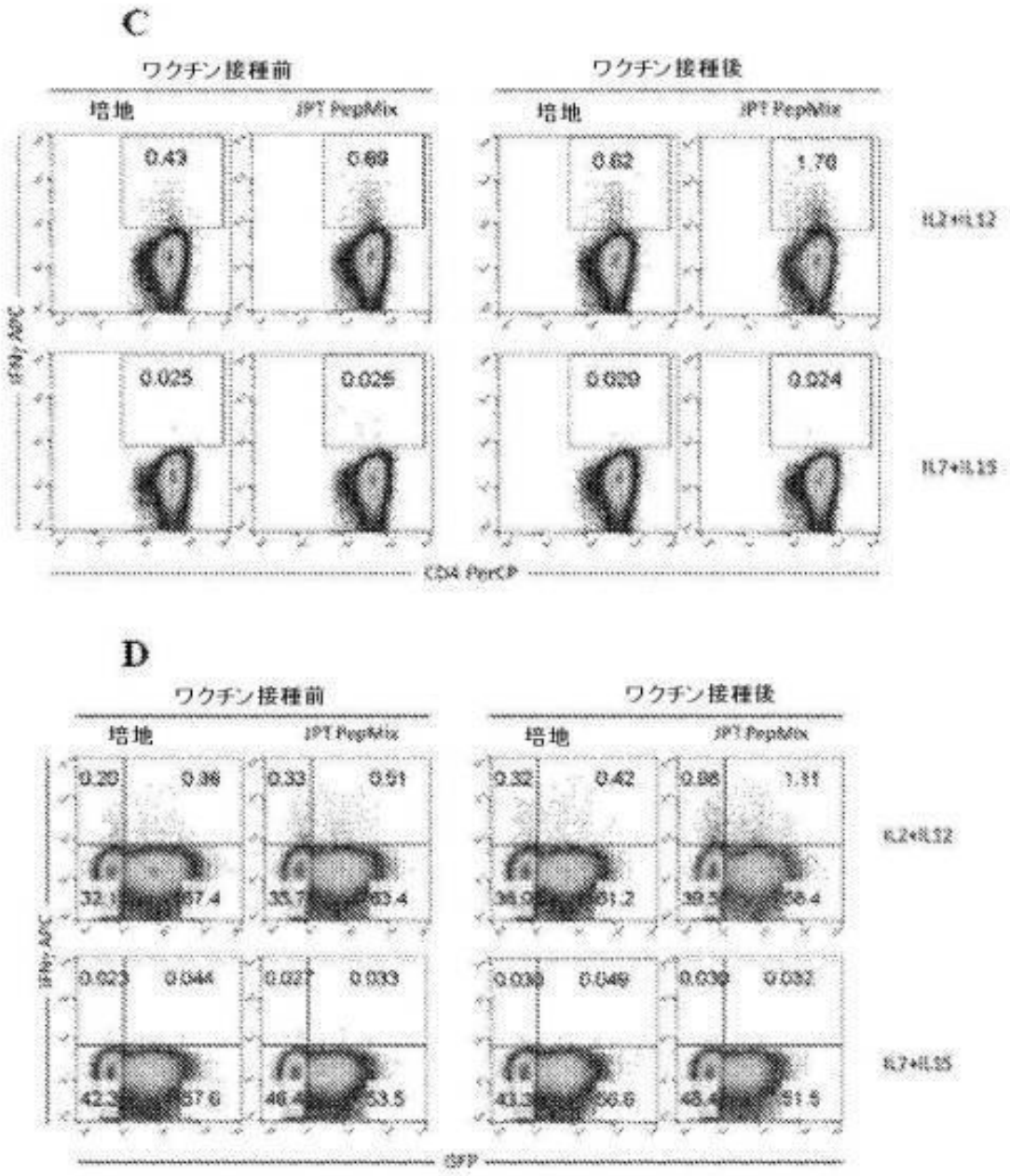

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き

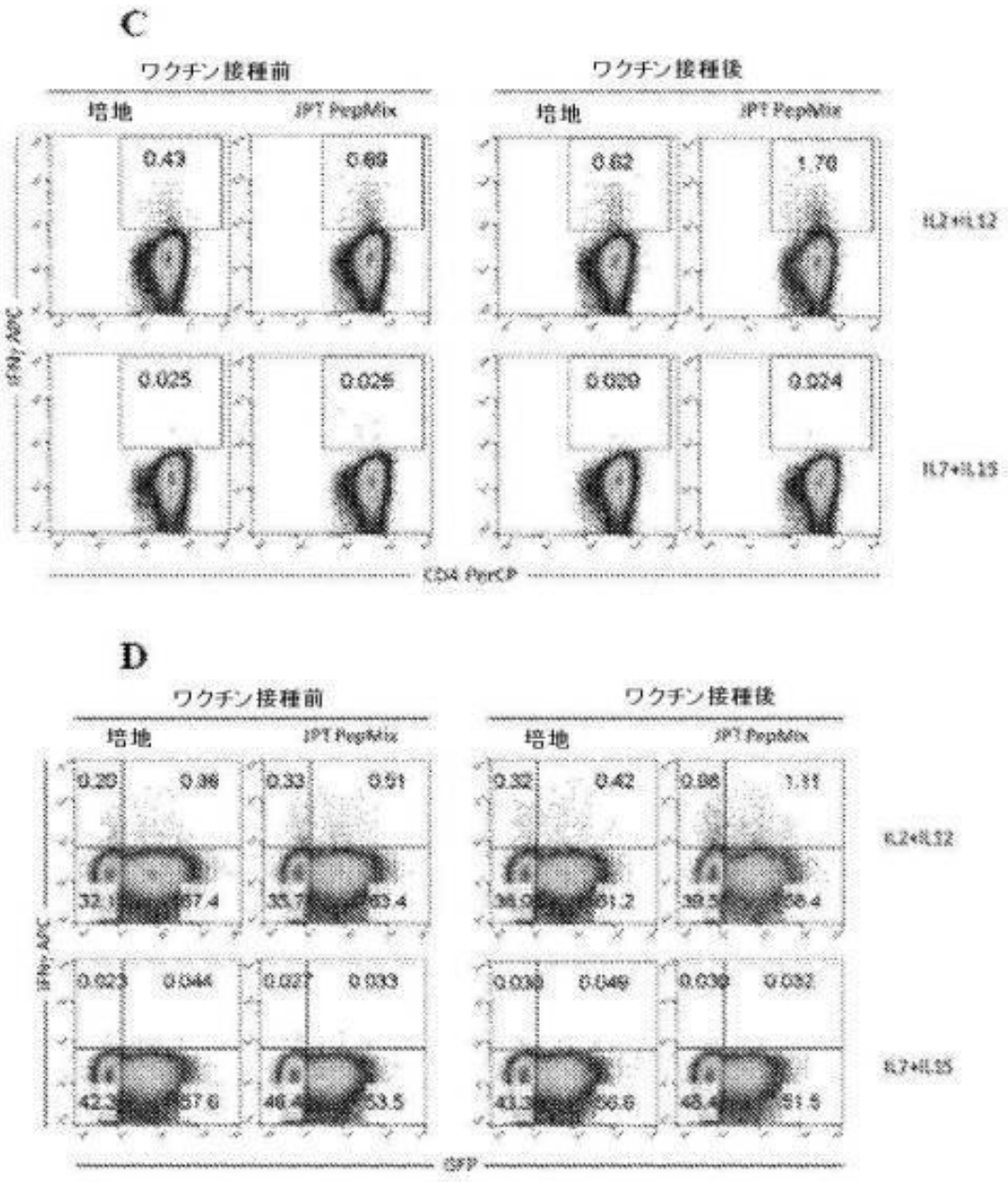
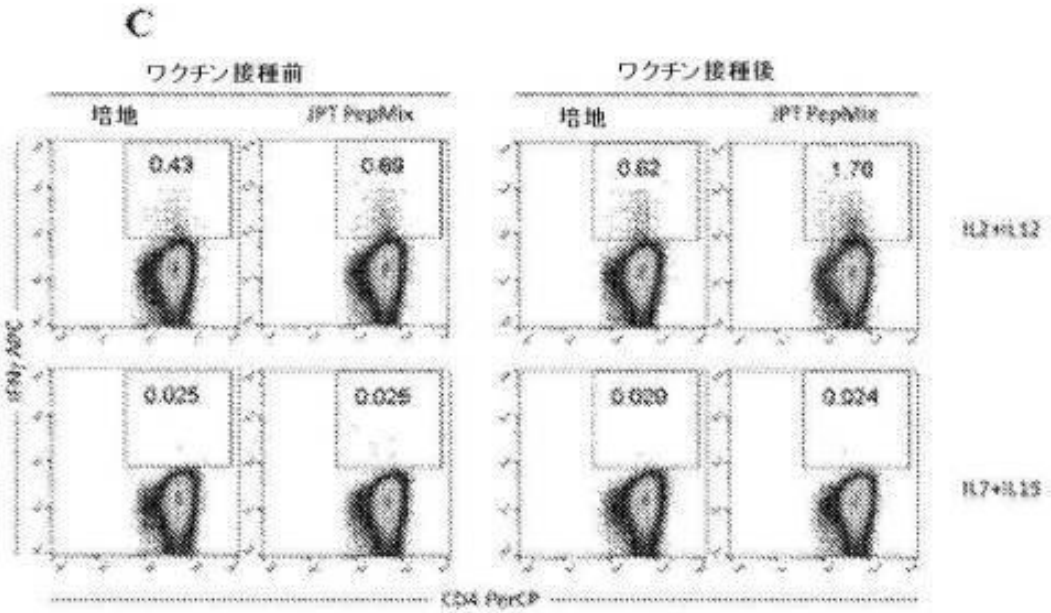

C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.76
IL2+IL12
0.025
0.025
0.020
0.024
IL7+IL15
IFNγ APC
CD4 PerCP
D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.51
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.030
0.049
0.030
0.032
IL7+IL15
IFNγ APC
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL13
0.025
0.025
0.020
0.024
IL7+IL15
CD4 PerCP

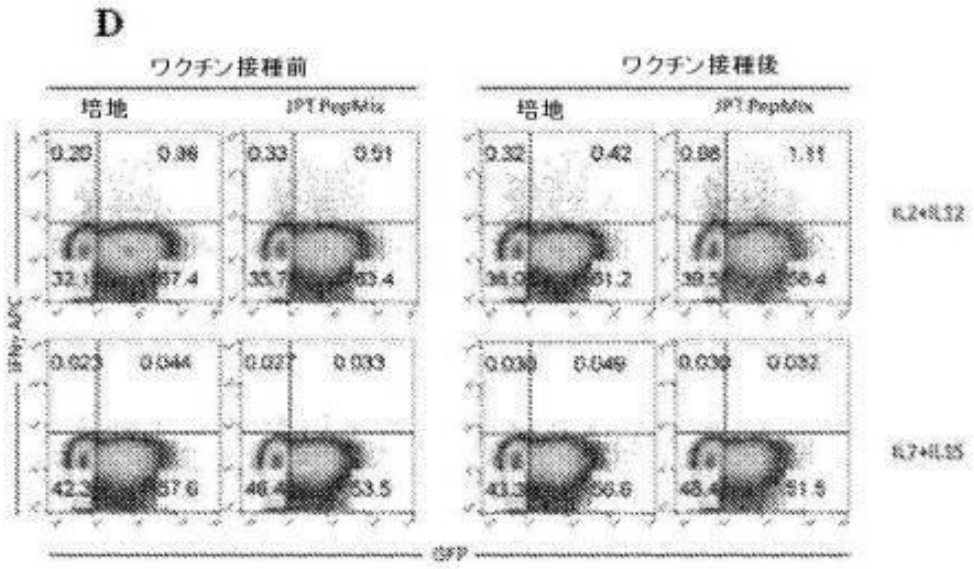
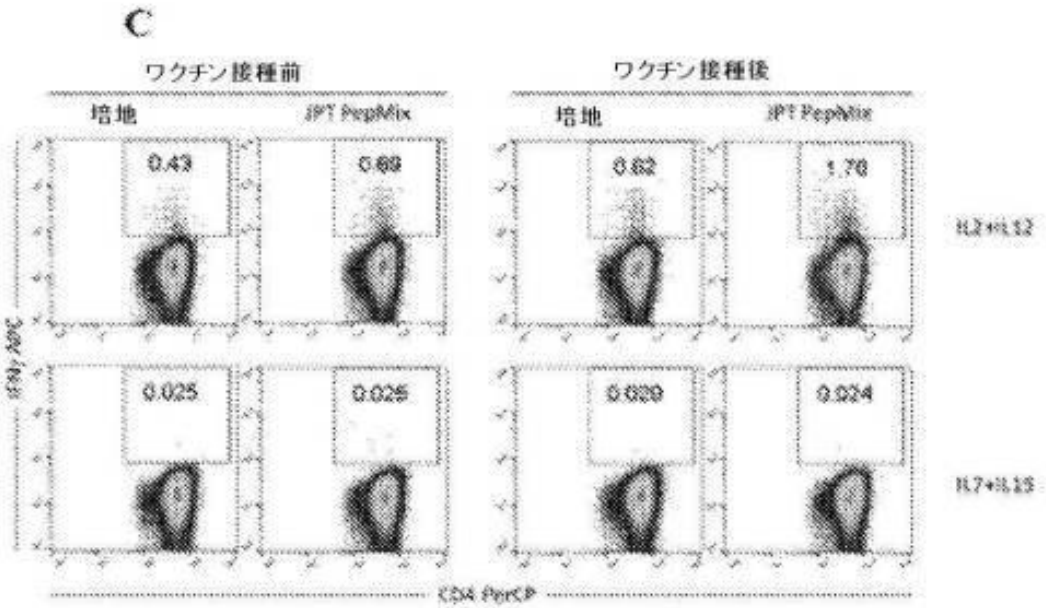

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.91
0.32
0.42
0.86
1.11
IL2+IL13
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
CFP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
0.025
0.025
0.020
0.024
IL2+IL12
IL7+IL15
IFNγ APC
CD4 PerCP

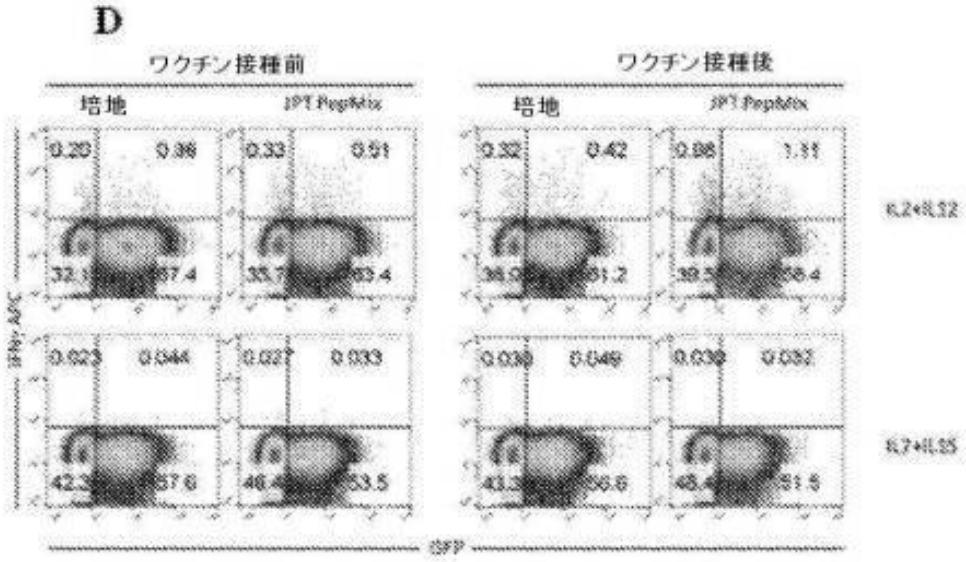
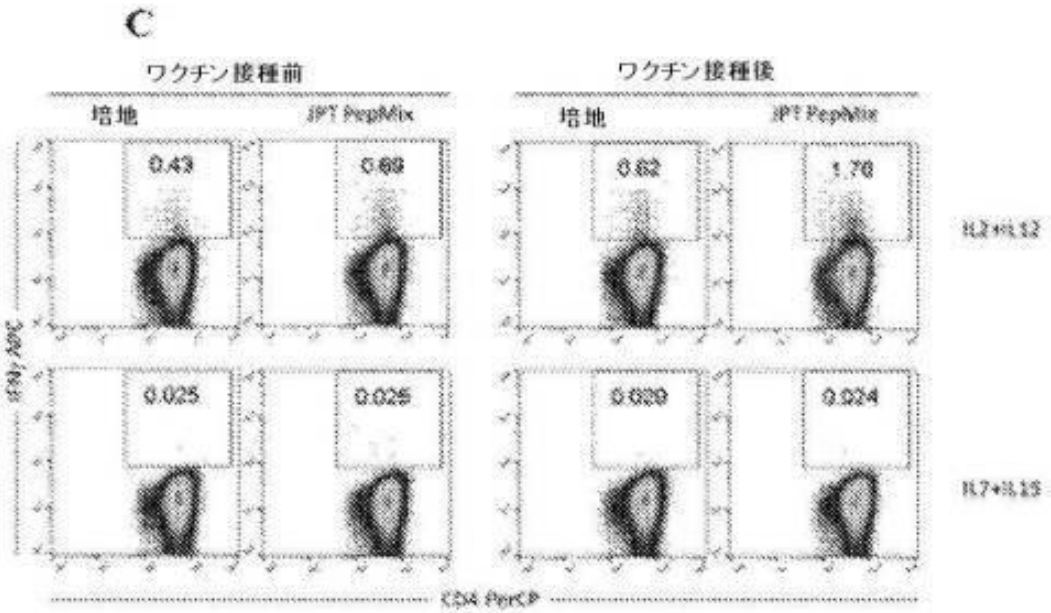

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.51
0.32
0.42
0.88
1.31
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL2+IL12
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL13
0.025
0.025
0.020
0.024
IL7+IL15
CD4 PerCP

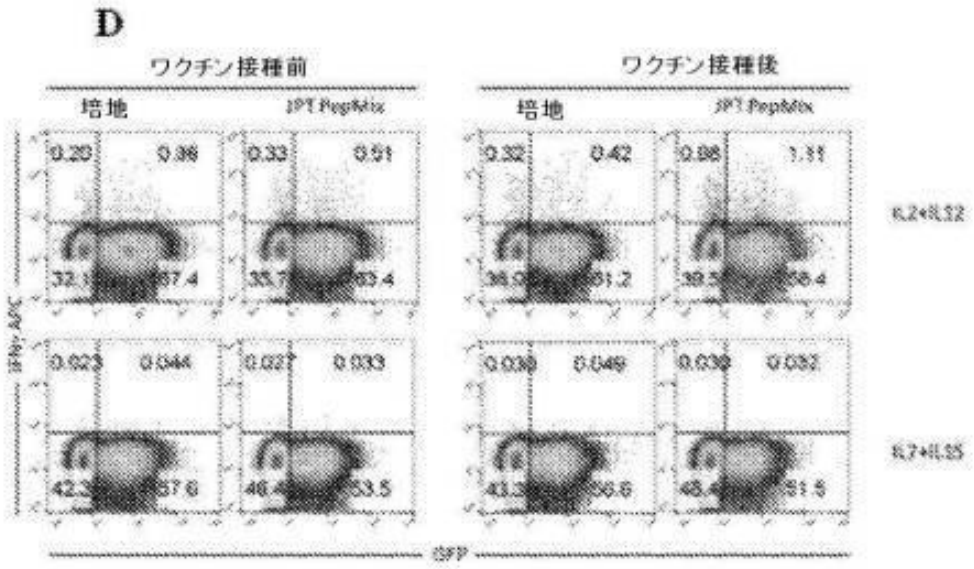

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.36
0.33
0.91
0.32
0.42
0.86
1.11
IL2+IL13
0.023
0.044
0.027
0.033
0.039
0.049
0.030
0.032
IL7+IL15
CFP

Figure 23 続き

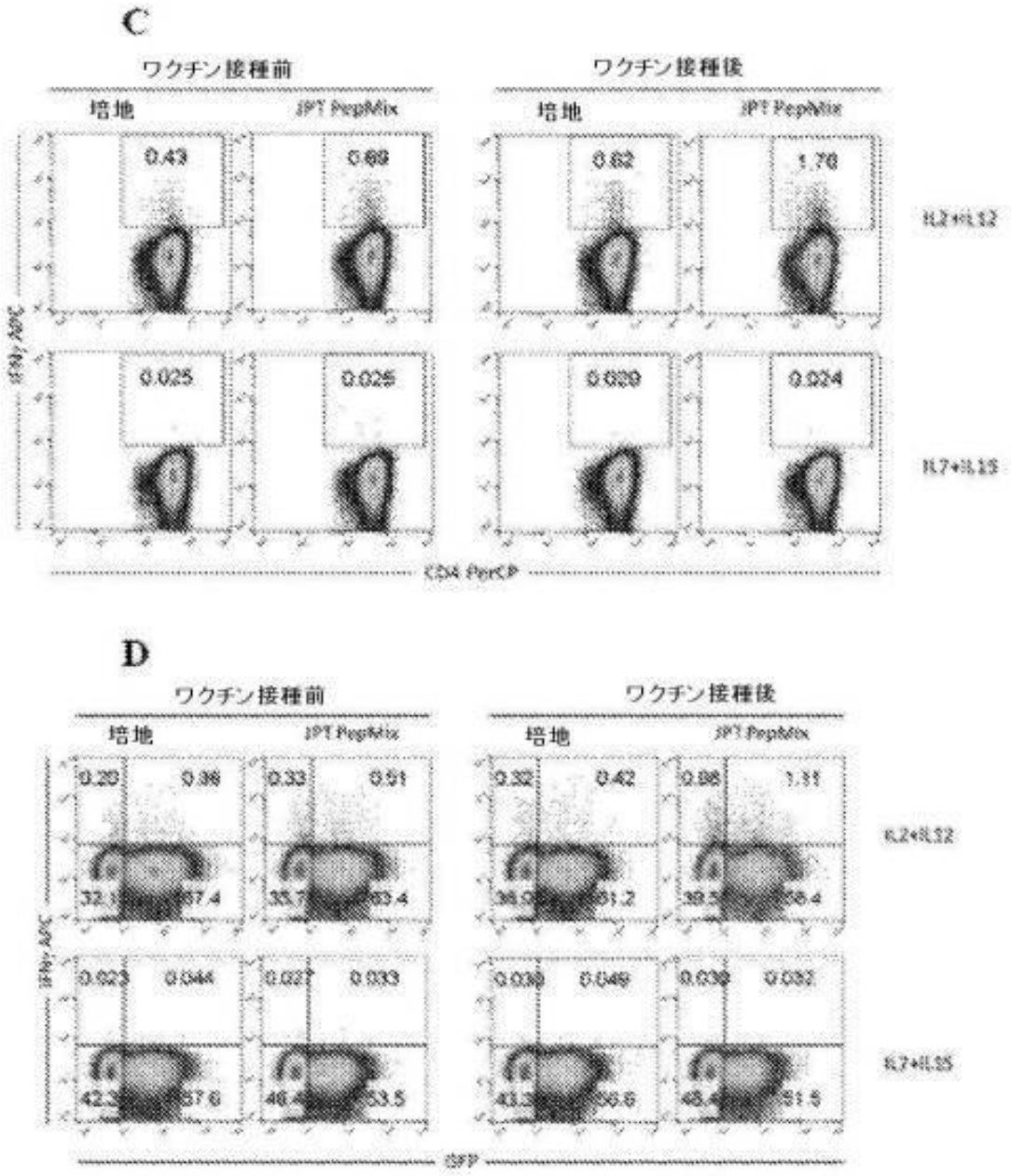


Figure 23 続き

Functional assay. Individual lentiviral vectors containing CCR5, Tat, or Vif shRNA sequences, as well as green fluorescent protein (GFP) under the control of the CMV earliest promoter for experimental purposes, are referred to as AGT103 / CMV-GFP. Lentiviral vectors were tested for their ability to knock down expression of CCR5, Tat, or Vif. Lentivirus particles were transduced into mammalian cells either in the presence or absence of polybrene. Cells were harvested after 2-4 days and proteins and

RNA were analyzed for CCR5, Tat, or Vif expression. After labeling cells with Western blot assay or specific fluorescent antibody (CCR5 assay), use either CCR5-specific antibody or isotype control antibody by analytical flow cytometry to compare the fluorescence of modified and unmodified cells. , Protein levels were tested.

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

Prior to transduction of lentivirus, the infectious virus titer was determined and the amount of virus to be added for appropriate multiplicity of infection (MOI) was calculated.

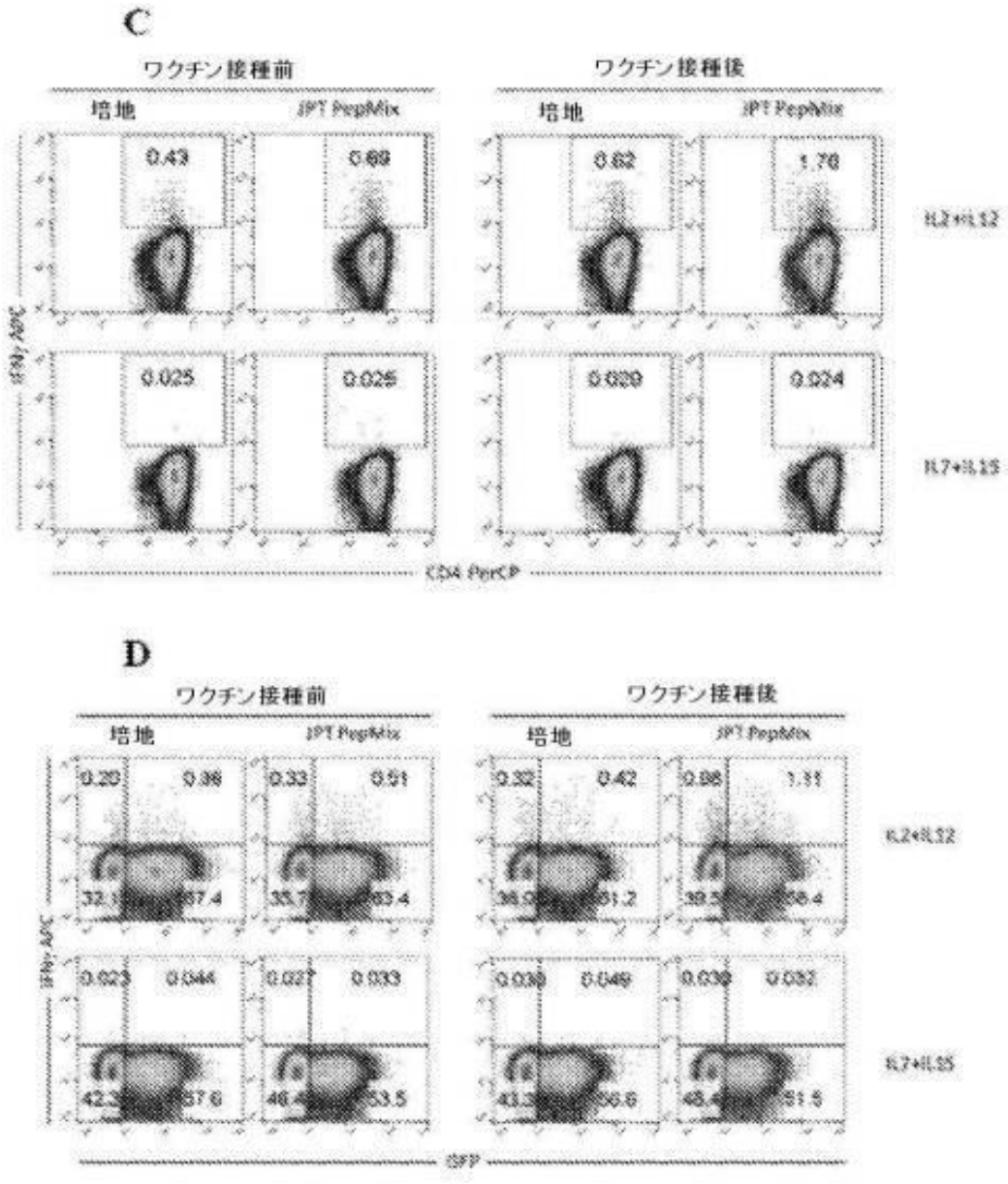


Figure 23 続き

Days 0-12: Antigen-specific enrichment. On day 0, the cryopreserved PBMCs were thawed, washed with 10 ml 37 ° C. medium at 1200 rpm for 10 minutes and resuspended in 37 ° C. medium at a concentration of $^{2 \times 10\ 6\ /\ ml.}$ The cells were cultured in a 24-well plate at 0.5 ml / well at 37 ° C. and 5% CO2. Cells were stimulated with a combination of reagents listed in Table 3 below to define optimal stimulation conditions.

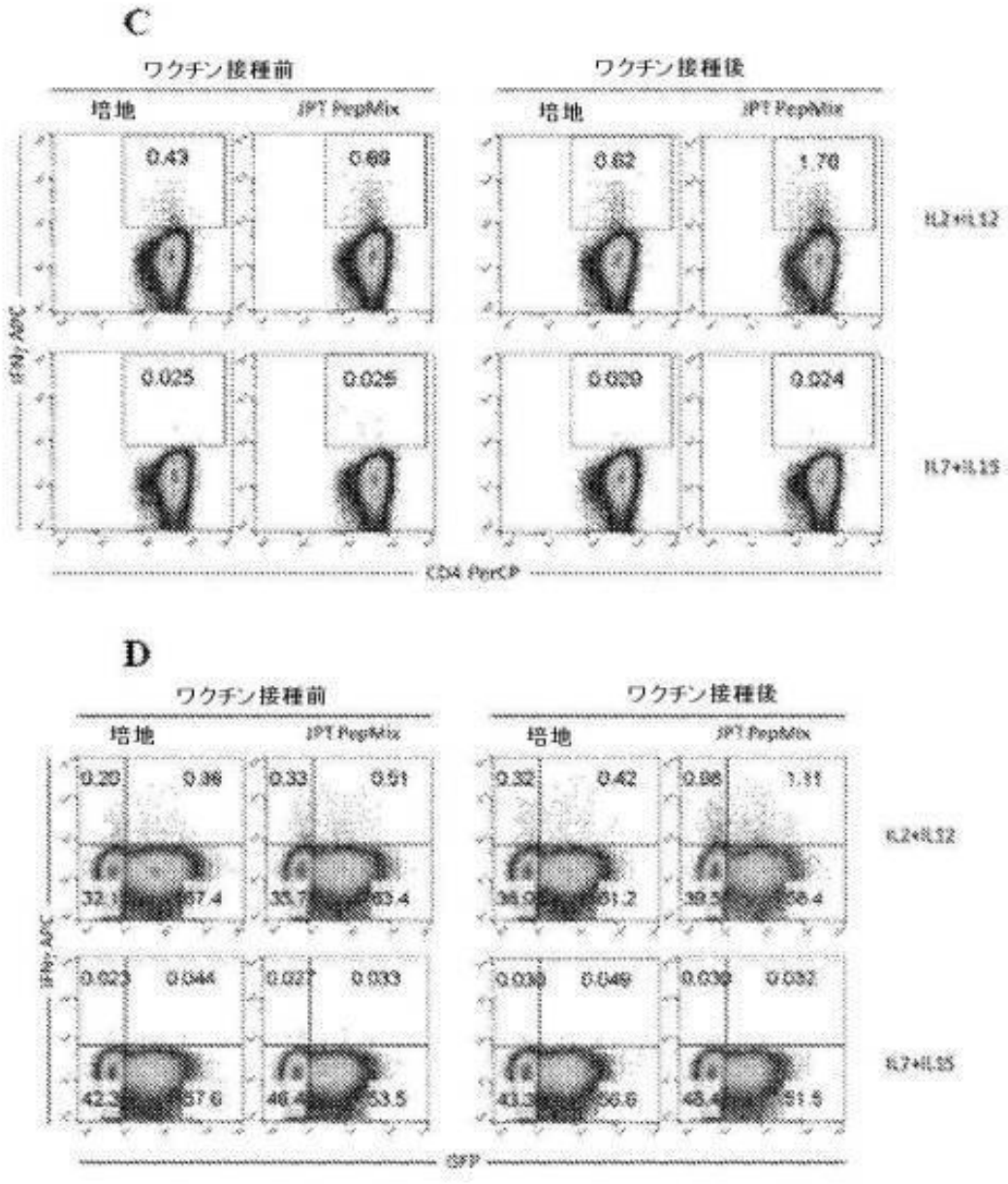


Figure 23 続き

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

Days 12-24: Non-specific expansion and lentivirus transduction. On day 12, stimulated cells were pipetted out of the plate and resuspended in fresh T cell culture medium at a concentration of 1 × 106 / ml. The resuspended cells were transferred to a T25 culture flask and stimulated with DYNABEADS® Human T-Activator CD3 / CD28 and in

addition the cytokines listed above, as described by the manufacturer. The flask was incubated in a vertical position.

On day 14, AGT103 / CMV-GFP was added at MOI20 and the cultures were returned to the incubator for 2 days. At this time, cells were collected by pipette operation, 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 those used in the prior step, and resuspended in viable cells 0.5×10^{6} cells per 1 ml.

Subjected 14-23 days, the number of cells was evaluated every 2 days, the cells were diluted to $0.5 \times {}^{10}6$ / ml in fresh medium. 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 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 at 1×10^{6} / ml for 1 day. Assays were performed to determine the frequency of antigen-specific T cells and cells transduced with lentivirus.

Amprenavir (0.5 ng / ml) was added to the culture on day 1 of stimulation and every other day during culture to prevent potential virus growth.

Antigen-specific T cells are investigated by intracellular cytokine staining of IFN gamma. Cultured cells after lentiviral transduction with peptide stimulation or after 1×10^{6} cells / ml, medium alone (negative control), (the individual peptide 5 [mu] g / ml) Gag peptide, or PHA (5 [mu] g / ml, positive Stimulated with (control). After 4 hours, BD GolgiPlug ™ (1: 1000, BD Biosciences) was added to block Golgi transport. After 8 hours, cells are washed and stained with extracellular antibody (CD3, CD4 or CD8; BD Biosciences) and intracellular antibody (IFN gamma; BD Biosciences) using the BD Cytofix / Cytoperm ™ kit as described by the manufacturer. bottom. Samples were analyzed on 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. Tests of CD3 + CD8 + GFP + IFN gamma + cells are included as controls.

These results show that T cells that are immune to the virus by transfecting CD4 T cells of the target T cell population with a lentivirus designed to specifically knock down the expression of HIV-specific proteins. It is shown that a proliferative population can be produced. This example serves as evidence of the concept that the disclosed lentiviral constructs can be used in combination with vaccination to result in functional cure of HIV patients.

(Example 4: Knockdown of CCR5 by experimental vector)
AGTc120 is a HeLa cell line that stably expresses large amounts of CD4 and CCR5. Transduction was performed on AGTc120 with or without LV-CMV-mCherry (red fluorescent protein mCherry expressed under the control of the CMV earliest promoter) or AGT103 / CMV-mCherry. Gene expression of the mCherry fluorescent protein was regulated by the CMV (cytomegalovirus earliest promoter) expression cassette. The LV-CMV-mCherry vector lacked microRNA clusters, whereas AGT103 / CMV-mCherry expressed therapeutic miRNAs for CCR5, Vif, and Tat.

As shown in FIG. 8A, the transduction efficiency was> 90%. After 7 days, cells were harvested, 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 mean fluorescence intensity (x-axis) of CCR5 APC against the most frequent normalized cell number (y-axis). After staining for cell surface CCR5, cells treated without lentivirus or with control lentivirus (expressing only the mCherry marker) showed no change in CCR5 density, whereas AGT103 (right section) showed CCR5 staining intensity. It was reduced to near the level of isotype control. Seven days later, cells were infected with or without the R5-tropic HIV reporter virus Bal-GFP. After 3 days, cells were collected and analyzed by flow cytometry. Over 90% of the cells were transduced. AGT103-CMV / CMVmCherry reduced CCR5 expression in transduced AGTc120 cells and blocked R5-tropic HIV infection compared to cells treated with the control vector.

FIG. 8B shows the relative insensitivity of transfected AGTc120 cells to HIV infection. As mentioned above, the lentiviral vector expresses the mCherry protein, and transduced cells infected with HIV (expressing GFP) appear as double-positive cells in the upper right quadrant of the false color flow cytometry dot plot. Become. In the absence of HIV (upper panel), GFP + cells were absent 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 the therapeutic AGT103 / CMV-mCherry vector was transduced into cells, only 0.83% of the cells appeared in the double positive quadrant indicating that the cells were transduced and infected.

Dividing 53.62 (ratio of double-positive cells containing control vector) by 0.83 (ratio of double-positive cells containing therapeutic vector) divides AGT103 from more than 65-fold protection from HIV in this experimental system. Is shown to have brought about.

(Example 5: regulation of CCR5 expression by shRNA inhibitor sequence in lentiviral vector)
Inhibitory RNA design. The sequence of the Homo sapiens chemokine receptor CCR5 (CCR5, NC 0000003.12) was used to search for potential siRNA or shRNA candidates that knock down CCR5 levels in human cells. Potential RNA interference sequences were selected from candidates selected by the Broad Institute program or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-IT RNA iDesigner. The shRNA sequence can be inserted into the plasmid immediately after the RNA polymerase III promoter, eg, H1, U6, or 7SK, to regulate shRNA expression. The shRNA sequence is inserted into a lentiviral vector using a similar promoter or embedded in a microRNA skeleton to allow expression by an RNA polymerase II promoter such as CMV or EF-1alpha. May be good. RNA sequences can also be synthesized as siRNA oligonucleotides and used independently of plasmids or lentiviral vectors.

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

Functional assay for CCR5 mRNA reduction: The assay for inhibition of CCR5 expression required co-transfection of the two plasmids. The first plasmid contains one of five different shRNA sequences directed against the CCR5 mRNA. The second plasmid contains the cDNA sequence of the human CCR5 gene. The plasmid was co-transfected into 293T cells. After 48 hours, cells were lysed and RNA was extracted using Qiagen's RNeasy kit. CDNA was synthesized from RNA using Invitrogen's SuperScript Kit. Samples were then analyzed by quantitative RT-PCR using an Applied Biosystems Step One PCR machine. Invitrogen's SYBR using a forward primer (5'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 93) and a reverse primer (5'-CCCCAAGAAGGTCAAGGTAATCA-3') (SEQ ID NO: 94) under standard conditions for polymerase chain reaction analysis. CCR5 expression was detected using Green. Beta actin gene using forward primer (5'-AGCGCGCGCTACAGCTTCA-3') (SEQ ID NO: 95) and reverse primer (5'-GGCGACGTAGCAGACGCTTCP-3') (SEQ ID NO: 96) under standard conditions for polymerase chain reaction analysis. Samples were normalized to mRNA for expression. Relative expression of CCR5 mRNA was determined by Ct values normalized to the level of actin messenger RNA for each sample. The results are shown in FIG.

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

(Example 6: Regulation of HIV component by shRNA inhibitor sequence in lentiviral vector)
Inhibitory RNA design.
Using the sequences of HIV1-type Rev / Tat (5'-GCGGAGACAGCGACGAAGAGC-3') (SEQ ID NO: 9) and Gag (5'-GAAGAAAGATGACAGCAT-3') (SEQ ID NO: 11),
Rev / Tat:
(5'GCGGAGACAGCGACGAAGAGCTTCAGAGAGCTCTTCGTCGCTGTCTCCGCTTTT-3') (SEQ ID NO: 10), and Gag:
(5'GAAGAAATGATGAGAGACATTTCAAGAGAGACTGTCATCATCATTTTCTTCTTTTT-3') (SEQ ID NO: 12) shRNA was designed. These were synthesized as described above and cloned into a plasmid.

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

Functional Assay for ShRNA Targeting of Rev / Tat or Gag mRNA: We used plasmid co-transfection, where the shRNA plasmid degrades luciferase messenger RNA in co-transfected cells and reduces luminescence intensity. It was tested whether it could be made to. A shRNA control (scrambled sequence) was used to establish maximum light yields from cells transfected with luciferase. When a luciferase construct containing a Rev / Tat target sequence inserted into the 3'-UTR (untranslated region of mRNA) was co-transfected with a Rev / Tat shRNA sequence, luminescence was reduced by approximately 90% and the shRNA sequence was potent. Functions were shown. Similar results were obtained when the luciferase construct containing the Gag target sequence in the 3'-UTR was co-transfected with the Gag shRNA sequence. These results indicate strong activity of the shRNA sequence.

As shown in FIG. 10A, knockdown of Rev / Tat target genes fused to the target mRNA sequence in the 3'UTR by transient transfection in 293T cells was measured by reduced luciferase activity. FIG. 10B shows a knockdown of the Gag target gene sequence fused to the luciferase gene. Results are shown as the mean ± SD of three independent transfection experiments, each performed in triplicate.

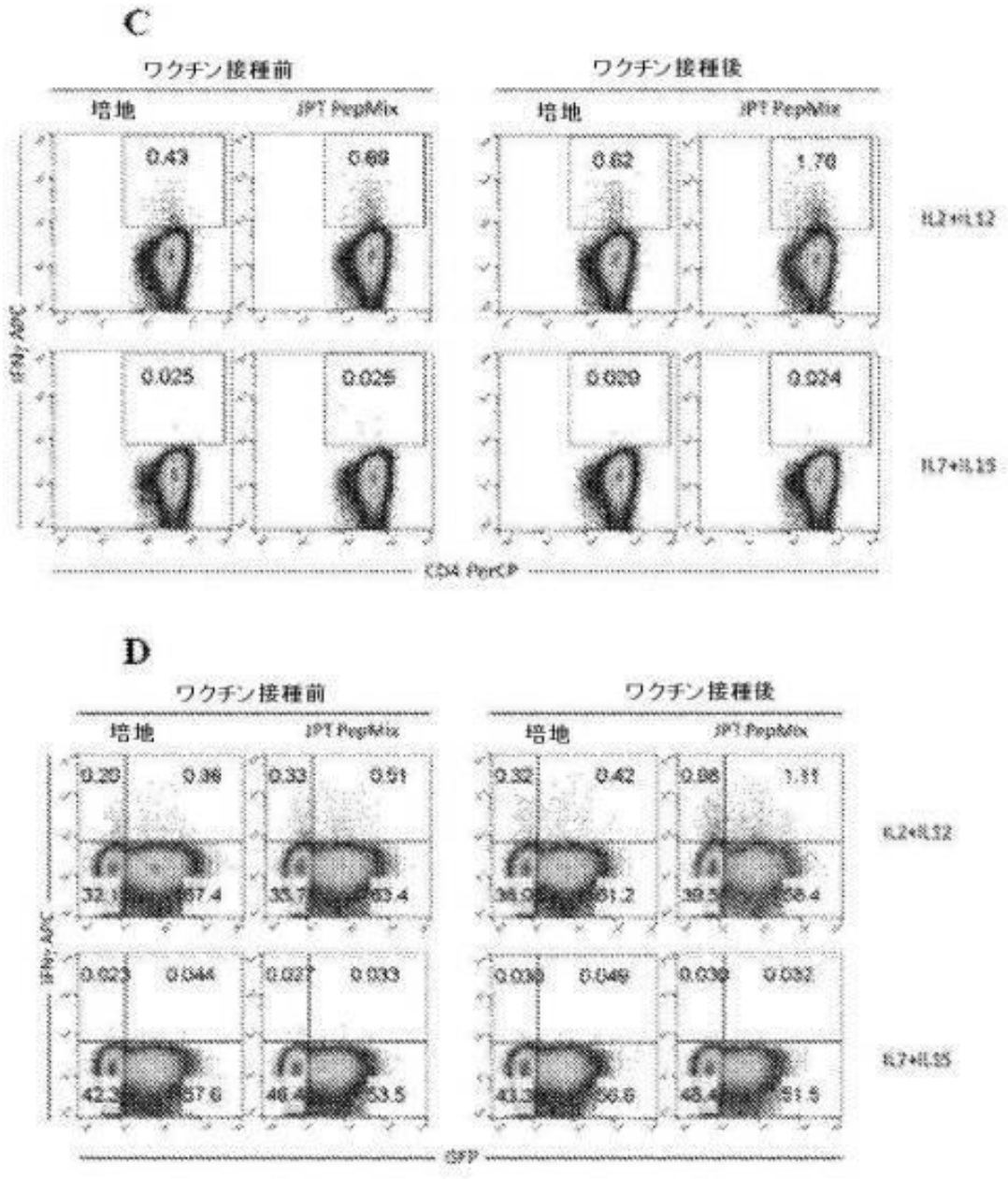


Figure 23 続き

(Example 7: AGT103 reduces Tat and Vif expression)

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

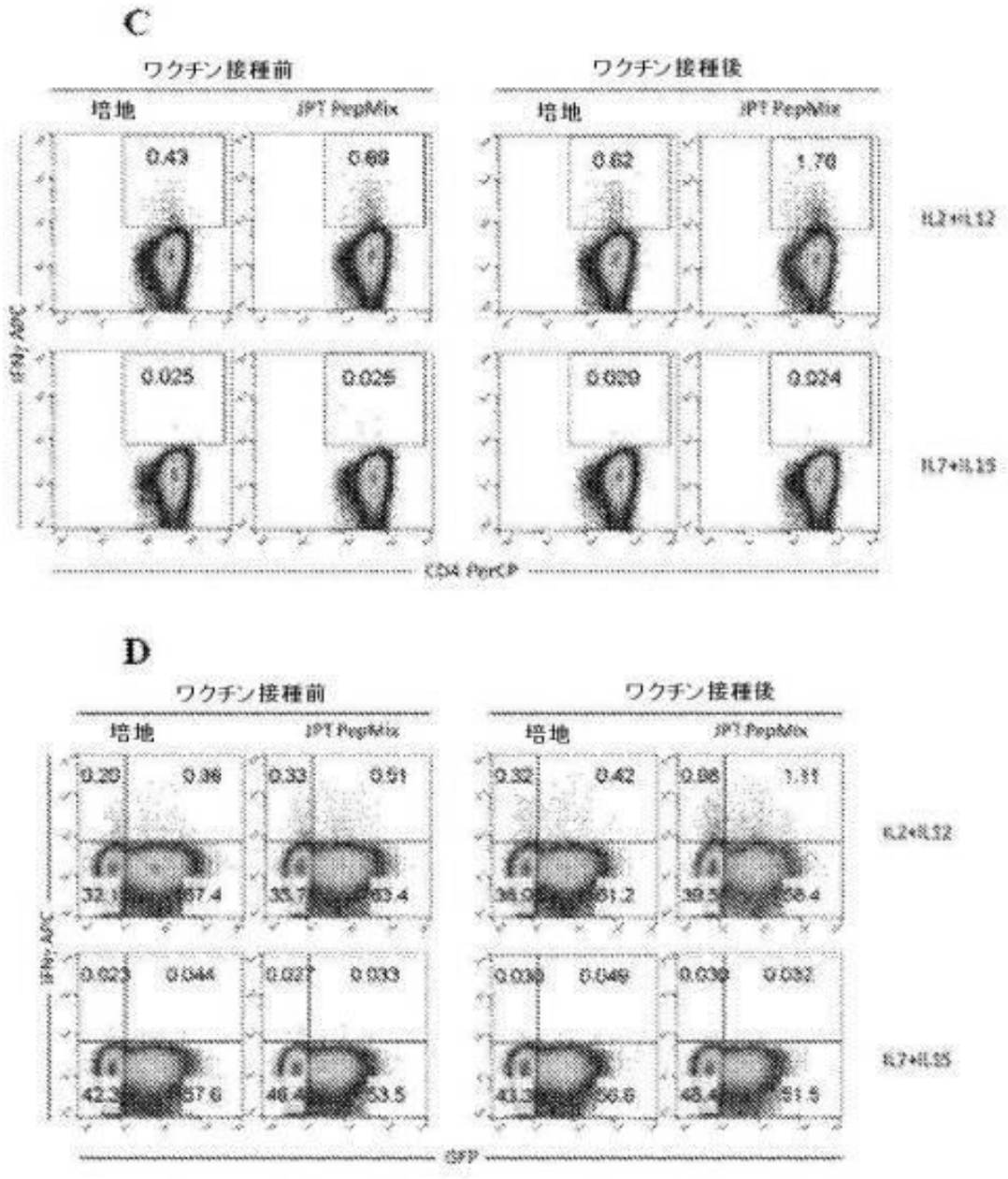


Figure 23 続き

AGT103 / CMV-GFP was transduced into a T lymphoblast-like cell line (CEM; CCRF-CEM; American Type Culture Collection Catalog No. CCL119). 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 the level of intact Tat sequences was tested using reverse transcriptase-polymerase chain reaction. As shown in FIG. 11, 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, for a total of> 4 minutes. It was a decrease of 1.

(Example 8: Regulation of HIV component by synthetic microRNA sequence in lentiviral vector)
Inhibitory RNA design. The HIV-1 Tat and Vif gene sequences were used to search for potential siRNA or shRNA candidates that knock down Tat or Vif levels in human cells. Potential RNA interference sequences were selected from candidates selected by the Broad Institute program or siRNA or shRNA design programs such as Thermo Scientific's BLOCK-IT RNA iDesigner. Selected shRNA sequences that are most potent for Tat or Vif knockdown were embedded within the microRNA backbone to allow expression by RNA polymerase II promoters such as CMV or EF-Ialpha. RNA sequences can also be synthesized as siRNA oligonucleotides and used independently of plasmids or lentiviral vectors.

Construction of plasmid. 'Incorporate (SEQ ID NO: 7) to the backbone of miR185, Tat miRNA (5'-GGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG-3 Tat target sequence (5'-TCCGCTTCTTCCTGCCATAG-3)' produces a) (SEQ ID NO: 3), which the lentiviral vector It was inserted and expressed under the control of the EF-1 alpha promoter. Similarly, 'incorporate (SEQ ID NO: 6) to the backbone of miR21, Vif miRNA (5'-CATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCTGACCA-3 Vif target sequence (5'-GGGATGTGTACTTCTGAACTT-3)' produces a) (SEQ ID NO: 2), wrenches it It was inserted into a viral vector and expressed under the control of the EF-1alpha promoter. The resulting Vif / Tat miRNA expressing lentiviral vector was produced in 293T cells using a lentiviral vector packaging system. Vif and Tat miRNAs were embedded in microRNA clusters consisting of miR CCR5, miR Vif, and miR Tat, all expressed under the control of the EF-1 promoter.

Functional assay for inhibition of Tat mRNA accumulation by miR185Tat. For transduction into 293T cells, a lentiviral vector expressing miR185 Tat (LV-EF1-miR-CCR5-Vif-Tat) was used with an infection multiplicity equal to 5. Twenty-four hours after transduction, cells were transfected with a plasmid expressing HIV strain NL4-3 (pNL4-3) using Lipofectamine 2000 under standard conditions. After 24 hours, RNA was extracted and Tat messenger RNA levels were tested by RT-PCR using Tat-specific primers and compared to control actin mRNA levels.

Functional assay for inhibition of Vif protein accumulation by miR21 Vif. For transduction into 293T cells, a lentiviral vector expressing miR21 Vif (LV-EF1-miR-CCR5-Vif-Tat) was used with an infection multiplicity equal to 5. Twenty-four hours after transduction, cells were transfected with a plasmid (pNL4-3) expressing the HIV strain NL4-3 using Lipofectamine 2000. After 24 hours, cells were lysed and total soluble protein was tested to measure Vif protein content. Cytolysis was isolated by SDS-PAGE according to established techniques. The separated protein was transferred to a nylon membrane and probed with a Vif-specific monoclonal antibody or actin control antibody.

Tat knockdown was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing either a synthetic miR185 Tat or miR155 Tat microRNA, as shown in FIG. 12A. bottom. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine 2000 for 24 hours, then RNA was extracted for qPCR analysis with primers for Tat. As shown in FIG. 12B, Vif knockdown was tested in 293T cells transduced with either a control lentiviral vector or a lentiviral vector expressing the microRNA of synthetic miR21Vif. After 24 hours, the HIV vector pNL4-3 was transfected with Lipofectamine 2000 for 24 hours, then proteins were extracted for immunoblot analysis with antibodies against HIV Vif.

(Example 9: Regulation of CCR5 expression by synthetic microRNA sequences in lentiviral vector)
A wrench virus vector containing a synthetic miR30 sequence of CCR5 (AGT103: TGTAAACTGAGCTTGCTCTA (SEQ ID NO: 97), AGT103-R5-1: TGTAAACTGAGCTTGCTCGC (SEQ ID NO: 98), or AGT103-R5-2: CATAGATTGGACTTGACCA (SEQ ID NO: 99). -Transduced into CCR5 cells. 6 days later, CCR5 expression was determined by FACS analysis with APC-conjugated CCR5 antibody and quantified by mean fluorescence intensity (MFI). CCR5 levels were set to 100% LV-Represented as% CCR5 using a control. The target sequences for AGT103 and AGT103-R5-1 are in the same region as CCR5 target sequence No. 5. The target sequence for AGT103-R5-2 is CCR5 target sequence No. 1. Same. AGT103 (2% of total CCR5) reduces CCR5 levels compared to AGT103-R5-1 (39% of total CCR5), and AGT103-R5-2, which does not reduce CCR5 levels. Most useful. This data is demonstrated herein in FIG.

Example 10: Regulation of CCR5 expression by synthetic microRNA sequences in lentiviral vectors containing either long or short WPRE sequences)
Construction of vector. Lentiviral vectors often require RNA regulatory elements for optimal expression of therapeutic genes or gene constructs. A common option is to use the Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). The present inventors have a full length WPRE: (5'AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCG

TATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCC
ACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCT
CGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCT
TCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCC
GCCT-3 ') (SEQ ID NO: 32)
WPRE element and AGT103 containing, which is shortened
(5'AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTAATGCCTCTGTATCATGCTATTGCTTCCC
GTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCC
CACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGG
CTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTC-3 ') (SEQ ID NO: 80)
Was compared with the modified AGT103 vector containing.

Functional assay for regulation of cell surface CCR5 expression in response to long vs. short WPRE elements in a vector sequence. AGT 103 containing long or short WPRE elements was used to transduce CEM-CCR5 T cells with an infection multiplicity equal to 5. Cells were harvested 6 days after transduction and stained with a monoclonal antibody capable of detecting cell surface CCR5 protein. The antibody is conjugated to a fluorescent marker and the intensity of its staining is directly proportional to the level of CCR5 on the cell surface. The control lentivirus did not affect cell surface CCR5 levels, resulting in a single population with an average fluorescence intensity of 73.6 units. The conventional AGT 103 with a long chain WPRE element reduced CCR5 expression to an average fluorescence intensity level of 11 units. AGT 103 modified to incorporate the short chain WPRE element resulted in a single cell population with an average fluorescence intensity of 13 units. Therefore, substitution of the short chain WPRE element had little or no effect on the ability of AGT103 to reduce cell surface CCR5 expression.

As shown in FIG. 14, AGT103 containing either long-chain or short-chain WPRE sequences was transduced into CEM-CCR5 cells. Six days later, CCR5 expression was determined by FACS analysis using APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as% CCR5 using an LV-control set to 100%. Decreased CCR5 levels were similar with AGT 103 having either a short WPRE sequence (5.5% of total CCR5) or a long WPRE sequence (2.3% of total CCR5).

Example 11: Regulation of CCR5 expression by synthetic microRNA sequences in lentiviral vectors with or without WPRE sequence)
Construction of vector. To test whether WPRE is required for downregulation of CCR5 expression by AGT103, we constructed a modified vector without the WPRE element sequence.

A functional assay for the regulation of cell surface CCR5 expression depending on whether the AGT103 vector contains or does not contain a long WPRE element. To test whether WPRE is required for regulation of CCR5 expression levels by AGT103, we used a multiplicity of infection equal to 5 and used AGT103, or a modified vector lacking WPRE, to CEM-CCR5. Transduced into T cells. Cells were harvested 6 days after transduction and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. This monoclonal antibody is directly conjugated to a fluorescent marker and its staining intensity is directly proportional to the number of CCR5 molecules per cell surface. The lentivirus control vector did not affect cell surface CCR5 levels, resulting in a uniform population with an average fluorescence intensity of 164. All AGT 103s lacking a lentiviral vector (AGT103 having long-chain WPRE and expressing GFP marker protein), AGT103 lacking GFP but containing long-chain WPRE elements, or both GFP and WPRE. It was equally effective in regulating cell surface CCR5 expression. After removal of GFP, AGT103 with or without WPRE element was indistinguishable in terms of its ability to regulate cell surface CCR5 expression.

AGT103 with or without GFP and WPRE was transduced into CEM-CCR5 cells. Six days later, CCR5 expression was determined by FACS analysis using APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as% CCR5 using an LV-control set to 100%. Decreased CCR5 levels were similar with AGT103 with or without WPRE sequence (0% of total CCR5) or without (0% of total CCR5). This data is demonstrated in FIG.

(Example 12: Regulation of CCR5 expression by the CD4 promoter that regulates synthetic microRNA sequences in lentiviral vectors.)
Construction of vector. A modified version of AGT103 was constructed to test the effect of using alternative promoters to express microRNA clusters that suppress gene expression of CCR5, Vif, and Tat. Instead of the usual EF-1 promoter, we have sequence:
(5'TGTTGGGGTTCAAATTTGAGCCCCAGCTGTTAGCCCTCTGCAAAGAAAAAAAAAAAAAAAAAAAAGAACAAAGGGCCTAGATTTCCCTTCTGAGCCCCACCCTAAGATGAAGCCTCT
TCTTTCAAGGGAGTGGGGTTGGGGTGGAGGCGGATCCTGTCAGCTTTGCTCTCTCTGTGGCTGGCAGTTTCTCCAAAGGGTAACAGGTGTCAGCTGGCTGAGCCTAGGCTGAACCC
TGAGACATGCTACCTCTGTCTTCTCATGGCTGGAGGCAGCCTTTGTAAGTCACAGAAAGTAGCTGAGGGGCTCTGGAAAAAAGACAGCCAGGGTGGAGGTAGATTGGTCTTTGACTC

CTGATTTAAGCCTGATTCTGCTTAACTTTTTCCCTTGACTTTGGCATTTTCACTTTGACATGTTCCCTGAGAGCCTGGGGGGTGGGGAACCCAGCTCCAGCTGGTGACGTTTGGGGC
CGGCCCAGGCCTAGGGTGTGGAGGAGCCTTGCCATCGGGCTTCCTGTCTCTCTTCATTTAAGCACGACTCTGCAGA-3 ') (SEQ ID NO: 30)
Was used to replace the T cell-specific promoter for CD4 glycoprotein expression.

A functional assay comparing the EF-1 and CD4 gene promoters in terms of their potency in reducing the expression of the CCR5 protein on the cell surface. AGT103 modified by using the CD4 gene promoter instead of the conventional EF-1 promoter was used for transduction of CEM-CCR5 T cells. Cells were harvested 6 days after transduction and stained with a monoclonal antibody capable of recognizing cell surface CCR5 protein. This monoclonal antibody is conjugated to a fluorescent marker and the staining intensity is directly proportional to the level of the cell surface CCR5 protein. Transduction of control lentivirus resulted in a population of CEM-CCR5 T cells stained with a CCR5-specific monoclonal antibody, resulting in an average fluorescence intensity of 81.7 units. AGT103 modified using the CD4 gene promoter instead of the EF-1 promoter to express microRNA showed a broad staining distribution with an average fluorescence intensity approximately equal to 17.3 units. Based on this result, the EF-1 promoter is at least similar to and likely superior to the CD4 gene promoter in terms of microRNA expression. Depending on the desired target cell population, the EF-1 promoter is universally active in all cell types and the CD4 promoter is active only in T lymphocytes.

A lentiviral vector (AGT103) containing a CD4 promoter that regulates synthetic microRNA sequences of CCR5, Vif, and Tat was transduced into CEM-CCR5 cells. Six days later, CCR5 expression was determined by FACS analysis using APC-conjugated CCR5 antibody and quantified as mean fluorescence intensity (MFI). CCR5 levels were expressed as% CCR5 using an LV-control set to 100%. In cells transduced with LV-CD4-AGT103, CCR5 levels were 11% of total CCR5. This is equivalent to that observed with LV-AGT103 containing the EF1 promoter. This data is demonstrated in FIG.

(Example 13: Detection of HIV Gag-specific CD4 T cells)
Cells and reagents. Frozen viable peripheral blood mononuclear cells (PBMC) were obtained from a vaccine company. Data were obtained using representative specimens from HIV + individuals enrolled in an early clinical trial (clinical trials.gov NCT01378156) testing candidate HIV therapeutic vaccines. Two specimens were obtained for "pre-vaccination" and "post-vaccination" studies. Cell culture products, supplements, and cytokines were obtained from commercial sources. Thomasson, M.D. , S. L. Heath, B.I. Sweeton, K.K. Williams, P.M. Cunningham, B.I. F. Keele, S.M. Sen, B. E. Palmer, N. et al. Chomont, Y. Xu, R. Basu, M. et al. S. Hellerstein, S.M. Kwa and H. L. Robinson (2016 years), "DNA / MVA Vaccination of HIV-1 Infected Participants with Viral Suppression on Antiretroviral Therapy, followed by Treatment Interruption:. Elicitation of Immune Responses without Control of Re-Emergent Virus", PLoS One, 11 Volume (10 No.): Cells were tested for response to recombinantly modified Waxinia Ankara 62B of Geovax Corporation as described on page 0163164. Synthetic peptides representing the entire HIV-1 Gag protein were obtained from GeoVax and HIV (GAG) Ultra peptide sets were obtained from JPT Peptide Technologies GmbH (www.jpt.com), Berlin, Germany. HIV (GAG) Ultra contains 150 peptides, each 15 amino acids long and 11 amino acids overlapping. These were chemically synthesized, then purified and analyzed by liquid chromatography-mass spectrometry. Collectively, these peptides represent the major immunogenic regions of the HIV Gag polyprotein and are designed to provide an average coverage of 57.8% among known HIV strains. The peptide sequence is based on the HIV sequence database of Los Alamos National Laboratory (http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html). Peptides are provided as 25 micrograms of dry trifluoroacetic acid salt for each peptide, dissolved in approximately 40 microliters of DMSO, and then diluted with PBS to final concentration. Monoclonal antibodies for detecting CD4 and cytoplasmic IFN gamma were obtained from commercial sources and intracellular staining was performed on interferon gamma using BD Pharmingen's Intracellular Staining Kit. The peptide was resuspended in DMSO. Control conditions for DMSO only are included.

Functional assay for detecting HIV-specific CD4 + T cells. Frozen PBMCs were thawed, washed and resuspended in RPMI medium containing 10% fetal bovine serum, supplements, and cytokines. Cultured PBMCs collected before or after vaccination were subjected to DMSO control, MVA GeoVax (multiplicity of infection equal to 1 plaque forming unit per cell), Peptides GeoVax (1 microgram) over 20 hours in the presence of GolgiStop reagent. / Ml), or treated with HIV (GAG) Ultra peptide mixture (1 microgram / ml). Cells were collected, washed, immobilized, permeabilized and stained with a monoclonal antibody specific for cell surface CD4 or intracellular interferon gamma. Stained cells were analyzed with a FACSCalibur analytical flow cytometer and data were gated on a subset of CD4 + T cells. Highlighted cells within the boxed area are double positive and are designated as HIV-specific CD4 T cells based on interferon gamma expression after stimulation with MVA or peptide. The numbers in the boxed area indicate the percentage of all CD4s identified as HIV-specific. No strong response to DMSO or MVA was detected. The GeoVax peptide elicited fewer responding cells compared to the HIV (GAG) Ultra peptide mixture of JPT, but the differences were slight and not significant.

As shown in FIG. 17, PBMCs derived from HIV-positive patients before or after vaccination were subjected to DMSO (control), recombinant MVA (MVA GeoVax) expressing HIV Gag of GeoVax, Gag peptide of GeoVax (Pep GeoVax,). Stimulated with Gag peptide pool 1 (also referred to herein as Gag peptide pool 1) or JPT's Gag peptide (HIV (GAG) Ultra, also referred to herein as Gag peptide pool 2) for 20 hours. IFNg production was detected by intracellular staining and flow cytometry using

standard protocols. Flow cytometric data were gated against CD4 T cells. The number captured in the square is the percentage of all CD4 T cells designated as "HIV-specific" based on the cytokine response to antigen-specific stimuli.

(Example 14: Expansion and proliferation of HIV-specific CD4 T cells and transduction of lentivirus)
Design and testing of methods for enriching PBMCs to increase the proportion of HIV-specific CD4 T cells and transducing AGT103 into these cells to produce the cell product AGT103T.

We designed a protocol for ex vivo culture of PBMC (peripheral blood mononuclear cells) in HIV-positive patients who received the therapeutic HIV vaccine. In this example, the therapeutic vaccine is MVA 62-B (3 doses) expressing plasmid DNA expressing the HIV Gag, Pol, and Env genes followed by 2 doses of the same HIV Gag, Pol, and Env genes. It consisted of a modified vaccine senior Ankara number 62-B). This protocol is not specific for vaccine products and requires only sufficient levels of post-immunized HIV-specific CD4 + T cells. Venous blood was collected and PBMCs were purified by Ficoll-Paque density gradient centrifugation. Alternatively, PBMCs or defined cell traction may be prepared by positive or negative selection methods using antibody cocktails and fluorescence activation or magnetic bead sorting. Purified PBMCs are washed and cultured in standard medium containing supplements, antibiotics, and fetal bovine serum. To these cultures was added a pool of synthetic peptides representing potential T cell epitopes within the HIV Gag polyprotein. Cultures are prepared by adding interleukin 2 and interleukin 12 cytokines selected after testing the combination of interleukin 2 and interleukin 12, interleukin 2 and interleukin 7, interleukin 2 and interleukin 15. Complement. Peptide stimulation is followed by a culture period of approximately 12 days. During the 12-day culture, fresh medium and fresh cytokine supplements were added approximately once every 4 days.

The peptide stimulation period is designed to increase the frequency of HIV-specific CD4 T cells in PBMC cultures. These HIV-specific CD4 T cells were activated by prior therapeutic immunization. These may be re-stimulated and propagated by exposure to synthetic peptides. Our goal is to achieve that CD4 T cells, which exceed 1% or equal to 1% of the total, are HIV-specific by the end of the peptide-stimulated culture period.

Approximately 12 days after culture, cells are washed to remove residual material and then stimulated with synthetic beads modified with antibodies to the CD4 T cell surface proteins CD3 and CD28. This well-established method for polyclonal stimulation of T cells reactivates cells and makes them more sensitive to transduction of AGT103 lentivirus. Lentivirus transduction takes place approximately on the 13th day of culture and uses a multiplicity of infection between 1 and 5. After transfection, cells are washed to remove residual lentiviral vector, cultured in medium containing interleukin 2 and interleukin 12, fresh medium and cytokines approximately every 4 days until approximately 24th day of culture. Add once to.

During the culture period, the antiretroviral drug saquinavir is added at a concentration of approximately 100 nM to suppress any possible increase in HIV.

Approximately 24 days after culture, cells were harvested, washed, sampled for efficacy and release assay, and then the remaining cells were suspended in cryopreservation medium before AGT103 was transfected approximately. Frozen in a single aliquot of $^{\text{approximately } 1 \times 10\ 10}$ cells per dose containing 1×10^{8} HIV-specific CD4 T cells.

The efficacy of the cell product (AGT103T) is tested in one of two alternating efficacy assays. Efficacy assay 1 tests the average number of genomic copies (integrated AGT103 vector sequences) per CD4 T cell. The minimum efficacy for releasing the product is approximately 0.5 genomic copies per CD4 T cell. This assay uses magnetic bead-labeled monoclonal antibodies to perform positive selection of CD3-positive / CD4-positive T cells, extracts whole-cell DNA, and uses quantitative PCR reactions to sequence specific to the AGT103 vector. It is done by detecting. Efficacy assay 2 tests the average number of integrated genomic copies of AGT103 within a subpopulation of HIV-specific CD4 T cells. This assay is first accomplished by stimulating PBMCs with a pool of synthetic peptides representing the HIV Gag protein. The cells are then stained with a specific antibody reagent capable of binding to CD4 T cells and also capturing the secreted interferon gamma cytokines. CD4-positive / interferon gamma-positive cells are captured by magnetic bead selection, whole cell DNA is prepared, and the number of genomic copies of AGT103 per cell is determined by a quantitative PCR reaction. Efficacy-based release criteria using Assay 2 require the presence of greater than or equal to 0.5 genomic copies per HIV-specific CD4 T cell in the AGT103 cell product.

A functional test for enriching and transfecting HIV-specific CD4 T cells from PBMCs of HIV-positive patients who received the therapeutic HIV vaccine. The effect of therapeutic vaccination on the frequency of HIV-specific CD4 T cells was tested by peptide stimulation assay (FIG. 14 panel B). The frequency of HIV-specific CD4 T cells prior to vaccination was 0.036% in this representative individual. After vaccination, the frequency of HIV-specific CD4 T cells increased approximately 2-fold to a value of 0.076%. Response cells (HIV-specific) identified by the accumulation of cytoplasmic interferon gamma were detected only after specific peptide stimulation.

We also present that, of all CD4 T cells in the culture, HIV-specific and AGT103 are present when AGT103 characterization is performed after peptide stimulation to concentrate HIV-specific CD4 T cells. We tested whether our goal of producing approximately 1% of transgenics was achieved. In this case, we used an experimental version of AGT103 that expresses green fluorescent protein (see GFP). In FIG. 14, panel C, 1.11% of all CD4 T cells are HIV-specific (in response to peptide stimulation) by culture after vaccination after peptide stimulation (HIV (GAG) Ultra) and AGT103 transduction. It was demonstrated (based on the expression of interferon gamma) and that AGT103 was transduced (based on the expression of GFP).

Several patients in the Therapeutic HIV Vaccine Study were tested to assess their range of response to peptide stimuli and begin defining eligibility criteria for participation in the gene therapy arm in future human clinical trials. bottom. FIG. 18 Panel D compares pre- and post-vaccination specimens from four vaccine study participants and shows the frequency of HIV-specific CD4 T cells in these participants. In three cases, the vaccinated specimen exhibits HIV-specific CD4 T cell values that are greater than or equal to 0.076% of all CD4 T cells. The ability to reach this value was not expected from pre-vaccinated specimens. Because in both patients 001-004 and patients 001-006, the value of HIV-specific CD4 T cells before vaccination started from 0.02%, while the value of HIV-specific CD4 T cells after vaccination started from 0.02%. This is because the final value reached 0.12% and the other individual did not increase this value after vaccination. The three patients who responded well to the vaccine in increasing the frequency of HIV-specific CD4 T cells also showed substantial enrichment of HIV-specific CD4 T cells after peptide stimulation and culture. In the three cases shown in FIG. 18 Panel E, samples in which 2.07%, 0.72%, or 1.54% of all CD4 T cells were HIV-specific by peptide stimulation and subsequent culture, respectively. Was generated. These values indicate that in the majority of individuals responding to the therapeutic HIV vaccine, approximately 1% of all CD4 T cells are HIV-specific in the final cell product and AGT103 is transfected. It is shown that they have a sufficiently large ex vivo response to peptide stimulation to enable our goal of achieving.

As shown in FIG. 18, panel A describes a treatment schedule. Panel B demonstrates that PBMC was stimulated with a Gag peptide or DMSO control for 20 hours. IFN gamma production was detected by intracellular staining with FACS. $CD4^+$ T cells were gated for analysis. Panel C demonstrates that $CD4^+$ T cells were expanded and proliferated and transduced with AGT103-GFP using the method shown in Panel A. The expanded $CD4^+$ T cells were allowed to stand in fresh medium containing no cytokine for 2 days and restimulated with Gag peptide or DMSO control for 20 hours. IFN gamma production and GFP expression were detected by FACS. $CD4^+$ T cells were gated for analysis. Panel D demonstrates that the frequency of HIV-specific $CD4^+$ T cells (IFN gamma positive, before and after vaccination) was detected in 4 patients. Panel E demonstrates that post-vaccinated PBMCs from 4 patients were expanded and investigated for HIV-specific CD4 + T cells.

(Example 15: dose response)
Construction of vector. A modified version of AGT103 was constructed and tested for its effect on increasing dose response to AGT103 and cell surface CCR5 levels. AGT103 was modified to include a green fluorescent protein (GFP) expression cassette under the control of the CMV promoter. Transduced cells express miR30CCR5 miR21Vif miR185Tat microRNA clusters and emit green light due to GFP expression.

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

As shown in FIG. 19, Panel A demonstrates its effect on increasing dose response to increasing AGT103-GFP and cell surface CCR5 expression. At a multiplicity of infection equal to 0.4, only 1.04% of the cells are green (indicating transduction) and exhibit significantly reduced CCR5 expression. At multiplicity of infection equal to 1, CCR5low, GFP + cell count increased to 68.1% / at multiplicity of infection equal to 5, CCR5low, GFP + cell count increased to 95.7%. These data are presented in histogram form in Panel B of FIG. 19, which shows a normally distributed population in terms of CCR5 staining towards lower average fluorescence intensities with increasing doses of AGT103-GFP. The efficacy of AGT103-GFP is presented in graph form in Panel C of FIG. 19 showing the percentage of inhibition of CCR5 expression with increasing doses of AGT103-GFP. At multiplicity of infections equal to 5, CCR5 expression levels were reduced by more than 99%.

(Example 16: AGT103 efficiently transduces primary human $CD4^+$ T cells)
Transduction of primary CD4 T cells with AGT103 lentiviral vector. A modified AGT103 vector containing a green fluorescent protein marker (GFP) was used with an infection multiplicity of between 0.2 and 5 for transduction into purified primary human CD4 T cells.

Functional assay for transduction efficiency of AGT103 in primary human CD4 T cells. CD4 T cells were isolated from human PBMCs (HIV-negative donors) using magnetic bead-labeled antibodies and standard procedures. Purified CD4 T cells were exvivo-stimulated with CD3 / CD28 beads and cultured daily in medium containing interleukin 2 prior to AGT103 transduction. The relationship between lentiviral vector dose (multiplicity of infection) and transduction efficiency is demonstrated in Panel A of FIG. 20 with a multiplicity of infection equal to 0.2 in 9.27% of CD4 positive T cells. It has been shown that AGT103 was transduced and this value was increased by transducing AGT103 into 63.1% of CD4-positive T cells at a multiplicity of infection equal to 5. In addition to achieving efficient transduction of primary CD4-positive T cells, it is also necessary to quantify the number of genomic copies per cell. In FIG. 20 Panel B, whole cell DNA of primary human CD4 T cells transduced at several multiplicity of infections was tested by quantitative PCR to determine the number of genomic copies per cell. At a multiplicity of infection equal to 0.2, 0.096 genomic copies per cell were measured, which is in good agreement with 9.27% GFP-positive CD4 T cells in Panel A. A multiplicity of infection equal to 1 produced 0.691 genomic copies per cell, and a multiplicity of infection equal to 5 produced 1.245 genomic copies per cell.

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

(Example 17: AGT103 inhibits HIV replication in $^{\text{primary CD4 + T cells}}$)
Protection of cells from HIV infection by transducing AGT103 into primary human CD4-positive T cells. Therapeutic lentivirus AGT103 was used to transduce primary human CD4-positive T cells with an infection multiplicity of between 0.2 and 5 per cell. The transduced cells were then loaded with the CXCR4-tropic HIV strain NL4.3, which does not require cell surface CCR5 for invasion. This assay tests the efficacy of microRNAs on the Vif and Tat genes of HIV in preventing proliferative infections in primary CD4 positive T cells, but of HIV released from infected primary human CD4 T cells. Indirect methods are used to detect the amount.

Functional assay for protection of primary human CD4 + T cells by AGT103 from CXCR4-tropic HIV infection. CD4 T cells were isolated from human PBMCs (HIV-negative donors) using magnetic bead-labeled antibodies and standard procedures. Purified CD4 T cells were stimulated exvivo with CD3 / CD28 beads and using multiplicity of infections between 0.2 and 5 daily in medium containing interleukin 2 prior to AGT103 transduction. It was cultured. Two days after transduction, CD4 positive T cell cultures were loaded with HIV strain NL4.3 engineered to express green fluorescent protein (GFP). Primary CD4 T cell cultures transduced and exposed to HIV were maintained for 7 days before collecting HIV-containing cell-free cultures. This cell-free culture was used to infect the highly tolerated T cell line C8166 for 2 days. The proportion of C8166 cells infected with HIV was determined by detecting fluorescence of GFP by flow cytometry. In simulated lentivirus infection, for a dose of NL4.3 HIV with a multiplicity of infection of 0.1, an amount of HIV was released into the culture that could establish a proliferative infection in 15.4% of C8166 T cells. .. For a dose of 0.2 multiplicity of infection, AGT103, this value for HIV infection of C8166 cells was reduced to 5.3%, and for AGT103 with a multiplicity of infection equal to 1, only 3.19% of C8166 T cells were HIV. I was not infected. C8166 infection was further reduced to 0.62% after AGT103 transduction using an infection multiplicity equal to 5. There is a clear dose response relationship between the amount of AGT103 used for transduction and the amount of HIV released into the culture medium.

As shown in FIG. 21, $CD4^+$ T cells isolated from PBMCs were stimulated with CD3 / CD28 beads and IL-2 for 1 day to transduce AGT103 at various concentrations (MOI). After 2 days, the beads were removed and $CD4^+$ T cells were infected with 0.1 MOI of HIV NL4.3-GFP. After 24 hours, cells were washed 3 times with PBS and cultured with IL-2 (30 U / ml) for 7 days. At the end of the culture, the supernatant was collected and infected with HIV tolerant cell line C8166 for 2 days. HIV-infected C8166 cells (GFP positive) were detected by FACS. Viable HIV decreased with increasing multiplicity of infection of AGT103 (MOI 0.2 = 65.6%, MOI 1 = 79.3%, and MOI, as observed by reduced infection of C8166 cells. 5 = 96%).

(Example 18: AGT103 protects primary human $CD4^+$ T cells from HIV-induced depletion)
Transduction of AGT103 into primary human CD4 T cells to protect against HIV-mediated cell pathology and cell depletion. PBMCs were obtained from healthy HIV-negative donors, stimulated with CD3 / CD28 beads, then cultured daily in medium containing interleukin 2, and then AGT103 using an infection multiplicity of between 0.2 and 5. The trait was introduced.

Functional assay for protection of primary human CD4 T cells from HIV-mediated cell pathology by AGT103. Primary human CD4 T cells transduced with AGT103 were infected with HIV NL 4.3 strain (CXCR4 tropism), which does not require CCR5 for cell entry. When using CXCR4-tropic NL 4.3, only the effects of Vif and Tat microRNAs on HIV replication have been tested. The dose of HIV NL 4.3 was a multiplicity of infection of 0.1. One day after HIV infection, cells were washed to remove residual virus

and cultured in medium supplemented with interleukin 2. Cells were harvested every 3 days during 14-day culture and then stained with a monoclonal antibody specific for CD4 and directly conjugated to a fluorescent marker to determine the proportion of CD4 positive T cells in PBMCs. Made possible. Untreated CD4 T cells, or CD4 T cells transfected with a control lentiviral vector, are highly sensitive to HIV loading, and the proportion of CD4 positive T cells in PBMCs is 10 by day 14 of culture. It fell to less than%. In contrast, AGT103 had a dose-dependent effect on preventing cell depletion due to HIV loading. At AGT103 doses with a multiplicity of infection of 0.2, more than 20% of PBMCs were CD4 T cells by day 14 of culture, but this value is 50% of PBMCs at AGT103 doses with a multiplicity of infection equal to 5. Super increased to CD4-positive T cells by day 14 of culture. Again, AGT103 has a clear dose-responsive effect on HIV cytopathic effects in human PBMCs.

As shown in FIG. 22, PBMCs were stimulated with CD3 / CD28 beads and IL-2 for 1 day to transduce AGT103 at various concentrations (MOI). After 2 days the beads were removed and the cells were infected with 0.1 MOI HIV NL4.3. After 24 hours, cells were washed 3 times with PBS and cultured with IL-2 (30 U / ml). Cells were collected every 3 days and $^{\text{the frequency of CD4 +}}$ T cells was analyzed by FACS. After 14 days of exposure to HIV, LV-control transduced CD4 $^{+}$ T cells were reduced by 87%, AGT103 MOI0.2 by 60%, AGT103 MOI1 by 37%, and AGT103 MOI5 by 17%. ..

(Example 19: Generation of CD4 + T cell population enriched for HIV specificity and transduced with AGT103 / CMV-GFP)
The effect of therapeutic vaccination against HIV on the distribution of CD4 +, CD8 +, and CD4 + / CD8 + T cells was minimal. As shown in FIG. 23A, the CD4 T cell population is shown in the upper left quadrant of the analytical flow cytometry dot plot, changing from 52% to 57% of all T cells after a series of vaccinations. These are representative data.

Peripheral blood mononuclear cells from participants in the HIV therapeutic vaccine trial were cultured in medium +/- interleukin 2 / interleukin 12 or +/- interleukin 7 / interleukin 15 for 12 days. Some cultures were stimulated with a duplicate peptide (HIV (GAG) Ultra peptide mixture) representing the entire p55 Gag protein of HIV-1 as a source of epitope peptides for T cell stimulation. These peptides are 10 to 20 amino acids long and overlap 20-50% of their length to represent the entire Gag precursor protein (p55) of the HIV-1 BaL strain. The composition and sequence of the individual peptides may be adjusted to compensate for regional variation in the major circulating HIV sequence, or if detailed sequence information is available for individual patients receiving this therapy. 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 displayed here. Stimulation with the HIV (GAG) Ultra peptide mixture was similar to medium control for both pre-vaccination and post-vaccination samples, and the HIV (GAG) Ultra peptide mixture acted as a polyclonal mitogen rather than toxic to cells. It was shown that it was not. The results of this analysis can be found in FIG. 23B.

The HIV (GAG) Ultra peptide mixture and interleukin 2 / interleukin 12 resulted in optimal expansion and proliferation of antigen-specific CD4 T cells. As shown in the upper panel of FIG. 23C, cells secreting the cytokine (interferon gamma) were increased in the vaccinated specimens exposed to the HIV (GAG) Ultra peptide mixture. 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 response of CD4 T cells to the HIV antigen.

Finally, transfecting AGT103 / CMV-GFP into antigen-expanded CD4 T cells is an HIV-specific, HIV-resistant helper CD4 T cell that is required for injection into patients as part of the functional healing of HIV. Was produced (according to various other embodiments and embodiments, AGT103 alone is used; for example, clinical embodiments may not include the CMV-GFP segment). The upper panel of FIG. 23C shows the results of analysis of the CD4 + T cell population in the culture. The x-axis of FIG. 23C shows the emission of green fluorescent protein (GFP), indicating that AGT103 / CMV-GFP was transduced into individual cells. In the vaccinated sample, 1.11% of all CD4 T cells secreting both cytokines were recovered, these cells responded specifically to the HIV antigen, and AGT103 / CMV-GFP was traited. It was shown to have been introduced. This is the target cell population and is a clinical product intended for infusion and functional healing of HIV. The efficiency of cell expansion and proliferation during antigen stimulation and the subsequent polyclonal expansion and proliferation phase of ex vivo culture $^{\text{can produce } 4 \times 10^{8}}$ antigen-specific, lentivirus-transduced CD4 T cells. This is four times more than the target of cell production, which results in approximately 40 antigen-specific, HIV-resistant CD4 T cells per microliter of blood, or approximately 5.7% of all-circulating CD4 T cells. Will be achievable.

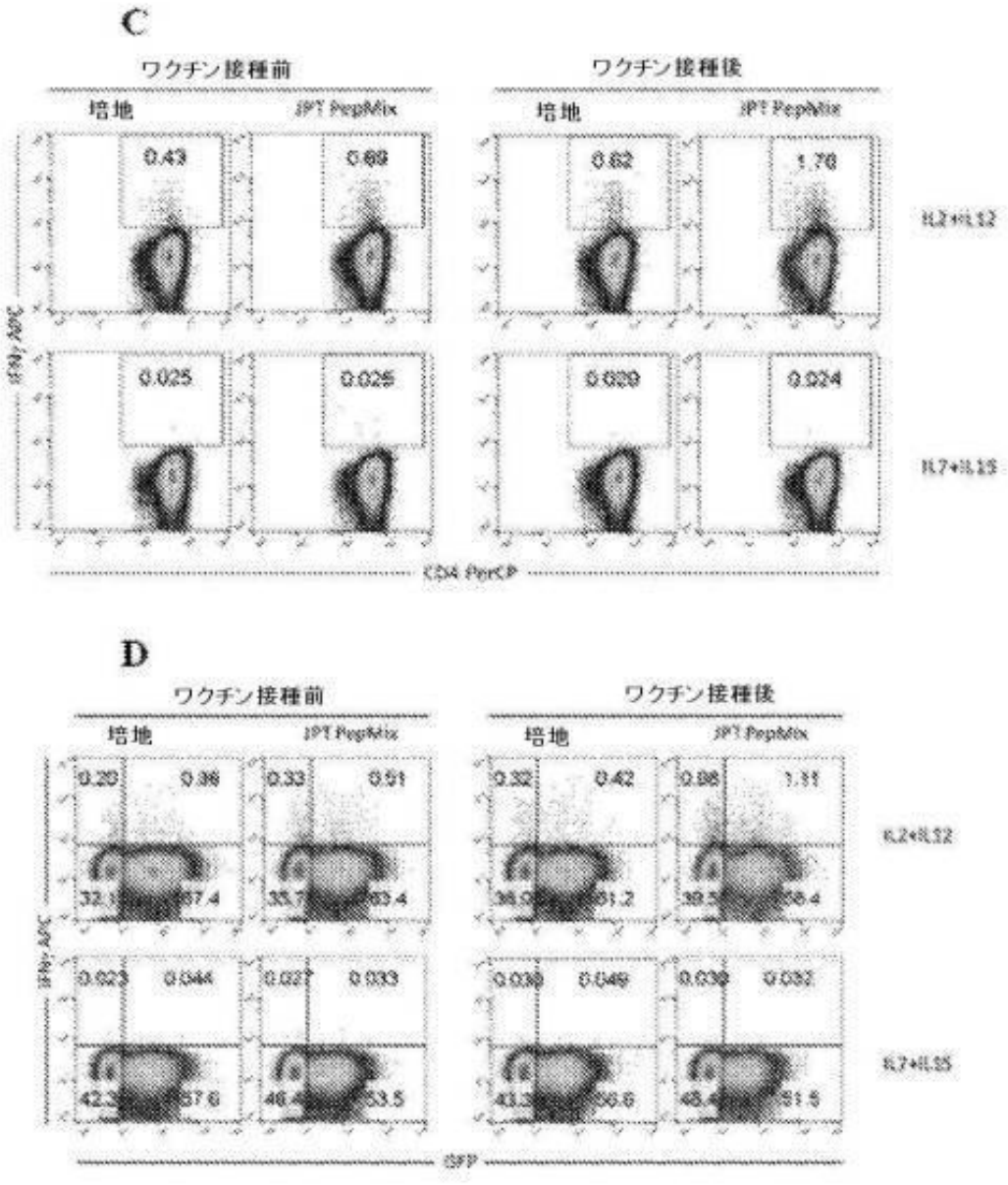


Figure 23 続き

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

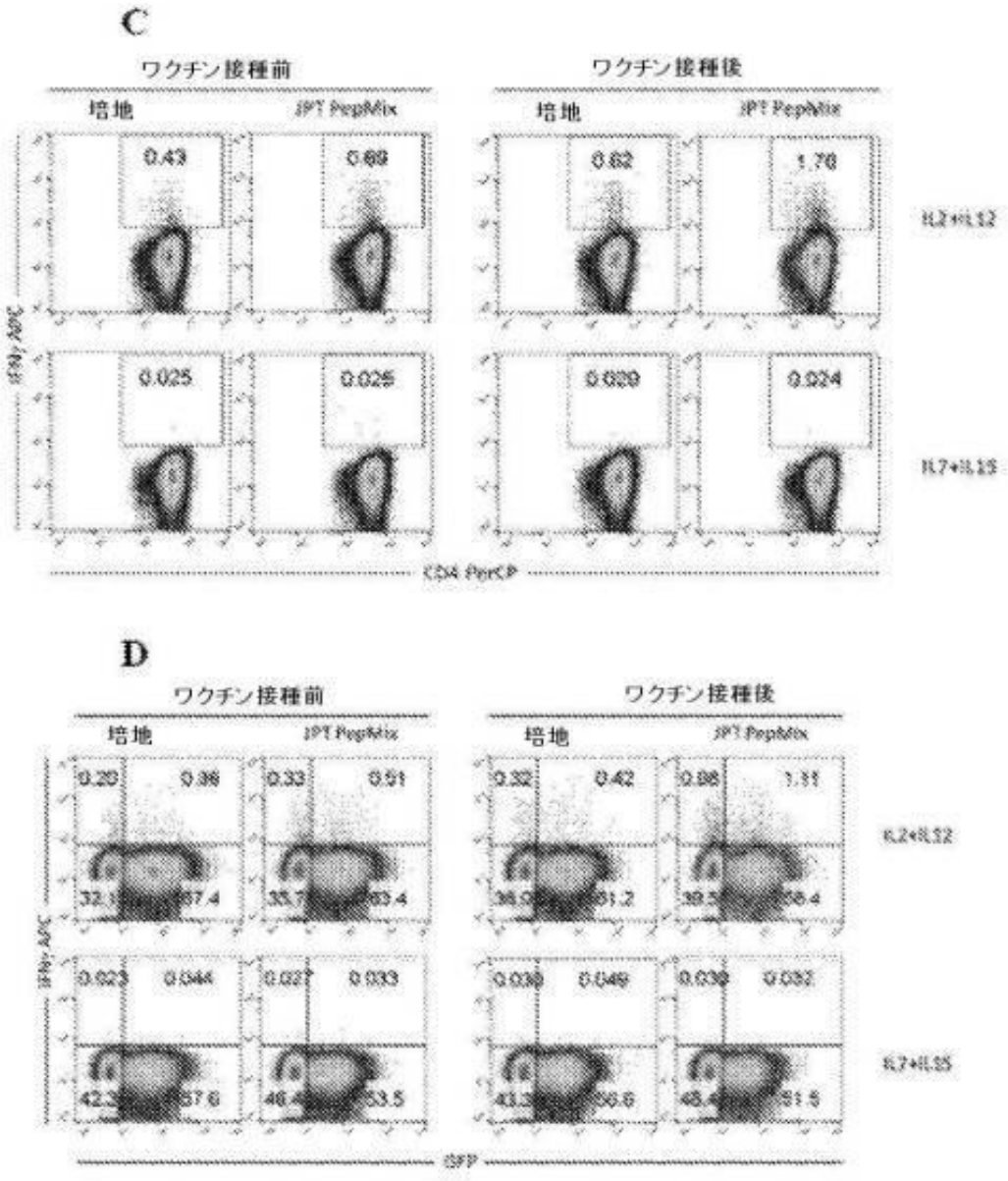


Figure 23 続き

(Example 20: Clinical study for the treatment of HIV)

AGT103T is a genetically modified autologous PBMC containing> 5 × 10 7 HIV-specific CD4 T cells, also transduced with the AGT103 lentiviral vector.

In a phase I clinical trial, while receiving cART, HIV infection, blood 1 mm ^{3} per CD4 + T cell count> 600 cells, and less than 200 per plasma 1ml copy of stable viral suppression was observed, Adult study participants will be tested for the safety and feasibility of injecting ex vivo-modified autologous CD4 T cells (AGT103T). All study participants will continue to receive their standard antiretroviral drug application during Phase I clinical trials. Up to 40 study participants receive two doses of

recombinantly modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol, and Env proteins by intramuscular injection every 8 weeks. Seven to ten days after the second immunization, blood samples are collected for in vitro testing and the frequency of CD4 + T cells in response to stimulation by a pool of overlapping synthetic peptides representing the HIV-1 Gag polypeptide is measured. .. Based on the measurement of the frequency of Gag-specific CD4 T cells, the top half of the respondents will be enrolled in the gene therapy arm and the bottom half of the responders will not continue the study. The cutoff for the top responders is predicted to be ≧ 0.065% of all CD4 T cells with a frequency of HIV-specific CD4 + T cells. Subjects enrolled in the gene therapy arm of our study were leukocyte depleted, followed by exvivo culture and stimulation with HIV Gag peptide and interleukin 2 and interleukin 12 for 12 days. Purification of PBMCs is then restimulated with beads modified with CD3 / CD28 bispecific antibodies (using Ficoll density gradient centrifugation or negative selection by antibody). To prevent the appearance of self-HIV in ex vivo cultures, the antiretroviral drug saquinavir is included at 100 nM. One day after CD3 / CD28 stimulation, AGT103 is transduced into cells with a multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which they expand and proliferate by polyclonal proliferation. The culture period is terminated by harvesting and washing cells, setting aliquots for efficacy and safety release assay, and resuspending the remaining cells in cryopreservation medium. The single dose is ≦ 1×10^{10} self-PBMCs. The efficacy assay measures the frequency of CD4 T cells that respond to peptide stimuli by expressing interferon gamma. Another release criterion is that the product must contain at least 0.5×10^{7} CD4 T cells that are HIV specific and are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell should not exceed 3. Five days prior to AGT103T infusion, subjects receive an infusion of ≤$1x10^{10}$ PBMCs containing genetically modified CD4 T cells after a dose of busulfuram (or Cytoxan) acclimation regimen.

Phase II studies will evaluate the efficacy of AGT103T cell therapy. Participants in Phase II studies were already enrolled in our Phase I study and had successful and stable engraftment of genetically modified autologous HIV-specific CD4 T cells. Individuals determined to have a clinical response defined as a positive change in the monitored parameters as described in efficacy assessment (1.3.) Are included. Study participants will be asked to add maraviroc to their existing antiretroviral drug application regimen. Maraviroc is a CCR5 antagonist that enhances the efficacy of gene therapy aimed at lowering CCR5 levels. Once maraviroc regimen was performed, subjects discontinued the previous anti-retroviral drug regimen and had plasma viral RNA levels of 10,000 per ml for 28 days or at two consecutive weekly blood draws. You will be asked to maintain only maraviroc monotherapy until you exceed it. If viremia is persistently high, participants are urged to return to their original antiretroviral drug regimen with or without maraviroc as recommended by their HIV care physician.

If participants' HIV remains suppressed for> 28 days during maraviroc monotherapy (less than 2,000 vRNA copies per ml plasma), it is sought to gradually reduce maraviroc dosing for 4 weeks, and then further. Intensive monitoring will take place over 28 days. Subjects who maintain HIV suppression with maraviroc monotherapy are considered to have functional cure. Subjects whose suppression of HIV is maintained even after withdrawal from maraviroc also have functional cure. Monthly monitoring for 6 months, followed by less intensive monitoring establishes the durability of functional healing.

Patient Selection Inclusion Criteria:
・ Age 18-60 years old.
-HIV infection recorded prior to study enrollment.
• Must be willing to follow the evaluation enforced by the study, including not changing the antiretroviral drug regimen during the study period (unless medically instructed).
-CD4 + T cell count per cubic millimeter> 600 cells (cells / mm3)
-> 400 cells / mm3 CD4 + T cell lowest point-HIV viral load <1,000 copies / milliliter (mL)

Exclusion criteria:
・ Some kind of viral hepatitis ・ Acute HIV infection ・ HIV viral load> 1,000 copies / mL
Active or recent (last 6 months) AIDS-defining complications • Any changes in HIV drug application within 12 weeks of study enrollment • Remission for at least 5 years, except for successfully treated basal cell carcinoma of the skin Not cancer or malignant disease-Current diagnosis of NYHA grade 3 or 4 congestive heart failure or uncontrolled angina or arrhythmia-History of bleeding disorders-Chronic steroid use for the last 30 days-Pregnancy or breastfeeding Medium-active drug or alcohol abuse-Serious illness over the last 30 days-Currently participating in another clinical trial or some previous genetic therapy

Safety evaluation / Acute injection reaction / Post-injection safety follow-up survey

Validity assessment-Phase I, number and frequency of modified CD4 T cells.
-Durability of modified CD4 T cells.

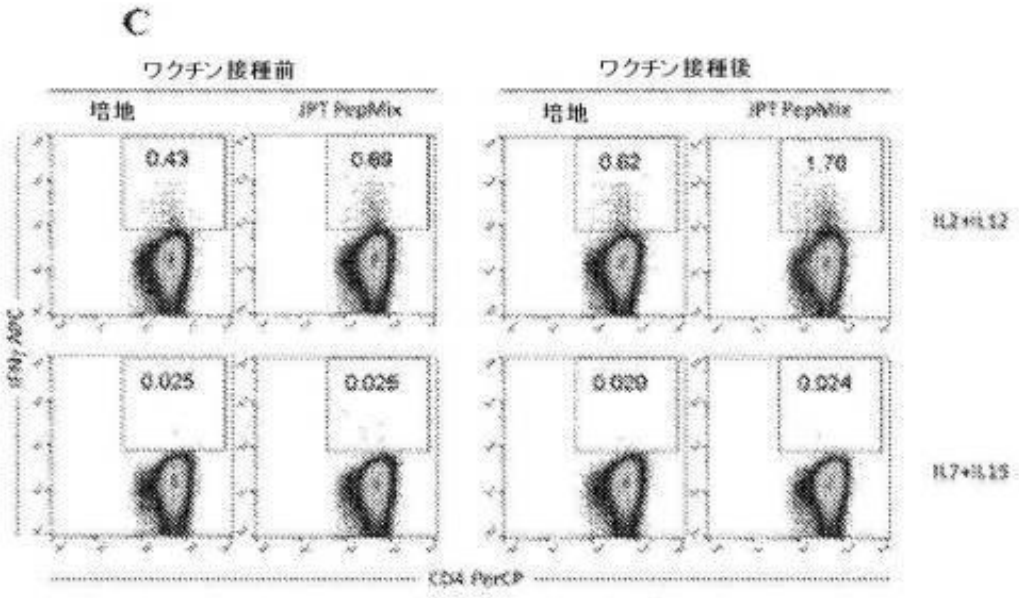
-In vitro response to Gag peptide restimulation as a measure of memory T cell function (ICS assay).
Multifunctional anti-HIV CD8 T cell response compared to pre- and post-vaccination time points.
Frequency of CD4 T cells producing double spliced HIV mRNA after in vitro stimulation.

Efficacy Assessment-Phase II / Gene Modified CD4 T Cell Number and Frequency.
· Maraviroc maintenance of monotherapy with viral suppression (per 1ml <2,000 pieces of vRNA copies, however vRNA copies per 1ml at successive weekly blood sampling twice if not exceeding 5×10^{4} is allowed).
・ Sustained virus suppression during and after withdrawal of maraviroc.
-Stable CD4 T cell number.

AGT103T consists of up to 1×10^{10} genetically modified autologous CD4 + T cells containing ≧ 5×10^{7} HIV-specific CD4 T cells and also transduced with the AGT103 lentiviral vector. In a phase I clinical trial, while receiving cART, HIV infection, blood 1 mm^{3} per CD4 + T cell count> 600 cells, and less than 200 per plasma 1ml copy of stable viral suppression was observed, The safety and feasibility of injecting ex vivo-modified autologous CD4 T cells (AGT103T) will be tested in adult study participants. Up to 40 study participants receive two doses of recombinant modified vaccinia Ankara (rMVA) expressing HIV Gag, Pol, and Env proteins by intramuscular injection every 8 weeks. Seven to ten days after the second immunization, blood samples are collected for in vitro testing and the frequency of CD4 + T cells in response to stimulation by a pool of overlapping synthetic peptides representing the HIV-1 Gag polypeptide is measured. .. Based on the measurement of the frequency of Gag-specific CD4 T cells, the top half of the respondents will be enrolled in the gene therapy arm and the bottom half of the responders will not continue the study. The cutoff for the top responders is predicted to be ≧ 0.065% of all CD4 T cells with a frequency of HIV-specific CD4 + T cells. Leukocyte depletion is performed on subjects enrolled in the gene therapy arm of our study and CD4 + T cells are enriched by negative selection. The enriched CD4 subset is mixed with 10% of the cell number of the CD4 negative subset to provide a source and antigen presenting cells. Concentrated CD4 T cells are stimulated with HIV Gag peptide and interleukin 2 and interleukin 12 for 12 days and then restimulated with beads modified with CD3 / CD28 bispecific antibodies. To prevent the appearance of self-HIV in ex vivo cultures, the antiretroviral drug saquinavir is included at 100 nM. One day after CD3 / CD28 stimulation, AGT103 is transduced into cells with a multiplicity of infection between 1 and 10. The transduced cells are cultured for an additional 7-14 days during which they expand and proliferate by polyclonal proliferation. The culture period is terminated by harvesting and washing cells, setting aliquots for efficacy and safety release assay, and resuspending the remaining cells in cryopreservation medium. The single dose is ≦ 1×10^{10} autologous cells enriched with a subset of CD4 + T cells. The efficacy assay measures the frequency of CD4 T cells that respond to peptide stimuli by expressing interferon gamma. Another release criterion is that the product must contain at least 0.5×10^{7} CD4 T cells that are HIV specific and are also transduced with AGT103. Another release criterion is that the number of AGT103 genome copies per cell should not exceed 3. Five days prior to AGT103T injection, subjects receive an injection of ≤$1x10^{10}$ concentrated and genetically modified CD4 T cells after a dose of Busulfram (or Cytoxan) acclimation regimen.

Phase II studies will evaluate the efficacy of AGT103T cell therapy. Participants in Phase II studies were already enrolled in our Phase I study and had successful and stable engraftment of genetically modified autologous HIV-specific CD4 T cells. Individuals determined to have a clinical response defined as a positive change in the monitored parameters as described in efficacy assessment (1.3.) Are included. Study participants will be asked to add maraviroc to their existing antiretroviral drug application regimen. Maraviroc is a CCR5 antagonist that enhances the efficacy of gene therapy aimed at lowering CCR5 levels. Once maraviroc regimen has been performed, the subject discontinues the previous anti-retroviral drug application regimen and plasma viral RNA levels per ml for 28 days or at two consecutive weekly blood draws are 10, You are required to maintain only maraviroc monotherapy until you exceed 000. If viremia is persistently high, participants are urged to return to their original antiretroviral drug regimen with or without maraviroc as recommended by their HIV care physician.

If participants' HIV remains suppressed for> 28 days during maraviroc monotherapy (less than 2,000 vRNA copies per ml plasma), it is sought to gradually reduce maraviroc dosing for 4 weeks, and then further. Intensive monitoring will take place over 28 days. Subjects who maintain HIV suppression with maraviroc monotherapy are considered to have functional cure. Subjects whose suppression of HIV is maintained even after withdrawal from maraviroc also have functional cure. Monthly monitoring for 6 months, followed by less intensive monitoring establishes the durability of functional healing.


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

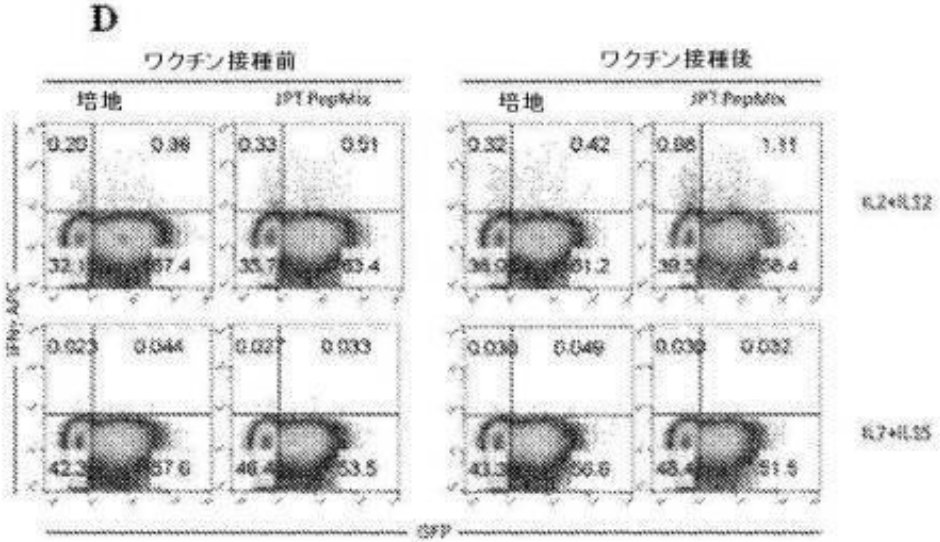
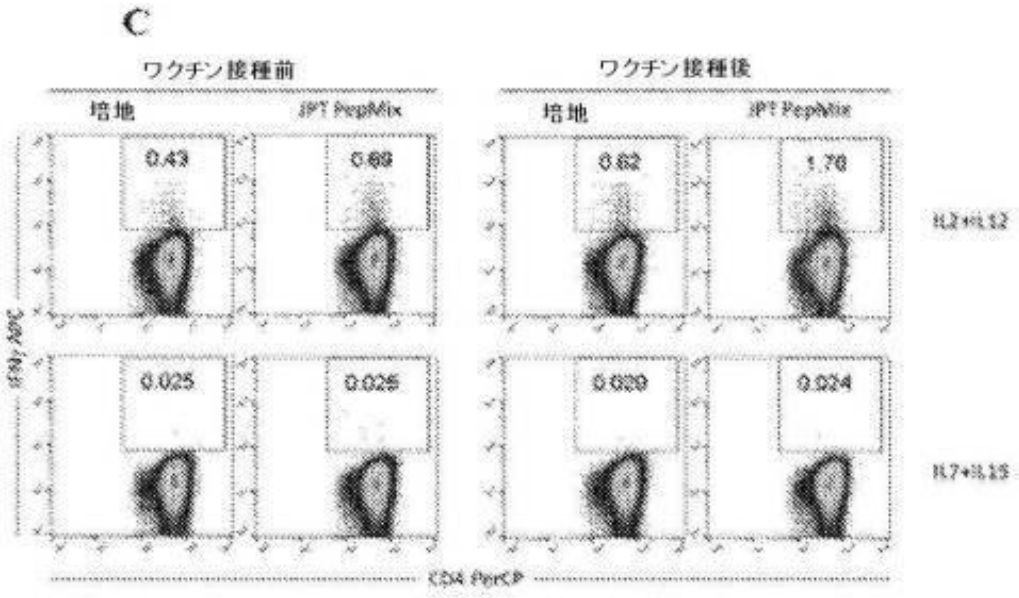

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

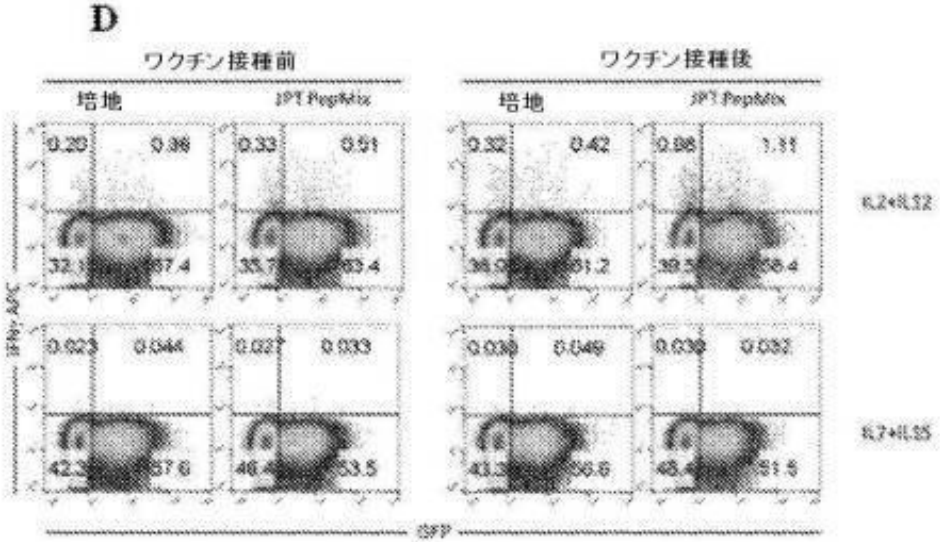
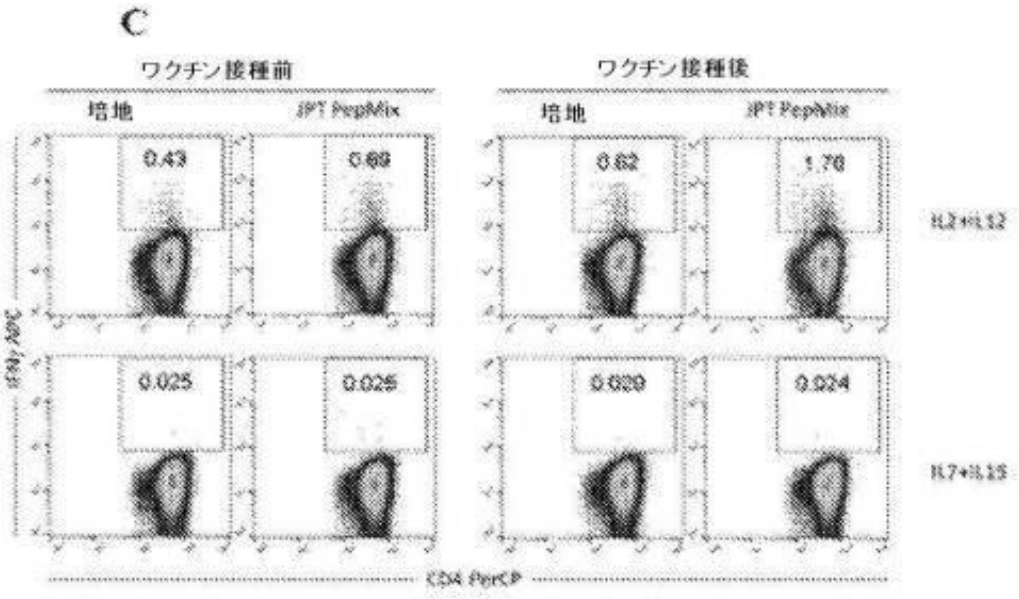

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

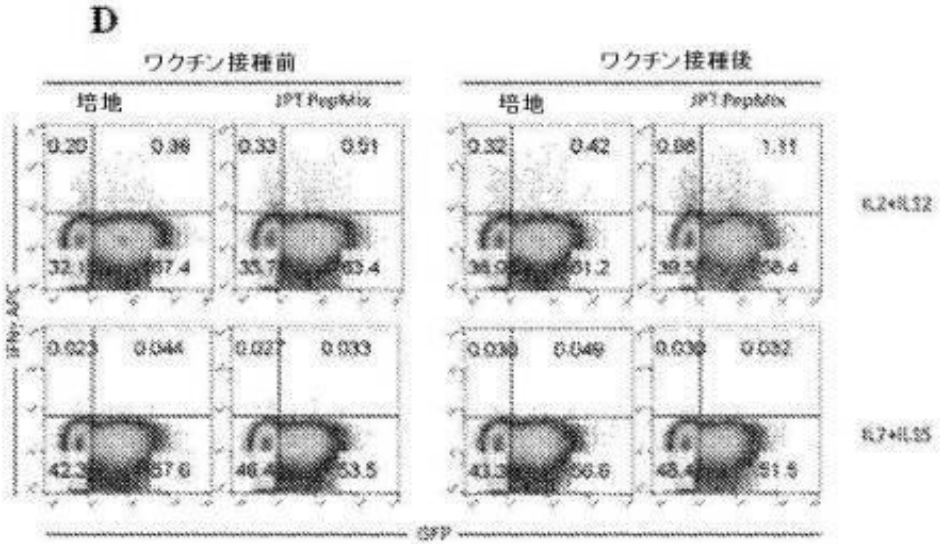
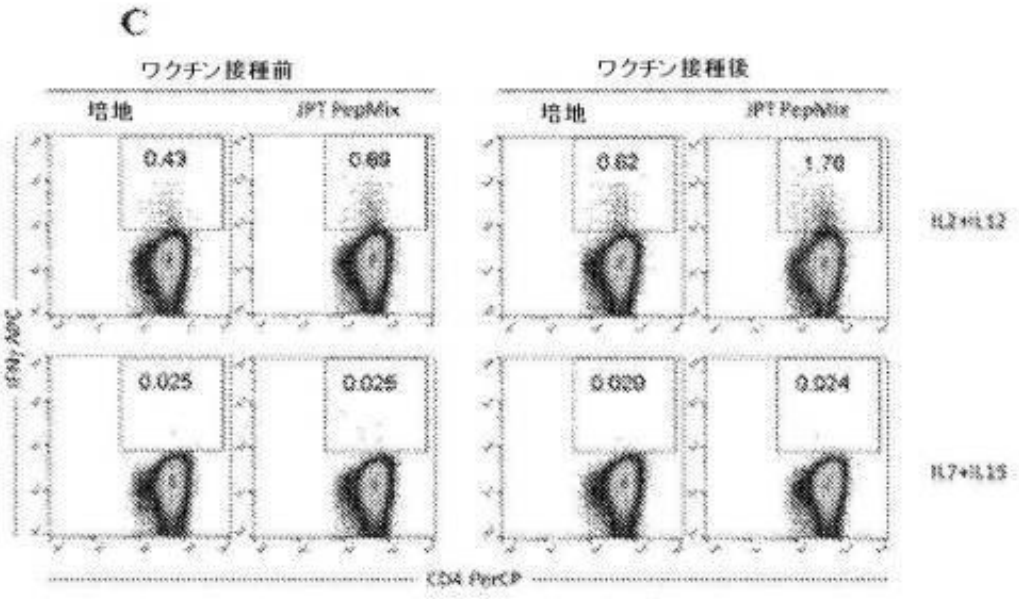

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

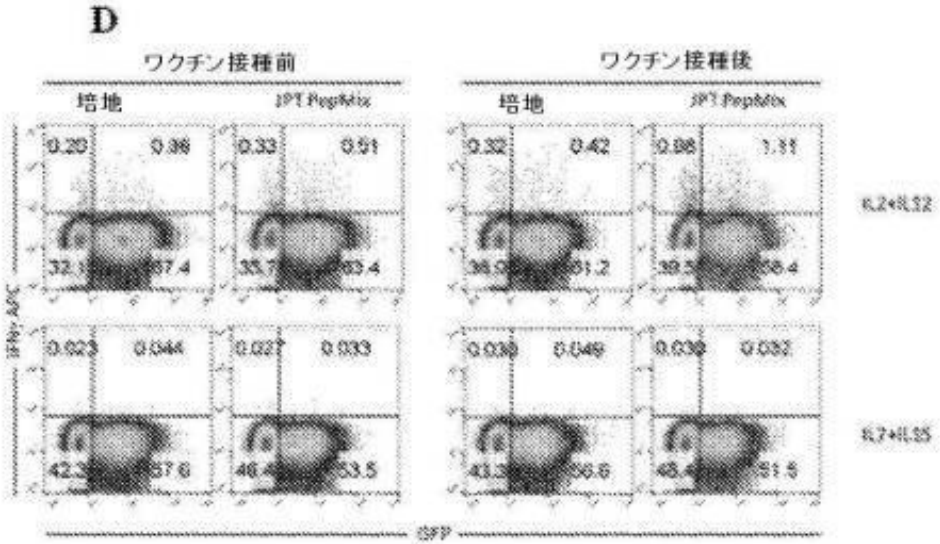
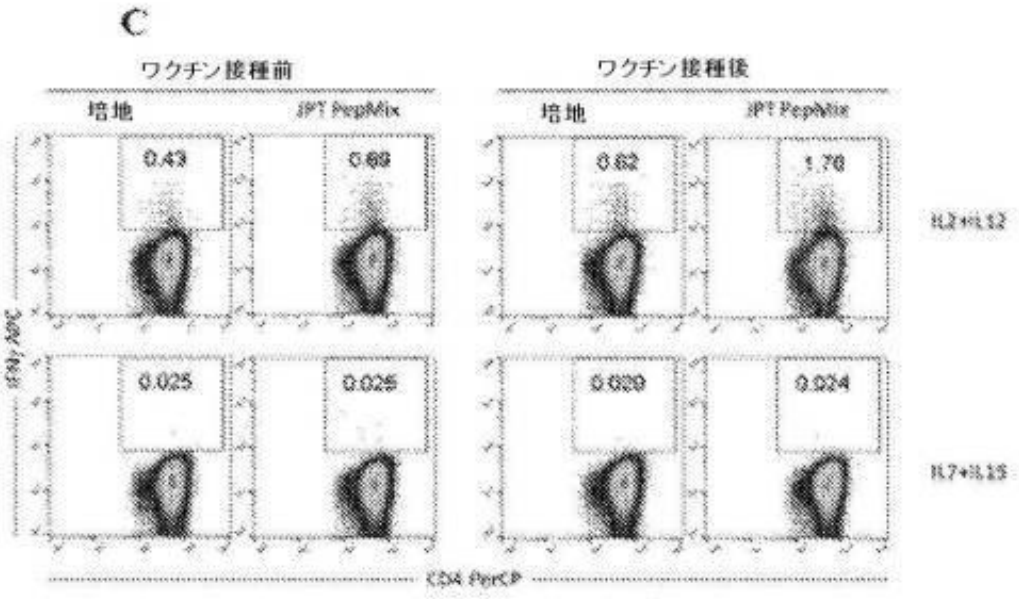

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

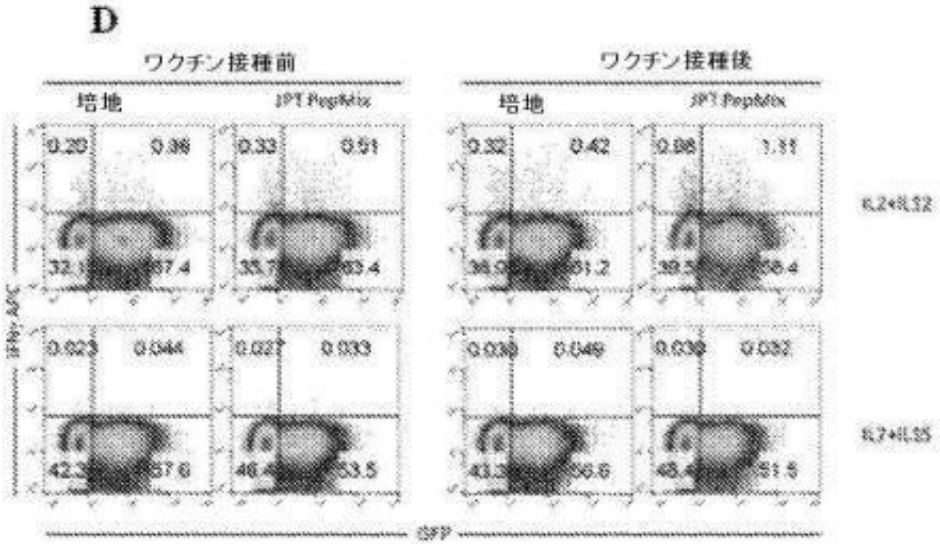
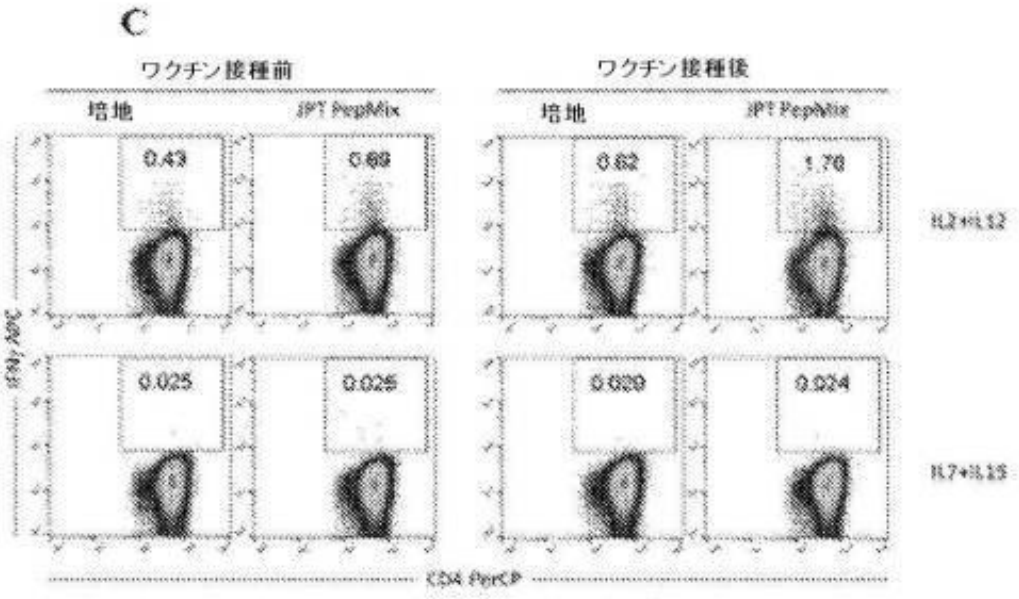

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

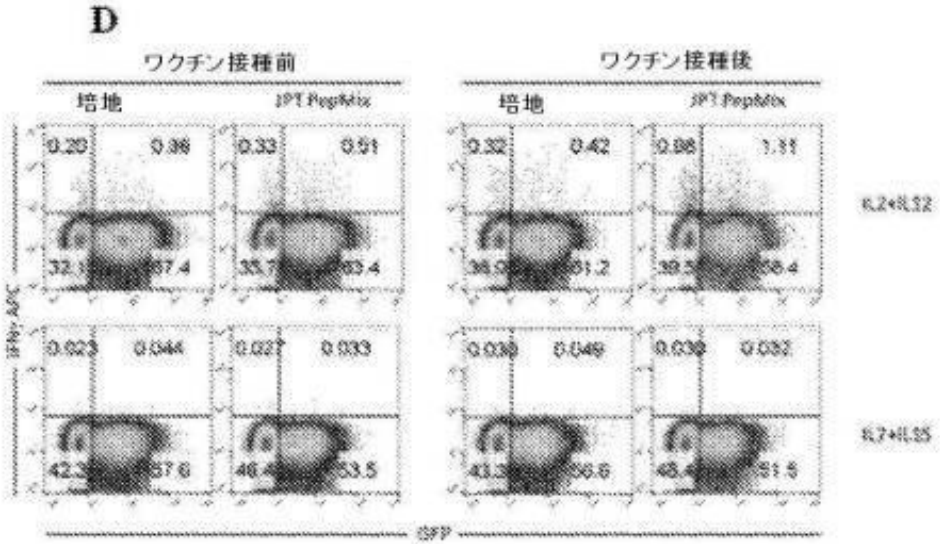
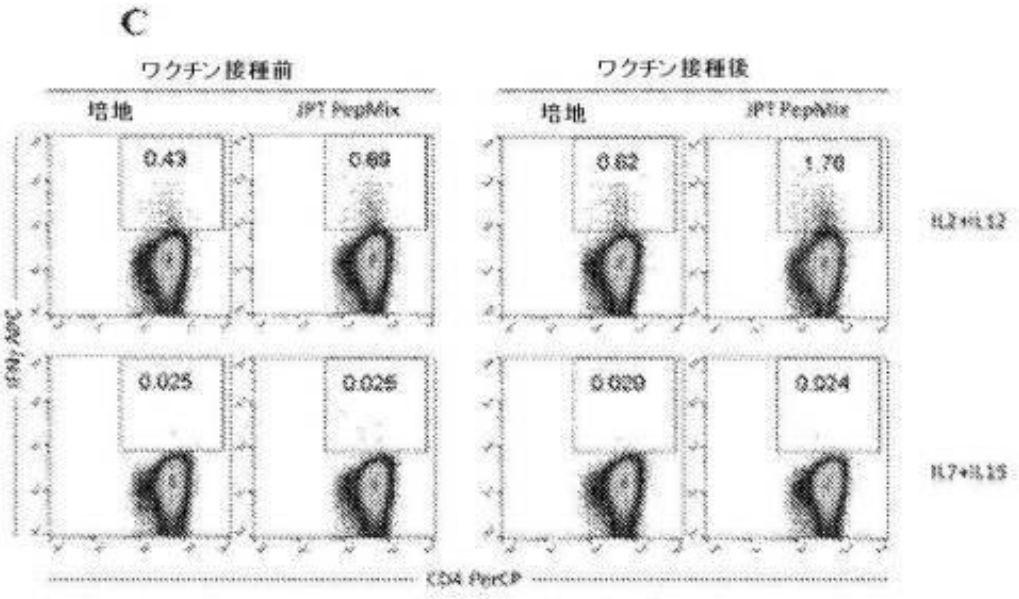

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

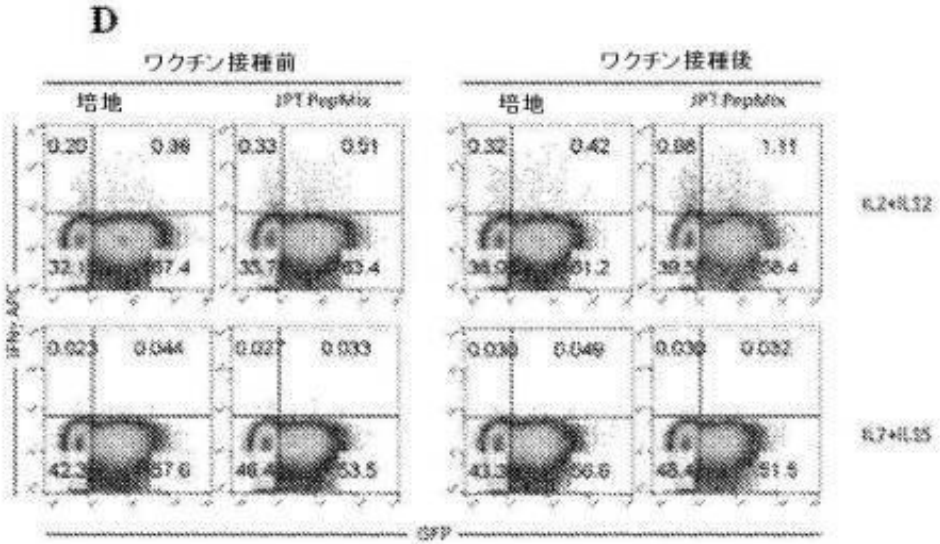
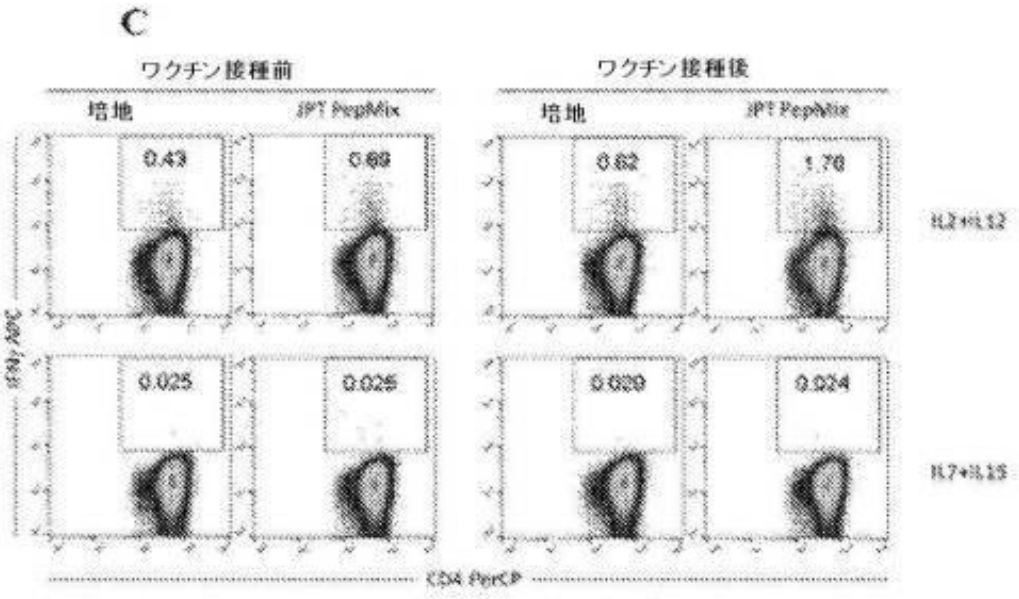

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

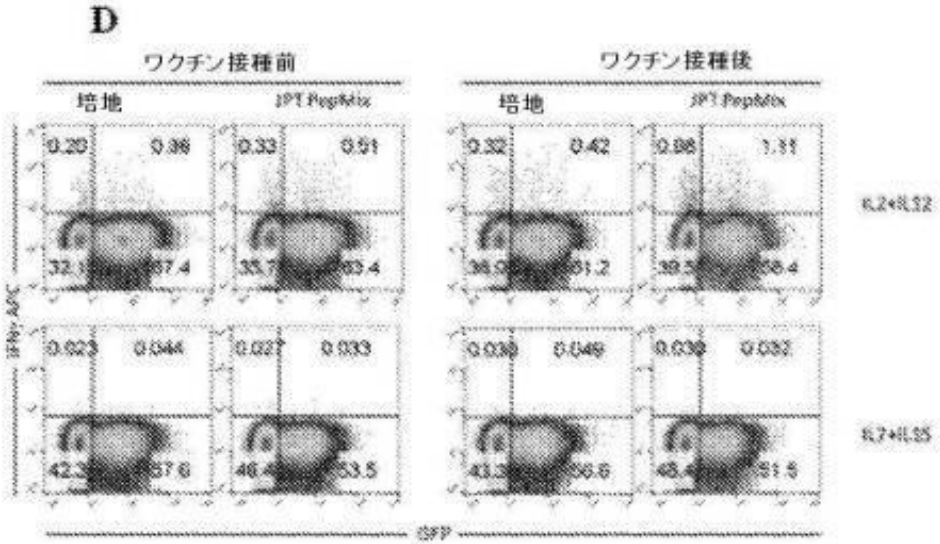
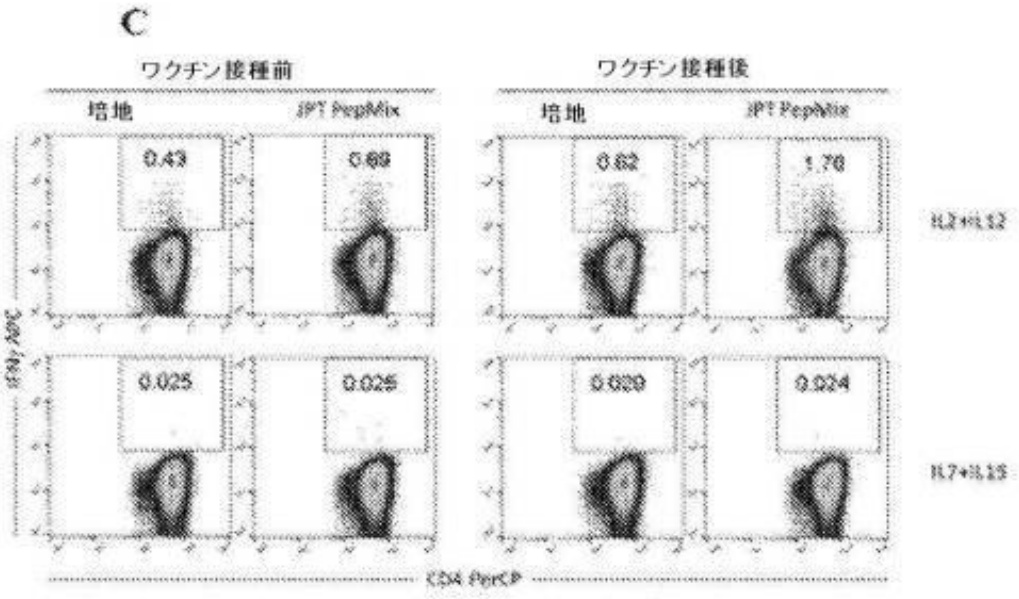

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

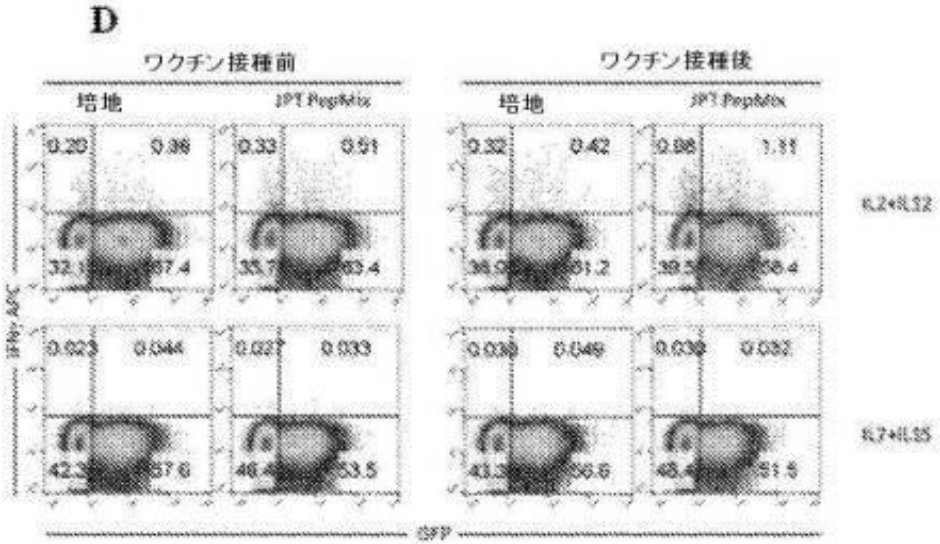
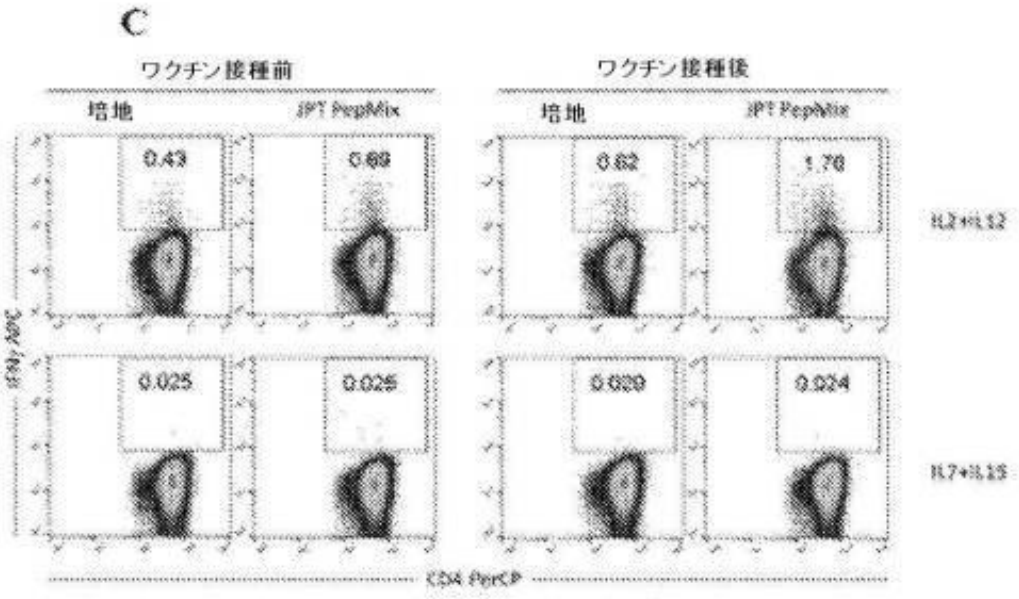

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

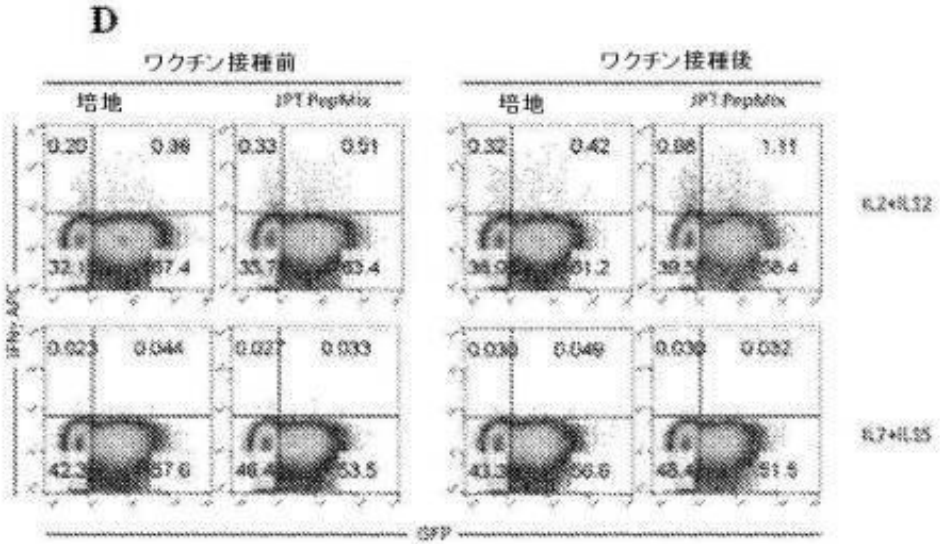
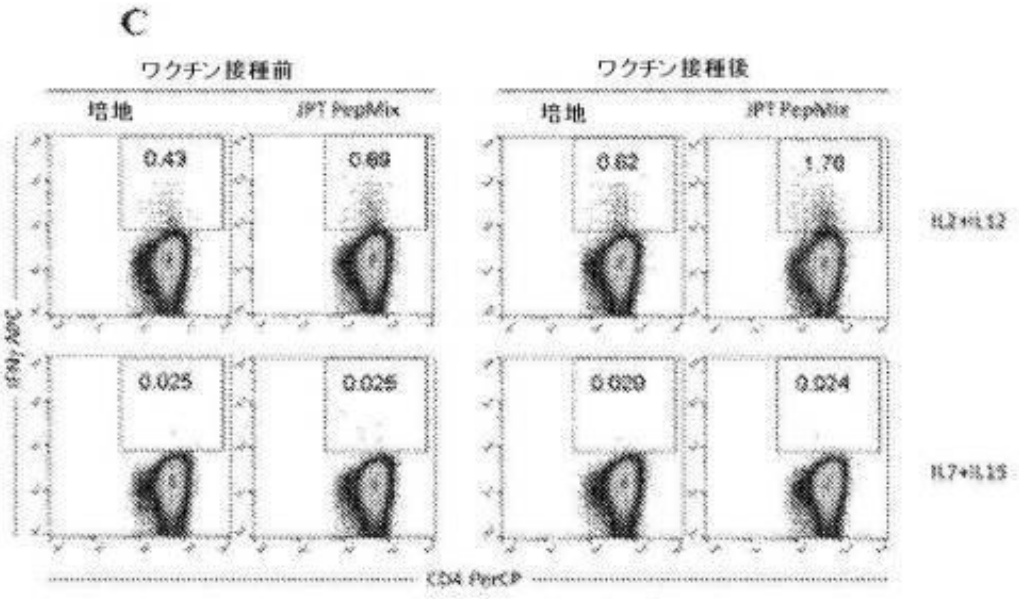

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

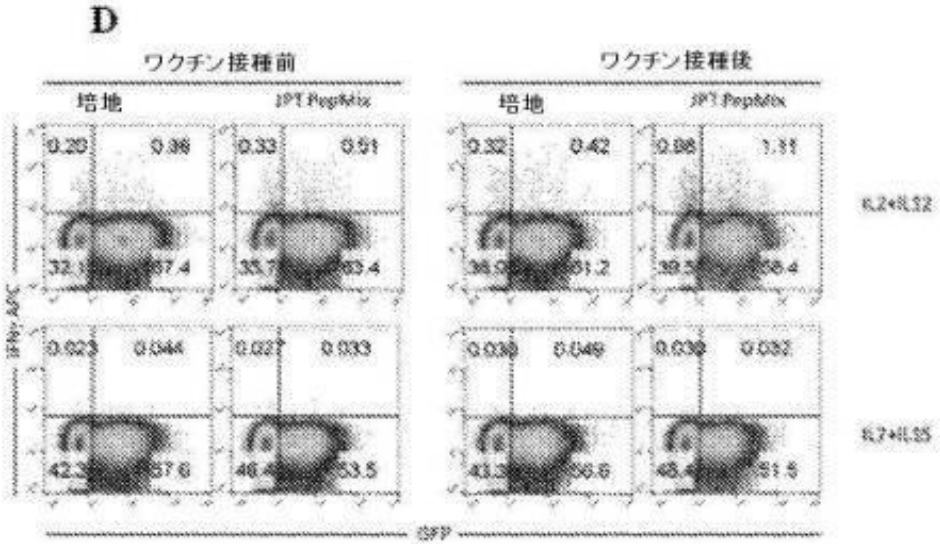
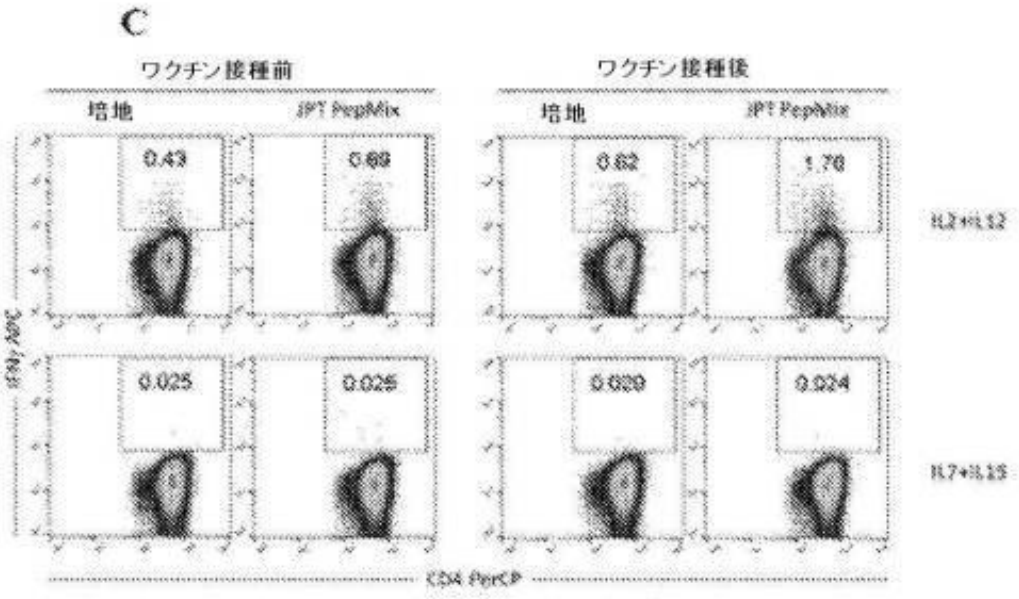

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

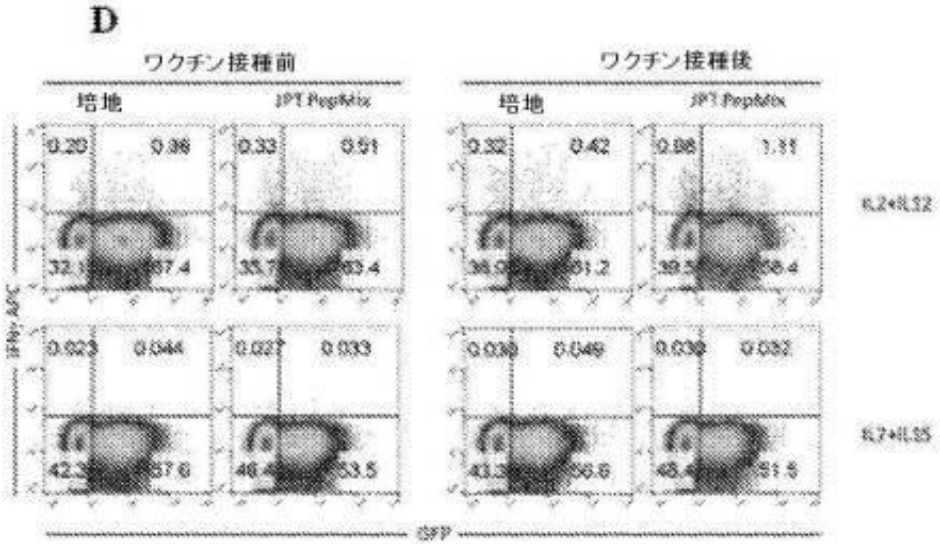
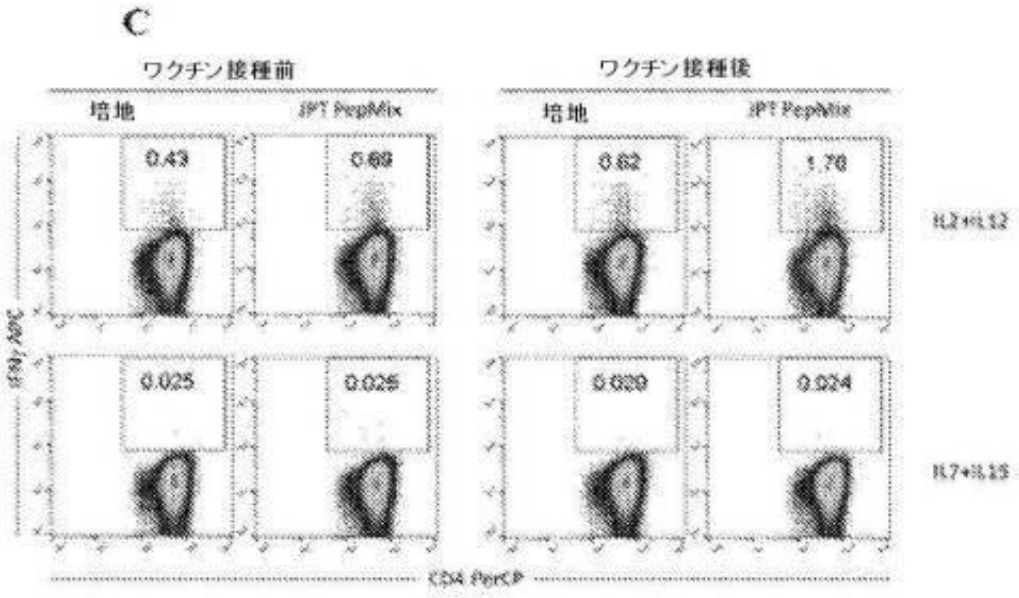

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

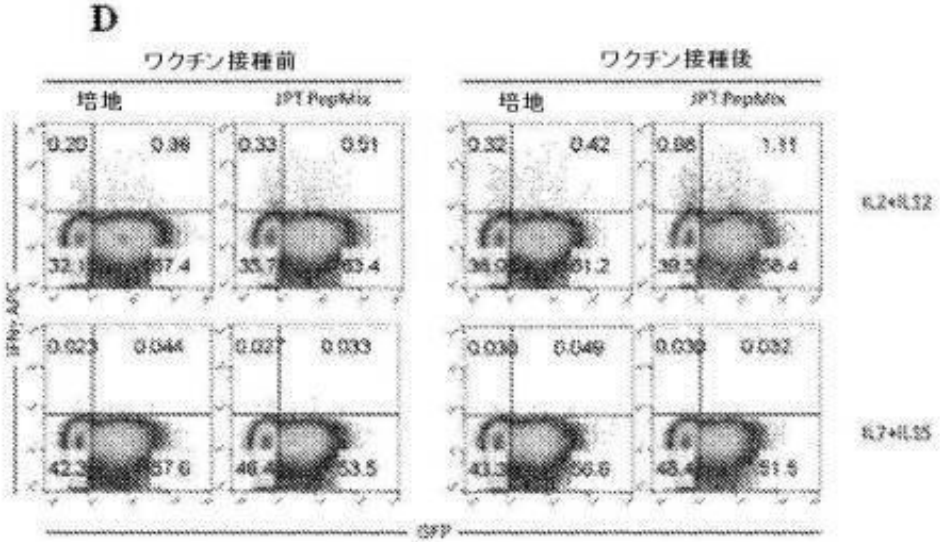
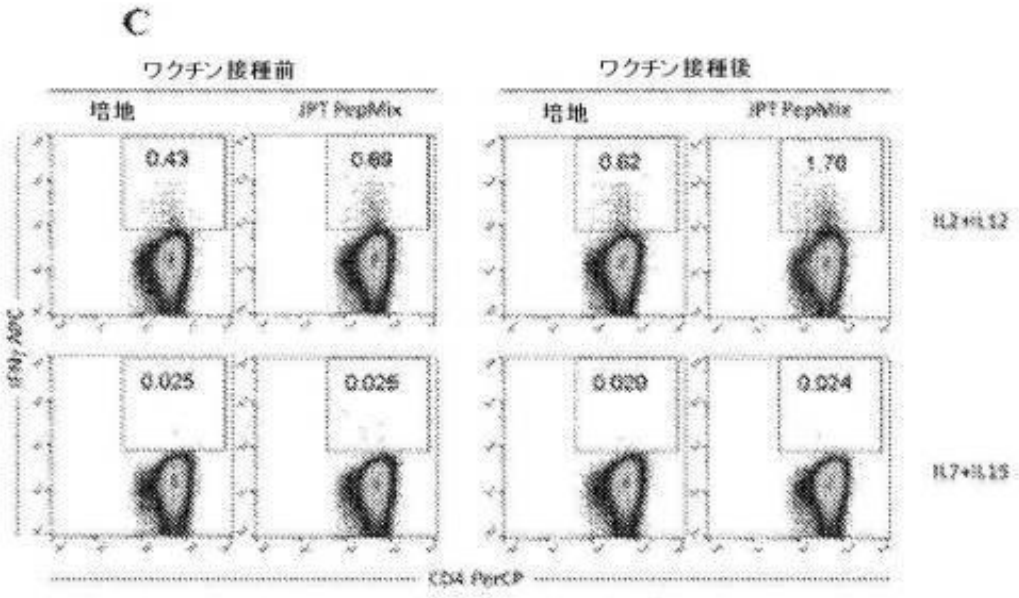

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

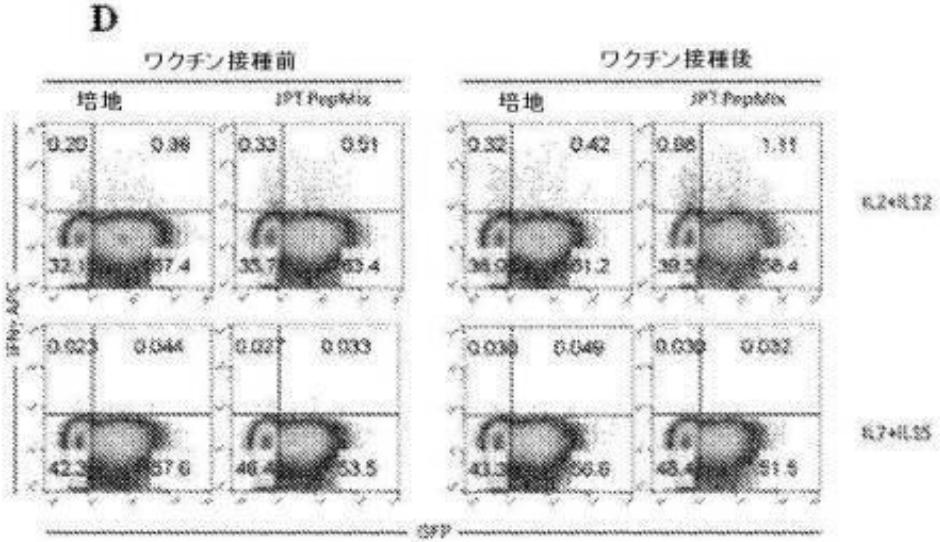
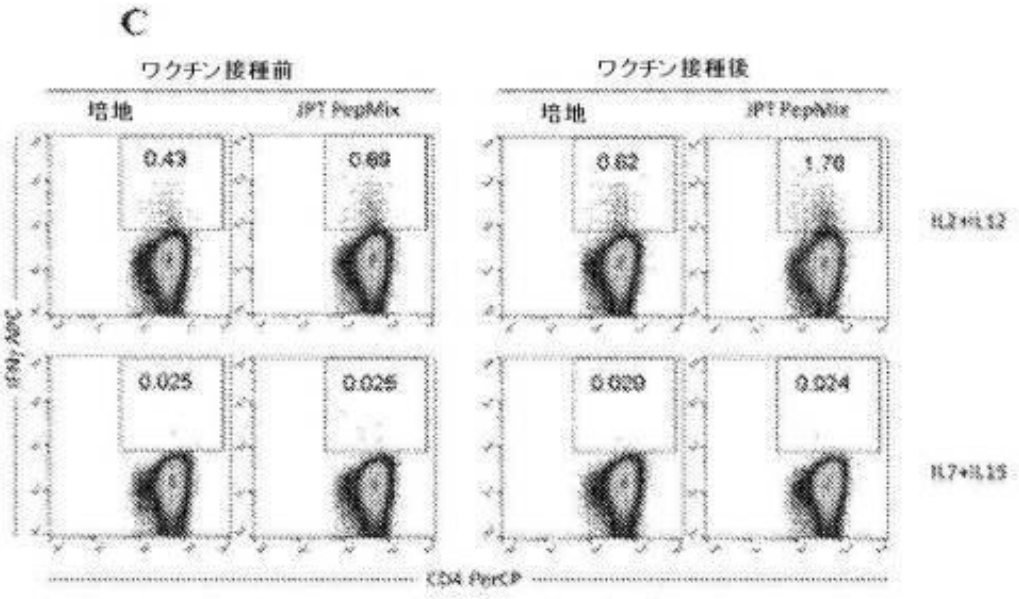

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

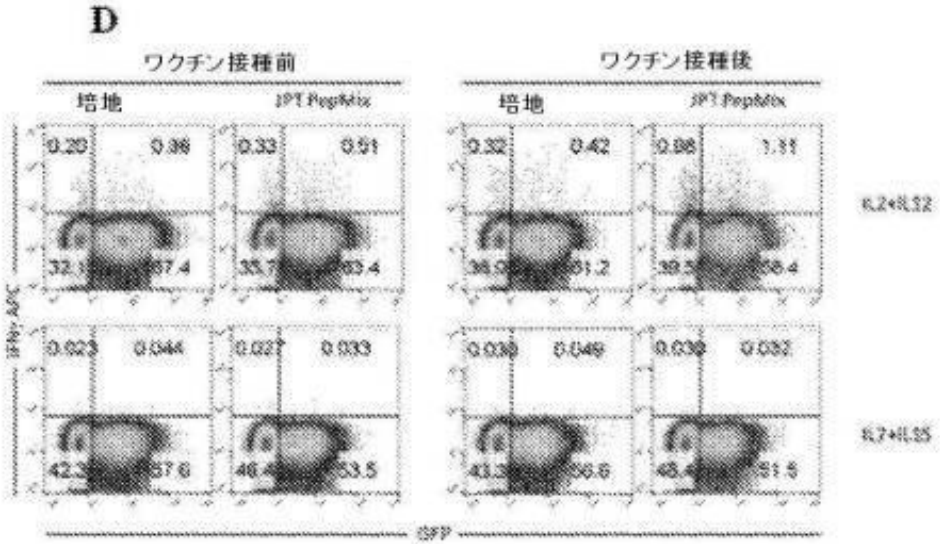
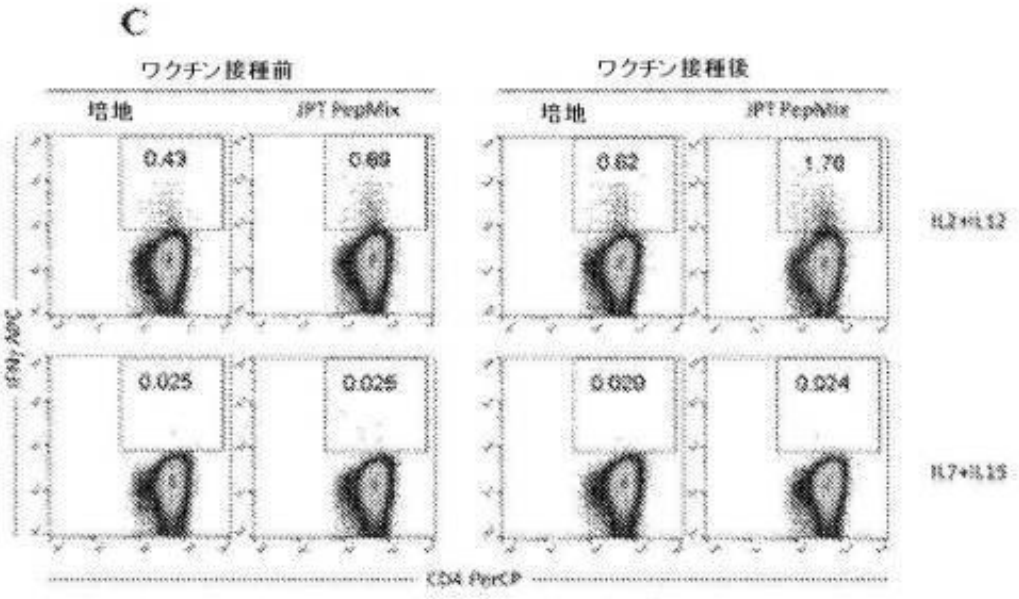

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

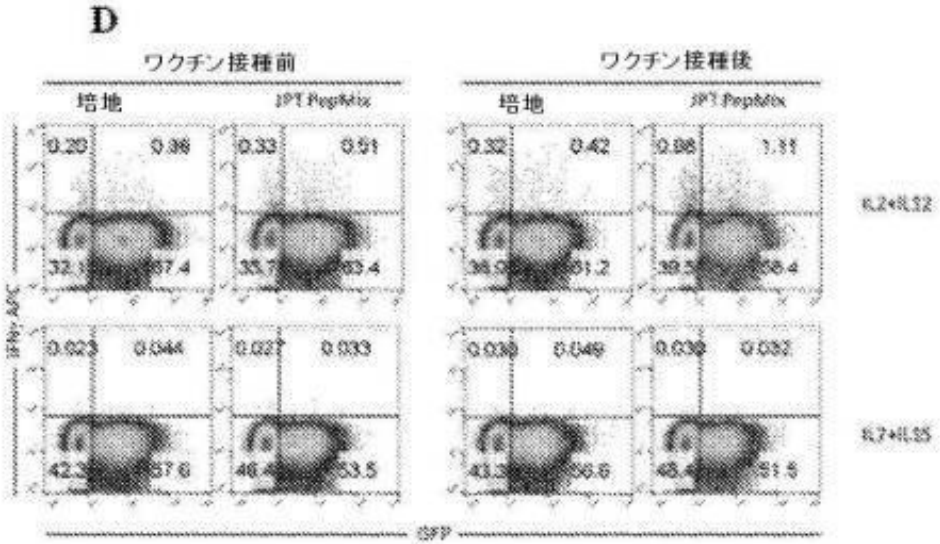
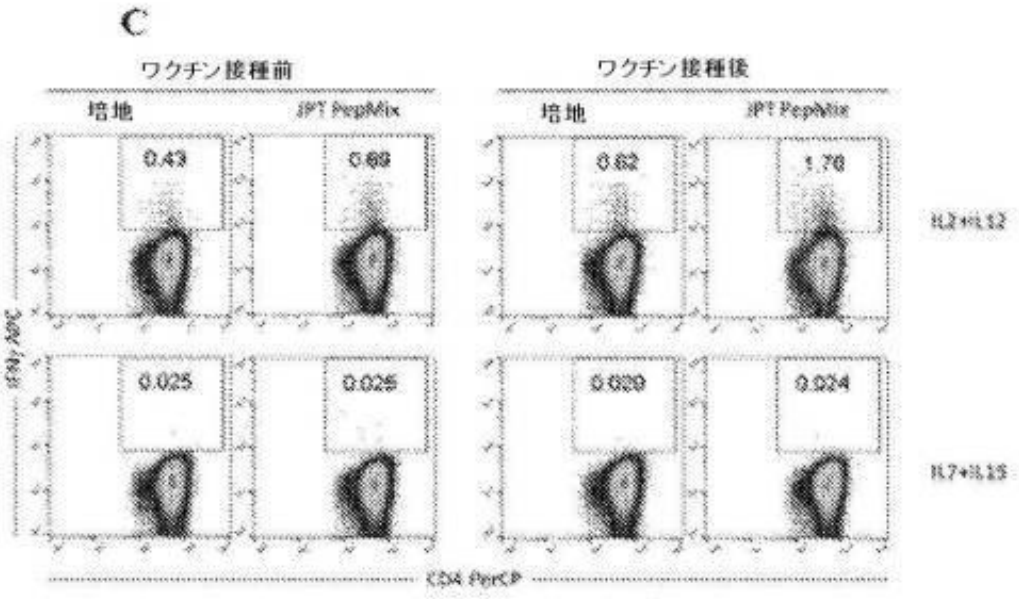

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

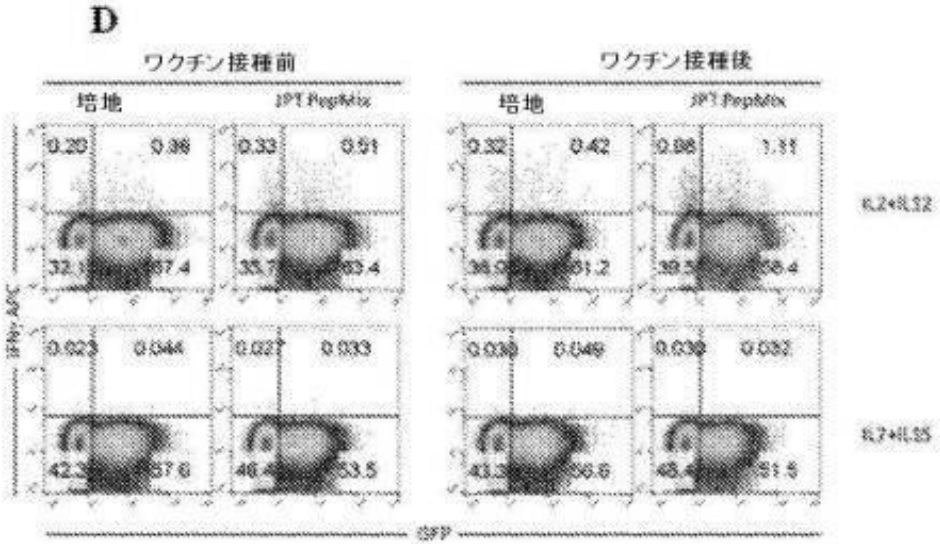
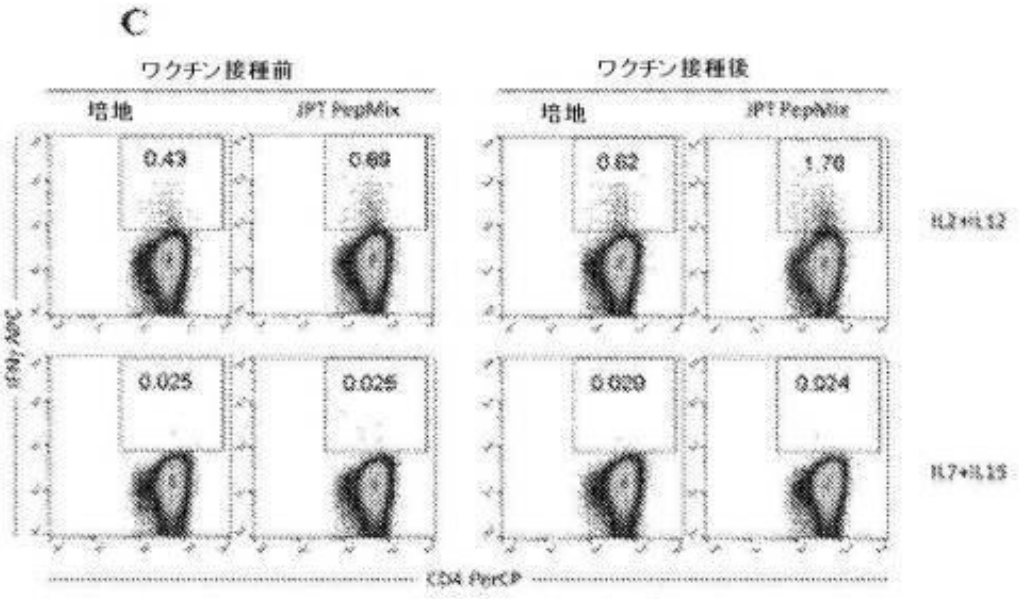

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

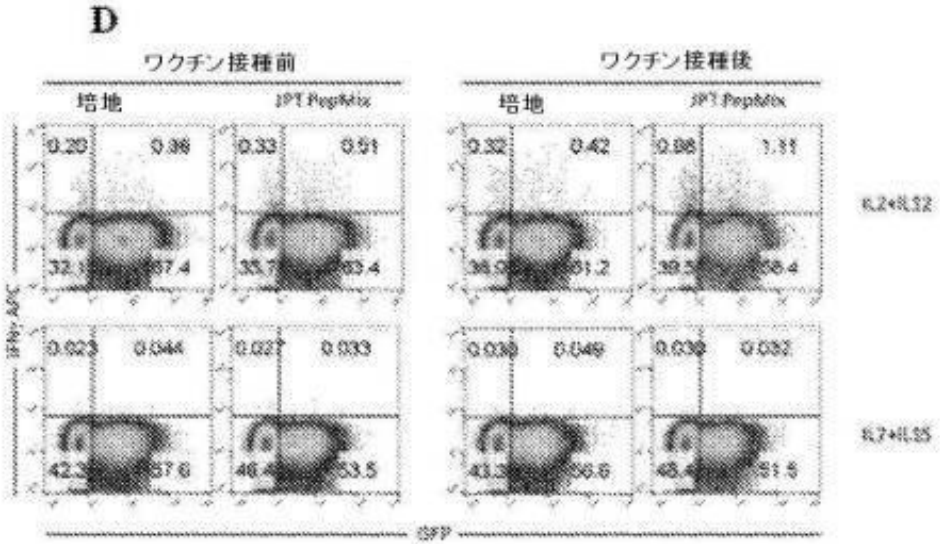
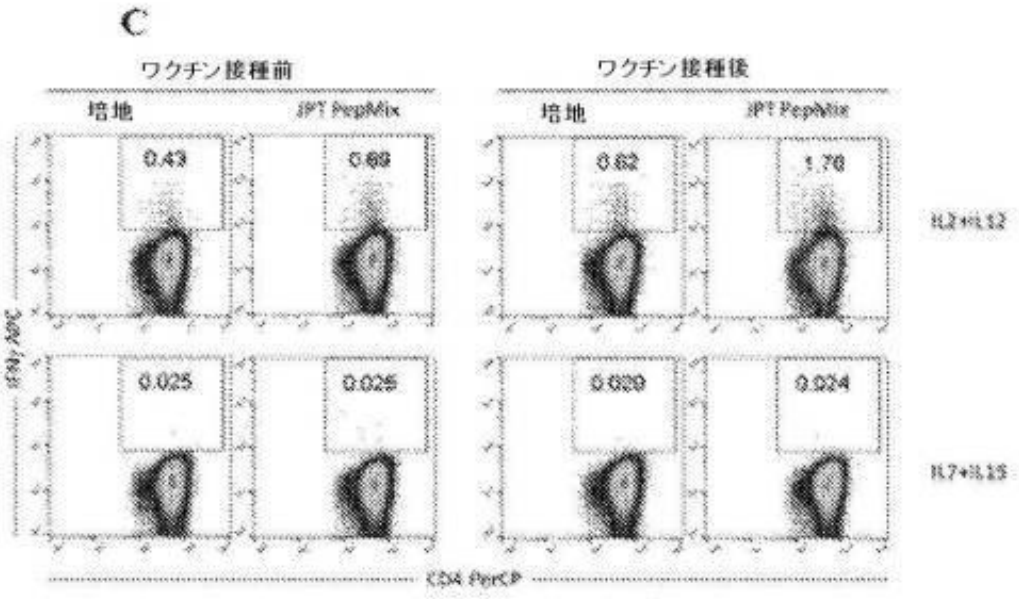

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

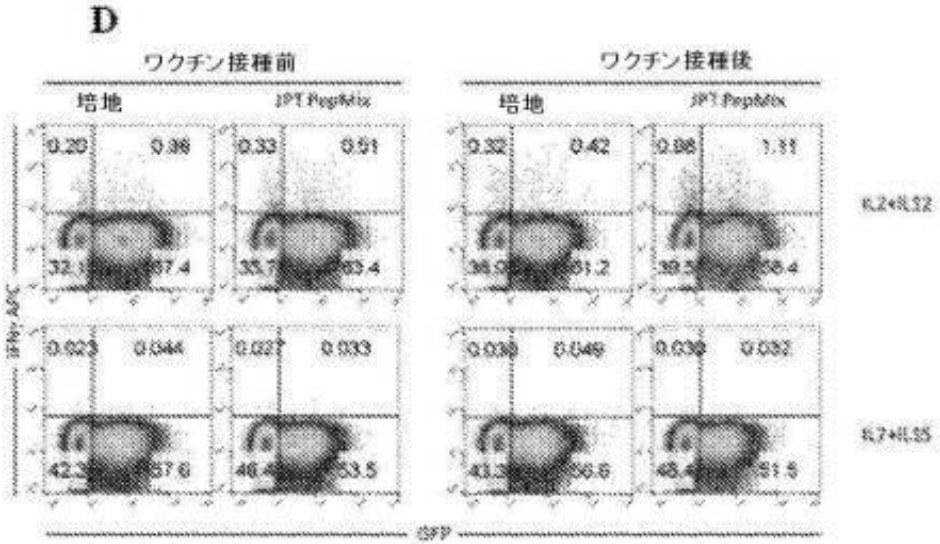
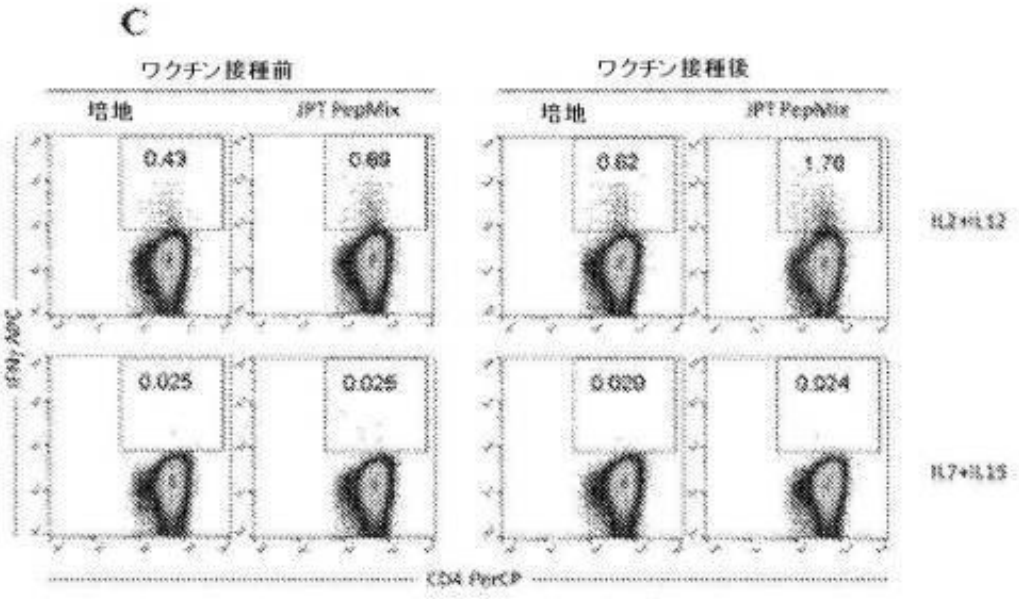

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

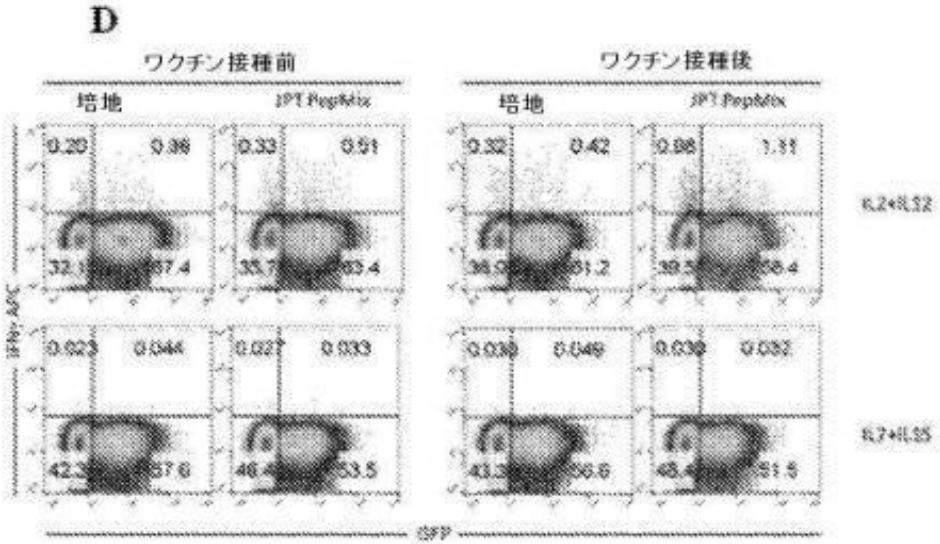
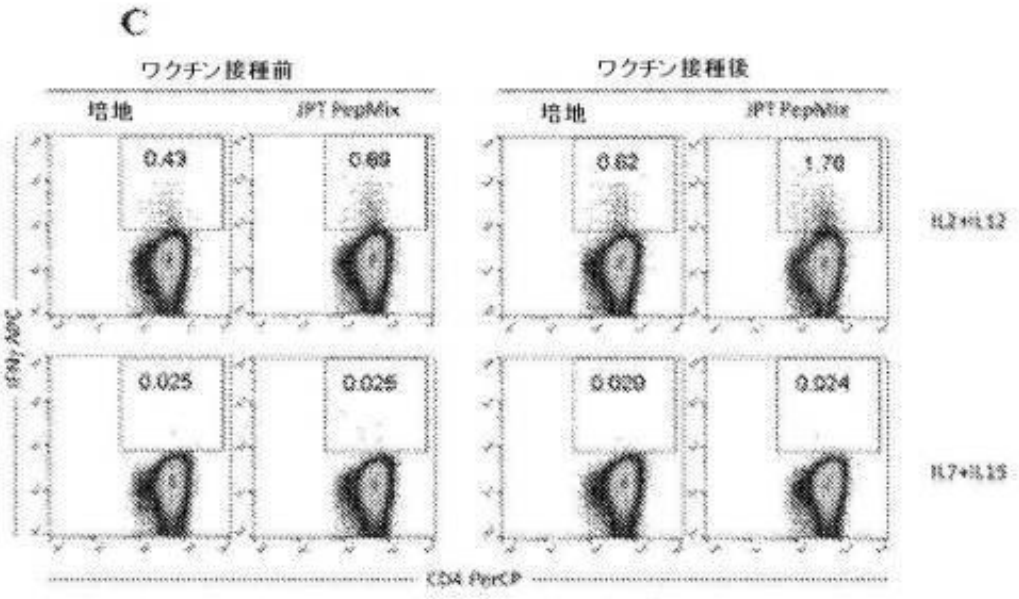

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

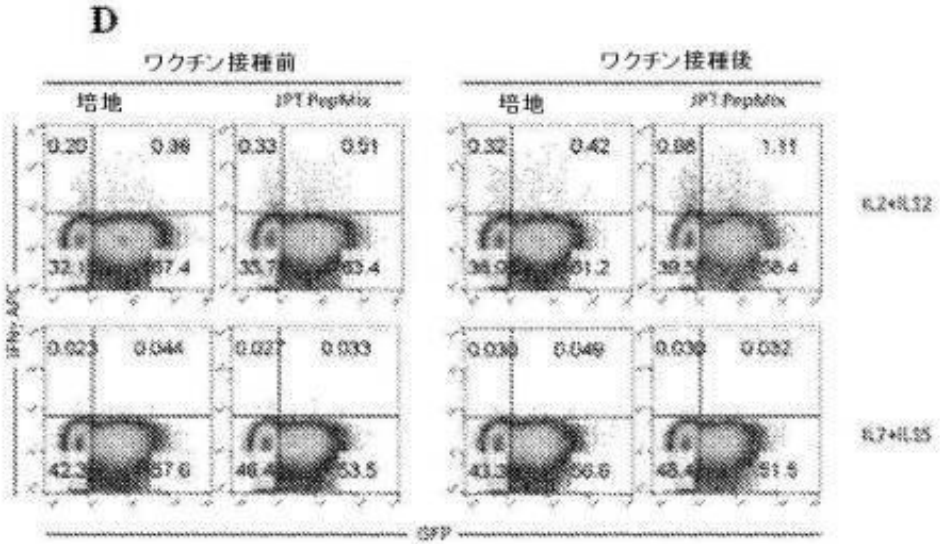
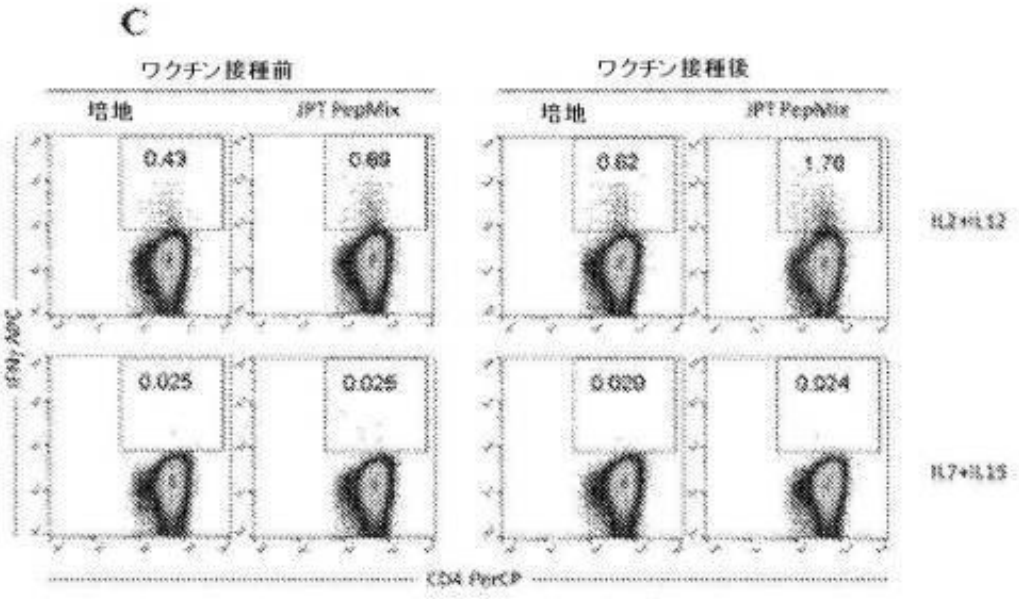

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

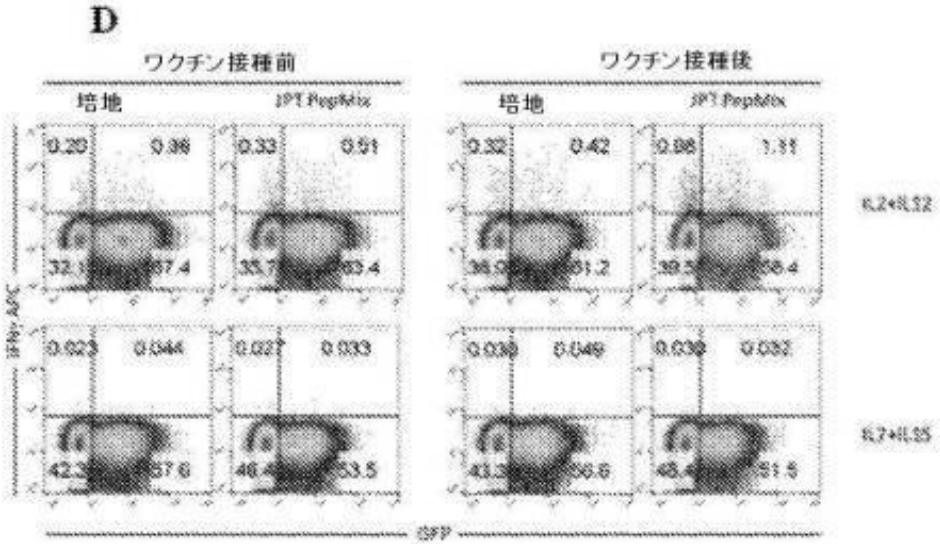
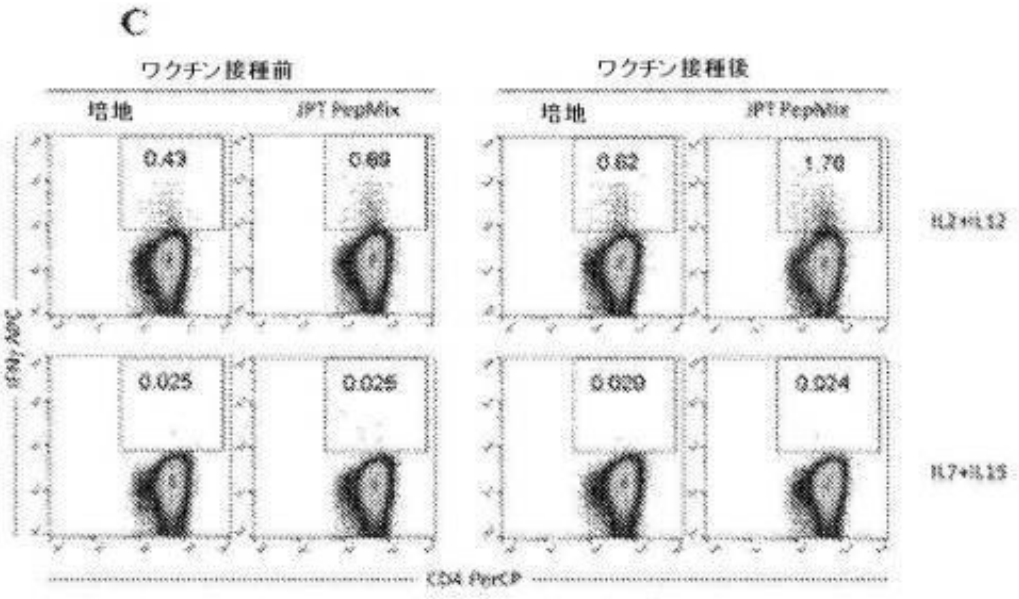

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

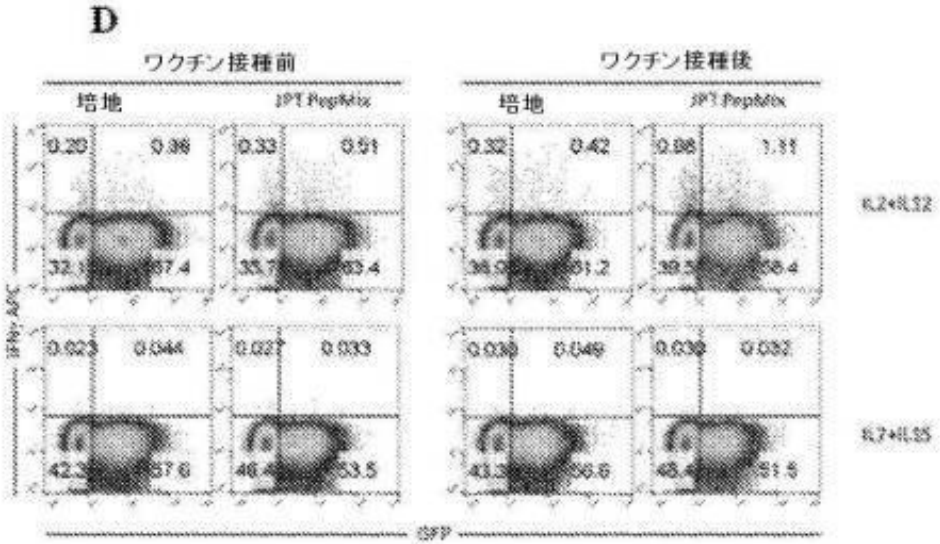
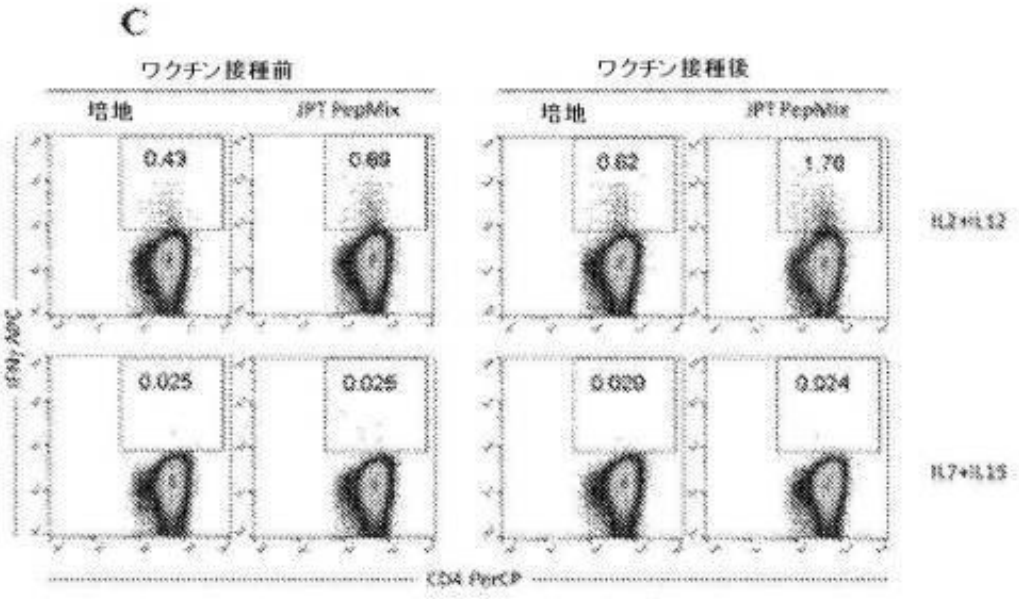

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

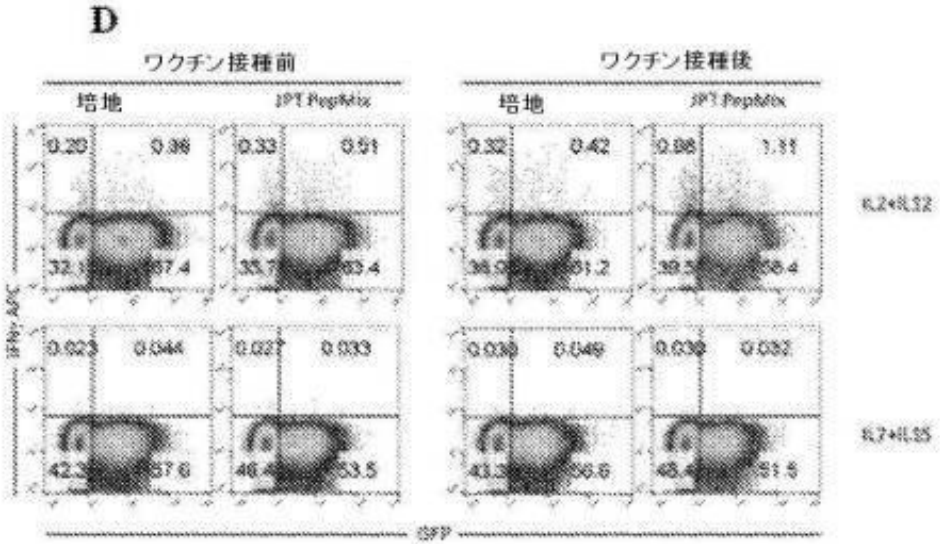
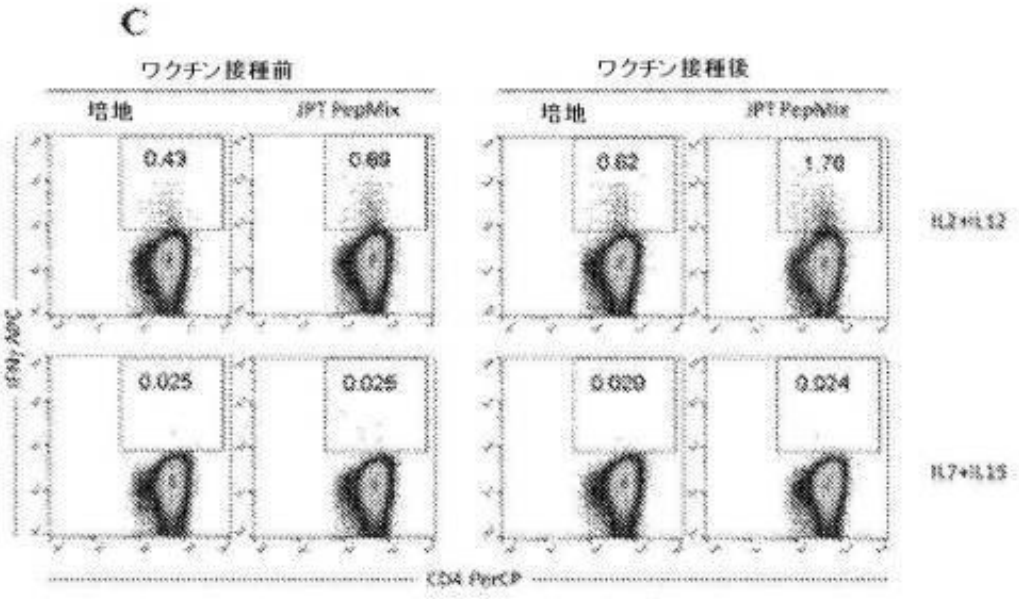

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

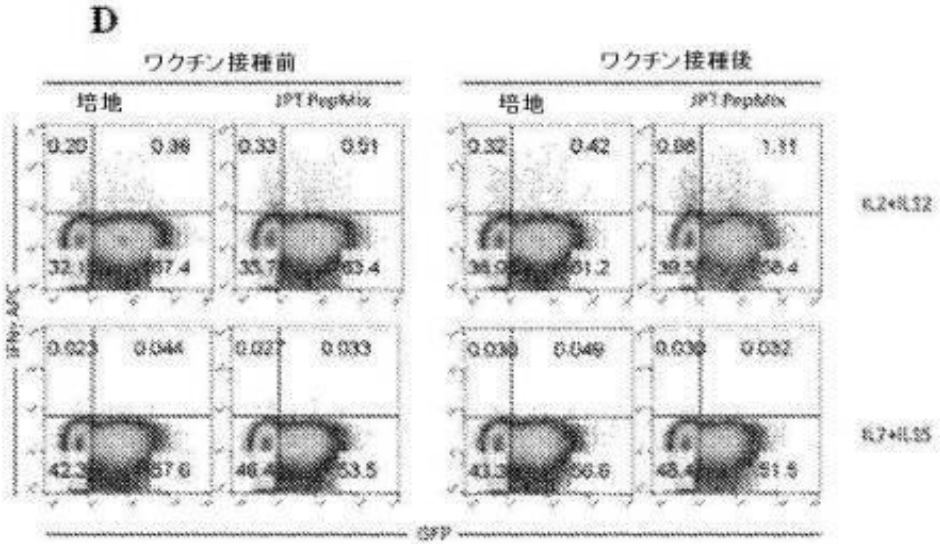
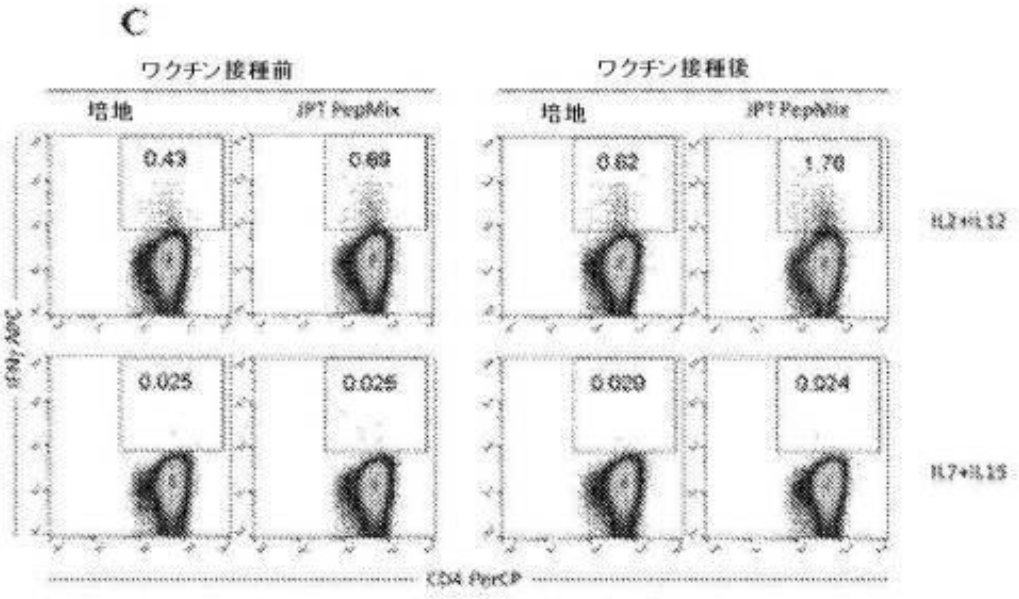

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

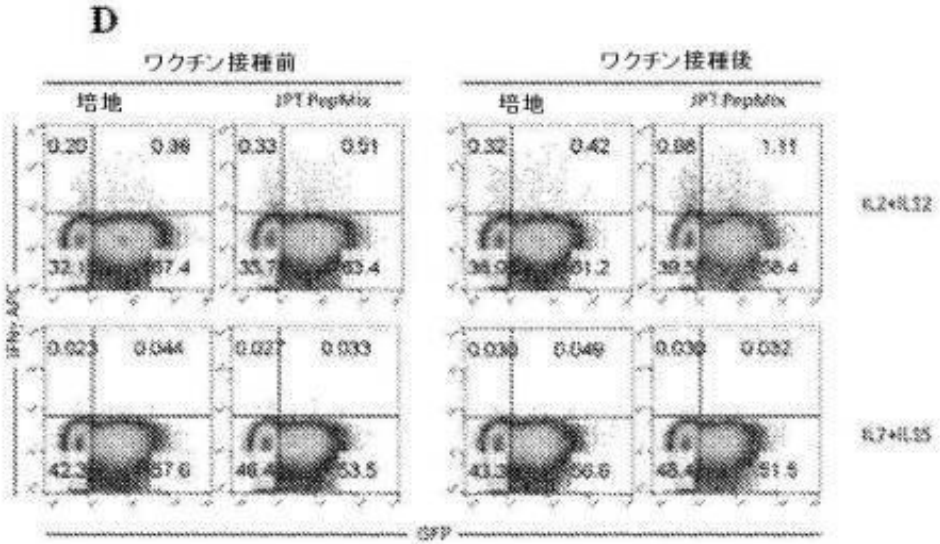
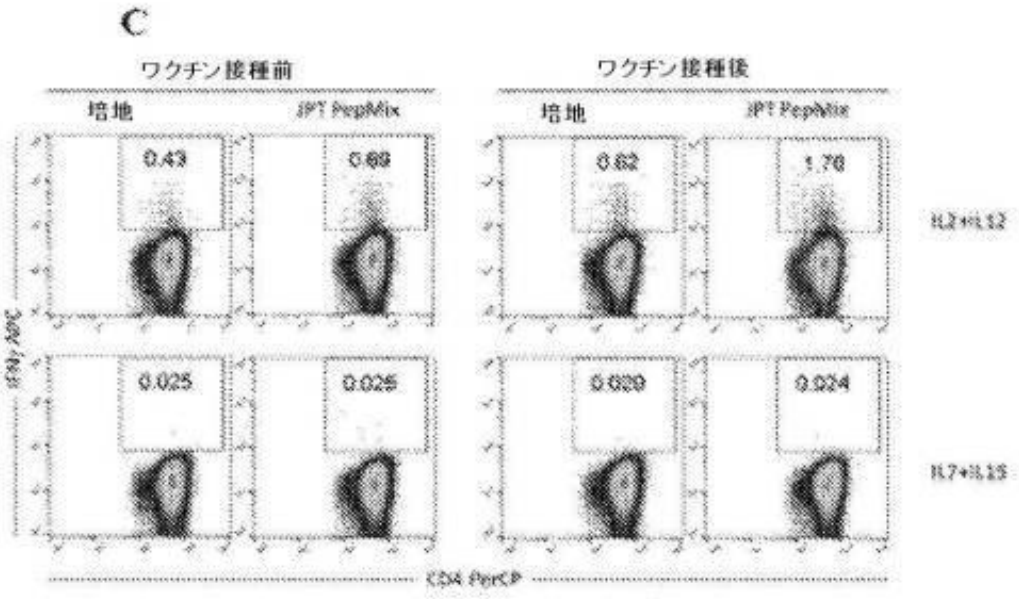

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

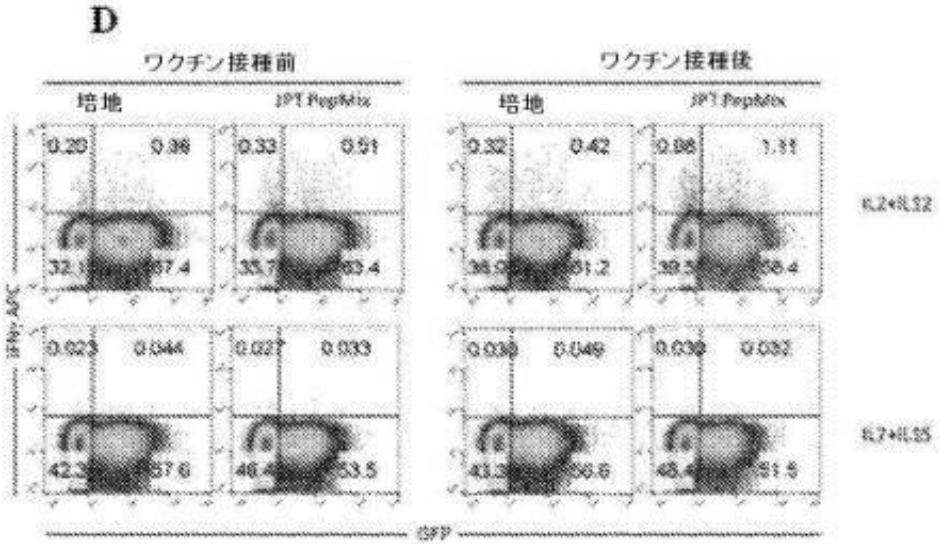
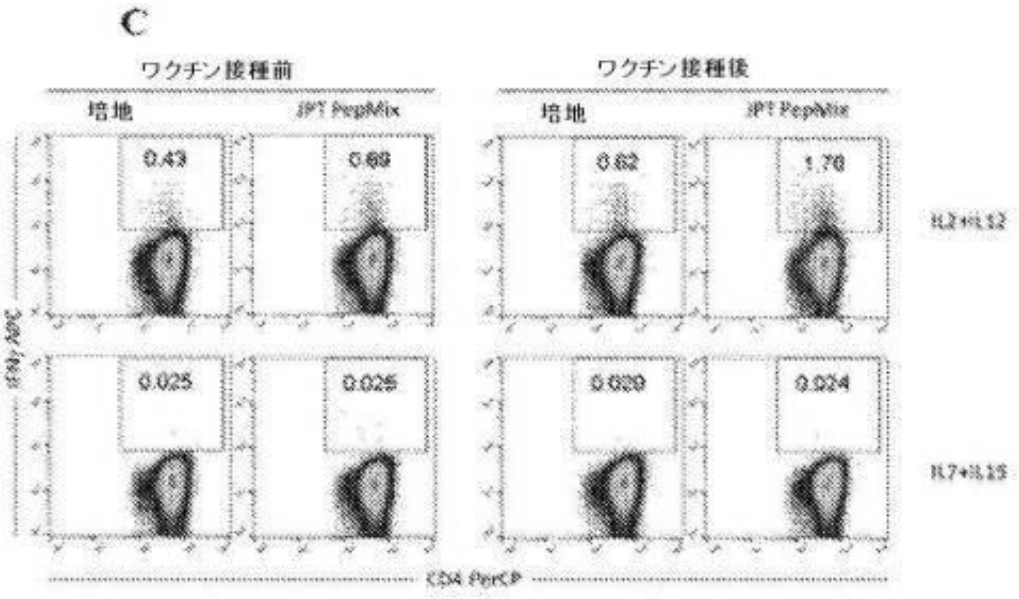

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

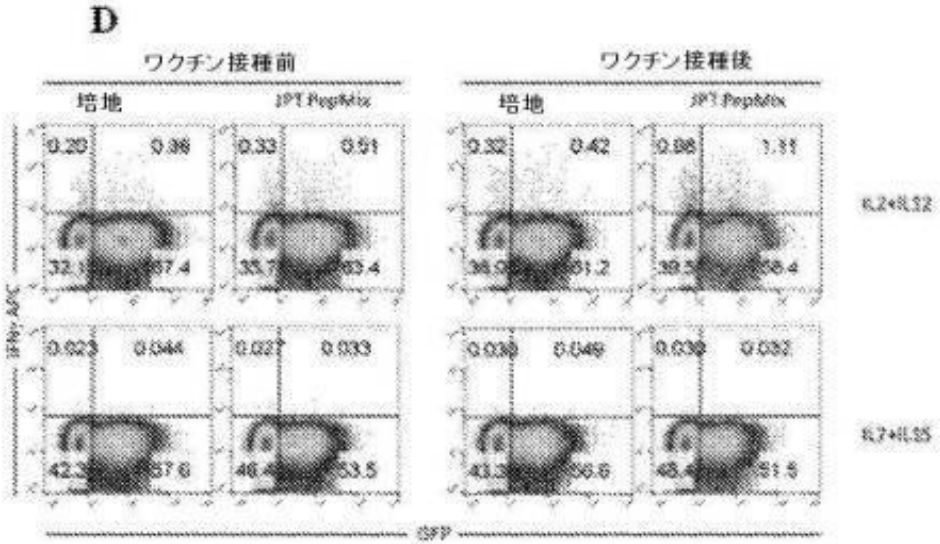
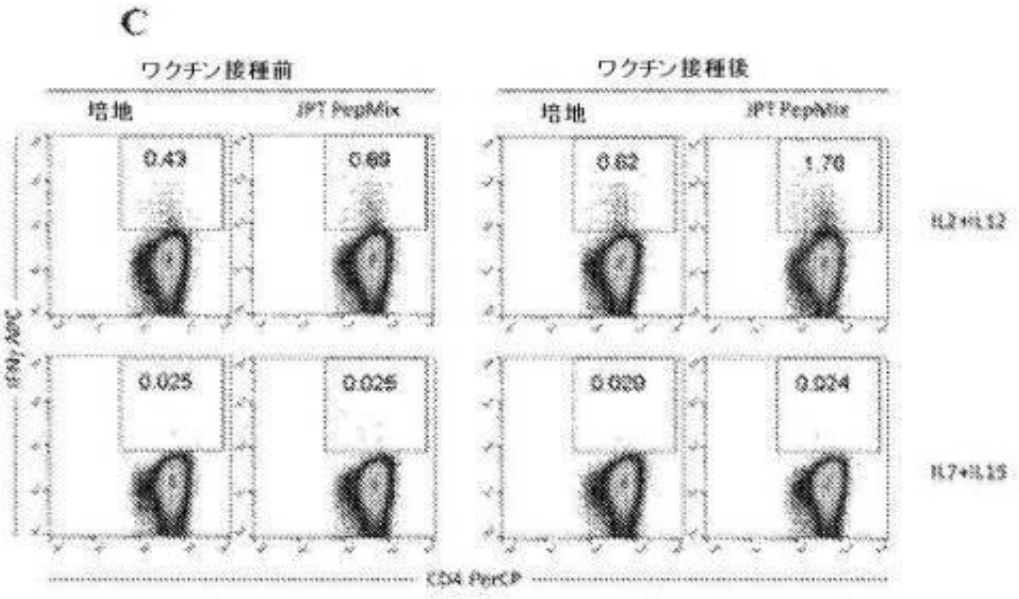

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

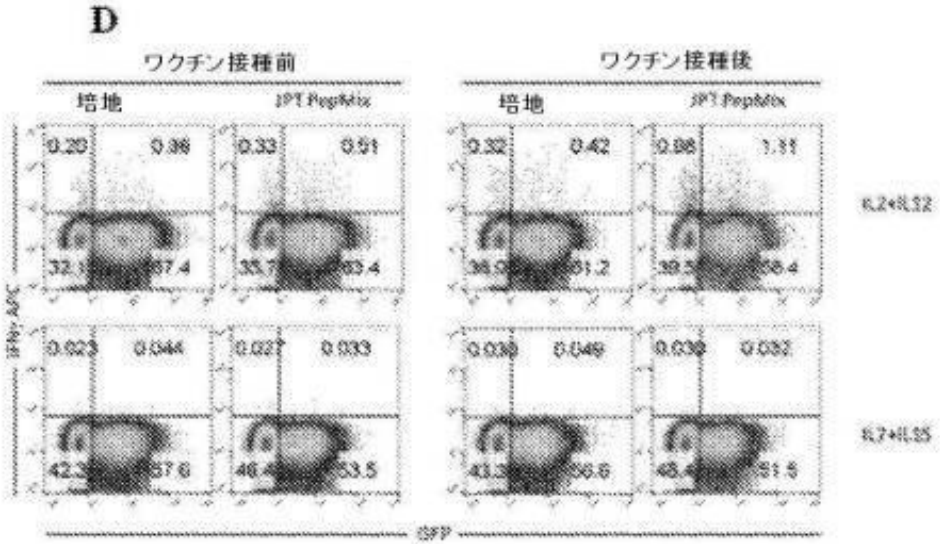
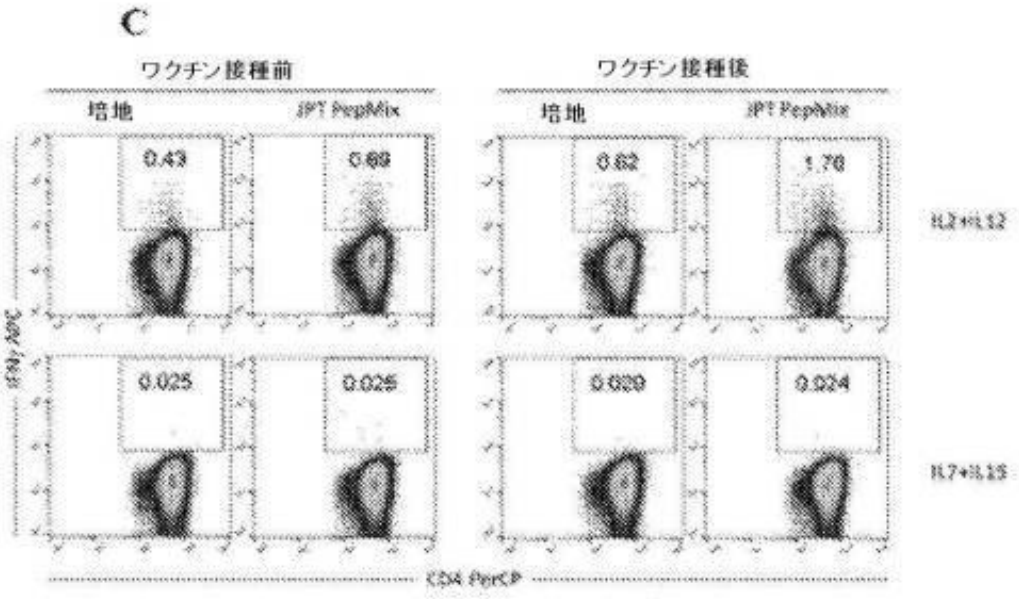

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

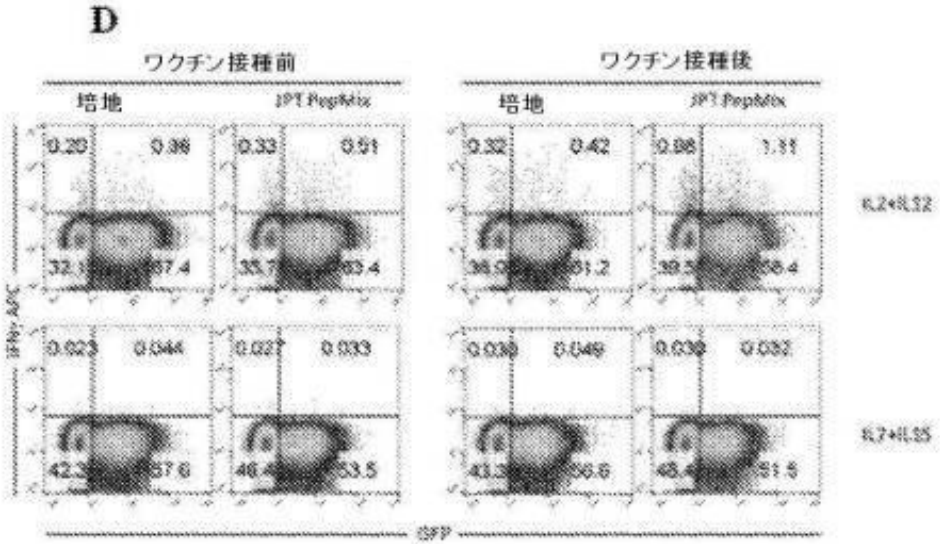
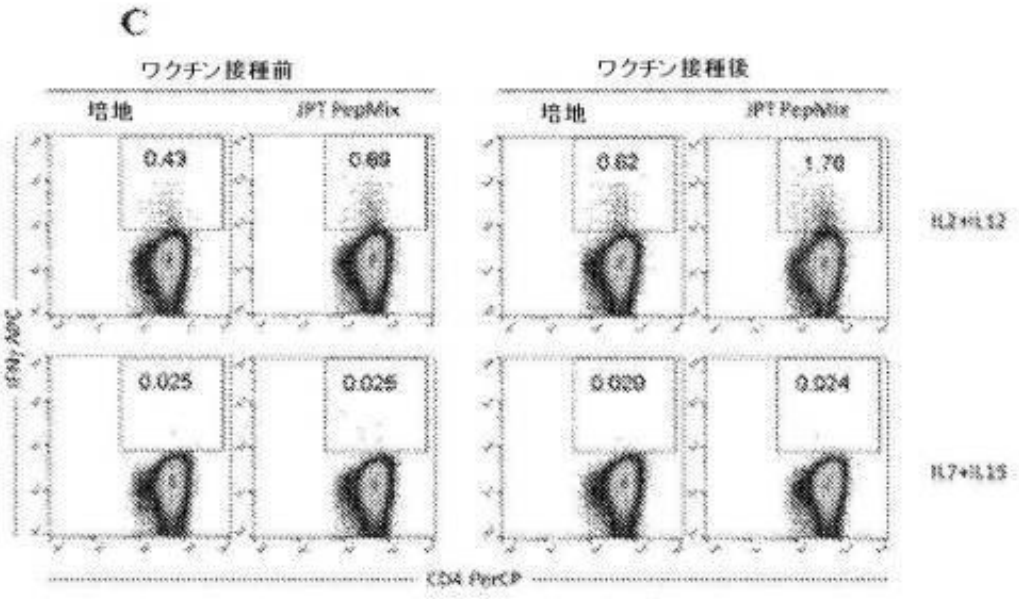

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

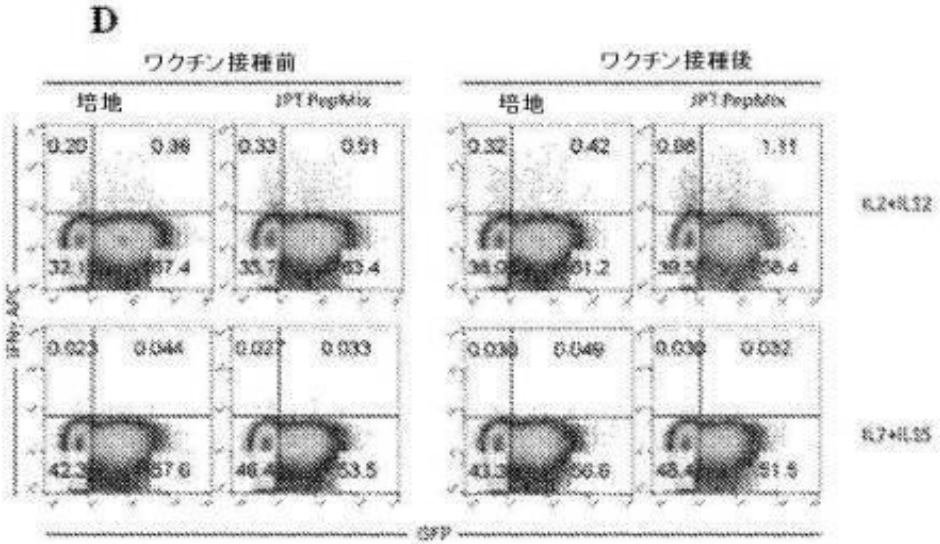
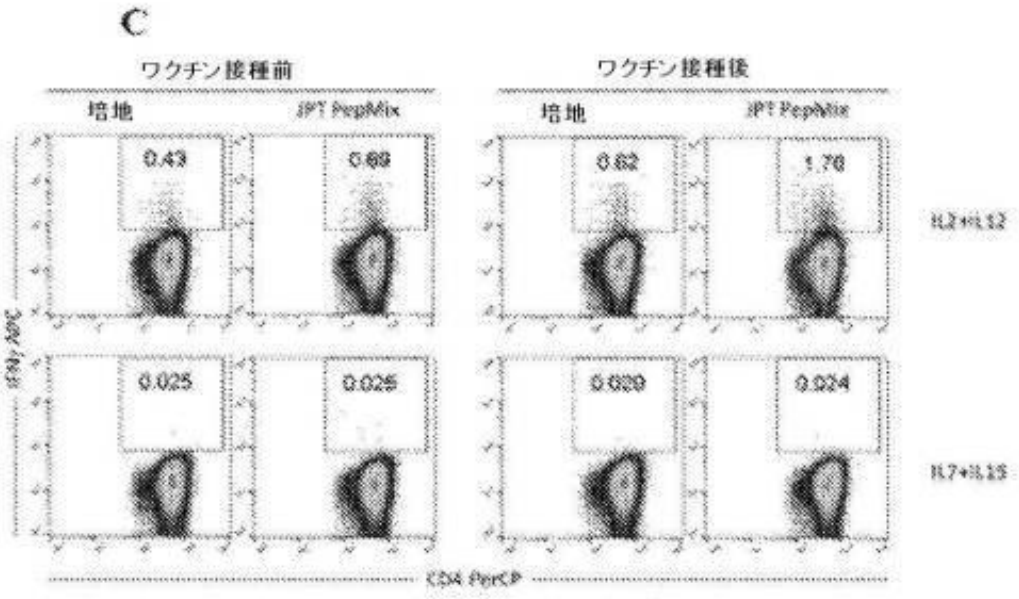

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

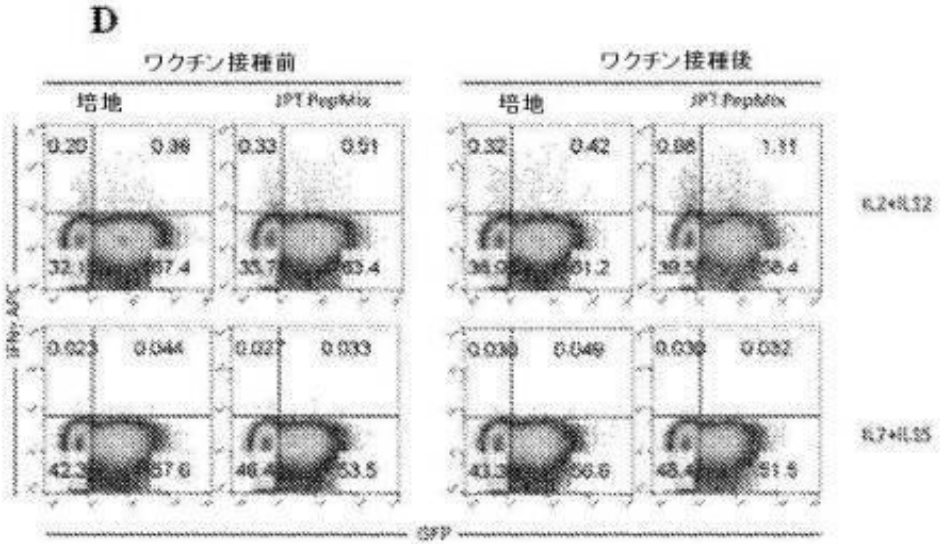
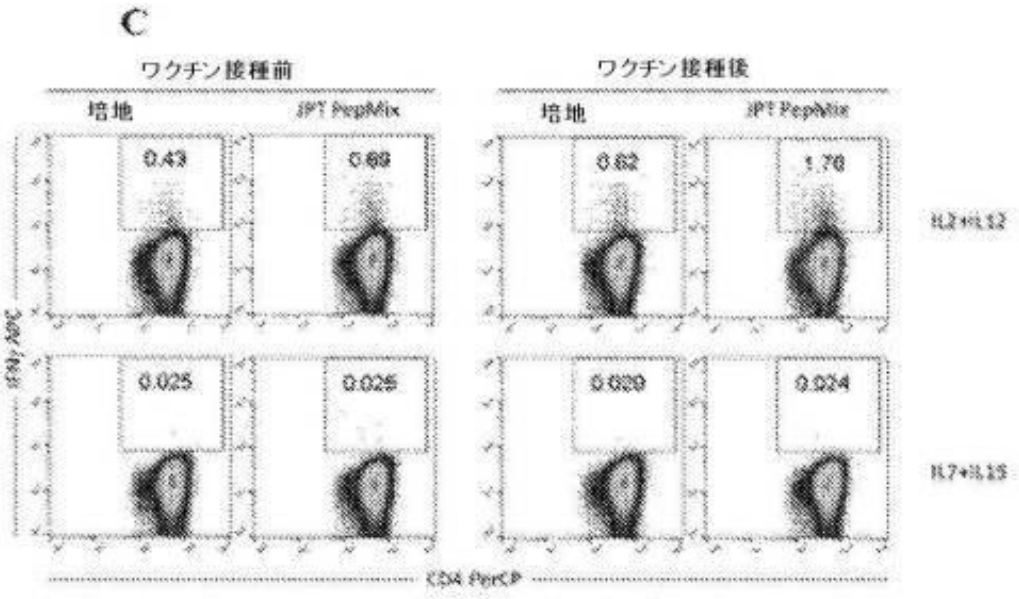

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

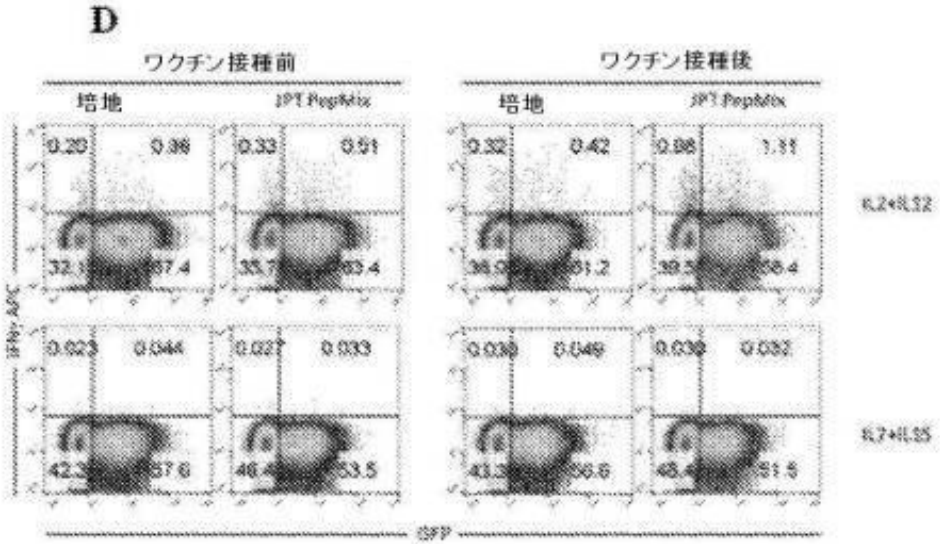
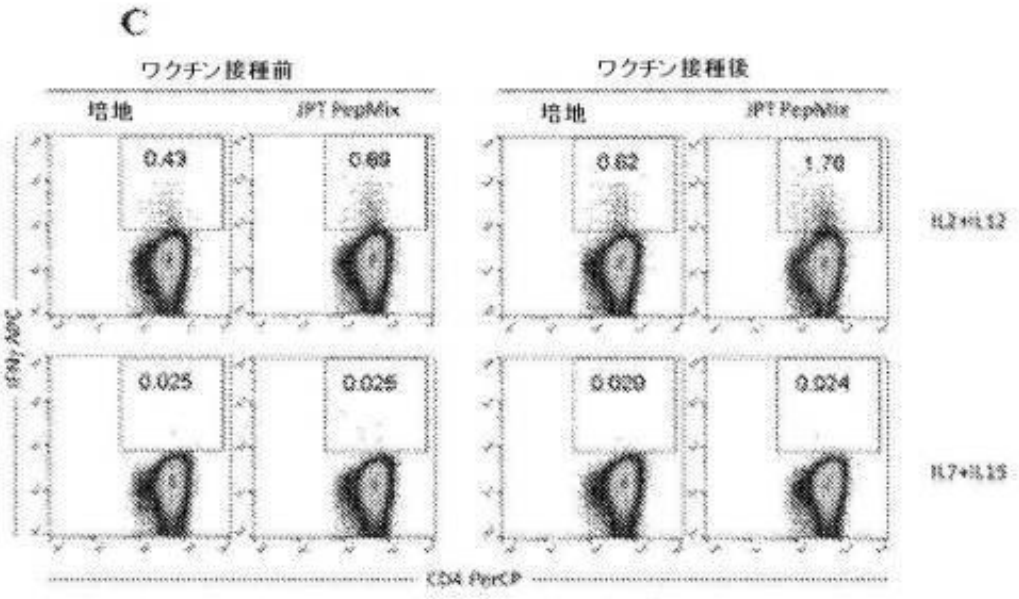

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

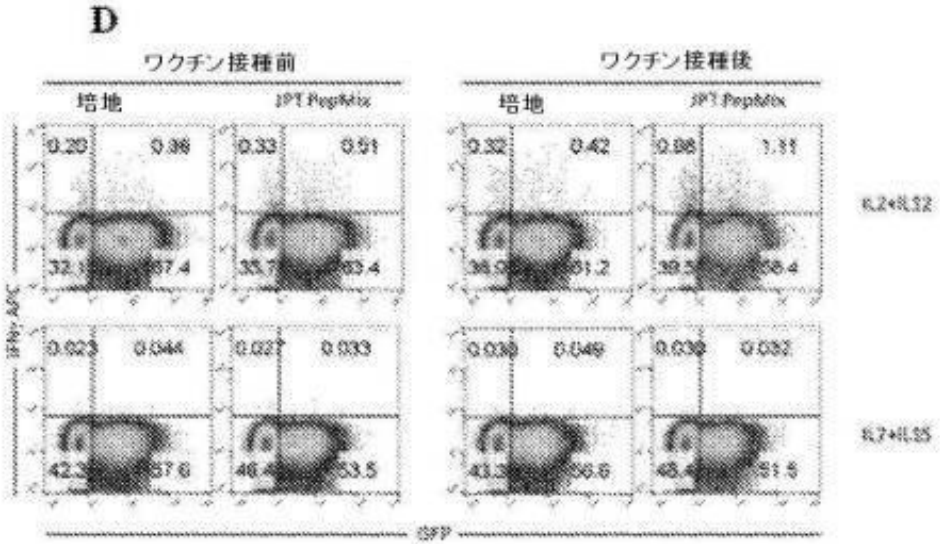
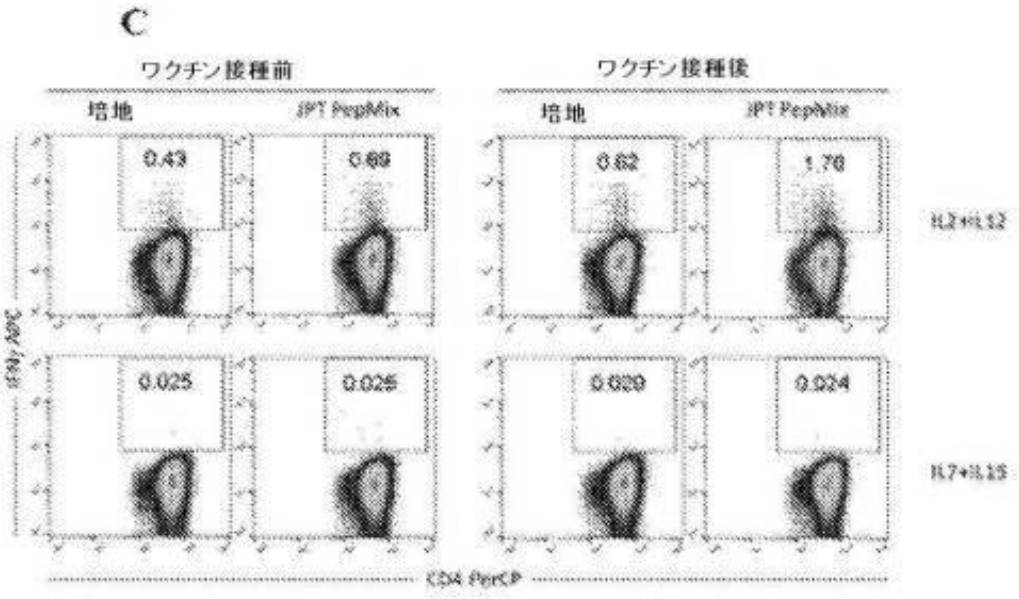

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

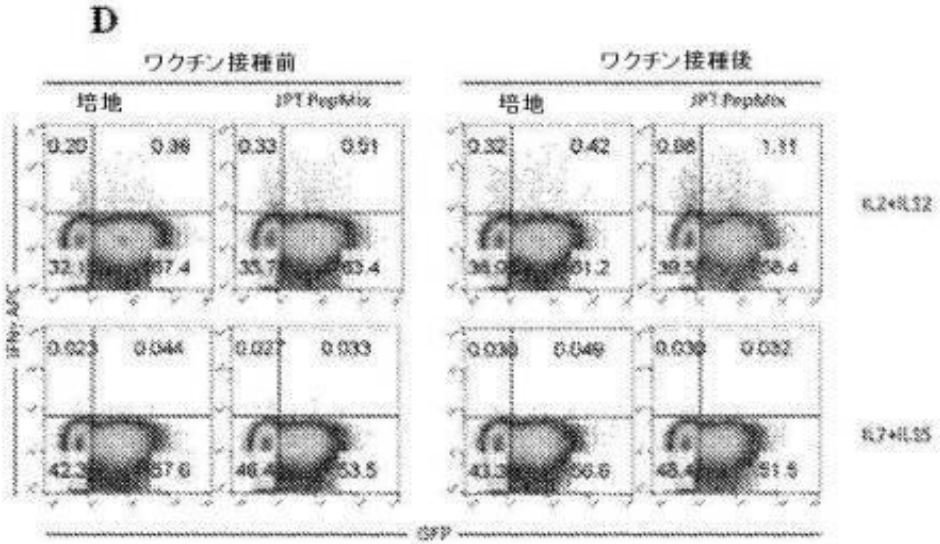
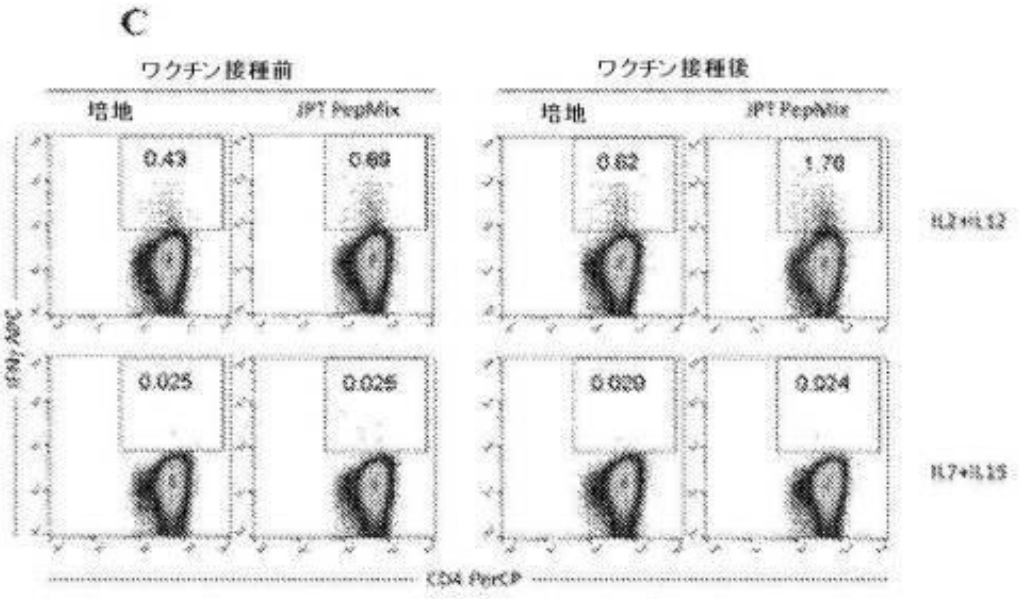

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

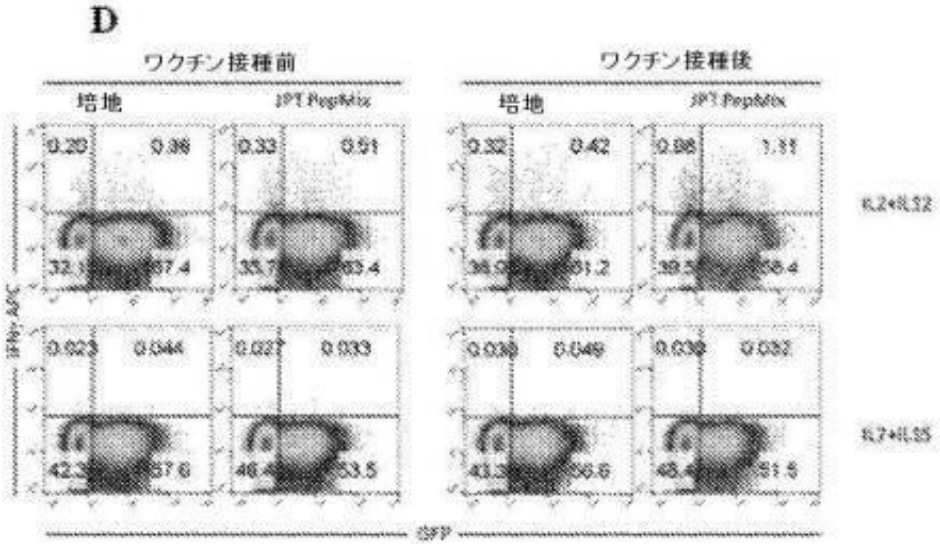
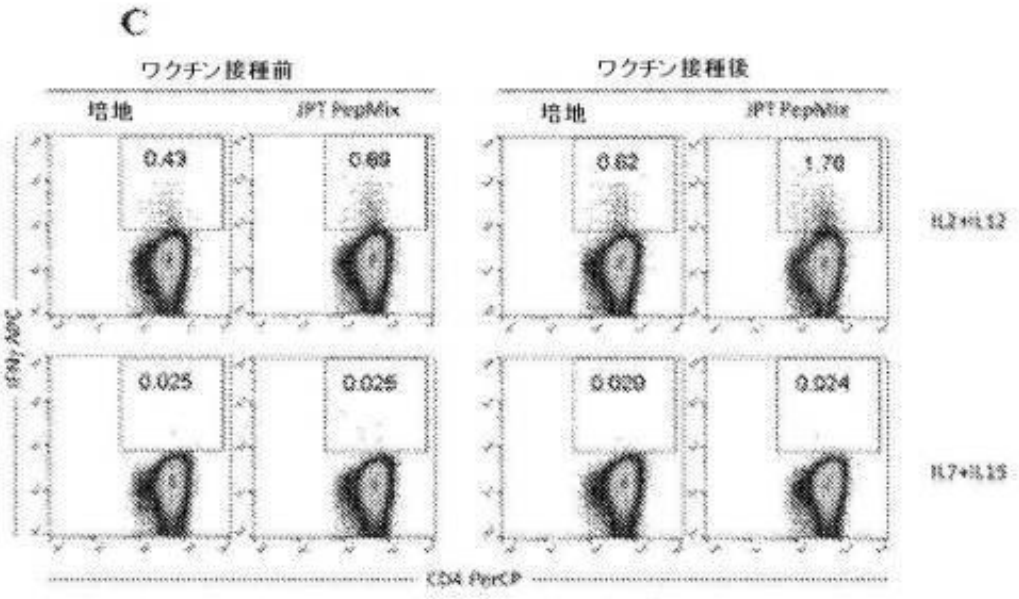

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

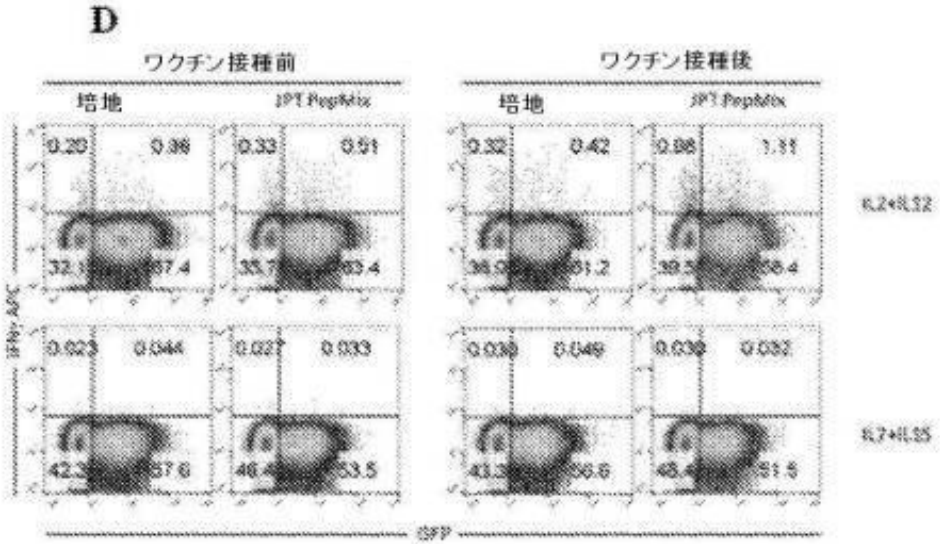
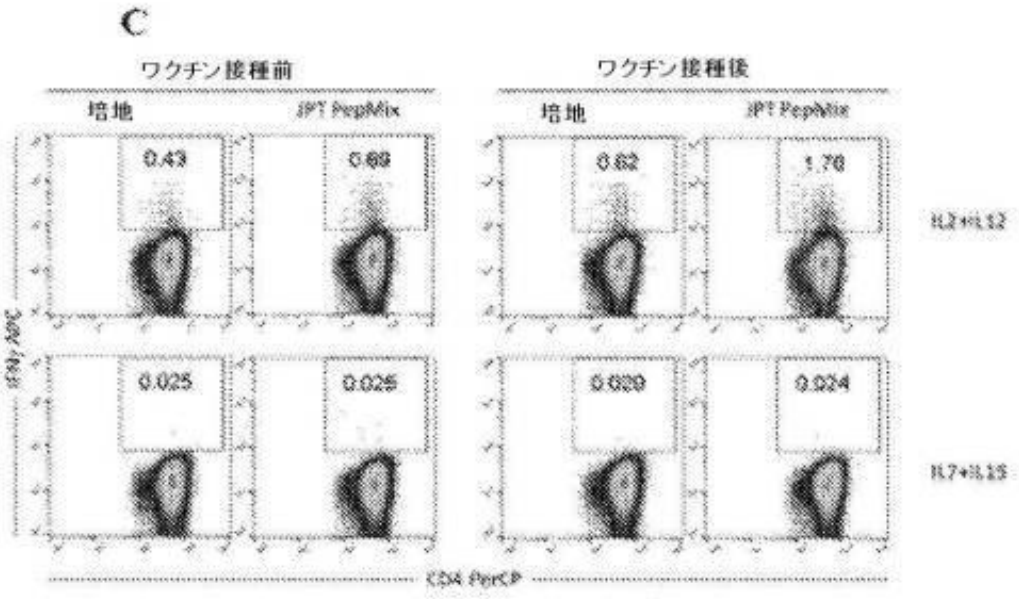

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

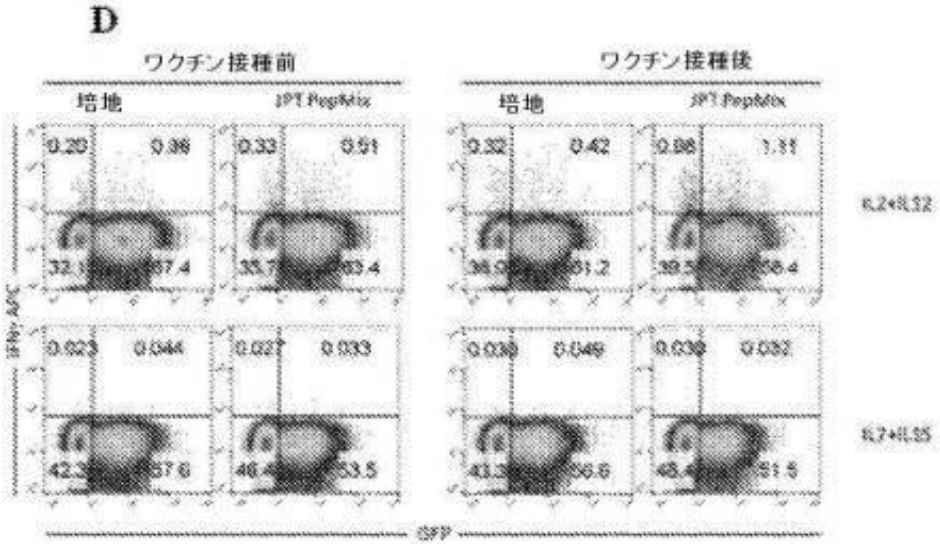
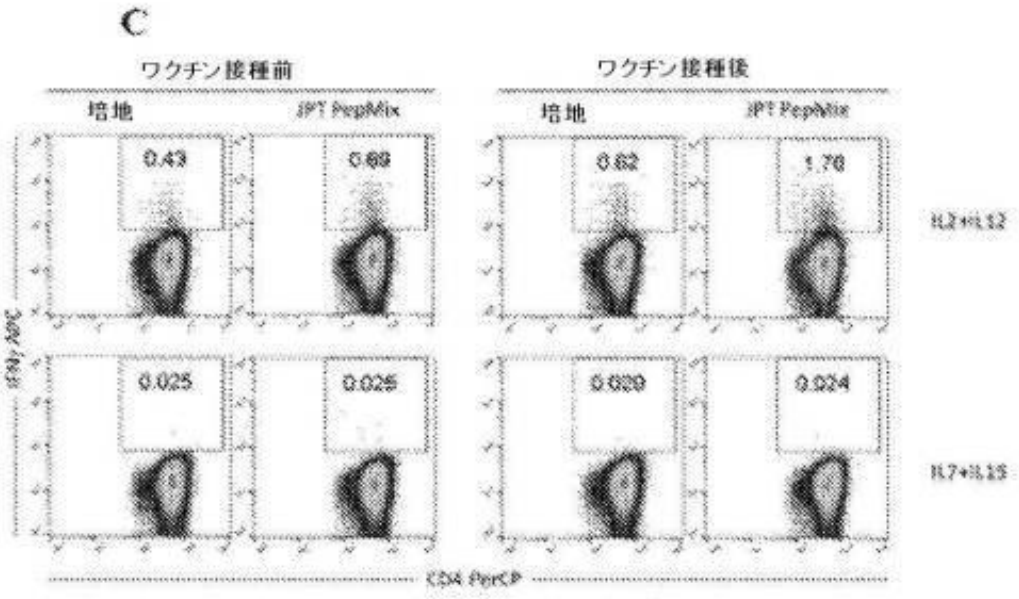

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

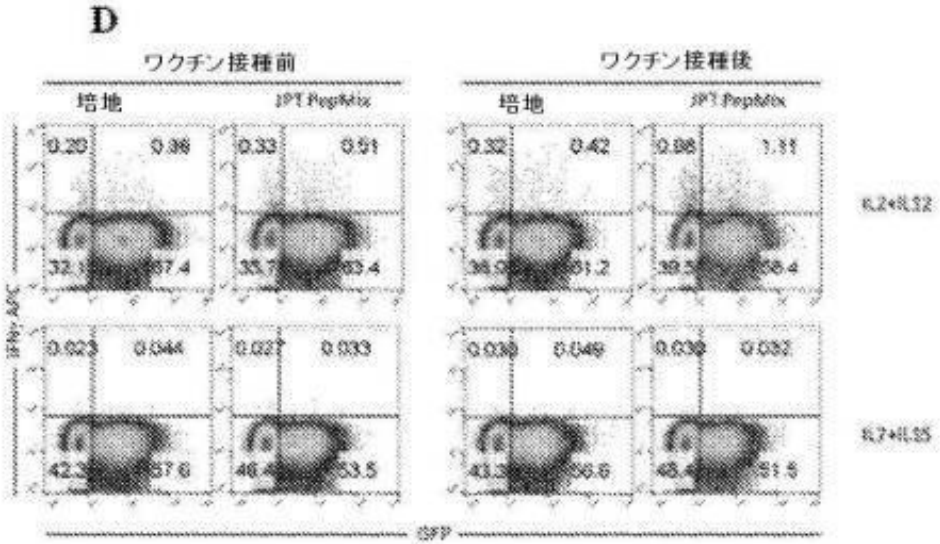
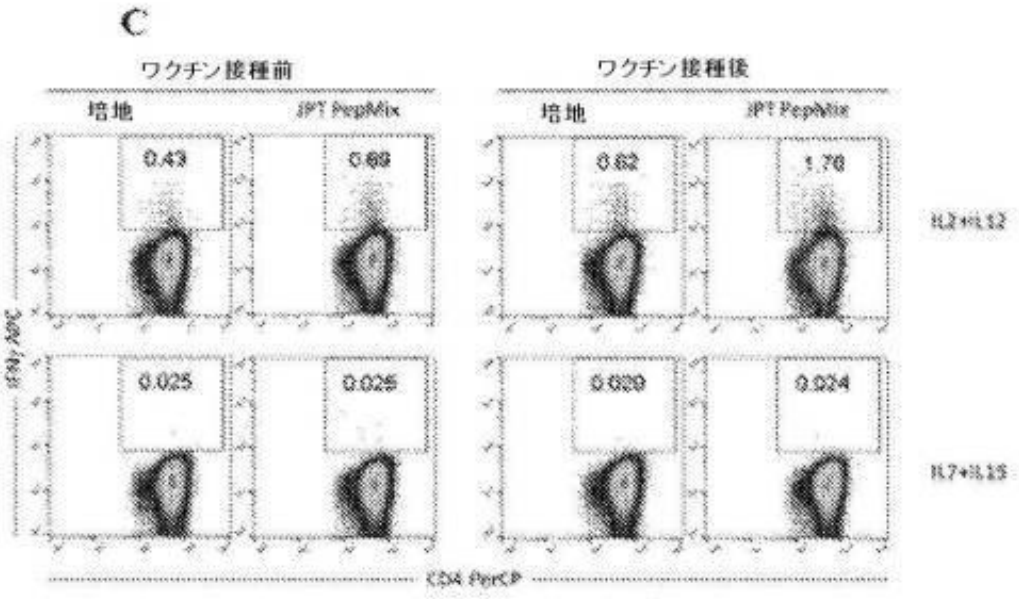

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

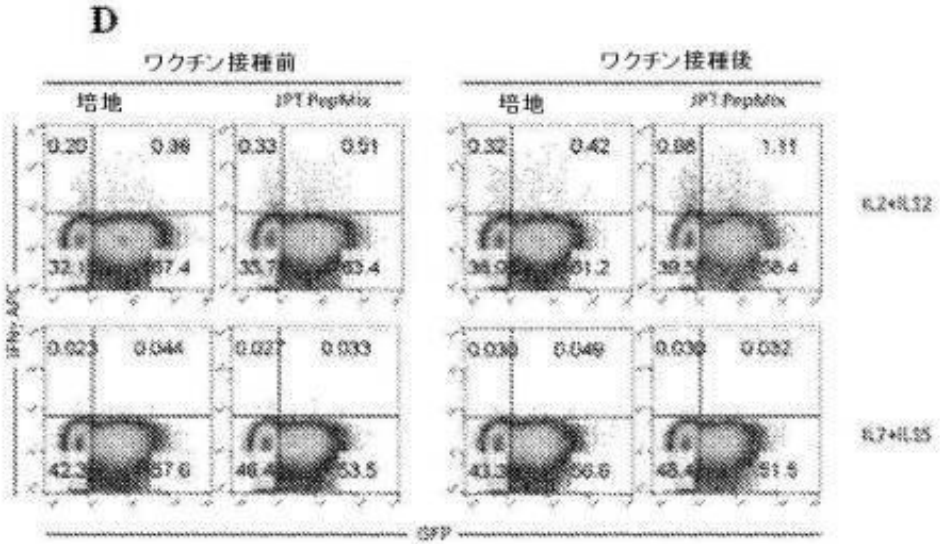
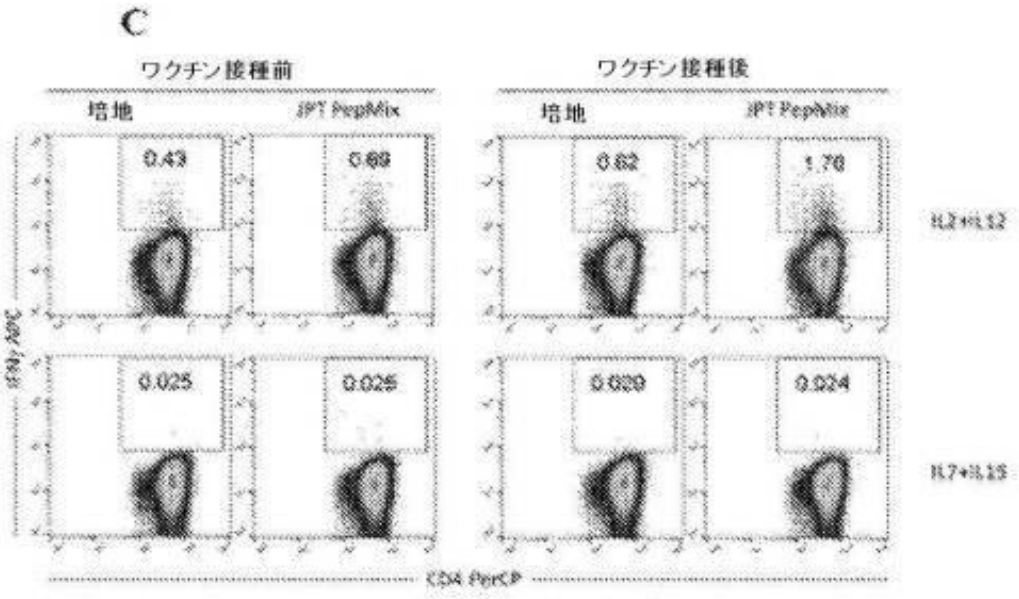

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

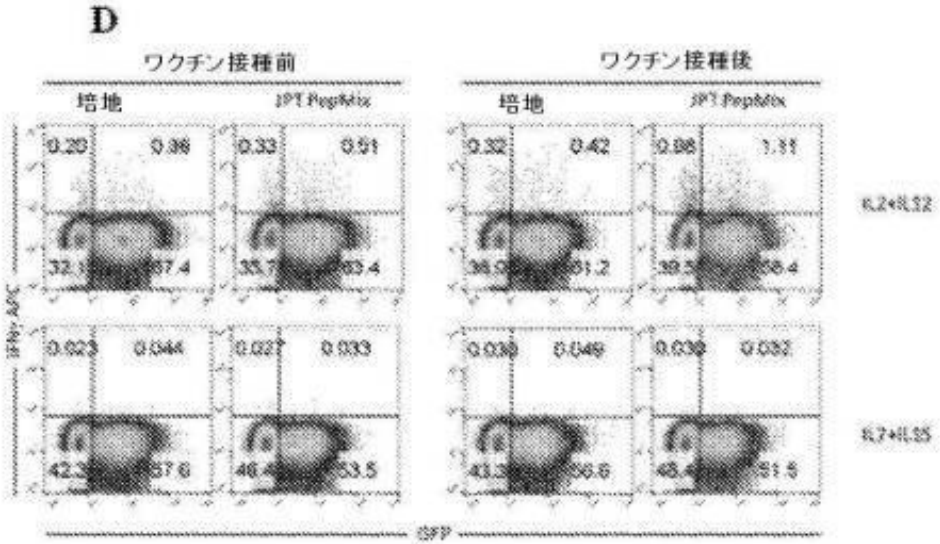
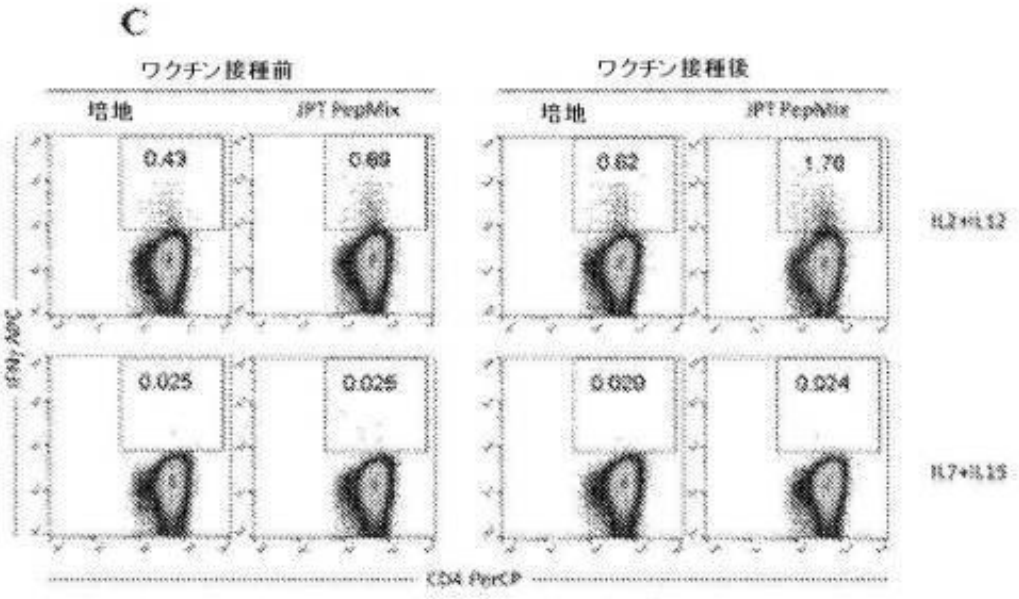

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

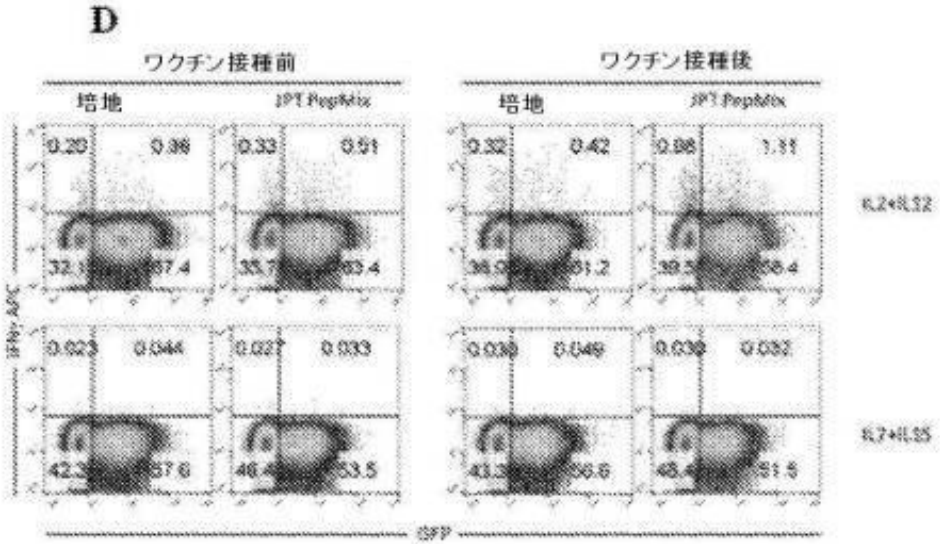
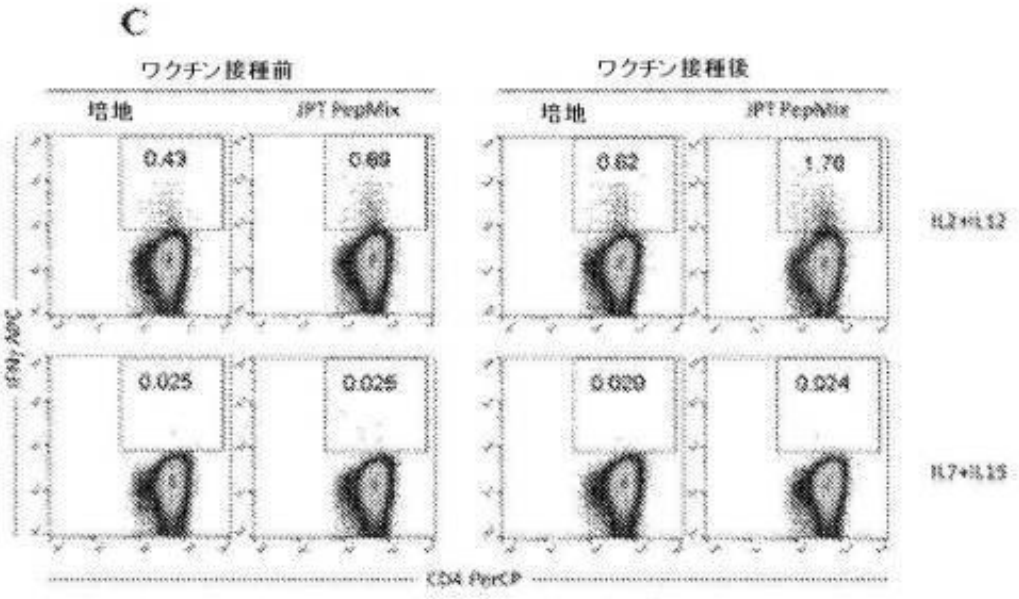

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

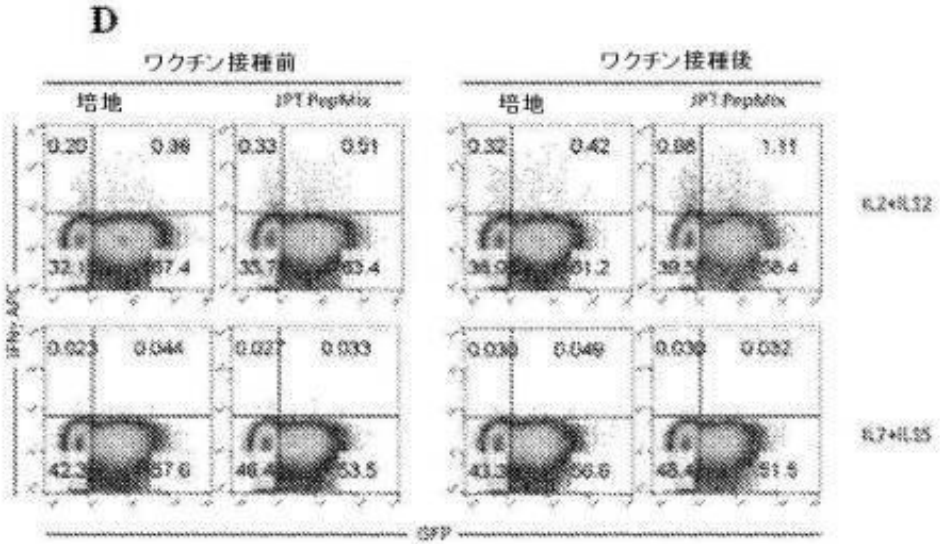
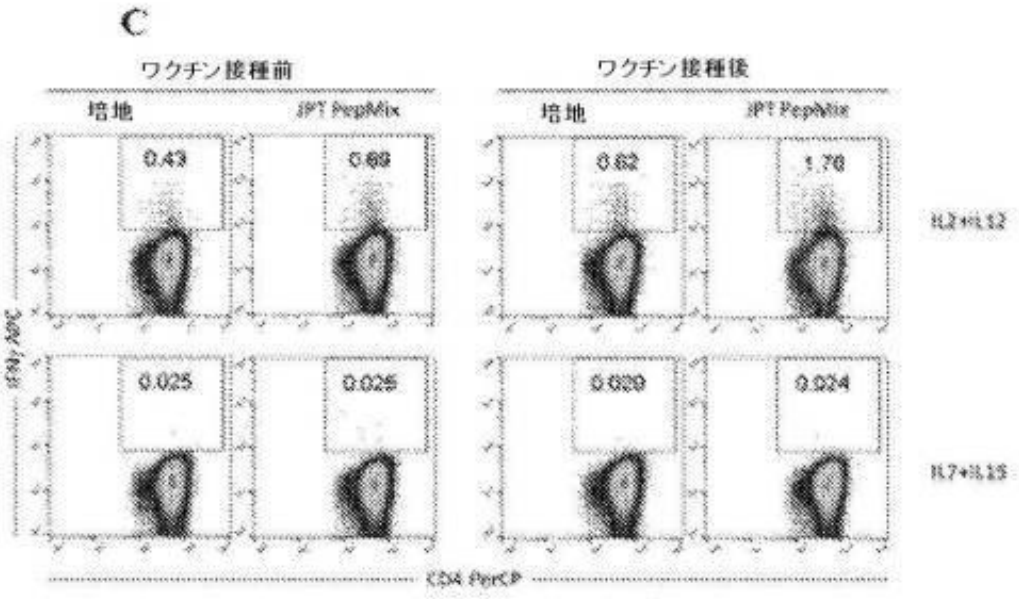

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

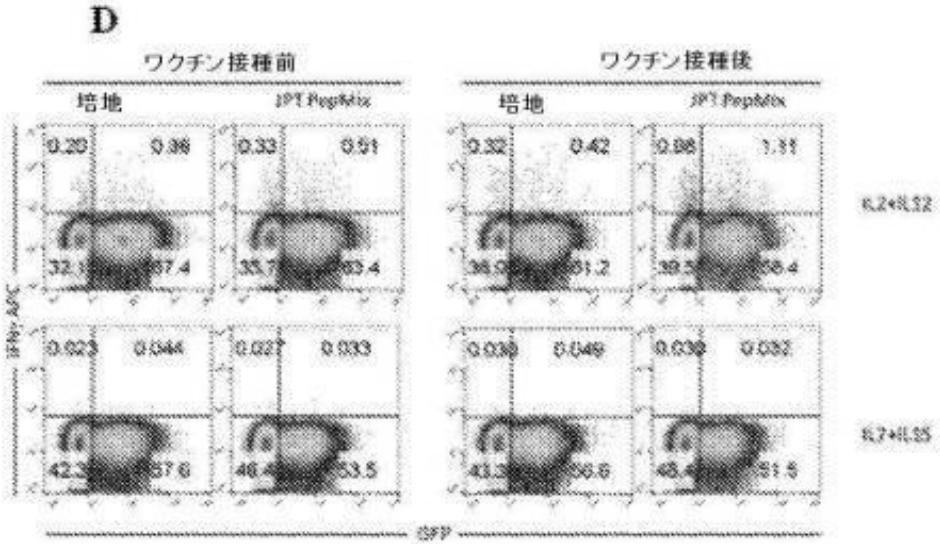
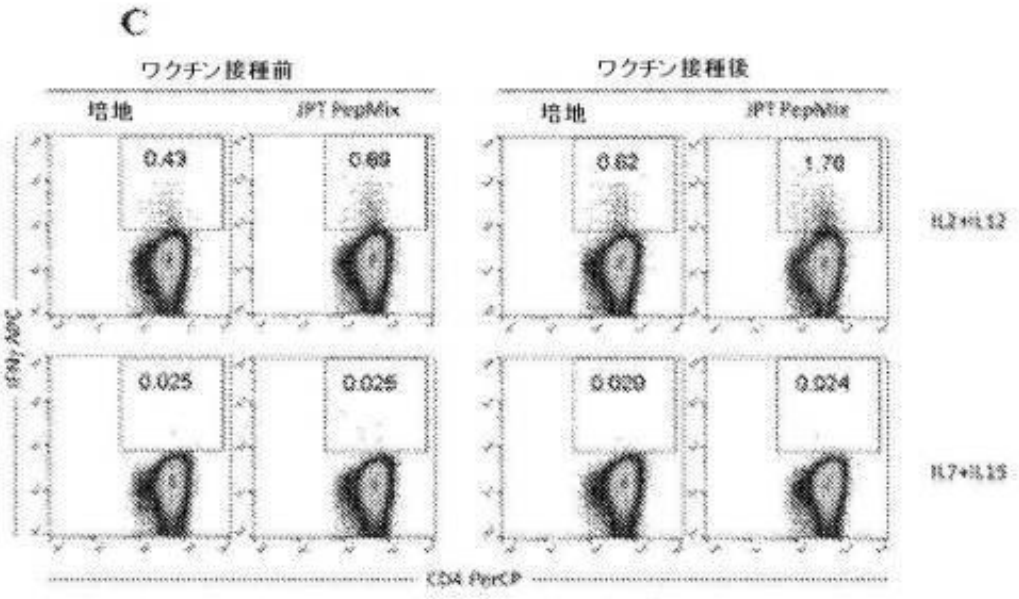

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

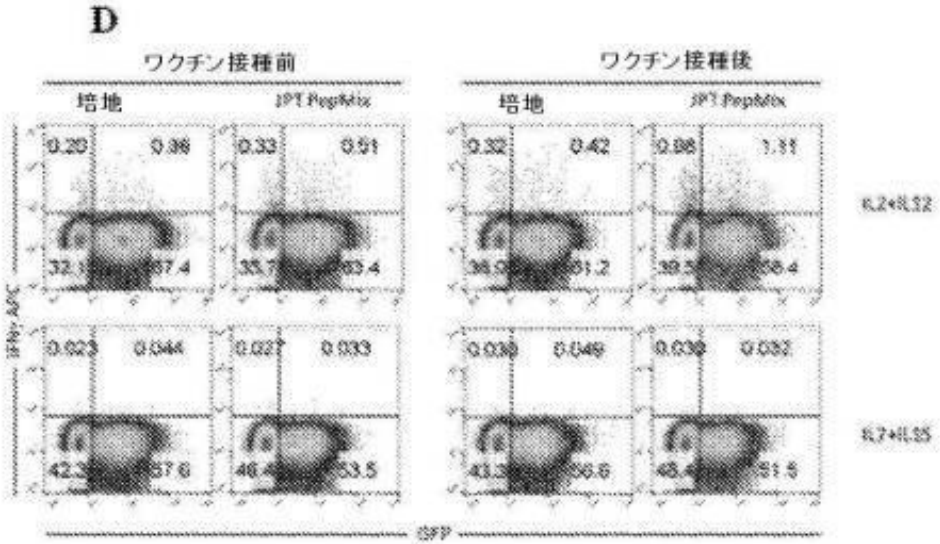
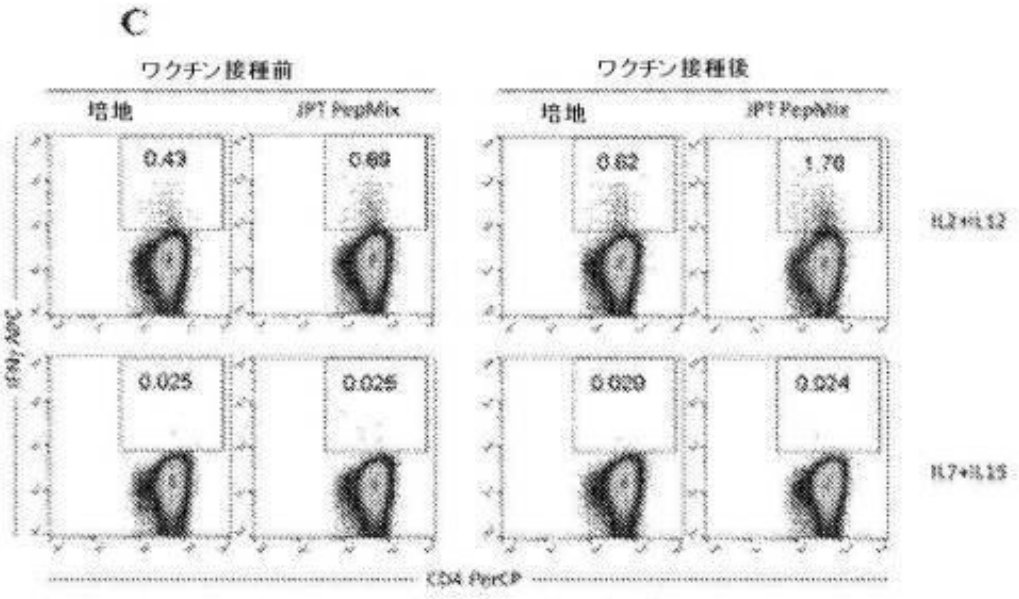

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

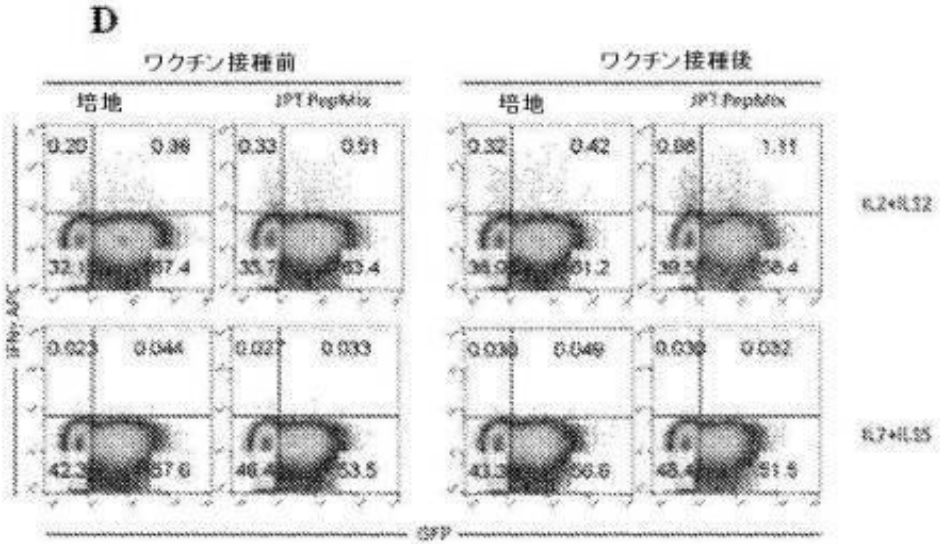
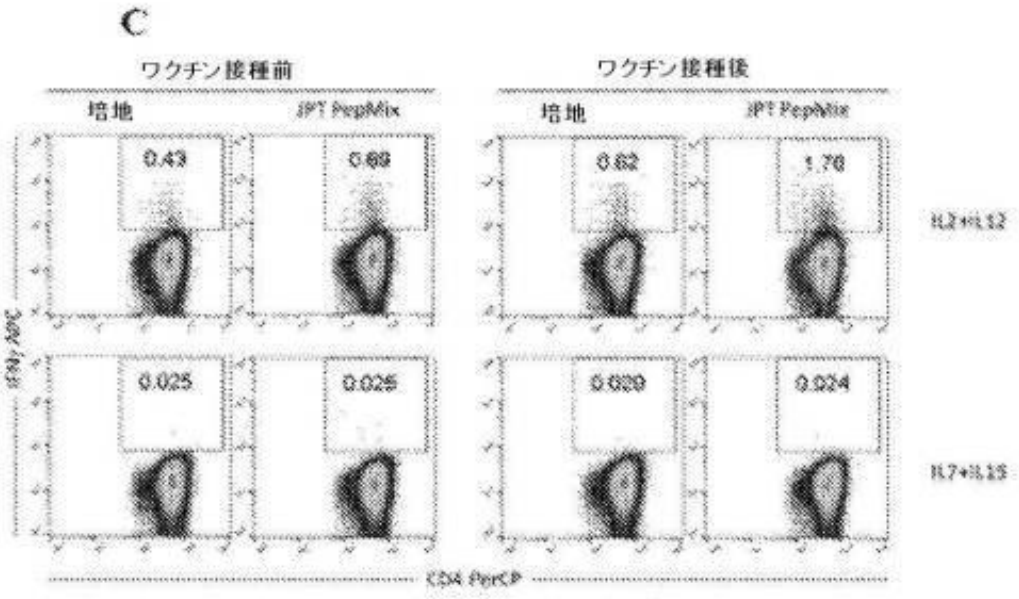

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

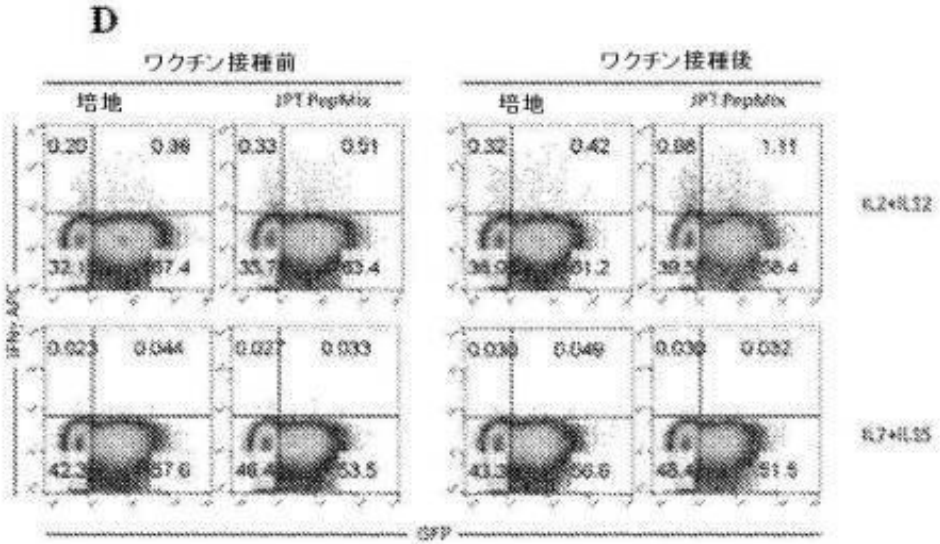
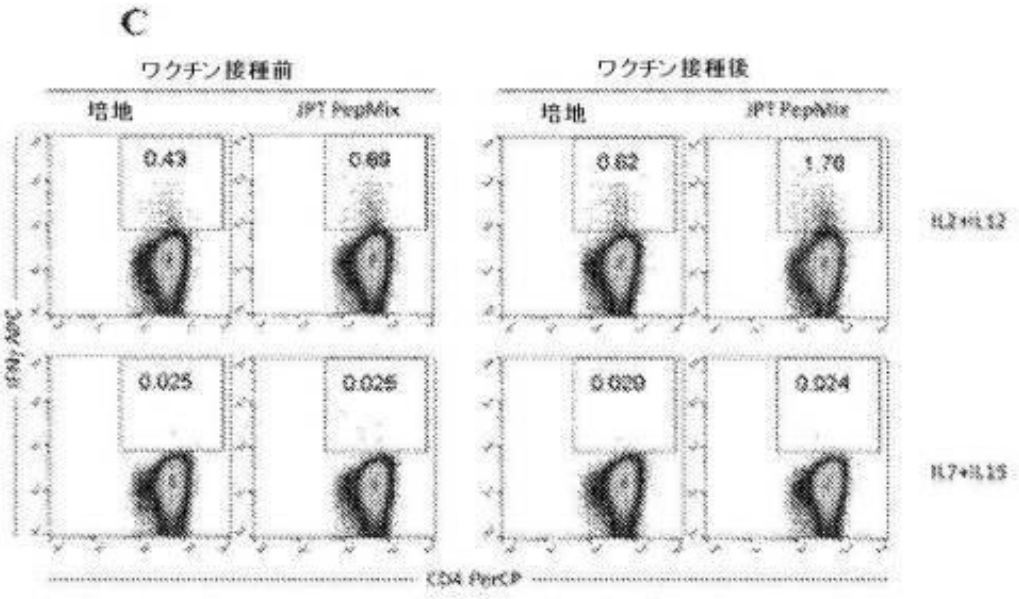

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

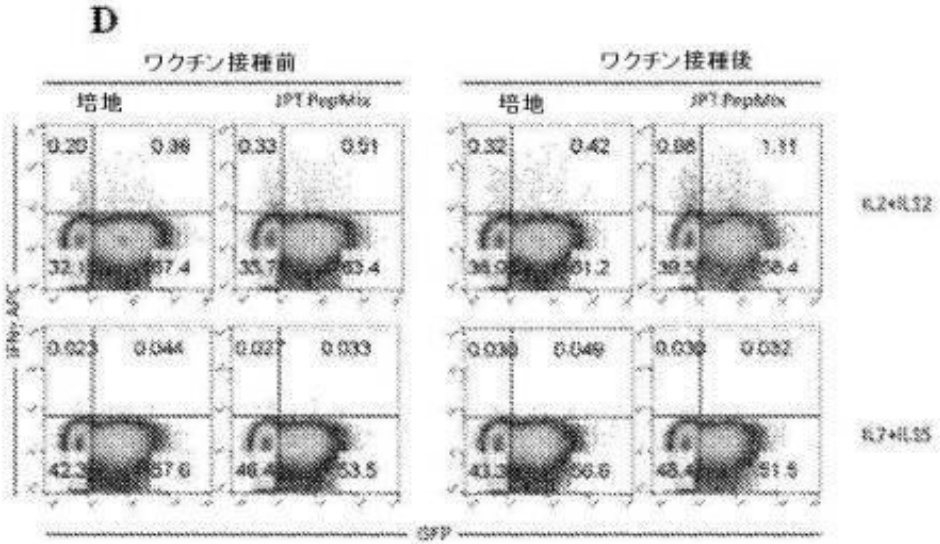
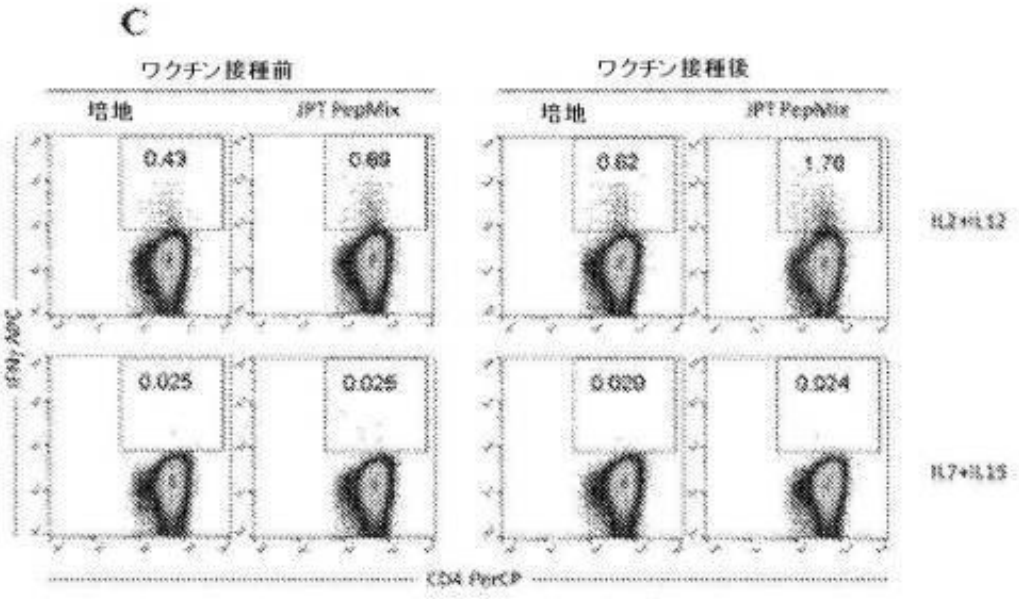

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

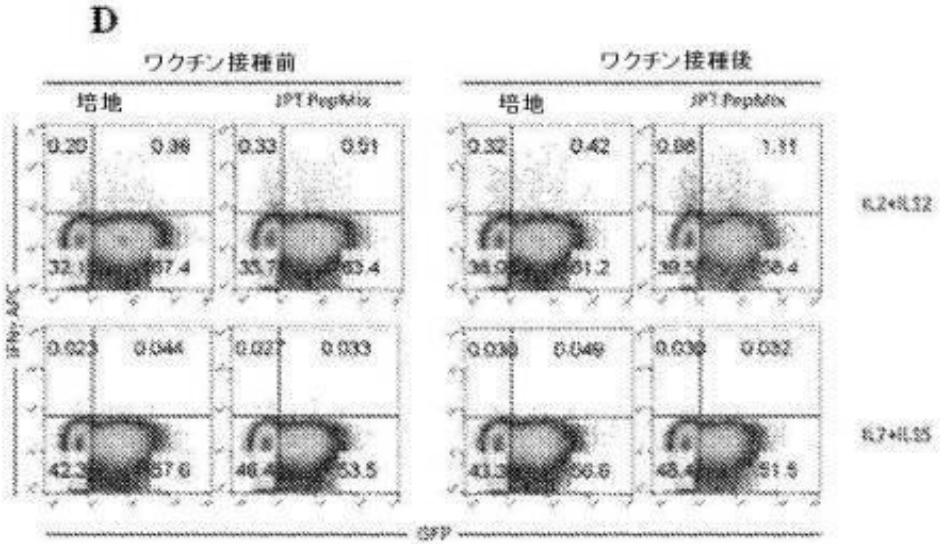
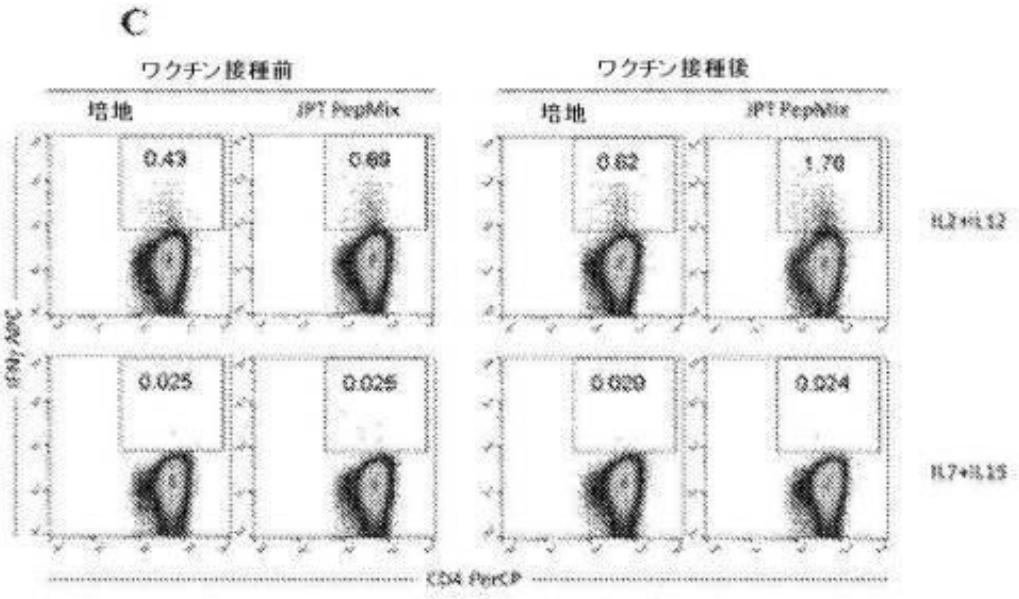

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

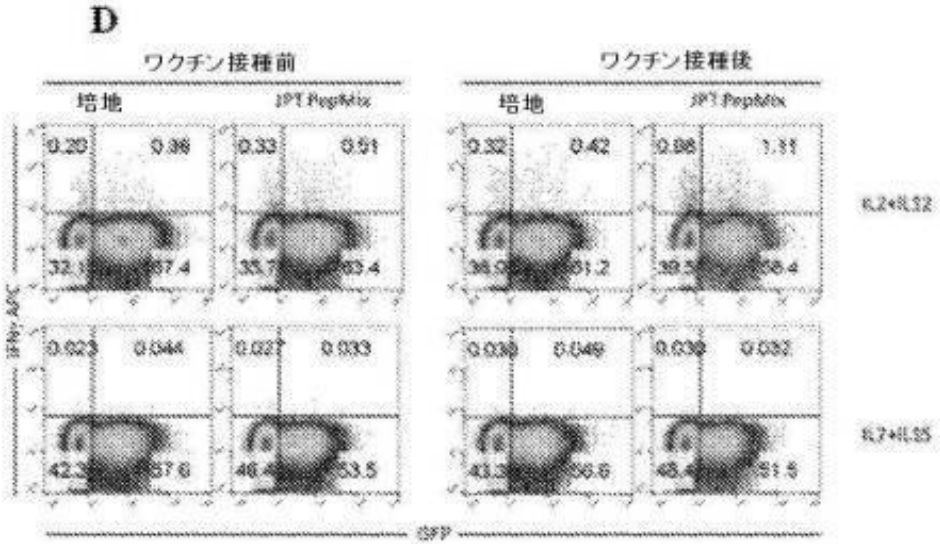
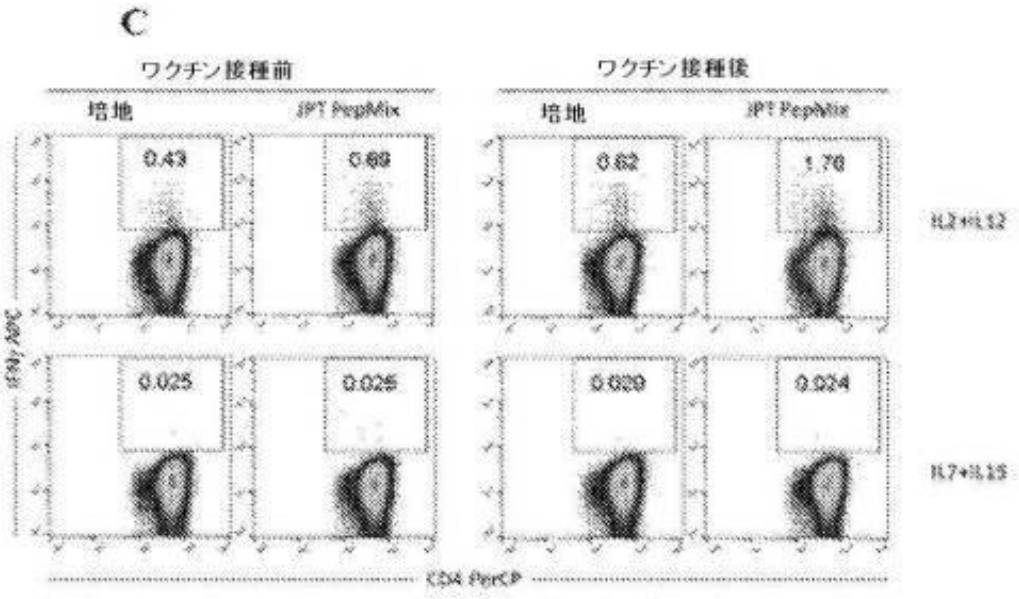

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

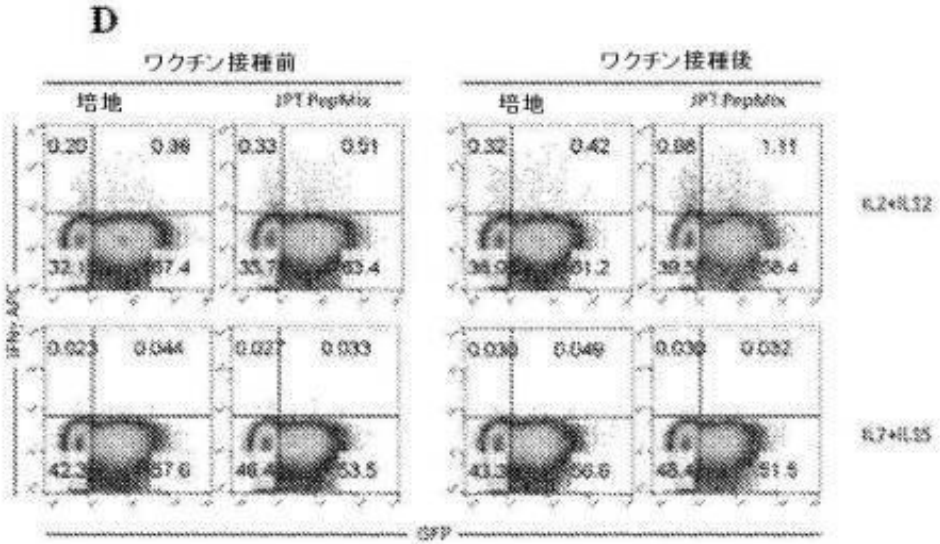
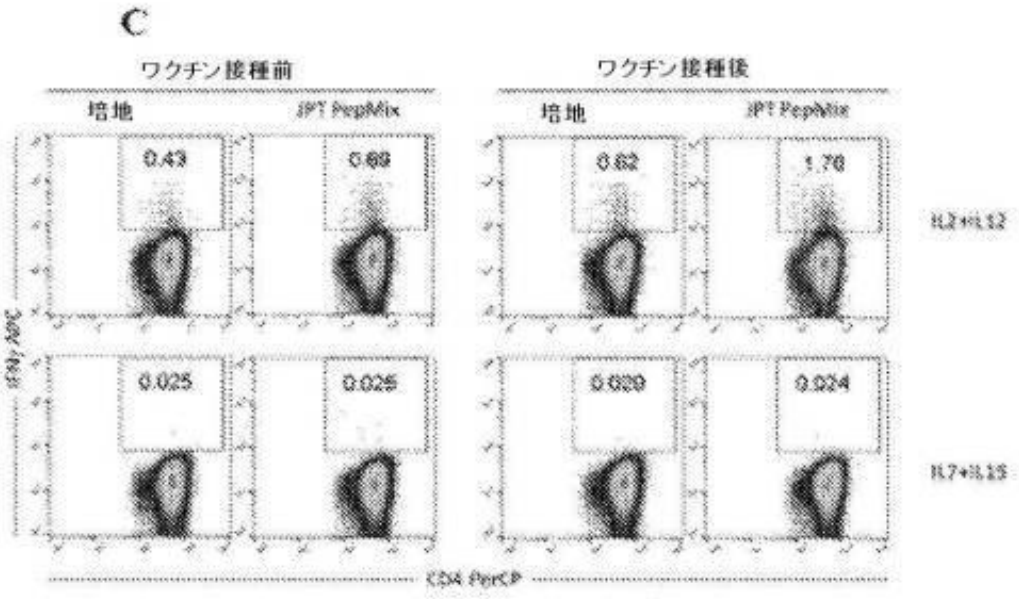

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

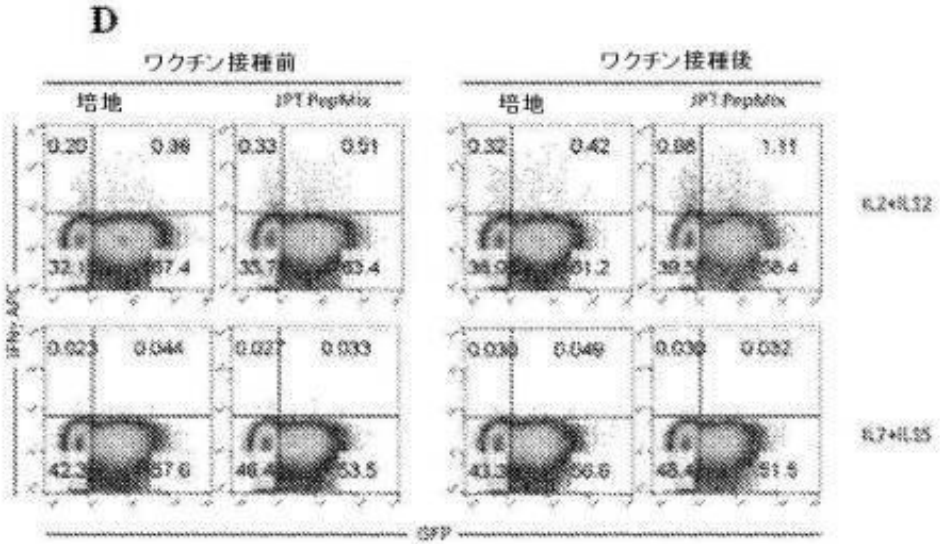
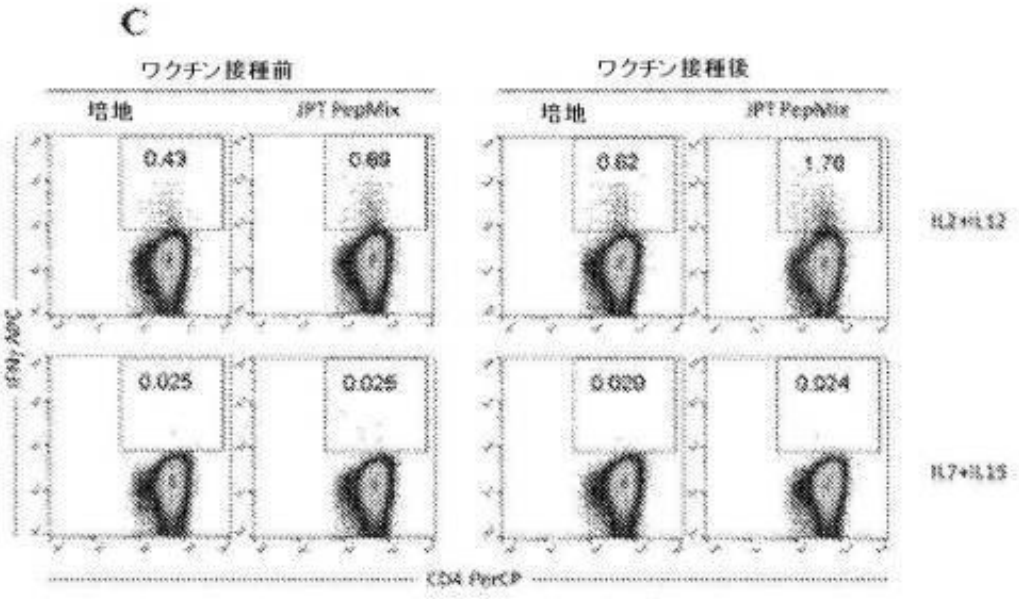

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

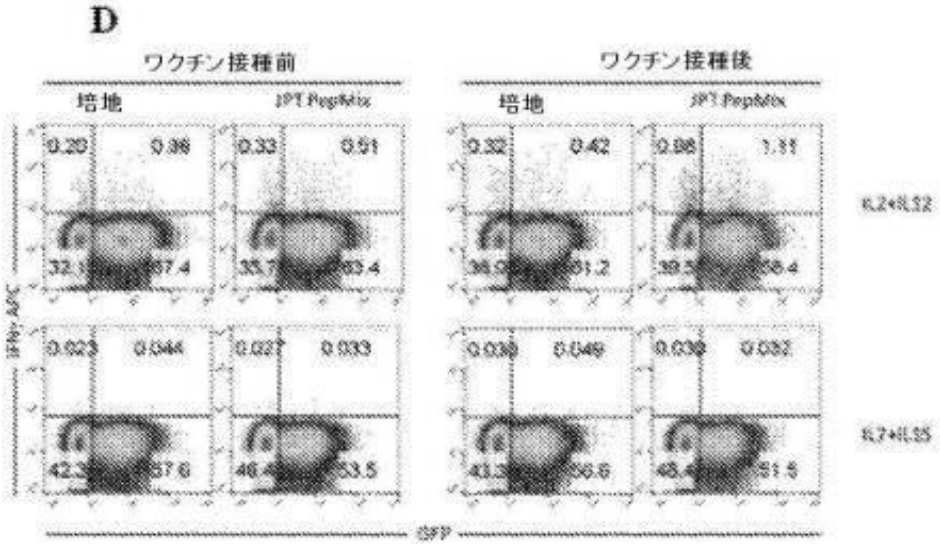
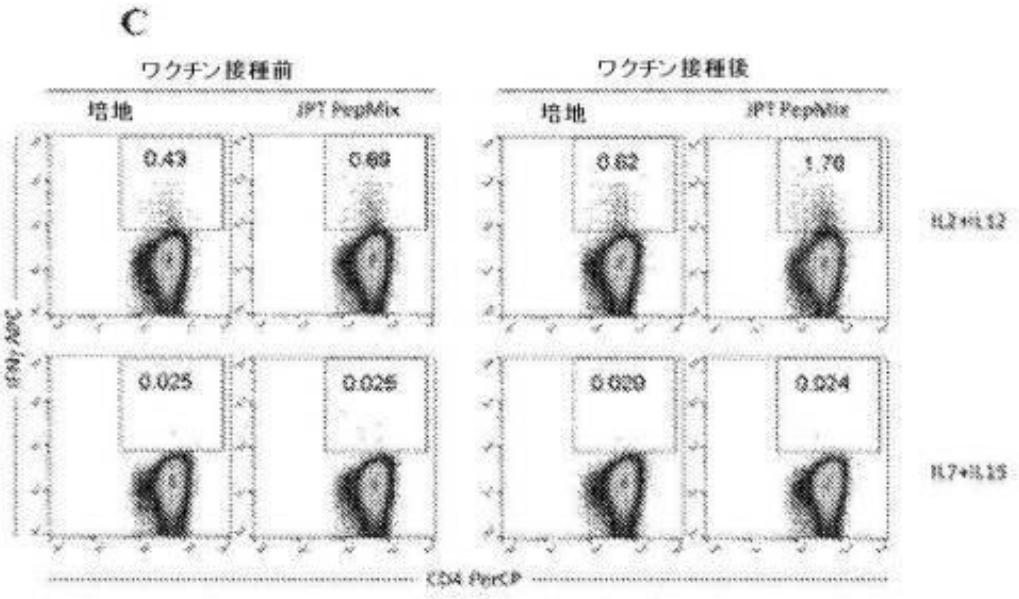

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き


C
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.43
0.69
0.62
1.70
IL2+IL12
0.025
0.025
0.029
0.024
IL7+IL15
IFNγ APC
CD4 PerCP

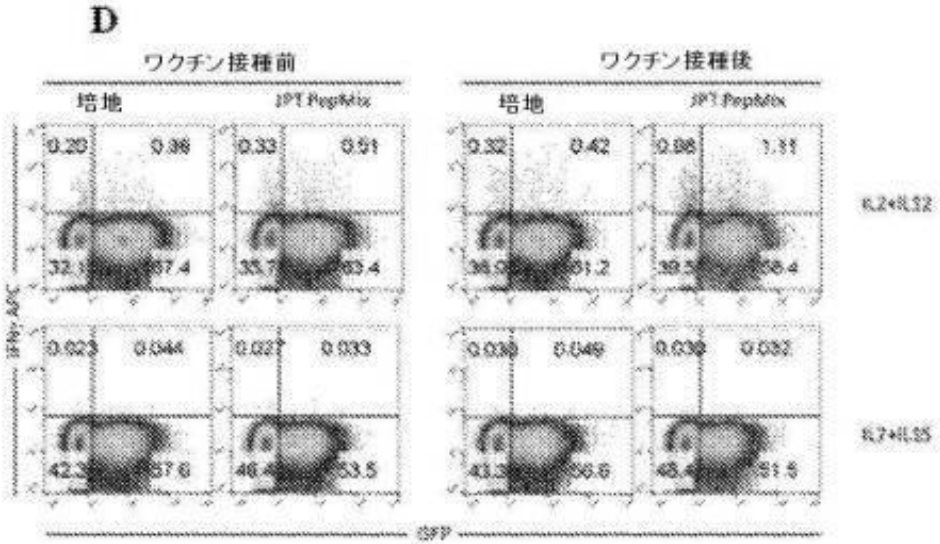

D
ワクチン接種前
ワクチン接種後
培地
JPT PepMix
0.20
0.38
0.33
0.91
0.32
0.42
0.98
1.31
IL2+IL12
0.023
0.044
0.027
0.033
0.039
0.049
0.039
0.032
IL7+IL15
IFNγ APC
CD8 PerCP

Figure 23 続き

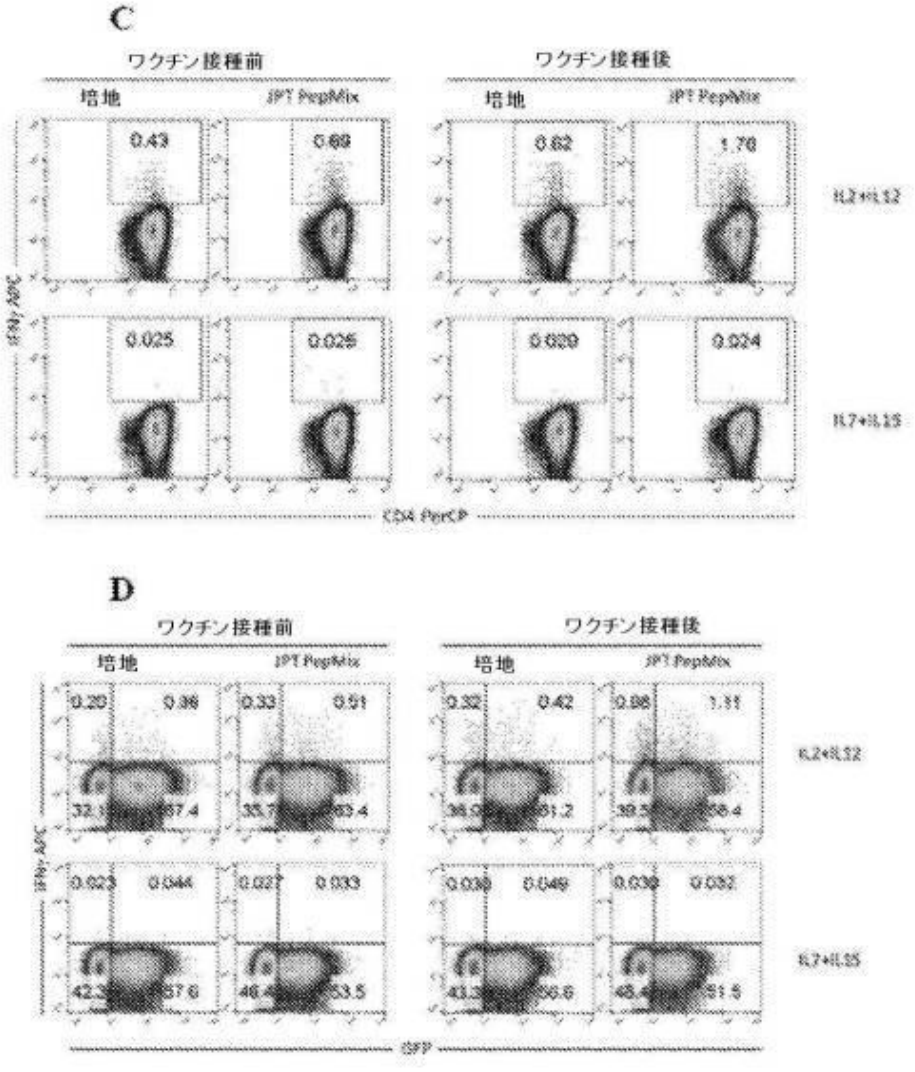


Figure 23 続き

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

Examples of embodiments of the present invention include the following items.

(Item 1)

A method of treating HIV-infected cells

(A) Ex vivo steps of contacting peripheral blood mononuclear cells (PBMCs) isolated from HIV-infected subjects with a therapeutically effective amount of stimulant.

(B) A step of exvivo transducing a viral delivery system encoding at least one genetic element into the PBMC, wherein the at least one genetic element is capable of inhibiting the production of the chemokine receptor CCR5. A step and a step comprising at least one small RNA capable of targeting a molecular RNA, or an HIV RNA sequence.

(C) A method comprising culturing the transduced PBMC for at least one day.

(Item 2)
The method of item 1, further comprising injecting the transduced PBMC into a subject.
(Item 3)
The method according to item 2, wherein the subject is a human.
(Item 4)
The method of item 1, wherein the stimulant comprises a peptide.
(Item 5)
The method of item 4, wherein the peptide comprises a gag peptide.
(Item 6)
The method of item 1, wherein the stimulant comprises a vaccine.
(Item 7)
6. The method of item 6, wherein the vaccine comprises an HIV vaccine.
(Item 8)
7. The method of item 7, wherein the HIV vaccine comprises an MVA / HIV62B vaccine or a variant thereof.
(Item 9)
The method of item 1, wherein the at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5 and at least one small RNA capable of targeting an HIV RNA sequence. ..
(Item 10)
The method of item 1 or 9, wherein the HIV RNA sequence comprises an HIV Vif sequence, an HIV Tat sequence, or a variant thereof.
(Item 11)
The method of item 1 or 9, wherein the at least one genetic element comprises microRNA or short RNA.
(Item 12)
11. The method of item 11, wherein the at least one genetic element comprises a microRNA cluster.
(Item 13)
The at least one genetic element
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
11. The method of item 11, comprising microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with.
(Item 14)
The at least one genetic element
AGGTATTTGCTGTTGACAGGTGAGCGACTGTAAACTGAGCTTGCTCACTGTGAAGCCACAGATTGGGTAGCAAGCAAGCAAGCATTACCGCTGCTACTGCCTCCGACTTCAGGGCTT (SEQ ID NO: 1)
The method according to item 11.
(Item 15)
The at least one genetic element
a.
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACACTCCGCACTGACATTTTTTGGTATTTTCATCTGACCA (SEQ ID NO: 2)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity, or
b.
GGGCTCGGCTCGAGCAGGGGGGGCGAGGATTCCGCTGTCTCTCCGCCATAGCGTGGTCCCCTCCCATGGCAGGCAGAAGCGCGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 3)
And at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity
11. The method of item 11, comprising microRNA having.
(Item 16)
The at least one genetic element

a.
CATCTCCATTGGCTGTACCACTTGTCTGGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGGAGTTCAGAAGAACACTCCGCACTGACATTTTTTGGTATTTTCATCTGACC
A (SEQ ID NO: 2); or
b.
GGGCTCGGCTCGAGCAGGGGGGGCGAGGATTCCGCTGTCTCTCCGCCATAGCGTGGTCCCCTCCCATGGCAGGCAGAAGCGCGCACCTTCCCTCCCAATGACCGCGTCTTCGTCG
(SEQ ID NO: 3)
14. The method of item 14.
(Item 17)
The microRNA cluster
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG
TCTTCGTC (SEQ ID NO: 31)
12. The method of item 12, comprising a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with.
(Item 18)
The microRNA cluster
AGGTATATTGCTGTTGACAGTGAGCGACTGTAAACTGAGCTTGCTCTACTGTGAAGCCACAGATGGGTAGAGCAAGCACAGTTTACCGCTGCCTACTGCCTCGGACTTCAAGGGGCT
TCCCGGGCATCTCCATGGCTGTACCACCTTGTCGGGGGATGTGTACTTCTGAACTTGTGTTGAATCTCATGGAGTTCAGAAGAACACATCCGCACTGACATTTTGGTATCTTTCATCT
GACCAGCTAGCGGGCCTGGCTCGAGCAGGGGGCGAGGGATTCCGCTTCTTCCTGCCATAGCGTGGTCCCCTCCCCTATGGCAGGCAGAAGCGGCACCTTCCCTCCCAATGACCGCG
TCTTCGTC (SEQ ID NO: 31)
The method according to item 12.
(Item 19)
A method of treating HIV infection in a subject,
(A) A step of immunizing the subject with an effective amount of the first stimulant.
(B) A step of removing leukocytes from the subject and purifying peripheral blood mononuclear cells (PBMC).
(C) Ex vivo contact of the PBMC with a therapeutically effective amount of the second stimulant.
(D) Ex vivo transduction of a virus delivery system encoding at least one genetic element into the PBMC.
(E) A method comprising culturing the transduced PBMC for at least one day.
(Item 20)
19. The method of item 19, further comprising injecting the transduced PBMC into the subject.
(Item 21)
19. The method of item 19, wherein the first stimulant and the second stimulant are the same.
(Item 22)
19. The method of item 19, wherein at least one of the first stimulant and the second stimulant comprises an HIV vaccine.
(Item 23)
22. The method of item 22, wherein the HIV vaccine comprises an MVA / HIV62B vaccine or a variant thereof.
(Item 24)
19. The method of item 19, wherein the virus delivery system comprises lentiviral particles.
(Item 25)
19. The method of item 19, wherein the at least one genetic element comprises a small RNA capable of inhibiting the production of the chemokine receptor CCR5, or at least one small RNA capable of targeting an HIV RNA sequence.

Patent Citations (153)

Publication number	Priority date	Publication date	Assignee	Title

Family To Family Citations				
US5668255A	1984-06-07	1997-09-16	Seragen, Inc.	Hybrid molecules having translocation region and cell-binding region
AU6014094A	1992-12-02	1994-06-22	Baylor College Of Medicine	Episomal vectors for gene therapy
WO1995002697A1	1993-07-13	1995-01-26	Rhone-Poulenc Rorer S.A.	Defective adenovirus vectors and use thereof in gene therapy
CA2265460A1	1996-09-11	1998-03-19	The Government Of The United States Of America, Represented By The Secre Tary, Department Of Health And Human Services	Aav4 vector and uses thereof
WO1999009139A1	1997-08-15	1999-02-25	Rubicon Laboratory, Inc.	Retrovirus and viral vectors
WO1999021979A1	1997-10-28	1999-05-06	Maxygen, Inc.	Human papillomavirus vectors
JP2002506652A	1998-03-20	2002-03-05	トラステイーズ・オブ・ザ・ユニバーシテイ・オブ・ペンシルベニア	Compositions and methods for helper-free production of recombinant adeno-associated virus
DK1115290T3	1998-10-01	2009-06-22	Univ Southern California	Retroviral gene delivery system and methods for its use
US6156514A	1998-12-03	2000-12-05	Sunol Molecular Corporation	Methods for making recombinant cells
US6410013B1	1999-01-25	2002-06-25	Musc Foundation For Research Development	Viral vectors for use in monitoring HIV drug resistance
WO2000072886A1	1999-05-26	2000-12-07	Dana-Farber Cancer Institute, Inc.	Episomally replicating lentiviral vectors
AU2001257611A1	2000-04-28	2001-11-12	Avigen, Inc.	Polynucleotides for use in recombinant adeno-associated virus virion production
AU2001261515A1	2000-05-12	2001-11-26	The Regents Of The University Of California	Treatment of human papillomavirus (hpv)-infected cells
WO2001091802A1	2000-05-30	2001-12-06	Baylor College Of Medicine	Chimeric viral vectors for gene therapy
NO314588B1 *	2000-09-04	2003-04-14	Bionor Immuno As	HIV peptides, antigens, vaccine composition, immunoassay test kits and a method for detecting antibodies induced by HIV
US7122181B2	2000-12-19	2006-10-17	Research Development Foundation	Lentiviral vector-mediated gene transfer and uses thereof
US20030119770A1	2001-08-02	2003-06-26	Zhennan Lai	Intercellular delivery of a herpes simplex virus VP22 fusion protein from cells infected with lentiviral vectors
WO2003015708A2	2001-08-18	2003-02-27	Myriad Genetics, Inc	Composition and method for treating hiv infection

US7737124B2	2001-09-13	2010-06-15	California Institute Of Technology	Method for expression of small antiviral RNA molecules with reduced cytotoxicity within a cell
WO2003040311A2	2001-10-25	2003-05-15	The Government Of The United States Of America As Represented By The Secretary Of Health And Human Services	Efficient inhibition of hiv-1 viral entry through a novel fusion protein including of cd4
US20070203333A1	2001-11-30	2007-08-30	Mcswiggen James	RNA interference mediated inhibition of vascular endothelial growth factor and vascular endothelial growth factor receptor gene expression using short interfering nucleic acid (siNA)
CA2479530A1	2002-03-20	2003-10-02	Massachusetts Institute Of Technology	Hiv therapeutic
US20040142416A1	2002-04-30	2004-07-22	Laipis Philip J.	Treatment for phenylketonuria
WO2004037847A2	2002-05-07	2004-05-06	Chiron Corporation	Hiv envelope-cd4 complexes and hybrids
US20040161412A1	2002-08-22	2004-08-19	The Cleveland Clinic Foundation	Cell-based VEGF delivery
DK1545204T3	2002-09-06	2016-11-14	The Government Of The Us Secretary Dept Of Health And Human Services	Immunotherapy with in vitro selected antigen-specific lymphocytes following non-myeloablative lymphodepletive chemotherapy
JP2006505288A	2002-11-04	2006-02-16	ユニバーシティー オブ マサチューセッツ	Allele-specific RNA interference
AU2003283174A1	2002-12-11	2004-06-30	Cytos Biotechnology Ag	Method for protein production
TW200502391A	2003-05-08	2005-01-16	Xcyte Therapies Inc	Generation and isolation of antigen-specific t cells
WO2004104591A2	2003-05-23	2004-12-02	Institut National De La Sante Et De La Recherche Medicale	Improvements to gamma delta t cell-mediated therapy
EP1644508A1	2003-07-11	2006-04-12	Cytos Biotechnology AG	Gene expression system
US20050019927A1	2003-07-13	2005-01-27	Markus Hildinger	DECREASING GENE EXPRESSION IN A MAMMALIAN SUBJECT IN VIVO VIA AAV-MEDIATED RNAi EXPRESSION CASSETTE TRANSFER
US20050138677A1	2003-09-16	2005-06-23	Pfister Herbert J.	Transgenic animal model for the treatment of skin tumors
WO2005028634A2	2003-09-18	2005-03-31	Emory University	Improved mva vaccines
WO2005033282A2	2003-10-01	2005-04-14	Pharmacia & Upjohn Company Llc	Polyamide compositions and therapeutic methods for treatment of human papilloma virus
US20080039413A1	2003-10-21	2008-02-14	Morris David W	Novel compositions and methods in cancer
JPWO2005051927A1	2003-11-26	2007-12-06	株式会社クレハ	Method for culturing CD4-positive T cells by stimulation culture of HIV-1-infected peripheral blood mononuclear cells, and HIV-1 growth inhibitor

EP1753777B1	2004-02-25	2014-05-07	Dana-Farber Cancer Institute, Inc.	METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HIV INFECTION USING TRIM5a
EP1737956A2	2004-03-01	2007-01-03	Massachusetts Institute of Technology	Rnai-based therapeutics for allergic rhinitis and asthma
TWI439284B	2004-04-09	2014-06-01	Abbvie Biotechnology Ltd	Multiple-variable dose regimen for treating tnfα-related disorders
US20080227736A1	2004-06-03	2008-09-18	Regents Of The University Of California,	Targeting Pseudotyped Retroviral Vectors
WO2006012221A2	2004-06-25	2006-02-02	The Regents Of The University Of California	Target cell-specific short interfering rna and methods of use thereof
WO2006023491A2	2004-08-16	2006-03-02	The Cbr Institute For Biomedical Research, Inc.	Method of delivering rna interference and uses thereof
WO2006039721A2	2004-10-08	2006-04-13	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods for bone resorption diseases, cancer, bone pain, immune disorders, and infectious diseases
EP1647595A1 *	2004-10-15	2006-04-19	Academisch Medisch Centrum bij de Universiteit van Amsterdam	Nucleic acids against viruses, in particular HIV
WO2006048215A1	2004-11-02	2006-05-11	Istituto Di Ricerche Di Biologia Molecolare P Angeletti Spa	Adenoviral amplicon and producer cells for the production of replication-defective adenoviral vectors, methods of preparation and use thereof
US7790446B2	2005-02-11	2010-09-07	Kosagen Cell Factory Oü	Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products
CN101160055A	2005-02-16	2008-04-09	莱蒂恩公司	Lentiviral vectors and their use
EP2573185A3	2005-02-16	2013-06-05	Lentigen Corporation	Lentiviral vectors and their use
DK2002003T3	2005-05-27	2016-03-21	Ospedale San Raffaele Srl	Gene vector comprising miRNA
WO2007015122A1	2005-08-02	2007-02-08	Genexel, Inc.	Therapy for alzheimer’s disease
US20070032443A1	2005-08-02	2007-02-08	Jaeseob Kim	Therapy for Alzheimer's disease
WO2007056388A2	2005-11-07	2007-05-18	The General Hospital Corporation	Compositions and methods for modulating poly (adp-ribose) polymerase activity
WO2007133674A2	2006-05-12	2007-11-22	Lentigen Corporation	Lentiviral vector compositions, methods and applications
US8535897B2	2006-06-19	2013-09-17	The Trustees Of Columbia University In The City Of New York	Assays for non-apoptotic cell death and uses thereof
US20080003225A1	2006-06-29	2008-01-03	Henri Vie	Method for enhancing the antibody-dependent cellular cytotoxicity (ADCC) and uses of T cells expressing

				CD16 receptors
WO2008008719A2	2006-07-10	2008-01-17	Alnylam Pharmaceuticals, Inc.	Compositions and methods for inhibiting expression of the myc gene
EP1878440A1	2006-07-13	2008-01-16	INSERM (Institut National de la Santé et de la Recherche Médicale)	Methods and compositions for increasing the efficiency of therapeutic antibodies using gamma delta cell activator compounds
CN101516365A	2006-07-26	2009-08-26	诺瓦提斯公司	Inhibitors of undecaprenyl pyrophosphate synthase
US20080199961A1	2006-08-25	2008-08-21	Avi Biopharma, Inc.	ANTISENSE COMPOSITION AND METHOD FOR INHIBITION OF miRNA BIOGENESIS
WO2008100292A2	2006-10-16	2008-08-21	Genelux Corporation	Modified vaccinia virus strains for use in diagnostic and therapeutic methods
ES2639568T3 *	2007-01-23	2017-10-27	Janssen Pharmaceutica Nv	Method to design a drug regimen for HIV-infected patients
CA2682694A1	2007-04-12	2008-10-23	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods with enhanced potency for multiple targets including fpps, ggpps, and dpps
US20080293142A1	2007-04-19	2008-11-27	The Board Of Regents For Oklahoma State University	Multiple shRNA Expression Vectors and Methods of Construction
EP2008656A1	2007-06-28	2008-12-31	Bergen Teknologioverforing AS	Compositions for the treatment of hyperphenylalaninemia
US8673477B2	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
WO2009026328A2 *	2007-08-21	2009-02-26	Immune Disease Institute, Inc.	Methods of delivery of agents to leukocytes and endothelial cells
BRPI0821998A2	2008-01-16	2019-08-27	Opal Therapeutics Pty Ltd	immunomodulation compositions and uses thereof.
GB0802754D0	2008-02-14	2008-03-26	Inst Superiore Di Sanito	Antisense RNA targetting CXCR4
EP2090659A1	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.	Infectious particle, process for its preparation and use thereof
WO2009120947A1	2008-03-28	2009-10-01	Virxsys Corporation	Lentivirus-based immunogenic vectors
GB0810209D0	2008-06-04	2008-07-09	Cambridge Entpr Ltd	Pluripotency associated epigenetic factor
US8629334B2	2008-07-16	2014-01-14	University Of Florida Research Foundation, Inc.	Viral-based transient-expression vector system for trees
WO2010022195A2	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system
EP2342321B1	2008-09-17	2018-04-11	Isogenis, Inc.	Construction of fully-deleted adenovirus-based gene

				delivery vectors and uses thereof
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
WO2010051521A1	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection
US8734795B2	2008-10-31	2014-05-27	Biogen Idec Ma Inc.	Light targeting molecules and uses thereof
WO2011071476A2	2008-11-14	2011-06-16	Life Technologies Corporation	Compositions and methods for engineering cells
EP2191834A1	2008-11-26	2010-06-02	Centre National De La Recherche Scientifique (Cnrs)	Compositions and methods for treating retrovirus infections
WO2010117974A2	2009-04-09	2010-10-14	Stemcyte Inc.	Hiv-resistant stem cells and uses thereof
EP2419113B1	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
EP2425001A4	2009-04-30	2012-11-14	Univ California	Combination anti-hiv vectors, targeting vectors, and methods of use
EP3329772B1	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
SG178909A1	2009-10-08	2012-04-27	Bavarian Nordic As	Generation of a broad t-cell response in humans against hiv
US20120027725A1	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules to treat liver cancer
CN101805750B	2009-12-29	2011-11-30	浙江大学	Construction and application of farnesyl pyrophosphoric acid synthetase RNA (Ribonucleic Acid) interference recombinant lentivirus vector
CN102782136A *	2010-02-18	2012-11-14	爱默蕾大学	Vectors expressing HIV antigens and GM-CSF and related methods for generating an immune response
WO2011119942A1	2010-03-25	2011-09-29	Vistagen Therapeutics, Inc.	Induction of ips cells using transient episomal vectors
WO2011133687A2	2010-04-20	2011-10-27	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-adrenergic receptor degradation
LT2561078T	2010-04-23	2019-01-10	Cold Spring Harbor Laboratory	NOVEL STRUCTURALLY DESIGNED shRNAs
US20110293571A1	2010-05-28	2011-12-01	Oxford Biomedica (Uk) Ltd.	Method for vector delivery
WO2012020757A1	2010-08-10	2012-02-16	タカラバイオ株式会社	Production method for cell populations
US20130281493A1	2010-10-07	2013-10-24	The Trustees Of The University Of Columbia In The City Of New York	Method for Treating Cancer Harboring a p53 Mutation

WO2012061075A2	2010-10-25	2012-05-10	The Regents Of The University Of California	Hiv resistant and functional hematopoietic stem/progenitor cells and macrophages from induced pluripotent stem cells
WO2012115980A1	2011-02-22	2012-08-30	California Institute Of Technology	Delivery of proteins using adeno-associated virus (aav) vectors
JP2014511704A	2011-04-13	2014-05-19	イミュニカム・エイビイ	Method for priming T cells
US9226976B2	2011-04-21	2016-01-05	University Of Massachusetts	RAAV-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
EP2782596A4	2011-11-22	2015-07-29	Philadelphia Children Hospital	Virus vectors for highly efficient transgene delivery
US9745631B2	2011-12-20	2017-08-29	Dana-Farber Cancer Institute, Inc.	Methods for diagnosing and treating oncogenic kras-associated cancer
BR112014019431A8	2012-02-07	2017-07-11	Global Bio Therapeutics Usa Inc	COMPARTMENTALIZED METHOD OF DELIVERY OF NUCLEIC ACID AND COMPOSITIONS AND USES THEREOF
WO2013174404A1	2012-05-23	2013-11-28	Ganymed Pharmaceuticals Ag	Combination therapy involving antibodies against claudin 18.2 for treatment of cancer
AU2013273483A1	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
WO2014016817A2	2012-07-17	2014-01-30	Universite De Geneve	Nucleic acids for down-regulation of gene expression
CA2922005A1	2012-09-27	2014-04-03	Population Diagnostics, Inc.	Methods and compositions for screening and treating developmental disorders
JP6391582B2	2012-11-13	2018-09-19	コディアック バイオサイエンシズ インコーポレイテッド	Methods for delivering therapeutic agents
CA2892448A1	2012-12-05	2014-06-12	Sangamo Biosciences, Inc.	Methods and compositions for regulation of metabolic disorders
US9642921B2	2012-12-20	2017-05-09	Tocagen Inc.	Cancer combination therapy and recombinant vectors
WO2014117050A2	2013-01-26	2014-07-31	Mirimus, Inc.	Modified mirna as a scaffold for shrna
CN103184224A	2013-04-03	2013-07-03	衡阳师范学院	Triple minRNA for resisting virus infection of aids and construction method thereof
WO2014187881A1	2013-05-21	2014-11-27	Max-Planck Gesellschaft zur Förderung der Wissenschaften e.V.	Isoforms of gata6 and nkx2-1 as markers for diagnosis and therapy of cancer and as targets for anti-cancer therapy
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
WO2015042308A2	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors

AU2014340083B2	2013-10-22	2019-08-15	Translate Bio, Inc.	mRNA therapy for phenylketonuria
CN106459995B	2013-11-07	2020-02-21	爱迪塔斯医药有限公司	CRISPR-associated methods and compositions using dominant grnas
EP2878674A1	2013-11-28	2015-06-03	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)	Stable episomes based on non-integrative lentiviral vectors
GB201322091D0	2013-12-13	2014-01-29	Cambridge Entpr Ltd	Modified serpins for the treatment of bleeding disorders
CA2946312A1	2014-04-23	2015-10-29	Juno Therapeutics, Inc.	Methods for isolating, culturing, and genetically engineering immune cell populations for adoptive therapy
DK3851537T3	2014-04-25	2024-03-18	Genethon	TREATMENT OF HYPERBILIRUBINAMIA
PL3689899T3	2014-04-25	2022-01-31	2Seventy Bio, Inc.	Mnd promoter chimeric antigen receptors
CA2955254A1	2014-08-29	2016-03-03	Immunomedics, Inc.	Identification of cancer genes by in-vivo fusion of human cancer cells and animal cells
SI3197472T1	2014-09-22	2022-01-31	Tanea Medical Ab	Recombinant phe-free proteins for use in the treatment of phenylketonuria
MA40783A *	2014-10-03	2017-08-08	Los Alamos Nat Security Llc	HIV VACCINES CONTAINING ONE OR MORE POPULATION EPISENSUS ANTIGENS
AU2015329696A1	2014-10-10	2017-04-27	The United States Of America, As Represented By The Secretary, Department Of Health And Human Services	Methods to eliminate cancer stem cells by targeting CD47
CN107405357B	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
JP2017534284A *	2014-10-27	2017-11-24	インターナショナル エイズ バクシーン イニシアティブ	Genetically stable replicable Sendai virus vector containing and expressing an optimized HIV gene
WO2016069716A1	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds
WO2016186708A1	2015-05-18	2016-11-24	Calimmune, Inc.	Gene therapeutic for the treatment of hiv and uses thereof
GB201509202D0	2015-05-28	2015-07-15	Ge Healthcare Bio Sciences Ab	Semi-static cell culture
JP6924487B2	2015-06-10	2021-08-25	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-embedded virus delivery system and how to use it
WO2017007994A1 *	2015-07-08	2017-01-12	American Gene Technologies International Inc.	Hiv pre-immunization and immunotherapy

JP6780870B2	2015-08-13	2020-11-04	北昊干細胞与再生医学研究院有限公司Ｂｅｉｈａｏ ＳｔｅｍＣｅｌｌ Ａｎｄ ＲｅｇｅｎｅｒａｔｉｖｅＭｅｄｉｃｉｎｅ Ｒｅｓｅａｒｃｈ Ｉｎｓｔｉｔｕｔｅ Ｃｏ．， Ｌｔｄ．	Induced expanded pluripotent stem cells, how to make and use
CN105112370B	2015-08-25	2019-02-05	杭州优善生物科技有限公司	A kind of method and its application of stimulated in vitro peripheral blood gamma delta T cells high efficiently multiplying
JP7059179B2	2015-10-20	2022-04-25	アンスティチュ ナショナル ドゥ ラ サンテ エ ドゥ ラ ルシェルシュ メディカル	Methods and products for genetic engineering
US11389546B2	2015-12-09	2022-07-19	Modernatx, Inc.	Heterologous UTR sequences for enhanced mRNA expression
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
WO2017173453A1	2016-04-01	2017-10-05	The Brigham And Women's Hospital, Inc.	Stimuli-responsive nanoparticles for biomedical applications
JP7173548B2	2016-06-08	2022-11-16	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
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
WO2018025923A1	2016-08-03	2018-02-08	国立大学法人鹿児島大学	Anti-htlv-1 drug and therapeutic agent for htlv-1-associated myelopathy/tropical spastic paraparesis (ham/tsp)
KR20190100318A	2016-12-30	2019-08-28	더 트러스티스 오브 더 유니버시티 오브 펜실바니아	Gene therapy to treat phenylketonuria
EP3565564A4	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
CN110621322A	2017-02-08	2019-12-27	达纳-法伯癌症研究所有限公司	Modulatable endogenous protein degradation with heterobifunctional compounds
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
US20200181645A1	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells

CN111433368A	2017-10-02	2020-07-17	美国基因技术国际有限公司	Vector with promoter and enhancer combination for treating phenylketonuria
WO2020011247A1	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
KR20220068954A	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression

* Cited by examiner, † Cited by third party

Cited By (16)

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
CN112105731A	2018-03-30	2020-12-18	日内瓦大学	micro-RNA expression constructs and uses thereof

JPWO2020027094A1 *	2018-07-31	2021-09-16	サイアス株式会社	A method for producing a regenerated T cell population via iPS cells
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
US20220211845A1 *	2019-05-08	2022-07-07	The Wistar Institute Of Anatomy And Biology	Dna encoded il-36 gamma as an adjuvant
CN112048523A *	2019-06-05	2020-12-08	南京艾德免疫治疗研究院有限公司	Method for preparing high-titer lentiviral vector by conventional centrifugation
IL296096A *	2020-03-03	2022-11-01	American Gene Tech Int Inc	On demand expression of exogenous factors in lymphocytes to treat hiv

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

Similar Documents

Publication	Publication Date	Title
JP6971492B2	2021-11-24	HIV preimmunization and immunotherapy
US20210121561A1	2021-04-29	Methods of producing cells resistant to hiv infection
JP7260170B2	2023-04-18	HIV immunotherapy without prior immunization step
JP2021138721A	2021-09-16	Hiv pre-immunization and immunotherapy
JP2021519069A	2021-08-10	Method for producing genetically modified lymphocytes

Priority And Related Applications

Child Applications (1)

Application	Priority date	Filing date	Relation	Title
JP2021174409A	2016-07-08	2021-10-26	Division	Hiv pre-immunization and immunotherapy

Priority Applications (1)

Application	Priority date	Filing date	Title
JP2021174409A	2016-07-08	2021-10-26	Hiv pre-immunization and immunotherapy

Applications Claiming Priority (7)

Application	Filing date	Title
US201662360185P	2016-07-08	
US62/360,185	2016-07-08	
US201662385864P	2016-09-09	
US62/385,864	2016-09-09	
US201662409270P	2016-10-17	
US62/409,270	2016-10-17	
PCT/US2017/013019	2017-01-11	Hiv pre-immunization and immunotherapy

Legal Events

Date	Code	Title	Description
2020-01-07	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20200107
2020-03-25	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20200325
2020-03-25	A871	Explanation of circumstances concerning accelerated examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A871 **Effective date**: 20200325
2020-05-25	A975	Report on accelerated examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A971005 **Effective date**: 20200525
2020-06-12	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20200612
2020-09-14	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20200914
2020-09-30	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523

			Effective date: 20200930
2020-11-12	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20201112
2021-01-07	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20210107
2021-03-08	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20210308
2021-05-28	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20210528
2021-08-06	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20210806
2021-09-07	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20210907
2021-09-15	TRDD	Decision of grant or rejection written	
2021-09-27	A01	Written decision to grant a patent or to grant a registration (utility model)	**Free format text**: JAPANESE INTERMEDIATE CODE: A01 **Effective date**: 20210927
2021-10-28	A61	First payment of annual fees (during grant procedure)	**Free format text**: JAPANESE INTERMEDIATE CODE: A61 **Effective date**: 20211026
2021-11-05	R150	Certificate of patent or registration of utility model	**Ref document number**: 6971492 **Country of ref document**: JP **Free format text**: JAPANESE INTERMEDIATE CODE: R150
2023-06-30	R154	Certificate of patent or utility model (reissue)	**Free format text**: JAPANESE INTERMEDIATE CODE: R154

Concepts

machine-extracted

Download Filter table

Name	Image	Sections	Count	Query match
immunotherapy		title,description	12	0.000
Human immunodeficiency virus		claims,description	365	0.000
cell		claims,description	352	0.000
T-lymphocyte		claims,description	263	0.000
vector		claims,description	243	0.000
method		claims,description	136	0.000
MicroRNAs		claims,description	111	0.000
peripheral blood mononuclear cell		claims,description	102	0.000
microRNA		claims,description	100	0.000
particle		claims,description	72	0.000
Lentivirus		claims,description	69	0.000
mixture		claims,description	49	0.000
manufacturing process		claims,description	48	0.000
Virion infectivity factor		claims,description	40	0.000
packaging method and process		claims,description	38	0.000
inhibitory effect		claims,description	37	0.000
4-amino-1-[(2r)-6-amino-2-[[(2r)-2-[[(2r)-2-[[(2r)-2-amino-3-phenylpropanoyl]amino]-3-phenylpropanoyl]amino]-4-methylpentanoyl]amino]hexanoyl]piperidine-4-carboxylic acid		claims,description	35	0.000
CCR5		claims,description	29	0.000
targeting		claims,description	29	0.000
miRNA		claims,description	28	0.000
stimulant		claims,description	13	0.000
culturing		claims,description	11	0.000
Envelope protein		claims,description	10	0.000

Protein X	claims,description	10	0.000
transducing effect	claims,description	9	0.000
Syncytin-1	claims	6	0.000
primary T-cell	claims	3	0.000

Show all concepts from the description section

Data provided by IFI CLAIMS Patent Services