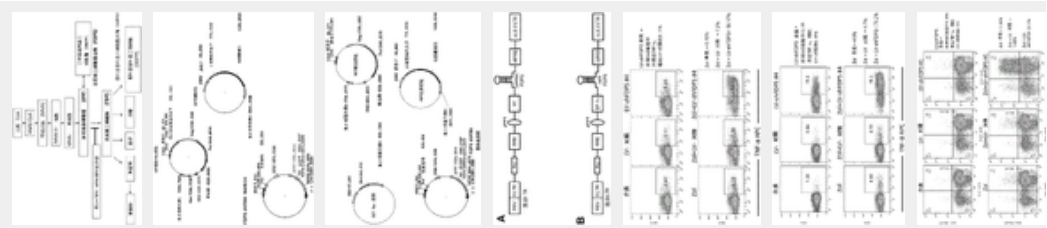
Methods and compositions for activating gamma-delta T cells

Abstract

The present invention relates generally to methods and compositions for gene therapy and immunotherapy for activating γ δ T cells, particularly useful for treating various cancers and infectious diseases.

Images (15)



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Current Assignee : American Gene Technologies International Inc

Worldwide applications

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

Application CN201780017712.6A events

2017-01-13 • Application filed by American Gene Technologies International Inc

2017-01-13 • Priority to CN202211395043.5A

2017-01-13 • Priority to CN202010396594.8A

2018-11-23 • Publication of CN108883100A

2022-11-25 • Application granted

2022-11-25 • Publication of CN108883100B

Status • Active

2037-01-13 • Anticipated expiration

Info: Patent citations (152), Non-patent citations (2), Cited by (14), Legal events, Similar documents, Priority and Related Applications

External links: Espacenet, Global Dossier, Discuss

Claims (32)

Hide Dependent

1. A viral vector for use in the treatment of a mevalonate pathway related disorder, said viral vector comprising:

a. at least one encoded shRNA capable of inhibiting the production of an enzyme of the mevalonate pathway, said enzyme being farnesyl diphosphate synthase (FDPS), the sequence of said shRNA being selected from the group consisting of:

i.GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT(SEQ ID NO:1);

ii.GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT(SEQ ID NO:2);

GCCATGTTACATGGCAAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTTT (SEQ ID NO: 3); or

GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTTGTTTT (SEQ ID NO: 4); or

b. At least one encoded microRNA capable of inhibiting the production of an enzyme of the mevalonate pathway which is farnesyl diphosphate synthase (FDPS), the sequence of said microRNA being selected from the group consisting of:

i.AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT(SEQ ID NO:5);

ii.AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT(SEQ ID NO:6);

iii.TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA(SEQ ID NO:7);

iv.CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA(SEQ ID NO:8);

<xnotran> v.CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); </xnotran> Or

vi.GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG(SEQ ID NO:10)。

2. The viral vector of claim 1, wherein the viral vector is a lentiviral vector.

3. The viral vector of any one of claims 1-2, wherein the viral vector is an adeno-associated viral vector.

4. A lentiviral particle produced by a packaging cell and capable of infecting a target cell, the lentiviral particle comprising: an envelope protein capable of infecting a target cell, and the lentiviral vector of claim 2.

5. The lentiviral particle of claim 4, wherein the envelope protein is targeted to an endocytic compartment of a target cell.

6. The lentiviral particle of claim 4, wherein the target cell is a cancer cell selected from one or more of the following: angiosarcoma, B-cell lymphoma, burkitt's lymphoma, breast cancer, bladder cancer, head and neck cancer, cervical cancer, colorectal cancer, endometrial cancer, ewing's sarcoma, fibrosarcoma, glioma, gastrinoma, gastric cancer, hepatocellular carcinoma, kaposi's sarcoma, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, medulloblastoma, mesothelioma, myxofibrosarcoma, multiple myeloma, high risk myelodysplastic syndrome, nasopharyngeal cancer, neuroblastoma, neurofibroma, lung cancer, ovarian cancer, osteosarcoma, pancreatic

cancer, pheochromocytoma, prostate cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, schwannoma, squamous cell carcinoma of the head and neck, testicular tumor, thyroid cancer, urothelial cancer, and wilm's tumor.

7. The lentiviral particle of claim 6, wherein the cancer cell is selected from one or more of: hodgkin lymphoma or non-hodgkin lymphoma.

8. The lentiviral particle of claim 6, wherein the cancer cell is selected from one or more of the following: astrocytoma, chronic lymphocytic leukemia, myeloid leukemia, acute lymphocytic leukemia, mucosa-associated lymphoid tissue B-cell lymphoma, anaplastic large cell lymphoma, mantle cell lymphoma, hepatoblastoma, laryngeal carcinoma, esophageal carcinoma, non-small cell lung carcinoma, and small cell lung carcinoma.

9. The lentiviral particle of claim 6, wherein the cancer cell is selected from one or more of the following: glioblastoma, acute myelogenous leukemia, chronic myelogenous leukemia, laryngeal squamous cell carcinoma, and esophageal squamous cell carcinoma.

10. The lentiviral particle of claim 4, wherein the target cell is one or more cancer cells present in hepatocellular carcinoma.

11. The lentiviral particle of claim 4, wherein the target cell is capable of activating γ δ T cells upon infection with the lentiviral particle.

12. A pharmaceutical composition for treating a mevalonate pathway related disorder, said pharmaceutical composition comprising: a lentiviral particle produced by a packaging cell and capable of infecting a target cell, the lentiviral particle comprising: an envelope protein capable of infecting a target cell, and the lentiviral vector of claim 2.

13. The pharmaceutical composition of claim 12, wherein the pharmaceutical composition is administered with an effective amount of an aminobisphosphonate.

14. The pharmaceutical composition of claim 13, wherein the aminobisphosphonate is zoledronic acid.

15. The pharmaceutical composition of claim 12, wherein the target cell is one or more cancer cells present in a cancer selected from one or more of the following: angiosarcoma, B-cell lymphoma, burkitt's lymphoma, breast cancer, bladder cancer, head and neck cancer, cervical cancer, colorectal cancer, endometrial cancer, ewing's sarcoma, fibrosarcoma, glioma, gastrinoma, gastric cancer, hepatocellular carcinoma, kaposi's sarcoma, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, medulloblastoma, mesothelioma, myxofibrosarcoma, multiple myeloma, high risk myelodysplastic syndrome, nasopharyngeal cancer, neuroblastoma, neurofibroma, lung cancer, ovarian cancer, osteosarcoma, pancreatic cancer, pheochromocytoma, prostate cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, schwannoma, squamous cell carcinoma of the head and neck, testicular tumor, thyroid cancer, urothelial cancer, and wilm's tumor.

16. The pharmaceutical composition of claim 15, wherein the cancer cell is selected from one or more of the following: hodgkin lymphoma or non-hodgkin lymphoma.

17. The pharmaceutical composition of claim 15, wherein the cancer cells are selected from one or more of the following: astrocytoma, chronic lymphocytic leukemia, myeloid leukemia, acute lymphocytic leukemia, mucosa-associated lymphoid tissue B-cell lymphoma, anaplastic large cell lymphoma, mantle cell lymphoma, hepatoblastoma, laryngeal carcinoma, esophageal carcinoma, non-small cell lung carcinoma, and small cell lung carcinoma.

18. The pharmaceutical composition of claim 15, wherein the cancer cell is selected from one or more of the following: glioblastoma, acute myelogenous leukemia, chronic myelogenous leukemia, laryngeal squamous cell carcinoma, and esophageal squamous cell carcinoma.

19. The pharmaceutical composition of claim 12, wherein the target cell is present in hepatocellular carcinoma.

20. The pharmaceutical composition of claim 12, wherein the target cell is capable of activating γ δ T cells following infection with the lentiviral particle.

21. Use of an immunotherapy-based composition in the manufacture of a medicament for treating cancer in a patient, wherein the immunotherapy-based composition comprises a lentiviral particle comprising:

a. envelope proteins capable of infecting cancer cells; and

b. the lentiviral vector of claim 2.

22. The use of claim 21, wherein the cancer is selected from one or more of the following: angiosarcoma, B-cell lymphoma, burkitt's lymphoma, breast cancer, bladder cancer, head and neck cancer, cervical cancer, colorectal cancer, endometrial cancer, ewing's sarcoma, fibrosarcoma, glioma, gastrinoma, gastric cancer, hepatocellular carcinoma, kaposi's sarcoma, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, medulloblastoma, mesothelioma, myxofibrosarcoma, multiple myeloma, high risk myelodysplastic syndrome, nasopharyngeal carcinoma, neuroblastoma, neurofibroma, lung cancer, ovarian cancer, osteosarcoma, pancreatic cancer, pheochromocytoma, prostate cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, schwann cell tumor, squamous cell carcinoma of the head and neck, testicular tumor, thyroid cancer, epithelial cancer, and wilm's tumor.

23. The use of claim 22, wherein the cancer cells are selected from one or more of the following: hodgkin lymphoma or non-hodgkin lymphoma.

24. The use of claim 22, wherein the cancer cells are selected from one or more of the following: astrocytoma, chronic lymphocytic leukemia, myeloid leukemia, acute lymphocytic leukemia, mucosa-associated lymphoid tissue B-cell lymphoma, anaplastic large cell lymphoma, mantle cell lymphoma, hepatoblastoma, laryngeal carcinoma, esophageal carcinoma, non-small cell lung carcinoma, and small cell lung carcinoma.

25. The use of claim 22, wherein the cancer cells are selected from one or more of the following: glioblastoma, acute myelogenous leukemia, chronic myelogenous leukemia, laryngeal squamous cell carcinoma, and esophageal squamous cell carcinoma.

26. The use of claim 21, wherein the cancer is leukemia.

27. The use of claim 21, wherein the cancer cells are capable of activating γ δ T cells in a subject following infection of the cancer cells with the immunotherapy-based composition.

28. The use of claim 27, wherein activating γ δ T cells comprises increasing Tumor Necrosis Factor (TNF) - α secretion by γ δ T cells.

29. The use of claim 21, wherein the immunotherapy-based composition is administered with an effective amount of an aminobisphosphonate.

30. The use of claim 29, wherein the aminobisphosphonate is zoledronic acid.

31. The use of claim 29, wherein the aminobisphosphonate is administered to the subject separately from the immunotherapy-based composition.

32. The use of claim 29, wherein the aminobisphosphonate is administered to a subject with the immunotherapy-based composition.

Description

Methods and compositions for activating gamma-delta T cells

Cross Reference to Related Applications

The present application claims priority from U.S. provisional patent application No. 62/279,474 entitled "methods and compositions for activating γ - δ T cells" filed on 2016, month 1, 15, which is incorporated herein by reference.

Technical Field

The present disclosure relates generally to the field of gene therapy and immunotherapy, and in particular to increased gamma delta ("GD") T cell activation.

Background

Human T cells are differentiated based on T cell receptor structure. The major population, including the CD4+ and CD8+ sub-populations, express receptors composed of alpha and beta chains. Smaller subsets express T cell receptors made by gamma and delta chains. γ δ ("GD") T cells constitute 3-10% of circulating lymphocytes, and the V δ 2+ subset constitutes 75% of GD T cells in blood. The V δ 2+ cells recognize non-peptide epitopes and do not require antigen presentation by major histocompatibility complex ("MHC") or human leukocyte antigens ("HLA"). Most of the V δ 2+ T cells also express the V γ 9 chain and are stimulated by exposure to 5-carbon pyrophosphate

compounds, which are intermediates in the mevalonate and non-mevalonate sterol/isoprenoid synthesis pathways. Reactions to isopentenyl pyrophosphate (5-carbon) are common in healthy humans.

Another subset of GD T cells, V δ 1+, accounts for a small percentage of T cells circulating in the blood, but V δ +1 cells are typically present in epithelial mucosa and skin.

In general, GD T cells have multiple functions, including killing tumor cells and pathogen infected cells. The ability to cytotoxicity, cytokine secretion and other effector functions is enhanced by unique T cell receptor ("TCR") stimulation consisting of two glycoprotein chains, γ and δ. The TCR of GD T cells has unique specificity and the cells themselves emerge with high cloning frequency, thus allowing a rapid innate response to tumors and pathogens.

Aminodiphosphonates ("ABP"), and other inhibitors of farnesyl diphosphate synthase ("FDPS"), located downstream of isopentenyl pyrophosphate ("1 PP") in the mevalonate pathway (see, e.g., figure 1), have been used to treat a variety of diseases, including cancer, particularly those involving bone metastases. ABP includes trade names, e.g.

(Novartis) and

(Merck).

ABP has also been used to stimulate GD T cells. This is probably because IPP begins to accumulate and geranylgeranyl pyrophosphate ("GGPP"), a downstream product of FDPS that inhibits inflammatory pathway activation, decreases when FDPS is inhibited in bone marrow cells. The reduction of GGPP removes inhibitors of the caspase-dependent inflammatory body pathway and allows secretion of mature cytokines including interleukin-beta and interleukin-18, the latter being particularly important for γ δ T cell activation.

Thus, when FDPS is blocked, increased IPP and decreased GGPP combine to activate V δ 2+ t cells. V δ 2+ cells activated by IPP or ABP will proliferate rapidly, express a variety of cytokines and chemokines, and can be used to destroy tumor cells or cells infected with pathogenic microorganisms in a cytotoxic manner.

However, ABP is associated with inflammation and osteonecrosis and has poor bioavailability due to their chemical nature. Also, IPP has a very short half-life and is difficult to synthesize. Both types of compounds require systemic administration in an individual. Thus, both ABP in general and IPP in particular leave many shortfalls for therapeutic purposes.

Disclosure of Invention

In one aspect, a method of activating GD T cells is provided. The method comprises infecting a target cell with a viral delivery system encoding at least one genetic element in the presence of GD T cells. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In an embodiment, the enzyme is FDPS. In embodiments, when the enzyme is inhibited in the target cell, the target cell subsequently activates GD T cells. In embodiments, the target cell is a cancer cell or a cell that has been infected with an infectious agent. In a preferred embodiment, activation of GD T cells results in GD T cells killing cancer cells or cells infected with an infectious agent. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate. In an embodiment, the aminobisphosphonate is zoledronic acid.

In another aspect, a method of treating cancer in a subject is provided. The method comprises administering to the subject a therapeutically effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, the cancer cell activates the GD T cell when the enzyme is inhibited in the cancer cell in the presence of the GD T cell, thereby treating the cancer. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate. In an embodiment, the aminobisphosphonate is zoledronic acid.

In another aspect, a method of treating an infectious disease in a subject is provided. The method comprises administering to the subject a therapeutically effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, when the enzyme is inhibited in a cell infected with an infectious agent in the presence of GD T cells, the infected cell activates the GD T cells, thereby treating the infected cell and the infectious disease. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate. In an embodiment, the aminobisphosphonate is zoledronic acid.

In another aspect, the at least one encoded genetic element comprises an shRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity to:

GTCCTGGAGTACAATGCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCGAGAAGTGCTGAACGAAATCCTGCTTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTGTTTTT (SEQ ID NO: 4). In a preferred embodiment, the shRNA comprises GTCCTGGAGTACAATGCCATTCGCCATTCTCGAGAATGCATGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCGAGAAGTGCTGAACGAAATCCTGCTTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTTTTT (SEQ ID NO: 4).

In another aspect, the at least one encoded genetic element comprises a microrna having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity to:

<xnotran> AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); </xnotran> <xnotran> AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGAC TTCAAGGGGCT (SEQ ID NO: 6); </xnotran> <xnotran> TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); </xnotran> <xnotran> CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA

(SEQ ID NO: 8); </xnotran> CATCTCCATGCTGTACCACTTGTCGGGACTTTCTCAGCCTCCTTCTGTCC
TGTTGAATCTCATGCAGAAGGAGGCGAGAAAGTCGAGAAGTCTGACATTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); <xnotran>
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG
(SEQ ID NO: 10). </xnotran> <xnotran> , RNA
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
(SEQ ID NO: 5); </xnotran> <xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
(SEQ ID NO: 6); </xnotran> <xnotran>
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); </xnotran>
<xnotran>
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA
(SEQ ID NO: 8); </xnotran> <xnotran>
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ
ID NO: 9); </xnotran> <xnotran>
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG
(SEQ ID NO: 10). </xnotran>

In another aspect, a viral vector comprising at least one encoded genetic element is provided. The at least one encoded genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme involved in the mevalonate pathway is farnesyl diphosphate synthase (FDPS). In embodiments, the at least one encoded genetic element comprises a microrna or shRNA.

In another aspect, the at least one encoded genetic element comprises an shRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity to a sequence described below. In a preferred embodiment, the shRNA comprises SEQ ID NO 1; 2, SEQ ID NO; 3, SEQ ID NO; or SEQ ID NO 4.

In another aspect, the at least one encoded genetic element comprises a microrna having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity to: 5, SEQ ID NO; 6, SEQ ID NO; 7 in SEQ ID NO; 8 in SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10. In a preferred embodiment, the microRNA comprises SEQ ID NO 5; 6, SEQ ID NO; 7 in SEQ ID NO; 8 in SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10.

In embodiments, the viral vector consists of any vector that is capable of efficiently transducing a small RNA into a target cell. In some embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated viral vector.

In another aspect, the viral vector includes a second encoded genetic element. In embodiments, the second genetic element comprises at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from: IL-18, TNF-alpha, interferon-gamma, IL-1, IL-2, IL-15, IL-17 and IL-12. In an embodiment, at least one chemokine is a CC chemokine or a CXC chemokine. In a further embodiment, the at least one chemokine is RANTES.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector for expressing at least one envelope plasmid of an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol and rev genes. When the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, lentiviral particles are produced by the packaging cell. In embodiments, the lentiviral particle is capable of infecting a target cell and inhibiting an enzyme involved in the mevalonate pathway in the target cell. In an embodiment, the enzyme involved in the mevalonate pathway is FDPS. In an embodiment, the lentiviral vector system comprises a first helper plasmid for expressing the gag and pol genes, and a second helper plasmid for expressing the rev gene. In embodiments, it is preferred that the envelope protein is optimized for infecting a target cell. In embodiments, the target cell is a cancer cell. In other embodiments, the target cell is a cell infected with an infectious agent.

Drawings

FIG. 1 depicts an overview of the major steps in the mevalonate pathway for the biosynthesis of steroids and isoprenoids.

FIG. 2 depicts an exemplary 3-vector lentiviral vector system in circularized form.

FIG. 3 depicts an exemplary 4-vector lentiviral vector system in circularized form.

FIG. 4 depicts: (A) A linear map of a lentiviral vector expressing an FDPS shRNA targeting sequence; and (B) a linear map of lentiviral vectors expressing synthetic micrornas with FDPS targeting sequences.

FIG. 5 depicts data demonstrating activation of V δ 2+ T cells with THP-1 leukemia cells with lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 6 depicts data demonstrating activation of V δ 2+ T cells with THP-1 leukemia cells with lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 7 depicts data demonstrating activation of V δ 2+ T cells with PC3 prostate cancer cells with lentivirus expressing FDPS shRNA #1 (SEQ ID NO: 1), as described herein.

FIG. 8 depicts data demonstrating activation of V δ 2+ T cells with PC3 prostate cancer cells with lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 9 depicts data demonstrating activation of V δ 2+ T cells with HepG2 cancer cells with lentiviruses expressing FDPS shRNA #1 (SEQ ID NO: 1) or FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 10 depicts data demonstrating activation of V δ 2+ T cells with THP-1 leukemia cells with lentivirus expressing miR30FDPS #1 (SEQ ID NO: 5), as described herein.

Figure 11 depicts data demonstrating the percentage of specific lysis versus the E: T ratio under various experimental conditions as described herein.

Figure 12 depicts data demonstrating that lentiviral delivered shRNA-based RNA interference targets the human FDPS gene.

Figure 13 depicts data demonstrating that lentiviral-delivered miR-based RNA interference targets the human FDPS gene.

FIG. 14 depicts data demonstrating activation of V δ 2+ T cells with HepG2 cancer cells with adeno-associated virus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 15 depicts immunoblot data demonstrating the lack of RAP1 prenylation (prenylation) in cells transduced with LV-shFDPS and treated with zoledronic acid.

Detailed Description

Summary of the disclosure

The present disclosure relates to gene therapy constructs and their delivery to cells resulting in the inhibition of farnesyl diphosphate synthase ("FDPS") that is required for the conversion of isopentenyl phosphate (IPP) to Farnesyl Diphosphate (FDP), such as that shown in fig. 1. In embodiments, one or more viral vectors are provided with micrornas or short homologous RNAs (shrnas) targeting FDPS, thereby reducing the expression level of the enzyme. Viral vectors include lentiviral vectors and AAV vectors. The result of modulating FDPS expression is an increase in the accumulation of IPP, a stimulator of GD T cell proliferation and differentiation. Thus, the constructs provided herein are useful for activating GD T cells, and for treating cancer and infectious diseases.

Definition and interpretation

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Furthermore, unless otherwise indicated, singular terms include the plural and plural terms include the singular. Generally, the nomenclature and techniques used in cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. Unless otherwise indicated, the methods and techniques of the present invention are generally performed following conventional methods well known in the art and as described in several general or more specific references that are cited and discussed throughout the present specification. See, e.g., sambrook J. And Russell D. [molecular cloning: a Laboratory Manual, third edition, cold Spring Harbor Laboratory Press (Cold Spring Harbor Laboratory Press, cold Spring Harbor, N.Y.), (2000); ausubel et al, protocol for molecular biology, eds: outline of Methods for Current Protocols in Molecular Biology (Short Protocols in Molecular Biology: A Complex of Methods from Current Protocols in Molecular Biology), wiley, john & Sons publishing company (2002); harlow and Lane, using antibodies: a Laboratory Manual (use Antibodies: A Laboratory Manual); cold spring harbor laboratory Press of cold spring harbor, N.Y. (1998); and Coligan et al,

short Protocols in Protein Science, wiley, john & Sons publishing company (2003). Any enzymatic reactions and purification techniques are performed according to the manufacturer's instructions or methods commonly used in the art or described herein. Nomenclature used in analytical chemistry, synthetic organic chemistry, and medical and pharmaceutical chemistry, and laboratory methods and techniques described herein are those well known and commonly used in the art.

As used in the specification and the appended claims, the singular forms "a", "an", and "the" are used interchangeably and are intended to include the plural forms and fall within the meaning unless the context clearly indicates otherwise. As used herein, "and/or" is meant to encompass any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative ("or").

All numerical labels, such as pH, temperature, time, concentration and molecular weight, including ranges, are approximate values, which vary (+) or (-), in increments of 0.1. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". The term "about" includes the exact value of "X" in addition to small increments of "X" (e.g., "X +0.1" or "X-0.1"). It is also to be understood that, although not always explicitly stated, the reagents described herein are exemplary only and that equivalents thereof are known in the art.

As used herein, the term "about" will be understood by one of ordinary skill in the art and will vary to some extent depending on the context in which it is used. If the term is not clear to one of ordinary skill in the art in the context in which the term is used, "about" would mean up to plus or minus 10% of the particular term.

The term "administering" or "administering" an active agent is understood to mean providing the active agent to a subject in need of treatment in a form that can be introduced into the subject in a therapeutically useful form and in a therapeutically effective amount.

As used herein, the term "comprising" or "comprises" is intended to mean that the compositions and methods include the elements mentioned, but not to exclude other elements. When used to define compositions and methods, "consisting essentially of 8230 \8230: \8230;" consisting of "shall mean to exclude other elements having any essential significance to the compositions and methods. "consisting of 8230; \8230composition" means that trace elements and essential process steps are excluded in excess of other ingredients of the claimed composition. Embodiments defined by each of these converted terms are within the scope of the present invention. It is therefore intended that the methods and compositions may include additional steps and components (including), or alternatively, non-critical steps and compositions (consisting essentially of), or only the method steps or compositions (consisting of).

As used herein, "expression," "expression," or "encoding" refers to the process by which a polynucleotide is transcribed into mRNA and/or the process by which transcribed mRNA is subsequently translated into a peptide, polypeptide, or protein. Expression may include splicing of mRNA or other forms of post-transcriptional or post-translational modification in eukaryotic cells.

The term "farnesyl diphosphate synthase" may also be referred to herein as FDPS, and may also be referred to herein as farnesyl pyrophosphate synthase or FPPS.

The term "γ δ T cells" may also be referred to herein as γ δ T cells, or further GD T cells. The term "γ δ T cell activation" or "γ δ T cell activation" refers to any measurable biological phenomenon associated with γ δ T cells and representative of such T cells being activated. Non-limiting examples of such biological phenomena include an increase in cytokine production, a change in the qualitative or quantitative composition of a cell surface protein, an increase in T cell proliferation and/or an increase in T cell effector function, such as killing a target cell or assisting another effector cell in killing a target cell.

The terms "individual," "subject," and "patient" are used interchangeably herein and refer to any individual mammalian subject, e.g., bovine, canine, feline, equine, or human.

The term "miRNA" refers to microrna, and may also be referred to herein as "miR.

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

In the context of two or more nucleic acid or polypeptide sequences, the term "percent identity" refers to two or more sequences or subsequences that have a specified percentage of nucleotide or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to those skilled in the art), or by visual inspection. Depending on the application, "percent identity" may exist over the regions of the sequences being compared, e.g., over the functional domains, or over the entire length of the two sequences being compared. For sequence comparison, one sequence is typically used as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm

program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity of the test sequence relative to the reference sequence based on the specified program parameters.

Optimal sequence alignments can be made for comparison, for example, by the local homology algorithm of Smith and Waterman, adv.appl.math.2:482 (1981); homology alignment by Needleman and Wunsch, J.mol.biol.48:443 (1970); similarity search by Pearson and Lipman, proc.nat' l.acad.sci.usa 85 (1988); the algorithms (GAP, BESTFIT, FASTA and TFASTA in Wisconsin Genetics Software Package (Wisconsin Genetics Software Package) of the Genetics computing Group (Genetics Computer Group), the scientific Dau (Science Dr.), madison, wis.) are executed by Computer or by visual inspection.

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

Percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available from http:// www. GCG. Com), using NWSgapdna. CMP matrices and GAP weights 40, 50, 60, 70 or 80, length weights 1,2,3,4, 5 or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of e.meyers and w.miller (cabaos, 4-11-17 (1989)), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J.mol.biol. (48): 444-453 (1970)) algorithm, which has been incorporated into the GAP program in the GCG software package (available from http:// www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, with GAP weights of 16, 14, 12, 10, 8, 6, or 4, and length weights of 1,2,3,4, 5, or 6.

Nucleic acid and protein sequences of the present disclosure can also be used as "query sequences" to search public databases, for example, to identify related sequences. Such searches can be run using the NBLAST and XBLAST programs (version 2.0) of Altschul et al, 1990, J.mol.biol., 215. A BLAST nucleotide search (score =100, word length = 12) can be performed using the NBLAST program to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches (score =50, word length = 3) can be performed using the XBLAST program to obtain amino acid sequences homologous to the protein molecules of the invention. To obtain gapped alignments for comparison purposes, gapped BLAST can be utilized as described in Altschul et al, 1997, nucleic Acids Res.,25 (17): 3389-3402. When utilizing BLAST and gapped BLAST programs, the default parameters for each program (e.g., XBLAST and NBLAST) can be used. See http:// www.ncbi.nlm.nih.gov.

The term "pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

As used herein, "pharmaceutically acceptable carrier" means and includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, which are physiologically compatible. The compositions can include pharmaceutically acceptable salts, such as acid addition salts or base addition salts (see, e.g., berge et al (1977) J Pharm Sci 66.

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, which is typically less than about 200 nucleotides or less in length and has silencing or interfering functions. In other embodiments, the small RNA is about 175 nucleotides or less in length, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less. Such RNAs include micrornas (mirnas), small interfering RNAs (sirnas), double-stranded RNAs (dsrnas), and short hairpin RNAs (shrnas). The "small RNA" of the present disclosure should be capable of inhibiting or knocking down gene expression of a target gene, typically through a pathway that results in the destruction of the target gene mRNA.

The term "therapeutically effective amount" refers to a sufficient amount of an active agent of the present disclosure, in a suitable composition and in a suitable dosage form, to treat or prevent the symptoms, progression, or complication of onset of a given ailment, injury, disease, or condition in a patient. The "therapeutically effective amount" depends on the condition of the patient or its severity, the age, weight, etc. of the subject being treated. The therapeutically effective amount may vary depending on any of a number of factors, including, for example, the route of administration, the condition of the subject, and other factors understood by those skilled in the art.

As used herein, the term "therapeutic vector" includes, but is not limited to, a lentiviral vector or an AAV vector.

"treating" is intended to target and combat a disease state, i.e., to ameliorate or prevent a disease state. Thus, the particular treatment will depend on the disease state to be targeted and the current or future state of the drug treatment and therapeutic method. Treatment may have associated toxicity.

The term "treatment" or "treating" generally refers to an intervention that attempts to alter the natural course of the subject being treated, and may be used prophylactically or during clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of a disease, alleviating symptoms, suppressing, reducing or inhibiting any direct or indirect pathological consequences of a disease, ameliorating or alleviating a disease state, and causing remission or improving prognosis.

Description of the disclosed aspects

In one aspect, a method of activating GD T cells is provided. The method comprises infecting a target cell with a viral delivery system encoding at least one genetic element in the presence of GD T cells. In embodiments, the at least one encoded genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In an embodiment, the enzyme is FDPS. In embodiments, the GD T cell is activated by the target cell when the enzyme is inhibited in the target cell. In embodiments, the target cell is a cancer cell or a cell that has been infected with an infectious agent. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA.

In embodiments, the at least one encoded genetic element comprises an shRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more percent identity to:

GTCCTGGAGTACAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCGAGAAGTGCTGAACGAAATCCTGCTTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTTTTT (SEQ ID NO: 4).

In a preferred embodiment, the shRNA comprises

GTCCTGGAGTACAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCGAGAAGTGCTGAACGAAATCCTGCTTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTGTTTTT (SEQ ID NO: 4).

In another aspect, the at least one encoded genetic element comprises a microrna having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more percent identity to:

<xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); </xnotran> <xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); </xnotran> <xnotran>
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); </xnotran>
<xnotran>
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); </xnotran> Cattccattggctgtaccaccttgtcgggactttctcaggcctccttctgcc tgttgaatctcatggcagaagggcgagaaagtctgacatttggtatctttcattgacca (SEQ ID NO: 9);
<xnotran>
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). </xnotran>

In a preferred embodiment, the microRNA comprises

<xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); </xnotran> <xnotran>

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); </xnotran> <xnotran> TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); </xnotran> <xnotran> CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); </xnotran> Cattccattggctgtaccaccttgtcgggactttctcaggcctccttctgcctg ttgaatctcatggcagaagggcgagaaagtctgacatttggtatctttcattgacca (SEQ ID NO: 9); <xnotran> GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). </xnotran>

In another aspect, the target cell is also contacted with an aminobisphosphonate. In a preferred embodiment, the aminobisphosphonate is zoledronic acid.

In another aspect, a method of treating cancer in a subject is provided. The method comprises administering to the subject a therapeutically effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, the cancer cell activates the GD T cell when the enzyme is inhibited in the cancer cell in the presence of the GD T cell, thereby treating the cancer. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA.

In another aspect, a method of treating an infectious disease in a subject is provided. The method comprises administering to the subject a therapeutically effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, when the enzyme is inhibited in the presence of GD T cells and in cells infected with an infectious agent, the infected cells activate GD T cells, thereby treating the infected cells and the infectious disease. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises a microrna or shRNA.

In embodiments, the at least one encoded genetic element comprises a nucleotide sequence identical to SEQ ID No. 1; 2, SEQ ID NO; 3, SEQ ID NO; or SEQ ID NO. 4, shRNA having a percent identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more. In a preferred embodiment, the shRNA comprises SEQ ID NO:1; the amino acid sequence of SEQ ID NO:2; the amino acid sequence of SEQ ID NO:3; or SEQ ID NO:4.

in other embodiments, the at least one encoded genetic element comprises a microrna having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more percent identity to: 5, SEQ ID NO; 6 is SEQ ID NO; 7 in SEQ ID NO; 8 in SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10. In a preferred embodiment, the microRNA comprises SEQ ID NO 5; 6 is SEQ ID NO; 7, SEQ ID NO; 8 is SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10.

In another aspect, a viral vector comprising at least one encoded genetic element is provided. The at least one encoded genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme involved in the mevalonate pathway is farnesyl diphosphate synthase (FDPS). In embodiments, the at least one encoded genetic element comprises a microrna or shRNA.

In another aspect, the at least one encoded genetic element comprises a nucleotide sequence substantially identical to SEQ ID No. 1; 2, SEQ ID NO; 3, SEQ ID NO; or SEQ ID NO. 4, shRNA having a percent identity of at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more. In a preferred embodiment, the shRNA comprises SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3; or SEQ ID NO:4.

in another aspect, the at least one encoded genetic element comprises a microrna having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, or more percent identity to: 5, SEQ ID NO; 6, SEQ ID NO; 7 in SEQ ID NO; 8 is SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10. In a preferred embodiment, the microRNA comprises SEQ ID NO 5; 6, SEQ ID NO; 7, SEQ ID NO; 8 in SEQ ID NO; 9, SEQ ID NO; or SEQ ID NO 10.

In embodiments, the viral vector includes any vector that can efficiently transduce small RNAs. In some embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated virus (AAV) vector.

In another aspect, the viral vector comprises a second encoded genetic element. In embodiments, the second genetic element comprises at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from: IL-18, TNF-alpha, interferon-gamma, IL-1, IL-2, IL-15, IL-17 and IL-12. In an embodiment, at least one chemokine is a CC chemokine, a CXC chemokine, a CX3 chemokine, or an XC chemokine. In a further embodiment, at least one chemokine is CC chemokine RANTES.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector for expressing at least one envelope plasmid of an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol and rev genes. When the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, lentiviral particles are produced by the packaging cell. In embodiments, the lentiviral particle is capable of infecting a target cell and inhibiting an enzyme involved in the mevalonate pathway in the target cell. In an embodiment, the enzyme involved in the mevalonate pathway is FDPS. In an embodiment, the lentiviral vector system comprises a first helper plasmid for expressing the gag and pol genes, and a second helper plasmid for expressing the rev gene. In embodiments, it is preferred that the envelope protein is optimized for infecting a target cell. In embodiments, the target cell is a cancer cell. In other embodiments, the target cell is a cell infected with an infectious disease.

Cancer treatment

The compositions and methods provided herein are useful for treating cancer. The cell, tissue or target may be a cancerous cell, a cancerous tissue, or a subject or patient diagnosed or at risk of developing a disease or condition. In certain aspects, the cell may be an epithelial cell, an endothelial cell, a mesothelial cell, a glial cell, a stromal cell or a mucosal cell. The cancer cell population can include, but is not limited to, brain, neuronal, blood, endometrial, meninges, esophageal, lung, cardiovascular, liver, lymph, breast, bone, connective tissue, fat, retina, thyroid, gland, adrenal, pancreas, stomach, intestine, kidney, bladder, colon, prostate, uterus, ovary, cervix, testis, spleen, skin, smooth muscle, cardiac muscle, or striated muscle cells. In another aspect, the cancer includes, but is not limited to, astrocytoma, acute myelogenous leukemia, anaplastic large cell lymphoma, acute lymphocytic leukemia, angiosarcoma, B-cell lymphoma, burkitt's lymphoma, breast cancer, bladder cancer, head and neck cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal cancer, endometrial cancer, esophageal squamous cell carcinoma, ewing's sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, gastric cancer, hepatoblastoma, hepatocellular carcinoma, kaposi's sarcoma, hodgkin's lymphoma, laryngeal squamous cell carcinoma, laryngeal cancer, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, mantle cell lymphoma, medulloblastoma, mesothelioma, myxofibrosarcoma, myeloid leukemia, mucosa-associated lymphoid tissue B-cell lymphoma, multiple myeloma, high risk myelodysplastic syndrome, nasopharyngeal carcinoma, neuroblastoma, neurofibroma, high-grade non-hodgkin's lymphoma, lung cancer, non-small cell lung cancer, ovarian cancer, esophageal cancer, osteosarcoma, pancreatic cancer, pheochromocytoma, prostate cancer, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, schwann cell tumor, small cell lung cancer, head and neck squamous cell carcinoma, testicular tumor, thyroid cancer, urothelial cancer, and wilm's tumor.

The compositions and methods provided herein are also useful for treating NSCLC (non-small cell lung cancer), pediatric malignancies, cervical and other tumors caused or facilitated by Human Papillomavirus (HPV), melanoma, barrett's esophagus (premalignant syndrome), adrenal and skin cancers, and autoimmune, neoplastic skin diseases.

Infectious diseases

The compositions and methods disclosed herein are useful for treating infectious diseases. The term "infectious disease" includes any disease caused by an infectious agent. "infectious agent" includes any exogenous pathogen, including but not limited to bacteria, fungi, viruses, mycoplasma and parasites. Infectious agents that can be treated with the compositions provided by the present disclosure include any art-recognized infectious organism that causes disease in an animal, including organisms such as bacteria of gram-negative or gram-positive cocci or bacilli, DNA and RNA viruses, including but not limited to DNA viruses such as papilloma viruses, parvoviruses, adenoviruses, herpes viruses, and vaccinia viruses, and RNA viruses such as arenaviruses, coronaviruses, rhinoviruses, respiratory syncytial viruses, influenza viruses, picornaviruses, paramyxoviruses, reoviruses, retroviruses, and rhabdoviruses. Examples of fungi that can be treated with the compositions and methods of the present disclosure include fungi that are mold or yeast-like growths, including, for example, fungi that cause diseases such as tinea, histoplasmosis, blastomycosis, aspergillosis, cryptococcosis, sporotrichosis, coccidioidomycosis, paracoccidioidomycosis, and candidiasis. The compositions and methods provided herein are useful for treating parasitic infections, including but not limited to infections caused by somatic tapeworms, blood flukes, tissue roundworms, amoeba and plasmodium, trypanosomes, leishmania and toxoplasma species.

Method for activation of GD T cells

Provided herein are compositions and methods for activating GD T cells in an individual, and methods for treating tumors and infectious diseases. For example, in embodiments, the compositions and methods provided herein can be used in methods of treating all known cancers, as activated GD T cells contain natural mechanisms for tumor immune surveillance (see, e.g., pauza et al 2014 Frontiers in immunol.5. Also, in embodiments, the compositions and methods provided herein are useful for treating infectious diseases, including but not limited to flavivirus, influenza virus, human retrovirus, mycobacteria, plasmodium and various other viral, fungal and bacterial infections. (see, e.g., pauza and Cairo,2015 Cell Immunol.296 (1).

Typically, the vector system is administered to an individual to transfect or transduce a target cell population with the disclosed constructs to decrease expression of FDPS, and in other embodiments, to increase expression of chemokines or cytokines. Administration and transfection/transduction may occur in vivo or ex vivo, with the transfected cells subsequently administered back to the subject in the latter case.

Administration of the disclosed vectors and transfection or transduction of the disclosed constructs into cells of a subject results in decreased expression of FDPS, increased expression of cytokines or chemokines, accumulation of IPP, and in many cases, a decrease in the growth rate of genetically modified tumor cells. All of these features work together to activate and co-localize GD T cells to the tumor or site of infection.

The disclosed methods may also increase the ability of NK cells to recognize and destroy tumor cells and/or infected cells. Crosstalk (crosstalk) between GD T cells and NK cells is an important aspect of the regulation of immune and inflammatory responses. In addition, GD T cells are known to initiate dendritic cell maturation, recruit B cells and macrophages, and participate in various cytolytic activities, such as secretion of interferon-gamma and TNF-alpha.

In embodiments, the disclosed compositions and methods provided herein include forms of gene therapy for activating GD T cells at the pathological site of a tumor or infectious disease. In one aspect, the compositions and methods provided herein activate GD T cells and support their proliferative, differentiation, and functional capacity by promoting the production of specific cytokines required for cytolytic activity that can kill cancer cells or treat infectious diseases.

In embodiments, the gene therapy sequence (e.g., FDPS shRNA) is carried by a therapeutic vector, including but not limited to a viral vector, such as a lentivirus or adeno-associated virus, although other viral vectors may also be suitable. Gene therapy constructs may also be delivered in the form of DNA or RNA, including but not limited to plasmid forms. In embodiments, the disclosed gene therapy constructs may also be delivered in the form of protein-nucleic acid complexes or lipid-nucleic acid complexes and mixtures of these agents. For example, the protein-nucleic acid complex may comprise the nucleic acid of interest in a complex with a cationic peptide such as lysine and arginine. The lipid-nucleic acid complex may comprise lipid emulsions, micelles, liposomes and/or mixtures of neutral and cationic lipids, such as DOTMA, DOSPA, DOTAP and DMRIE.

In embodiments, the therapeutic vector may comprise a single construct or at least two, at least three, at least four, or at least five different constructs. When more than one construct is present in the vector, the constructs may be identical, or they may be different. For example, the constructs may differ in their promoters, the presence or absence of integrational elements, and/or their sequences. In some embodiments, the therapeutic vector will comprise at least one construct encoding a small RNA capable of knocking down FDPS expression. In embodiments, the therapeutic vector will also encode specific cytokines and/or chemokines, including but not limited to TNF- α, interferon- γ, IL-1, IL-2, IL-15, IL-17, IL-18, or IL-12. In some embodiments, a single construct can encode a small RNA capable of knocking down the expression of FDPS and specific cytokines or chemokines, including but not limited to TNF- α, interferon- γ, IL-1, IL-2, IL-15, IL-17, IL-18, or IL-12.

In embodiments, the viral vector may introduce a nucleic acid construct that integrates into the host chromosome. Alternatively, transient delivery vectors can be used to prevent chromosomal integration and limit the longevity of gene therapy constructs.

In embodiments, the disclosed constructs and vectors comprise short homologous region RNAs ("shrnas"), micrornas ("mirnas"), or sirnas capable of reducing or knocking-down expression of FDPS and/or geranyl pyrophosphate synthase ("GPPS") and/or farnesyl transferase ("FT") genes. By down-regulating these genes that control steroid and isoprenoid synthesis, levels of isopentenyl pyrophosphate ("IPP") are increased. Elevation and accumulation of IPP is a known mechanism to increase GD T cell activation. Furthermore, down-regulation of these pyrophosphate synthase genes eliminates important negative regulators of inflammatory body function, which in turn leads to increased expression of cytokines important for GD T cell activation and effector cell function.

In embodiments, the disclosed constructs are regulated by specific promoters capable of producing interleukin-2 and/or interleukin-15 to maintain GD T cell proliferation. In addition, the disclosed constructs can be regulated by specific promoters, capable of producing interleukin-1 β and/or interleukin-18 and/or interferon- γ, required for

GD T cell differentiation and for obtaining all effector cell functions. Desirable effector cell functions include the ability to direct cytotoxic cell killing of tumors and/or infected cells, the ability to secrete beneficial cytokines and/or chemokines, the ability to increase expression of NK receptors required to recognize cancer or infected cells, and the ability to increase expression of Fc receptors required for binding of targeting antibodies to co-localize GD T cells with cancer or infected cell targets.

In embodiments, the disclosed methods activate GD T cells, resulting in an indirect effect of increased ability of NK cells to attack and destroy cancer, tumor, or infected cells. Activation of NK cells requires stimulation of GD T cell proliferation and differentiation, and expression of a 4-1BBL co-stimulatory ligand, which is required for coordination with the 4-1BB co-stimulatory receptor on NK cells. This form of crosstalk is believed to be an important mechanism for activating NK cells and is achieved herein through the action of the disclosed methods and compositions.

On the other hand, cross-talk between GD T cells and NK cells is an important mechanism to eliminate inflammatory dendritic cells accumulated in diseased tissues. Separately, neither GD T cells nor NK cells can destroy dendritic cells, but once the above cross-talk interaction occurs, NK cells are altered to produce cytotoxicity to inflammatory dendritic cells. This immune-regulatory mechanism depends on the strong activation and proliferation of GD T cells.

In embodiments, the disclosed methods for activating GD T cells further comprise the step of inhibiting pathological inflammatory responses, which may include cell proliferation leading to atherosclerosis, chronic immune activation that stimulates tumor growth, autoimmune diseases, including psoriasis and other manifestations in the epidermis, inflammatory diseases of the central nervous system, arthritis, and other diseases of unregulated immune response.

In embodiments, the therapeutic carrier is administered concurrently with an aminobisphosphonate drug (ABP) drug to achieve synergistic activation of γ δ T cells. Synergy may allow alternating, varying or reduced doses of ABP, and may reduce adverse reactions to ABP, including acute inflammatory reactions and chronic diseases.

Constructs for GD T cell activation

Inhibition of FDPS leads to IPP accumulation, leading to activation of V δ 2+ GD T cells and expression of IL-18, which is also important in activating GD T cells. Inhibition of farnesyl transferase results in a reduction in prenylation of the protein. The disclosed constructs can be transfected or transduced into specific target cells, such as tumor cells or infected cells, where they can express RNA sequences (i.e., siRNA, shRNA or microRNA) that will inhibit translation of FDPS and encode and express cytotoxic cytokines or chemokines.

Disclosed herein are constructs for reducing expression of FDPS and/or FT, increasing expression of cytokines, and increasing expression of chemokines, including RANTES. For example, in some embodiments, the construct can encode interferon-gamma, IL-1, IL-2, IL-15, IL-17, IL-18, or IL-12.

The expression of cytokines and chemokines, such as those listed above, will result in the local cytotoxic destruction of tumor cells or cells infected with pathogenic organisms. Thus, expression of these constructs by tumor cells or infected cells will produce unwanted cells that assist in their own destruction.

Likewise, if the disclosed constructs are expressed in tumor cells or infected cells, decreasing the expression of FDPS and FT will result in activation and recruitment of GD T cells to the tumor site or site of cell infection. Increasing the expression of RANTES will further attract GD T cells to the expected tissue location. Because GD T cells can kill a wide range of tumors of epithelial origin as well as many leukemias and lymphomas, and are also capable of producing high levels of the anti-tumor cytokine IFN γ, recruitment of GD T cells to the tumor site can be a particularly effective means of inducing anti-tumor immunity.

The reduced expression of FDPS can be achieved by shRNA, microrna, siRNA or other methods known in the art. For example, according to SEQ ID NO: 1. 2,3 or 4, or variants thereof, can be used in the disclosed constructs and methods, this example is not limiting. The coding regions for RNA for reducing expression of FDPS and FT, and the coding regions for cytokines and chemokines may be in the same construct or on different constructs.

A classical approach for the production of recombinant polypeptides or gene regulatory molecules comprising small RNAs is the use of stable expression constructs. These constructs are based on chromosomal integration of a transduced expression plasmid (or at least a portion thereof) into the genome of a host cell, short-term plasmid transfection or a non-integrating viral vector with a limited half-life. The sites of gene integration are generally random, and the number and proportion of genes integrated at any particular site is generally unpredictable; similarly, non-integrating plasmids or viral vectors also produce nuclear DNA, but these materials often lack sequences required for DNA replication and continuous maintenance. Thus, constructs relying on chromosomal integration result in permanent maintenance of the recombinant gene, which may exceed the treatment interval.

An alternative to stable expression constructs for gene expression is transient expression constructs. Expression of the latter gene expression constructs is based on non-integrated plasmids, and thus expression is often lost when cells undergo division or the plasmid vector is disrupted by endogenous nucleases.

The disclosed constructs are preferably episomal constructs that are transiently expressed. Episomal constructs degrade or dilute over time such that they do not permanently alter the genome of the subject nor integrate them into the chromosome of the target cell. Episomal replication processes typically involve host cell replication machinery and viral trans-acting factors.

Avoiding chromosomal integration reduces certain barriers to gene delivery in vivo. However, even integration-defective constructs can have background frequency of integration, and any DNA molecule can find rare homologies for recombination with host sequences; however, these integration rates are very rare and often of no clinical significance.

Thus, in some embodiments, the disclosed vectors support active gene and/or small RNA delivery over a period of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, or about 12 weeks. In some embodiments, the disclosed vectors support active gene and/or small RNA delivery over a period of about 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months or more. Any combination of these time periods may also be used in the methods of the invention, for example 1 month and 1 week, or 3 months and 2 weeks.

However, in some embodiments, the construct comprises an integration element dependent on the retroviral integrase gene such that the construct is integrated into the chromosome of the subject. Reverse transcription and transposition are additional examples of mechanisms by which mobile genetic elements integrate or insert into chromosomes. Plasmids can integrate into chromosomes by recombination, and gene editing techniques (including CRISPR and TALENs) utilize guide RNA sequences and alter chromosomal loci through gene conversion mechanisms.

The construct may contain specific promoters for expression of cytokines involved in maintaining GD T cells (i.e., IL-2, IL-7, IL-17, and IL-15). For example, promoters that may be incorporated into the disclosed constructs include, but are not limited to, TATA-box promoters, cpG-box promoters, CCAAT-box promoters, TTGACA-box promoters, BRE-box promoters, INR-box promoters, AT-based promoters, CG-based promoters, ATCG-compact promoters, ATCG-balanced promoters, medium ATCG-promoters, low AT promoters, low CG promoters, AT-spike promoters, and CG-spike promoters. Referring to Gagniuc and Ionescu-Tirgovist, eukaryotic genomes can display up to 10 general classes of gene promoters (Eukaryotic genes mass exclusion up to 10 genetic classes of gene promoters), BMC GENOMICS 13 (2012).

Therapeutic vectors

The constructs may be delivered by known transfection and/or transduction vectors, including but not limited to lentiviral vectors, adeno-associated viruses, poxviruses, herpesvirus vectors, protein and/or lipid complexes, liposomes, micelles, and the like.

The viral vector can preferentially target the cell type (i.e., tumor cell or bone marrow cell) that can be used in the disclosed methods. Due to the specific viral envelope-host cell receptor interactions and the viral mechanisms of gene expression, viral vectors can be used to transduce genes into target cells. As a result, viral vectors have been used as vehicles for gene transfer into many different cell types, including whole embryos, fertilized eggs, isolated tissue samples, in situ tissue targets, and cultured cell lines. The ability to introduce and express foreign genes in cells can be used for studies of gene expression, elucidation of cell lines and the potential to provide therapeutic intervention, such as gene therapy, induction of somatic reprogramming of pluripotent stem cells, and various types of immunotherapy. Viral components from viruses such as papovaviridae (e.g., bovine papilloma virus or BPV) or herpesviridae (e.g., epstein Barr virus or EBV) or hepadnaviridae (e.g., hepatitis b virus or HBV) or poxvirus vectors including vaccinia can be used in the disclosed vectors.

Lentiviral vectors are a preferred type of vector for the disclosed compositions and methods, but the disclosure is not particularly limited to lentiviral vectors. Lentiviruses are a class of viruses that can deliver large amounts of viral nucleic acid into a host cell. Lentiviruses are characterized by their unique ability to infect/transduce non-dividing cells, and upon transduction, lentiviruses integrate their nucleic acids into the chromosome of the host cell.

Infectious lentiviruses have three major genes, gag, pol, and env, which encode virulence proteins, and two regulatory genes, including tat and rev. Depending on the particular serotype and virus, there may be additional helper genes encoding proteins involved in regulating, synthesizing and/or processing viral nucleic acid and other replication functions.

In addition, lentiviruses contain a Long Terminal Repeat (LTR) region, which can be about 600nt long. The LTR may be segmented into U3, R and U5 regions. The LTRs can mediate retroviral DNA integration into the host chromosome through the action of integrases. Alternatively, the LTR may be used to circularize viral nucleic acid in the

absence of a functional integrase.

Viral proteins involved in the early stages of lentivirus replication include reverse transcriptase and integrase. Reverse transcriptase is a virally encoded RNA-dependent DNA polymerase. The enzyme uses the viral RNA genome as a template for the synthesis of complementary DNA copies. Reverse transcriptase also has rnase H activity to destroy the RNA template. Integrase binds host DNA and viral cDNA produced by reverse transcriptase. Integrase the LTR is treated prior to insertion of the viral genome into the host DNA. Tat acts as a transactivator during transcription to enhance initiation and extension. The rev response element functions post-transcriptionally, regulating mRNA splicing and transport to the cytoplasm.

Typically, viral vectors comprise glycoproteins, and various glycoproteins can provide specific affinities. For example, VSVG peptides can increase transfection into bone marrow cells. Alternatively, the viral vector may also have a targeting moiety, such as an antibody, attached to its capsid peptide. The targeting antibody may be specific for an antigen that is overexpressed on tumors, such as HER-2, PSA, CEA, M2-PK, and CA19-9.

Other viral vector specificities are also known in the art and can be used to target specific cell populations. For example, poxvirus vectors target macrophages and dendritic cells.

Lentiviral vector system

Lentivirus virions (particles) are expressed from a vector system that encodes essential viral proteins to produce virions (viral particles). There is at least one vector comprising a nucleic acid sequence encoding a lentiviral pol protein necessary for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol protein is expressed from multiple vectors. Vectors also exist that contain a nucleic acid sequence encoding a lentiviral gag protein necessary for the formation of a viral capsid operably linked to a promoter. In one embodiment, the gag nucleic acid sequence is located on a different vector than at least some of the pol nucleic acid sequences. In another embodiment, the gag nucleic acid is located on a separate vector from all pol nucleic acid sequences encoding the pol protein.

Many modifications can be made to the vector which are used to generate particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to, deletion of the U3 region of the LTR, tat deletion and Matrix (MA) deletion.

gag, poi and env vectors do not contain nucleotides of the lentiviral genome that package lentiviral RNA, called lentiviral packaging sequences.

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

In another embodiment, the envelope protein is not from a lentivirus, but from a different virus. The resulting particles are referred to as pseudoparticles. By appropriate selection of the envelope, almost any cell can be "infected". For example, env genes encoding envelope proteins targeting the endocytic compartment can be used, such as the genes of the following viruses: influenza virus, VSV-G, alphavirus (simmerk forest virus, sindbis virus), arenavirus (lymphocytic choriomeningitis virus), flavivirus (tick-borne encephalitis virus, dengue virus, hepatitis c virus, GB virus), rhabdovirus (vesicular stomatitis virus, rabies virus), paramyxovirus (mumps or measles) and orthomyxovirus (influenza virus). Other envelopes that may preferably be used include those from Moloney leukemiSup>A viruses such as MLV-E, MLV-A and GALV. When the host cell is a primary cell, the latterAn envelope is particularly preferred. Other envelope proteins may be selected depending on the desired host cell. For example, targeting specific receptors such as dopamine receptors can be used for brain delivery. Another target may be vascular endothelium. These cells can be targeted using a filovirus envelope. For example, ebola virus GP becomes GP and GP through post-transcriptional modification $_2$ A glycoprotein. In another embodiment, different lentiviral capsids with pseudotyped envelopes may be used (e.g., FIV or SHIV [U.S. Pat. No. 5,654,195]]). The SHIV pseudotype vector can be easily used in animal models, such as monkeys.

As detailed herein, lentiviral vector systems typically include at least one helper plasmid comprising at least one of the gag, pol, or rev genes. Each of the gag, pol and rev genes may be provided on a separate plasmid, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol and rev genes are provided on the same plasmid (e.g., fig. 2). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (e.g., fig. 3). Thus, both 3-vector and 4-vector systems can be used to produce lentiviruses, as described in the examples section and elsewhere herein. The therapeutic vector, the envelope plasmid and at least one helper plasmid are transfected into a packaging cell line. A non-limiting example of a packaging cell line is the

293T/17HEK cell line. When the therapeutic vector, the envelope plasmid and at least one helper plasmid are transfected into a packaging cell line, lentiviral particles are ultimately produced.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system comprises a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing the gag, pol and rev genes, wherein lentiviral particles are produced by the packaging cell line when the lentiviral vector, the envelope plasmid and the at least one helper plasmid are transfected into the packaging cell line, wherein the lentiviral particles are capable of inhibiting the production of chemokine receptor CCR5 or are capable of targeting HIV RNA sequences.

In another aspect, and as detailed in fig. 2, a lentiviral vector (which is also referred to herein as a therapeutic vector) can comprise the following elements: hybrid 5' Long terminal repeat (RSV/5 ' LTR) (SEQ ID NOS: 11-12), psi sequence (RNA packaging site) (SEQ ID NO: 13), RRE (Rev-response element) (SEQ ID NO: 14), cPPT (polypurine tract) (SEQ ID NO: 15), H1 promoter (SEQ ID NO: 16), FDPS shRNA (SEQ ID NO:1,2,3, 4), woodchuck post-transcriptional regulatory element (WPRE) (SEQ ID NO: 17) and 3' delta LTR (SEQ ID NO: 18). In another aspect, sequence variation by substitution, deletion, addition, or mutation can be used to modify the sequence references herein.

In another aspect, as detailed herein, helper plasmids have been designed to include the following elements: the CAG promoter (SEQ ID NO: 19); HIV component gag (SEQ ID NO: 20); HIV component pol (SEQ ID NO: 21); HIV Int (SEQ ID NO: 22); HIV RRE (SEQ ID NO: 23); and HIV Rev (SEQ ID NO: 24). In another aspect, the helper plasmids may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variations by substitution, deletion, addition, or mutation can be used to modify the sequence references herein.

On the other hand, as detailed herein, the envelope plasmid is designed to include the following elements from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 25) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 26). In another aspect, sequence variations by substitution, deletion, addition, or mutation can be used to modify the sequence references herein.

In another aspect, plasmids used for lentiviral packaging can be modified with similar elements, and intron sequences can be removed without loss of vector function. For example, the following elements may be substituted for similar elements in a plasmid containing the packaging system: elongation factor-1 (EF-1), phosphoglycerate kinase (PGK) and ubiquitin C (UbC) promoters may be substituted for the CMV or CAG promoters. SV40 polyA and bGH polyA can replace rabbit beta globulin polyA. The HIV sequences in the helper plasmids can be constructed from different strains or clades of HIV. The VSV-G glycoprotein may be substituted with a membrane glycoprotein of: feline endogenous virus (RD 114), gibbon Ape Leukemia Virus (GALV), rabies virus (FUG), lymphocytic choriomeningitis virus (LCMV), fowl plague influenza virus type A (FPV), ross river alphavirus (RRV), murine leukemia virus 10A1 (MLV) or Ebola virus (EboV).

Notably, lentiviral packaging systems are commercially available (e.g., the Lenti-vpak packaging kit from OriGene technology, inc. of Rockville, md.) and may also be designed as described herein. Furthermore, it is within the skill of the art to substitute or modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the efficiency of production of lentiviral particles.

Dosage and formulation

The disclosed vectors allow for short, medium, or long term expression of a gene or sequence of interest and episomal maintenance of the disclosed vectors. Thus, the dosage regimen may vary depending upon the condition being treated and the method of administration.

In one embodiment, the transduction vector may be administered to a subject in need thereof at different doses. In particular, a subject may be administered approximately $\geqq 10^{6}$ One infectious dose (where 1 dose is required on average to transduce 1 target cell). More specifically, about $\geqq 10$ can be administered to the subject ^{7} About.gtoreq.10 ^{8} About.gtoreq.10 ^{9} Or about.gtoreq.10 ^{10} Individual infectious dose, or any number of doses between these values. The upper limit of the transduction vector dose will be determined for each disease indication and will depend on the toxicity/safety profile of each individual product or product batch.

In addition, the vectors of the present disclosure may be administered periodically, for example, once or twice a day, or any other suitable period of time. For example, the vector may be administered to a subject in need thereof once a week, once every other week, once every three weeks, once a month, once every three months, every six months, every nine months, once a year, every eighteen months, every two years, every thirty months, or once every three years.

In one embodiment, the disclosed vectors are administered as a pharmaceutical composition. In some embodiments, the pharmaceutical compositions comprising the disclosed carriers can be formulated into a variety of dosage forms, including but not limited to nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical use.

Each dosage form may contain various solubilizers, disintegrants, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. Pharmaceutical compositions containing the carrier may also be formulated for injection, insufflation, infusion or intradermal exposure. For example, injectable formulations can comprise the disclosed carriers in aqueous or non-aqueous solutions at a suitable pH and tonicity.

The disclosed vectors can be administered to a subject by direct injection into a tumor site or infection site. In some embodiments, the vector may be administered systemically. In some embodiments, the carrier may be administered to the tissue immediately surrounding the tumor or infection site through a guiding cannula.

The disclosed carrier compositions can be administered using any pharmaceutically acceptable method, e.g., intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenous, intradermal, intramuscular, subcutaneous, intraperitoneal), pulmonary, intravaginal, topical (locally), topical (topically), topical after scarification, mucosal administration, by aerosol, in a semi-solid medium such as agarose or gelatin, or by oral or nasal spray formulation.

In addition, the disclosed carrier compositions can be formulated into any pharmaceutically acceptable dosage form, such as solid dosage forms, tablets, pills, lozenges, capsules, liquid dispersions, gels, aerosols, pulmonary aerosols, nasal aerosols, ointments, creams, semi-solid dosage forms, solutions, emulsions, and suspensions. Further, the composition may be a controlled release formulation, a sustained release formulation, an immediate release formulation, or any combination thereof. In addition, the composition may be a transdermal delivery system.

In some embodiments, the pharmaceutical composition comprising the carrier may be formulated into a solid dosage form for oral administration, and the solid dosage form may be a powder, a granule, a capsule, a tablet or a pill. In some embodiments, the solid dosage form may include one or more excipients, such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose, or gelatin. In addition, solid dosage forms may include, in addition to excipients, lubricants, such as talc or magnesium stearate. In some embodiments, the oral dosage form may be an immediate release or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. Excipients used in modified release dosage forms are well known to those of ordinary skill in the art.

In a further embodiment, the pharmaceutical composition comprising the carrier may be formulated in a sublingual or buccal dosage form. Such dosage forms include sublingual tablets or solution compositions for sublingual administration, and buccal tablets placed between the cheek and gums.

In some embodiments, the pharmaceutical composition comprising the carrier may be formulated as a nasal dosage form. The dosage forms of the present invention comprise solutions, suspensions and gel compositions for nasal delivery.

In some embodiments, the pharmaceutical composition comprising the carrier may be formulated in a liquid dosage form for oral administration, such as a suspension, emulsion or syrup. In some embodiments, the liquid dosage form may include various excipients such as a humectant, a sweetener, an aromatic agent, or a preservative, in addition to conventional simple diluents such as water and liquid paraffin. In particular embodiments, the composition comprising the carrier may be formulated as appropriate for administration to a pediatric patient.

In some embodiments, the pharmaceutical composition may be formulated for parenteral administration, such as a sterile aqueous solution, suspension, emulsion, non-aqueous solution, or suppository. In some embodiments, the solution or suspension may include propylene glycol, polyethylene glycol, vegetable oils such as olive oil, or injectable esters such as ethyl oleate.

The dosage of the pharmaceutical composition may vary depending on the body weight, age, sex, administration time and mode, excretion rate and severity of disease of a patient.

In some embodiments, treatment of cancer is achieved by direct injection of the disclosed vector constructs into a tumor using a needle or an intravascular cannula. In some embodiments, the disclosed vectors are administered into the cerebrospinal fluid, blood or lymphatic circulation by intravenous or arterial cannulation or injection, intradermal delivery, intramuscular delivery, or injection into a drainage organ (organ) near the site of disease.

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

Examples

Example 1 development of a Lentiviral vector System

Lentiviral vector systems were developed as summarized in FIG. 4 (circularized form). Lentiviral particles were produced in 293T/17HEK cells (purchased from American Type Culture Collection, manassas, va.) after transfection with therapeutic vectors, envelope plasmids and helper plasmids. Transfection of 293T/17HEK cells producing functional viral particles the reagent poly (ethylenimine) (PEI) was used to increase the efficiency of plasmid DNA uptake. Plasmids and DNA were initially added separately to serum-free medium in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, the cell culture medium is collected and the lentiviral particles are purified by high speed centrifugation and/or filtration followed by anion exchange chromatography. The concentration of lentiviral particles can be expressed in transduction units per ml (TU/ml). Determination of TU was done by measuring HIV p24 levels in culture (p 24 protein incorporated into lentiviral particles), by quantitative PCR to measure viral DNA copy number per cell, or by infecting cells and using light (if the vector encodes a luciferase or fluorescent protein marker).

As described above, a 3-vector system (i.e., a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the carrier system is shown in figure 2. Briefly, and with reference to fig. 2, the topmost vector is the helper plasmid, which in this case includes Rev. The vector appearing in the middle of FIG. 2 is an enveloped plasmid. The lowermost vector is a therapeutic vector, as described herein.

More specifically with reference to FIG. 2, the helper + Rev plasmid includes the CAG enhancer (SEQ ID NO: 27); the CAG promoter (SEQ ID NO: 19); the chicken beta actin intron (SEQ ID NO: 28); HIV gag (SEQ ID NO: 20); HIV Pol (SEQ ID NO: 21); HIV Int (SEQ ID NO: 22); HIV RRE (SEQ ID NO: 23); HIV Rev (SEQ ID NO: 24); and rabbit beta globin poly A (SEQ ID NO: 29).

The envelope plasmid includes the CMV promoter (SEQ ID NO: 25); beta-globin intron (SEQ ID NO: 30); VSV-G (SEQ ID NO: 28); and rabbit beta globin poly A (SEQ ID NO: 31).

A2-vector lentiviral packaging system containing helper (+ Rev) and envelope plasmids was synthesized.

Materials and methods:

construction of helper plasmids: helper plasmids were constructed by initial PCR amplification of DNA fragments from pNL4-3HIV plasmid (NIH Aids reagent project) containing Gag, pol and integrase genes. Primers were designed to amplify fragments with EcoRI and NotI restriction sites, which can be used to insert the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5-.

The sequence of Gag, pol, integrase fragment is shown below:

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAG
GTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAG
CCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT
GAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAAT
TAGGAATACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTAC
CATACCTAGTATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCT
TTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGT
TGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAG
TACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTAT
GTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGTAT
TATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGAATG
AAGGGTGCCCACACTAATGATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAA
ACATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATAATA
GGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAA
TCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATATGCATTGGGAATCATTCAAGCACAACCAGATAAGAGT
GAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATTGGTC
AGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTTAACCTACCACCTGTAG
TAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAA
AAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAG
TAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAG

GAGTAATAGAATCTATGAATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAA
AGGGGGG
ATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGAC
AGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCAT
CAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA(SEQ ID NO:34)

next, a DNA fragment containing Rev, RRE and rabbit beta globin poly A sequences with XbaI and XmaI flanking restriction sites was synthesized by the MWG operon. The DNA fragment was then inserted into the plasmid at XbaI and XmaI restriction sites. The DNA sequence is shown below:

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGC
CCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTAC
CACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTA
AAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCA
GCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATC
AACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT
TTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGG
TGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCT
TTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTAT
GAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA
AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAAC
CATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGC
CTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATT
TCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG(SEQ ID NO:35)

finally, the CMV promoter of pcdna3.1 was replaced with the CAG enhancer/promoter and chicken β actin intron sequence. A DNA fragment containing the CAG enhancer/promoter/intron sequence with MluI and EcoRI flanking restriction sites was synthesized by the MWG operon. The DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence is shown below:

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGT
ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTC
ATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGG
GGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGT
TTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCG
CCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTG
TAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTG
TGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGG
GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCC
CCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGG
TGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTAT
GGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCG
GCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGG
CAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC(SEQ ID NO:36)

construction of VSV-G envelope plasmid:

the vesicular stomatitis indiana virus glycoprotein (VSV-G) sequence is synthesized from the MWG operon with flanking EcoRI restriction sites. The DNA fragment was then inserted into the pcdna3.1 plasmid (invitrogen) at the EcoRI restriction site and the correct orientation was determined by sequencing using CMV-specific primers.

The DNA sequence is as follows:

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAATTACC
ATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGC
TTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAACGAAA
CAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATGCAACTGTG
ACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCACAGTTCATCAACGGAAAATGCAGCAATTACATATGC
CCCACTGTCCATAACTCTACAACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCC
TGGGAAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGG
TTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGACGTTG
AGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCCTG
CTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACTACCACAGAAAG
GGAACTGTGGGATGACTGGGCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACATGGTATGTTGGAC
TCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAA
ATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTT
TGCATTAAATTAAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC(SEQ ID NO:37)

4-vector systems (i.e., 3-vector lentiviral packaging systems) were also designed and produced using the methods and materials described herein. A schematic of the 4-carrier system is shown in fig. 3. Briefly, and with reference to fig. 3, the topmost vector is the helper plasmid, in this case it does not include Rev. The second vector from the top was a separate Rev plasmid. The second vector from the bottom is an envelope plasmid. The bottommost vector is the aforementioned therapeutic vector.

Referring in part to FIG. 2, the helper plasmid includes the CAG enhancer (SEQ ID NO: 27); the CAG promoter (SEQ ID NO: 19); the chicken beta actin intron (SEQ ID NO: 28); HIV gag (SEQ ID NO: 20); HIV Pol (SEQ ID NO: 21); HIV Int (SEQ ID NO: 22); HIV RRE (SEQ ID NO: 23); and rabbit beta globin poly A (SEQ ID NO: 29).

The Rev plasmid contains the RSV promoter (SEQ ID NO: 38); HIV Rev (SEQ ID NO: 39); and rabbit beta globin poly A (SEQ ID NO: 29).

The envelope plasmid includes the CMV promoter (SEQ ID NO: 25); beta-globin intron (SEQ ID NO: 30); VSV-G (SEQ ID NO: 28); and rabbit beta globin poly A (SEQ ID NO: 29).

A3-vector lentiviral packaging system was synthesized containing helper, rev and envelope plasmids.

Materials and methods:

construction of Rev-free helper plasmids:

helper plasmids without Rev were constructed by inserting DNA fragments containing RRE and rabbit β -globin poly a sequences. This sequence was synthesized from the MWG operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A β globin sequence was then inserted into the helper plasmid at XbaI and XmaI restriction sites. The DNA sequence is shown below:

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGC
AGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA
CAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTT
TTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTG
GCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTT
AACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGC
TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCT
AACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCAT
CCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCC
ATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTT

GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
CCCGGG(SEQ ID NO:35)

construction of Rev plasmid:

the RSV promoter and HIV Rev sequences are synthesized by the MWG operon as a single DNA fragment, with flanking Mfel and XbaI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the Mfe and XbaI restriction sites, where the CMV promoter was replaced by the RSV promoter. The DNA sequence is as follows:

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCG
CTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGA
TTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTA
GCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTC
CATAGAAGACACCGGGACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATC
AAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG
GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGT
GGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA(SEQ ID NO:40)

plasmids for 2-vector and 3-vector packaging systems can be modified with similar elements and intron sequences can be removed without loss of vector function. For example, the following elements may be substituted for similar elements in 2-carrier and 3-carrier packaging systems:

a promoter: elongation factor-1 (EF-1) (SEQ ID NO: 41), phosphoglycerate kinase (PGK) (SEQ ID NO: 42) and ubiquitin C (UbC) (SEQ ID NO: 43) can be substituted for CMV (SEQ ID NO: 25) or CAG promoter (SEQ ID NO: 19). These sequences may be further altered by addition, substitution, deletion or mutation.

Poly A sequence: SV40 poly A (SEQ ID NO: 44) and bGH poly A (SEQ ID NO: 45) may be substituted for rabbit beta globin poly A (SEQ ID NO: 29). These sequences may be further altered by addition, substitution, deletion or mutation.

HIV Gag, pol and integrase sequences: the HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. For example, HIV Int (SEQ ID NO: 22) from Bal strain; HIV Gag (SEQ ID NO: 20); HIV Pol (SEQ ID NO: 21) can be interchanged with the gag, pol, and int sequences contained in the helper/helper + Rev plasmids described herein. These sequences may be further altered by addition, substitution, deletion or mutation.

Coating a film: the VSV-G glycoprotein may be substituted with a membrane glycoprotein from: feline endogenous virus (RD 114) (SEQ ID NO: 46), gibbon Ape Leukemia Virus (GALV) (SEQ ID NO: 47), rabies virus (FUG) (SEQ ID NO: 48), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 49), fowl plague influenza A virus (FPV) (SEQ ID NO: 50), ross river A virus (RRV) (SEQ ID NO: 51), mouse leukemia virus 10A1 (MLV) (SEQ ID NO: 52), or Ebola virus (Ebola virus) (SEQ ID NO: 53). The sequences of these envelopes are identified in the sequence section herein. In addition, these sequences may be further altered by addition, substitution, deletion or mutation.

In summary, a comparison and comparison of 3-vector to 4-vector systems can be made, in part, as follows. 3-vector Lentiviral vector System comprising: 1. helper plasmids: HIV Gag, pol, integrase and Rev/Tat;2. envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vectors: RSV 5'LTR, psi packaging signal, gag fragment, RRE, env fragment, cPPT, WPRE and 3' delta LTR. The 4-vector lentiviral vector system comprises: 1. helper plasmids: HIV Gag, pol, and integrase; rev plasmid: rev;3. envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vehicle: RSV 5'LTR, psi packaging signal, gag fragment, RRE, env fragment, cPPT, WPRE and 3' delta LTR. Sequences corresponding to the above-described elements are identified in the sequence listing section herein.

Example 2 development of Lentiviral vectors expressing FDPS

The purpose of this example was to develop FDPS lentiviral vectors.

Inhibitory RNA design: sequences of human farnesyl diphosphate synthase (FDPS) (NM — 002004.3) mRNA were used to search for potential siRNA or shRNA candidates to knock down FDPS levels in human cells. Potential RNA interference sequences are selected from candidates selected by siRNA or shRNA design programs, such as the GPP Web portal from the Broad institute (http:// portals. Branched. Organization. Org/GPP/public /) or the BLOCK-iT RNAi designer from Thermo science (https:// rnaidesigner. Thermofisher. Com/rnainexpress /). Separately selected shRNA sequences are inserted into lentiviral vectors, immediately 3' to RNA

polymerase III promoters, such as HI (SEQ ID NO: 16), U6 (SEQ ID NO: 54) or 7SK (SEQ ID NO: 55), to modulate shRNA expression. These lentiviral shRNA constructs were used to transduce cells and measure changes in specific mRNA levels. The shrnas that are most effective in reducing mRNA levels are individually embedded within a microrna backbone to allow expression through EF-1 α or CMV RNA polymerase II promoters. The microRNA backbone is selected from mirbase. The RNA sequence was also synthesized as a synthetic siRNA oligonucleotide and introduced directly into cells without the use of lentiviral vectors.

Vector construction: for FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by the Eurofins MWG operon. The overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for 1 hour at 37 ℃. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo science. The DNA concentration was measured, and the vector and oligonucleotide (3: 1 ratio) were mixed, annealed, and ligated. Ligation was performed with T4DNA ligase at room temperature for 30 minutes. 2.5 microliters of ligation mix was added to 25 microliters of STBL3 competent bacterial cells. The transformation was achieved after a heat shock at 42 degrees celsius. Bacterial cells were plated on ampicillin-containing agar plates and drug-resistant colonies (indicating the presence of ampicillin-resistant plasmids) were recovered and amplified in LB broth. To check for insertion of oligonucleotide sequences, plasmid DNA was extracted from harvested bacterial cultures using the Thermo scientific DNA mini prep kit. The insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using specific primers for the promoter regulating shRNA expression. Exemplary shRNA sequences knockdown FDPS were determined using the following target sequences:

GTCCTGGAGTACAATGCCATT (FDPS target sequence #1;

gtcctggagtacaatggcattgtactccagtgaactgacttgacttgacttttt (FDPS shRNA sequence #1;

gcaggattttcgttcagcactt (FDPS target sequence #2;

gcaggattttcgttcagcacttcgagaagaagtgctgaacgaaatcctgcttttt (FDPS shRNA sequence #2;

gccatgttacatggagatt (FDPS target sequence #3, seq ID no;

gccatgttacatgcaggaatttcgaaatttcctgccatgttacattggcttttt (FDPS shRNA sequence #3;

gcagaaggctgaggt (FDPS target sequence #4; and

gcagaaggctggagaaagtctcgagcttctcaggcctctcccttgcttttt (FDPS shRNA sequence # 4.

The shRNA sequences were then assembled into synthetic microRNAs (miRs) under the control of EF-1. Alpha. Promoter. Briefly, miR hairpin sequences, e.g., miR30, miR21 or miR185, as detailed below, were obtained from mirbase. The 19-22 mer shRNA target sequence was used to construct synthetic miR sequences. The miR sequences are arranged as antisense-hairpin loop sequences (specific for each microrna) -sense target sequences.

The following miR sequences were developed:

<xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (miR 30FDPS #1;SEQ ID NO:5) </xnotran>

<xnotran>
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (miR 30FDPS #2;SEQ ID NO:6) </xnotran>

<xnotran> TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (miR 30FDPS #3;SEQ ID NO:7) </xnotran>

<xnotran>
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA
</xnotran>

(miR 155FDPS #1;SEQ ID NO:8) </xnotran>

<xnotran>
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (miR 21FDPS #1;SEQ ID NO:9) </xnotran>

<xnotran>
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG (miR 185FDPS #1;SEQ ID NO:10) </xnotran>

Example 3: knockdown of FDPS by shRNA #4 in THP1 monocytic leukemia for 3 days

This example illustrates that knockdown of FDPS in THP1 monocytic leukemia cells by Lentivirus (LV) expressing FDPS shRNA #4 stimulates TNF- α expression in γ δ T cells, as shown in figure 5.

Transduction of THP1 cells with LV control or LV-FDPS shRNA #4 (1X 10) ^{5} One cell) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced THP-1 cells were incubated with 5X 10 ^{5} PBMC cells and IL-2 in round bottom 96 well plate co-culture for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. After staining for V.gamma.9 V.delta.12 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Delta.0 antibodies, cells were analyzed by flow cytometry. Viable cells were gated and selected for V.delta.2 + and TNF-. Alpha. + cells on dot blots. Activated cytotoxic V γ 9V δ 2T cells appear in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the LV-control stimulated 3.1% of TNF-. Alpha.expressing V.gamma.9V.delta.2T cells and LV-FDPS shRNA #4 5%. The LV-control stimulated 7.2% of TNF-. Alpha.expressing V.gamma.9Vdelta.2T cells and LV-FDPS shRNA #4 56.2% when treated with zoledronic acid.

Example 4: knockdown of FDPS by shRNA #4 in THP1 leukemia cells for 14 days

This example illustrates that knockdown of FDPS in THP1 leukemia cells by Lentivirus (LV) expressing FDPS shRNA #4 for 14 days stimulates TNF- α expression in GD T cells as shown in figure 6.

THP1 cells (1X 10) transduced with LV control or LV-FDPS shRNA #4 ^{5} Individual cells) for 14 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced THP-1 cells were incubated with 5X 10 ^{5} PBMC cells and IL-2 in round bottom 96 well plate co-culture for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. Cells were analyzed by flow cytometry after staining for V.gamma.9 V.delta.12 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Delta.0 antibodies. Viable cells were gated and selected for V.delta.2 + and TNF-. Alpha. + cells on dot blots. Activated cytotoxic V γ 9V δ 2T cells appear in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the LV-control stimulated 0.9% of TNF-. Alpha.expressing Vy 9 V.delta.2T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) 15.9%. Upon treatment with zoledronic acid, the LV-control stimulated 4.7% of TNF-. Alpha.expressing V.gamma.9Vdelta.2T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) 76.2%.

Example 5: knockdown of FDPS by shRNA #1 in PC3 prostate cancer cells for 3 days

This example illustrates that knockdown of FDPS in PC3 prostate cancer cells by Lentivirus (LV) expressing FDPS shRNA #1 stimulates TNF- α expression in GD T cells for 3 days, as shown in figure 7.

PC3 cells were transduced with LV control or LV-FDPS shRNA #1 (SEQ ID NO: 1) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced PC3 cells were incubated with 5X 10 ^{5} PBMC cells and IL-2 in round bottom 96 well plate co-culture for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. After staining for V.gamma.9 V.delta.2 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Alpha.antibodies, cells were analyzed by flow cytometry. Viable cells were gated and selected for V.delta.2 + and TNF-. Alpha. + cells on dot blots. Activated cytotoxic Vgamma 9 Vdelta 2T cells appear in flow cytometryIn the upper right quadrant of the cell map. Without zoledronic acid, the LV-control stimulated 0.2% of TNF-. Alpha.expressing Vy 9 V.delta.2T cells and LV-FDPS shRNA #1 stimulated 0.5%. Upon treatment with zoledronic acid, the LV-control stimulated 1.7% of TNF-. Alpha.expressing V.gamma.9 V.delta.2T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) 32.2%.

Example 6: knockdown of FDPS by shRNA #4 in PC3 prostate cancer cells for 3 days

This example illustrates that knockdown of FDPS in PC3 prostate cancer cells by Lentivirus (LV) expressing FDPS shRNA #4 for 3 days stimulated TNF- α expression in GD T cells, as shown in figure 8.

PC3 cells were transduced with LV control or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced PC3 cells were incubated with 5X 10^5 PBMC cells and IL-2 were co-cultured in round bottom 96 well plates for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. After staining for V.gamma.9 V.delta.12 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Delta.0 antibodies, cells were analyzed by flow cytometry. Viable cells were gated and selected for V.delta.2 + and TNF-. Alpha. + cells on dot blots. Activated cytotoxic V γ 9V δ 2T cells appear in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the LV-control stimulated 0.5% of TNF-. Alpha.expressing V.gamma.9V.delta.2T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) 1.9%. When treated with zoledronic acid, the LV-control stimulated 2.1% of TNF-. Alpha.expressing V.gamma.9V.delta.2T cells and LV-FDPS shRNA #4 28.7%.

Example 7: knockdown of FDPS in HepG2 hepatoma cells by shRNA #1 and #4 for 3 days

This example illustrates that knockdown of FDPS in HepG2 hepatoma cells by Lentiviruses (LV) expressing FDPS shRNA #1 (SEQ ID NO: 1) and shRNA #4 (SEQ ID NO: 4) for 3 days stimulates TNF- α expression in GD T cells, as shown in FIG. 9.

HepG2 cells were transduced with LV control, LV-FDPS shRNA #1 (SEQ ID NO: 1) or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced HepG2 cells were incubated with 5X 10^5 PBMC cellAnd IL-2 in round bottom 96 well plates for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. After staining for V.gamma.9 V.delta.12 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Delta.0 antibodies, cells were analyzed by flow cytometry. Viable cells were gated and V.delta.2 + and TNF-. Alpha. + cells were selected on dot blots. Activated cytotoxic V γ 9V δ 2T cells appear in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, LV-control stimulated 0.4% of TNF-. Alpha.expressing V.gamma.9Vdelta.2T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) and #4 (SEQ ID NO: 4) stimulated 0.7% and 0.9%, respectively. When treated with zoledronic acid, the LV-control stimulated 6.9% of TNF-. Alpha.expressing V.gamma.9Vdelta.2T cells and LV-FDPS shRNA #1 and #4 stimulated 7.6% and 21.1%, respectively.

Example 8: knockdown of FDPS in THP1 leukemia cells by microRNA-30 for 3 days

This example illustrates that knockdown of FDPS in THP1 leukemia cells by Lentivirus (LV) expressing FDPS targeted synthetic microrna-30 stimulates TNF- α expression in γ δ T cells for 3 days, as shown in figure 10.

Transduction of THP1 cells with LV control or LV-miR30FDPS #1 (SEQ ID NO: 5) (1X 10) ^{5} Cells) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced THP-1 cells were incubated with 5X 10^5 PBMC cells and IL-2 in round bottom 96 well plate co-culture for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. After staining for V.gamma.9 V.delta.12 and TNF-. Alpha.with fluorophore-conjugated anti-TCR-V.delta.2 and anti-TNF-. Delta.0 antibodies, cells were analyzed by flow cytometry. Viable cells were gated and selected for V.delta.2 + and TNF-. Alpha. + cells on dot blots. Activated cytotoxic V γ 9V δ 2T cells appear in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the LV-control stimulated 0.2% of TNF-. Alpha.expressing V.gamma.9V.delta.2T cells and LV-miR30FDPS 8.1%. When treated with zoledronic acid, the LV-control stimulated 5.3% of TNF-. Alpha.expressing V.gamma.9V.delta.2T cells and LV-miR30FDPS #1 (SEQ ID NO: 5) stimulated 67.3%.

Example 9E produced from a mixture of THP-1 cells, cultured human GD T cells and/or Zomet (Zol): ratio of T

This example demonstrates the results of mixing treated THP-1 monocyte-like tumor cells with cultured human GD T cells, as shown in fig. 11.

Monocyte-like cell line THP-1 was treated with control Lentiviral Vector (LV), LV suppressing farnesyl diphosphate synthase gene expression (LV-FDPS), zoledronic acid (Zol), or a combination. As shown in fig. 11, the legend is: a lentiviral control vector (LV-control), a lentiviral vector expressing microRNA down-regulated FDPS (LV-FPPS), zoeta (Zol), zoeta + lentiviral control (Zol + LV-control), or a Zoeta + lentiviral vector expressing microRNA down-regulated FPPS (Zol + LV-FPPS).

Human GD T cells were cultured from anonymous donors and cultured at 4: 1. 2:1 or 1: a ratio of 1 (GD T: THP-1) was added to the treated THP-1 cells for 4 hours. Cell killing was measured by fluorimetry. When THP-1 cells were treated with a combination of LV-FDPS and Zol, cytotoxic T cell killing of GD T cells was greatly increased compared to either treatment alone. LV-FDPS resulted in greater killing when LV-FDPS alone was compared to Zol alone, but was more than 3-fold less than tumor cell killing after combined treatment. The proportion is 4:1, combined LV-FDPS + Zol treatment caused nearly 70% of tumor cell killing; this is more than 3 times higher than the second best treatment (LV-FDPS only).

Example 10-shRNA-based RNA interference targeting lentiviral delivery of the human farnesyl diphosphate synthase (FDPS) Gene

HepG2 human hepatocyte cancer cells were infected with lentiviral vectors containing the H1 promoter and either non-targeting or four different FDPS shRNA sequences, as shown in FIG. 12.

After 48 hours, RNA was extracted from the cells and converted to cDNA. Expression of FDPS cDNA was determined by quantitative PCR using SYBR Green and FDPS primers. FDPS expression was normalized to actin levels for each sample.

FDPS-targeting lentiviral vectors were generated in 293T cells containing the HI promoter and non-targeting sequence (5

Or one of four different FDPS shRNA sequences

Gtcctggagtacaatggcattgtactccagtggacttttt (FDPS shRNA sequence #1;

gcaggattttcgttcagcacttcgagaagaagtgctgaacgaaatcctgcttttt (FDPS shRNA sequence #2;

gccatgttacatgcaggaatttcgaaatttcctgccatgttacattggcttttt (FDPS shRNA sequence #3; and

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT

(FDPS shRNA sequence # 4.

HepG2 human hepatocyte cancer cells were then infected with lentiviral vectors to determine the efficacy of FDPS knockdown. After 48 hours, RNA was extracted from the cells using RNeasy RNA isolation kit (Qiagen) and converted to cDNA using Superscript VILO cDNA synthesis kit (Thermo science). Expression of FDPS cDNA was determined by quantitative PCR using a SYBR Green PCR mixture (Thermo science) and FDPS primers (forward primer: 5 ` -AGGAATTGATGGCGAGAGG-3 ` (SEQ ID NO: 61) and reverse primer: 5 ` -CCCAAAAGAGGTCAAGGTAATCA-3 ` (SEQ ID NO: 62)) on the applied biosystems StepOne qPCR machine. FDPS expression was normalized to actin level using actin primers (forward primer: 5-. The relative FDPS RNA expression for the shCon samples was set at 100%. FDPS expression was reduced by 85% (FDPS sequence # 1), 89% (FDPS sequence # 2), 46% (FDPS sequence # 3) and 98% (FDPS sequence # 4).

Example 11 MiR-based RNA interference targeting lentiviral delivery of the human farnesyl diphosphate synthase (FDPS) Gene

As shown in FIG. 13, hepG2 human hepatocyte cancer cells were infected with lentiviral vectors containing the H1 promoter (SEQ ID NO: 16) and the FDPS shRNA #4 (SEQ ID NO: 4) sequence or the EF-1. Alpha. Promoter (SEQ ID NO: 41) and the miR 30-based FDPS sequence. After 48 hours, cells were lysed and immunoblotted using anti-FDPS (Thermo science) and anti-actin (Sigma) antibodies as protein loading controls.

More specifically, human parenchymal liver cells were infected with a lentiviral vector containing the H1 promoter (SEQ ID NO: 16) and the FDPS shRNA sequence GCAGAAGGAGGCTGAGAAGTCTCGAGACTTTCTCAGCCTCCTTCTTGCTTTTT (FDPS shRNA sequence #4 SEQ ID NO).

After 48 hours, cells were lysed with NP-40 lysis buffer and proteins were quantitated using Bio-Rad protein assay reagents. 50 μ g of the protein sample was electrophoresed on 4-12% bis-Tris gel (Thermo science) and transferred to a PVDF membrane (EMD Millipore). Immunoblotting was performed using anti-FDPS (Thermo science) and anti-actin (Sigma) antibodies as protein loading controls. The antibody was conjugated to an HRP-conjugated secondary antibody and detected using Immobilon Western ECL reagent (EMD Millipore Co.) with Licor c-Digit blot scanner. Densitometry of immunoblot strips was quantified with NIH image software. LV control with EF-1 promoter was set at 100%. FDPS protein expression is reduced by 68% (LV-shFDPS # 4), 43% (LV-miR FDPS # 1) and 38% (LV-miR FDPS # 3).

Example 12 knock-down of FDPS by adeno-associated Virus (AAV) expressing FDPS shRNA #4 in HepG2 hepatoma cells for 3 days

This example illustrates that knockdown of FDPS by adeno-associated virus (AAV) expressing FDPS shRNA #4 (SEQ ID NO: 4) in HepG2 hepatoma cells for 3 days stimulates TNF- α expression in GD-T cells (FIG. 14, panel B).

HepG2 cells were transduced with control or AAV-FDPS shRNA #4 (SEQ ID NO: 8) for 3 days. Two days after transduction, cells were treated with or without 1 μ M zoledronic acid. After 24 hours, the transduced HepG2 cells were incubated with 5X 10^5 PBMC cells and IL-2 were co-cultured in round bottom 96 well plates for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2T cells. In the case of using fluorophore-conjugated anti-TCR-V delta 2 and anti-TNF-alpha antibodies to V gamma 9V delta 2 and TNF-alphaAfter staining, cells were analyzed by flow cytometry. Viable cells were gated and V.delta.2 + and TNF-. Alpha. + cells were selected on dot blots. Activated cytotoxic V γ 9V δ 2T cells appeared in the upper right quadrant of the flow cytogram (fig. 14, panel B).

And (3) constructing an AAV vector. FDPS shRNA sequence #4 (SEQ ID NO: 4) was inserted into pAAV plasmid (Cell Biolabs). The FDPS oligonucleotide sequence containing BamHI and EcoRI restriction sites was synthesized by the Eurofins MWG operon. The overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees celsius to room temperature. pAAV was digested with the restriction enzymes BamHI and EcoRI at 37 ℃ for 1 hour. The digested pAAV plasmid was purified by agarose gel electrophoresis and extracted from the gel using the DNA gel extraction kit of Thermo science. The DNA concentration was measured, and the vector and oligonucleotide (3: 1 ratio) were mixed, annealed, and ligated. Ligation was performed with T4DNA ligase at room temperature for 30 minutes. 2.5 microliters of the ligation mixture was added to 25 microliters of STBL3 competent bacterial cells. The transformation was achieved after a heat shock at 42 degrees celsius. Bacterial cells were plated on agar plates containing ampicillin and drug resistant colonies (indicating the presence of ampicillin resistant plasmids) were recovered and amplified in LB broth. To check for insertion of oligonucleotide sequences, plasmid DNA was extracted from harvested bacterial cultures using the Thermo scientific DNA mini prep kit. The insertion of the shRNA sequence in pAAV plasmid was verified by DNA sequencing using specific primers for the promoter regulating shRNA expression. An exemplary AAV vector having an H1 promoter (SEQ ID NO: 16), shFDPS sequence (e.g., SEQ ID NO: 4), left inverted terminal repeat (left ITR; SEQ ID NO: 65), and right inverted terminal repeat (right ITR; SEQ ID NO: 66) can be set forth in Panel A of FIG. 14.

Producing the AAV particle. The AAV-FDPS shRNA plasmid was combined with plasmids pAAV-RC2 (cell Biolabs) and pHelper (cell Biolabs). The pAAV-RC2 plasmid contains Rep and AAV2 capsid genes, and pHelper contains adenovirus E2A, E4 and VA genes. To produce AAV particles, these plasmids were expressed in a 1:1:1 (pAAV-shFDPS: pAAV-RC2: pHelper) into 293T cells. To transfect cells in a 150mm dish (BD Falcon Co.), 10 micrograms of each plasmid was added together in 1ml of DMEM. In another tube, 60 microliters of the transfection reagent PEI (1 microgram/ml) (Polysciences Inc.) was added to 1ml DMEM. The two tubes were mixed together and incubated for 15 minutes. The transfection mixture was then added to the cells and the cells were harvested after 3 days. Cells were lysed by freeze/thaw lysis in dry ice/isopropanol. Benzonase nuclease (Sigma) was added to the cell lysate for 30 minutes at 37 degrees Celsius. The cell debris was then pelleted by centrifugation at 12,000rpm for 15 minutes at 4 degrees celsius. The supernatant was collected and then added to the target cells.

Example 13 reduction of RAP1 prenylation in cells transduced with LV-shFDPS and treated with zoledronic acid

This example illustrates that lentiviral-delivered shRNA targeting the human farnesyl diphosphate synthase (FDPS) gene and zoledronic acid synergistically inhibit farnesyl diphosphate production.

FDPS is an enzyme in the isoprenoid synthesis pathway that catalyzes the production of farnesyl diphosphate. Inhibition of FDPS enzymatic activity by zoledronic acid or reduction of protein expression by shRNA-mediated knock-down will result in reduced farnesyl diphosphate levels. Farnesyl diphosphate is required for farnesylation of cellular proteins. RAPIA is a protein modified by farnesylation and can be used as a biomarker for cellular farnesyl diphosphate levels. FDPS activity was measured using antibodies that specifically recognize reduced RAPIA farnesylation after transduction with LV-shFDPS alone or in combination with zoledronic acid. HepG2 human hepatocyte cancer cells were infected with lentiviral vectors containing the FDPS shRNA sequence # 4. For zoledronic acid treated cells, zoledronic acid (Sigma) was added over the last 24 hours. After 48 hours, cells were lysed with NP-40 lysis buffer and proteins were quantified using Bio-Rad protein assay reagents. 50 μ g of the protein sample was electrophoresed on 4-12% bis-Tris gel (Thermo science) and transferred to a PVDF membrane (EMD Millipore). Immunoblotting was performed using anti-FDPS (Thermo science), anti-RAP 1A (Santa Cruz) and anti-actin (Sigma) antibodies as protein loading controls. The antibody was conjugated to an HRP-conjugated secondary antibody and detected using Immobilon Western ECL reagent (EMD Millipore Co.) using a Licor c-Digit blot scanner. An increase in RAPIA band intensity was associated with decreased farnesylation. Degarnination of RAPIA occurred only in cells transduced with LV-shFDPS and treated with zoledronic acid.

Example 14 treatment of subjects with cancer

LV-FDPS is a gene drug delivered from lentiviral vectors by local administration to advanced unresectable liver parenchymal cell carcinoma

Phase I clinical trials will test the safety and feasibility of using ultrasound guided liver cannulae to deliver LV-FDPS to hepatocellular carcinoma (HCC) sites in patients without concomitant radiotherapy or chemotherapy. It is reasonable to predict that this study will successfully treat HCC. The study was an open label, 4x3 dose escalation (4 dose ranges with up to 3 subjects per dose) to determine the maximum tolerated dose of LV-FDPS for patients with stage III/IV unresectable HCC at 18 years of age or older.

LV-FDPS is a genetic therapy aimed at reducing the expression of farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that LV-FDPS modified tumor cells induce anti-tumor activity of human γ δ T cells, including the ability to kill tumors through cytotoxicity.

Subjects with lesions of interest having a longest diameter ≧ 1cm (as measured by helical CT) and a maximum diameter ≦ 4.9cm and satisfying the admission and exclusion criteria detailed below were enrolled for the next available administration category. A maximum of 3 subjects were enrolled per dose group. The dose is the number of transduction units of LV-FDPS delivered in a single bolus via an intrahepatic cannula, as described in product release standards, in a volume of no more than 25mL. Based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al, phase I clinical trial of thymidine kinase-based Gene therapy for advanced hepatocellular carcinoma (a phase I clinical trial of thymidine kinase-based Gene therapy in advanced hepatocellular carcinoma), 2010, cancer Gene ther.17 837-43), the minimum dose was 1×10^{9} Transduce the unit and increment by 10-fold to the next dose $1X10^{10}$ Transduction unit, next dose $1X10^{11}$ Transduction unit, and maximum dose is $1X10^{12}$ A transduction unit. The subject is recruited and treatedTreatment and assessment lasted 3 months. All safety assessments were completed for each group prior to enrollment and treatment of subjects at the next higher dose level. Recruitment and dose escalation continued until the maximum tolerated dose was reached or the study was terminated.

Cannulation was performed through the left subclavian artery until the catheter tip was at the appropriate hepatic artery junction. The cannula was guided by ultrasound examination as described (Lin et al, clinical efficacy of cisplatin, mitomycin C, interleukin and 5-Fluorouracil arterial perfusion chemotherapy on non-resectable advanced liver parenchymal cell carcinoma (Clinical effects of intra-articular infusion chemotherapy with cissplatin, mitomycin C, leucovor and 5-fluoroouracil for unresectable advanced hepatocellular carcinoma), 2004, J.Chin.Med.Assoc.67, 602-10.

Measurement of primary outcome

Safety is as follows: systematic and local area adverse events were ranked according to CTCAS and coded according to MedRA. Prior to dose escalation, adverse event data will be assessed for all subjects within a single dose range. The final safety assessment contained data for all dose ranges.

Measurement of secondary outcome

● Local administration and subsequent biopsy or autopsy to obtain post-tissue LV-FDPS lesion distribution and retention.

● Objective Response Rate (ORR) in targeted and measurable non-localized lesions (if present) by physical analysis, medical imaging or biopsy within 3 months after treatment.

● Levels of LV-FDPS in the blood 10 min, 30 min, 1 h and 1 day after local injection.

● Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT within 3 months after treatment.

No history in the interim analysis survived more than historical control (no LV-FDPS) patients.

Admission standard

● Over the age of 18 years, both males and females are included.

● Diagnosis is confirmed by histology or cytology or clinical criteria based on currently accepted parenchymal cell derived liver parenchymal cell cancers, where resection, transplantation or other potentially curative therapy is inappropriate at the time of screening.

● The treating physician determines that the lesion is suitable for local area targeted delivery.

● The target focus is a measurable disease with one-dimensional maximum diameter more than or equal to 1.0cm which is displayed by computed tomography; the maximum diameter is less than or equal to 5.0 cm.

● Kamofsky expressed as 60-80% of the ECOG value.

● The expected life is more than or equal to 12 weeks.

● Hematopoietic function: WBC is more than or equal to 2,500/mm ^{3} ; ANC≥1000/mm ^{3} (ii) a The hemoglobin is more than or equal to 8g/dL; the platelet count is more than or equal to 50,000/mm ^{3} (ii) a Coagulation INR is less than or equal to 1.3.

● AST and ALT <5 times ULN; ALPS <5 times ULN. Bilirubin is less than or equal to 1.5 times ULV; creatine is less than or equal to 1.5 times ULN and eGFR is more than or equal to 50.

● Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.

● The attending physician considers renal, cardiovascular and respiratory function to be adequate.

● And (3) immune function: the circulating V gamma 9V delta 2+ T cells are more than or equal to 30/mm ^{3} (ii) a Has no immunodeficiency.

● Serological and viral RNA tests are negative for HIV.

● Written informed consent.

Exclusion criteria

● The target lesion is adjacent to, surrounds, or infiltrates the blood vessel.

● Primary HCC suitable for resection, transplantation, or other potentially curative therapy.

● Liver surgery or chemoembolization within the past 4 months.

● Liver radiation or total body radiation therapy over the past 4 months.

● Chemotherapy for 4 weeks or any use of nitrosourea, mitomycin C or cisplatin.

● Currently or within the past 4 weeks receiving aminobisphosphonate treatment

● Study drug with a half-life of <5 drugs or within 4 weeks.

● Impaired wound healing due to diabetes.

● Serious mental illness, alcohol dependence or illegal drug use.

● Are reluctant to comply with research protocols and reporting requirements.

● The aminobisphosphonate treatment was performed within the last 4 months.

● Cardiovascular, cerebrovascular (stroke), immunological (except for hepatitis b or c virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system diseases of clinical significance; current brain disease; variceal bleeding requiring hospitalization or transfusion within the past 4 months.

● History of HIV or acquired immunodeficiency syndrome.

● Anti-retroviral drugs are currently or previously used for treatment.

● Pregnancy, lactation or refusal to use barrier or chemical contraceptives throughout the test and follow-up interval.

LV-FDPS is the adjuvant administration of aminobisphosphonate as a gene drug delivered by local administration from lentiviral vectors to advanced unresectable liver parenchymal carcinoma

Phase I clinical trials will test the safety and feasibility of using ultrasound guided liver cannulae to deliver LV-FDPS to hepatocellular carcinoma (HCC) sites in patients with aminobisphosphonate chemotherapy. It is reasonable to predict that this study will successfully treat HCC. The study was an open label, 4x3 dose escalation (4 dose range, up to 3 subjects per dose) to determine the maximum tolerated dose of LV-FDPS in patients with stage III/IV unresectable HCC 18 years old or older.

LV-FDPS is a genetic therapy aimed at reducing the expression of farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that LV-FDPS modified tumor cells induce anti-tumor activity of human γ δ T cells, including the ability to kill tumors through cytotoxicity. Previous experimental studies have also shown the possibility of a positive interaction between LV-FDPS and specific aminobisphosphonate drugs, which may be present in primary conditionsOr in metastatic disease. For this study, subjects will receive dose increments of LV-FDPS with continuous standard therapeutic doses using the recommendations of the physician and subject preferences

(pamidronate) which is a salt of,

(zoledronic acid) or

(risedronate).

The longest diameter of the target focus is more than or equal to 1cm (measured by spiral CT) and the maximum diameter<Subjects of 4.9cm and meeting the admission and exclusion criteria detailed below were enrolled and started in aminobisphosphonate therapy. After 30 days, the size of the target lesion was re-evaluated to ensure that the subject still met the initiation criteria for LV-FDPS. Subjects who did not have an objective clinical response to the aminobisphosphonate were enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects were enrolled per dose group and all subjects continued to use the aminobisphosphonate during the study unless otherwise suggested by the attending physician. The LV-FDPS dose is the number of transduction units of LV-FDPS delivered in a single bolus by intrahepatic intubation, as described in the product release standard, the volume not exceeding 25mL. Based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al, phase I clinical trial of thymidine kinase-based Gene therapy for advanced hepatocellular carcinoma (a phase I clinical trial of thymidine kinase-based Gene therapy in advanced hepatocellular carcinoma), 2010, cancer Gene ther.17 837-43), the minimum dose was 1×10^{9} Transduce the unit and increment by 10-fold to the next dose $1X10^{10}$ Transduction unit, next dose $1X10^{11}$ Transduction unit, and maximum dose is $1X10^{12}$ A transduction unit. Subjects were enrolled, treated and evaluated for 3 months. All safety assessments were completed for each group prior to enrollment and treatment of subjects at the next higher dose level. Recruitment and dose escalation continues until maximum tolerance is reachedSubject to dose or termination study.

Cannulation was performed through the left subclavian artery until the catheter tip was at the appropriate hepatic artery junction. The cannula was guided by ultrasound examination as described (Lin et al, clinical efficacy of cisplatin, mitomycin C, interleukin and 5-Fluorouracil arterial perfusion chemotherapy on terminal parenchymal carcinoma that cannot be surgically removed (Clinical effects of intra-acute infusion chemotherapy with cispain, mitomycin C, leucovor and 5-fluoroouracil for unresectable advanced hepatocellular carcinoma), 2004, J.Chin.Med.Assoc.67.602-10.

Measurement of primary outcome

Safety: systematic and local area adverse events were ranked according to CTCAS and coded according to MedRA. Prior to dose escalation, all subjects within a single dose range will be evaluated for adverse event data. The final safety assessment contained data for all dose ranges.

Measurement of secondary outcome

● Local administration followed by biopsy or autopsy to obtain LV-FDPS lesion distribution and retention after tissue.

● Objective Response Rate (ORR) in targeted and measurable non-localized lesions (if present) within 3 months after treatment by physical analysis, medical imaging or biopsy.

● Levels of LV-FDPS in the blood 10 min, 30 min, 1 h and 1 day after local injection.

● Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT within 3 months after treatment.

● Patients survived beyond the historical control (no LV-FDPS) without history in the interim analysis.

Admission standard

● Over the age of 18 years, both male and female are included.

● Diagnosis is confirmed by histology or cytology or clinical criteria based on currently accepted parenchymal cell derived liver parenchymal cell cancers, where resection, transplantation or other potentially curative therapy is inappropriate at the time of screening.

● The treating physician determines that the lesion is suitable for localized regional targeted delivery.

● The target focus is a measurable disease with one-dimensional maximum diameter more than or equal to 1.0cm, which is displayed by computed tomography; the maximum diameter is less than or equal to 5.0 cm.

● Kamofsky expressed as 60-80% of the ECOG value.

● The expected life is more than or equal to 12 weeks.

● Hematopoietic function: WBC is more than or equal to 2,500/mm ^{3} ; ANC≥1000/mm ^{3} (ii) a The hemoglobin is more than or equal to 8g/dL; platelet count is greater than or equal to 50,000/mm ^{3} (ii) a Coagulation INR is less than or equal to 1.3.

● AST and ALT <5 times ULN; ALPS <5 times ULN. Bilirubin is less than or equal to 1.5 times ULV; creatine is less than or equal to 1.5 times ULN and eGFR is more than or equal to 50.

● Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities. ● The attending physician considers renal, cardiovascular and respiratory functions to be adequate.

● And (3) immune function: the circulating V gamma 9V delta 2+ T cells are more than or equal to 30/mm ^{3} (ii) a Has no immunodeficiency disease.

● Serological and viral RNA tests are negative for HIV.

● Written informed consent.

Exclusion criteria

● Intolerance or reluctance to continue the adjuvant treatment of the aminodiphosphonates.

● The amino-diphosphonic acid drug has objective clinical response after treatment.

● The target lesion is adjacent to, surrounds, or infiltrates the blood vessel.

● Primary HCC suitable for resection, transplantation or other potentially curative therapy.

● Liver surgery or chemoembolization over the past 4 months.

● Liver radiation or whole body radiation therapy over the past 4 months.

● Chemotherapy (excluding aminobisphosphonates) or any use of nitrosourea, mitomycin C or cisplatin within 4 weeks.

● Study drug with a drug half-life of <5 or within 4 weeks.

● Impaired wound healing due to diabetes.

● Severe psychiatric illness, alcohol dependence or illegal drug use.

● Are reluctant to comply with research protocols and reporting requirements.

● Cardiovascular, cerebrovascular (stroke), immunological (except for hepatitis b or c virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system diseases of clinical significance; current brain disease; variceal bleeding requiring hospitalization or transfusion within the past 4 months.

● History of HIV or acquired immunodeficiency syndrome.

● Anti-retroviral drugs are currently or previously used for treatment.

● Pregnancy, lactation or refusal to use barrier or chemical contraceptives throughout the test and follow-up interval.

Example 15 treatment of subjects with Chronic viral liver disease

LV-FDPS is a gene drug delivered to the liver by local administration from a lentiviral vector for the treatment of hepatitis B, C, HIV or other liver viral infections

Phase I clinical trials will test the safety and feasibility of using ultrasound guided cannulae to deliver LV-FDPS to virally infected liver. It is reasonable to predict that this study will successfully treat liver infections. The study was an open label, 4x3 dose escalation (4 dose ranges with up to 3 subjects per dose) to determine the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with chronic viral liver disease and resistant to chemotherapy.

LV-FDPS is a genetic therapy aimed at reducing the expression of farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that LV-FDPS modified tumor cells induce human γ δ T cells, including the cytotoxic ability against virus infected cells.

Subjects diagnosed with liver viral infections, including hepatitis b, c, HIV or other viruses, were enrolled into the next available LV-FDPS dosing category. Maximum 3 pairs were recruited per dose groupSuch as a mouse. The LV-FDPS dose is the number of transduction units of LV-FDPS delivered in a single bolus via an intrahepatic cannula, and the volume does not exceed 25mL as described in the product release standard. Based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al, phase I clinical trial of thymidine kinase-based Gene therapy for advanced hepatocellular carcinoma (a phase I clinical trial of thymidine kinase-based Gene therapy in advanced hepatocellular carcinoma), 2010, cancer Gene ther.17 837-43), the minimum dose was 1×10^{9} Transduce the unit and increment by 10-fold to the next dose $1X10^{10}$ Transduction unit, the next dose is $1X10^{11}$ Transduction unit, and maximum dose is $1X10^{12}$ A transduction unit. Subjects were enrolled, treated and evaluated for 3 months. All safety assessments were completed for each group prior to enrollment and treatment of subjects at the next higher dose level. Enrollment and dose escalation continued until the maximum tolerated dose was reached or the study was terminated.

Cannulation was performed through the left subclavian artery until the catheter tip was at the appropriate hepatic artery junction. The cannula was guided by ultrasound examination as described (Lin et al, clinical efficacy of cisplatin, mitomycin C, interleukin and 5-Fluorouracil arterial perfusion chemotherapy on terminal parenchymal carcinoma that cannot be surgically removed (Clinical effects of intra-acute infusion chemotherapy with cispain, mitomycin C, leucovor and 5-fluoroouracil for unresectable advanced hepatocellular carcinoma), 2004, J.Chin.Med.Assoc.67.602-10.

Measurement of primary outcome

Safety: systematic and local area adverse events were ranked according to CTCAS and coded according to MedRA. Prior to dose escalation, all subjects within a single dose range will be evaluated for adverse event data. The final safety assessment contained data for all dose ranges.

Measurement of secondary outcome

● Local administration followed by biopsy or autopsy to obtain LV-FDPS lesion distribution and retention after tissue.

● Objective Response Rate (ORR) measured as Sustained Viral Response (SVR) in the organ or in the whole body within 3 months after treatment.

● Levels of LV-FDPS in the blood 10 min, 30 min, 1 h and 1 day after local injection.

● Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT within 3 months after treatment.

● No history in the interim analysis survived more than historical control (no LV-FDPS) patients.

Admission standard

● Over the age of 18 years, both males and females are included.

● Diagnosis is confirmed histologically or cytologically or based on currently accepted clinical criteria for chronic viral liver disease, and resection, transplantation or other potentially curative therapy is not appropriate at the time of screening.

● The treating physician determines that the lesion is suitable for localized regional targeted delivery.

● Kamofsky expressed as 60-80% of ECOG value.

● The expected life is more than or equal to 12 weeks.

● Hematopoietic function: WBC is more than or equal to 2,500/mm ^{3} ; ANC≥1000/mm ^{3} (ii) a The hemoglobin is more than or equal to 8g/dL; the platelet count is more than or equal to 50,000/mm ^{3} (ii) a Coagulation INR is less than or equal to 1.3.

● AST and ALT <5 times ULN; ALPS <5 times ULN. Bilirubin is less than or equal to 1.5 times ULV; creatine is less than or equal to 1.5 times ULN and eGFR is more than or equal to 50.

● Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.

● The attending physician considers renal, cardiovascular and respiratory functions to be adequate.

● And (3) immune function: the circulating V gamma 9V delta 2+ T cells are more than or equal to 30/mm ^{3} (ii) a Has no immunodeficiency.

● Serological and viral RNA tests are negative for HIV.

● Written informed consent.

Exclusion criteria

● Chronic viral diseases suitable for excision, transplantation or other potentially curative therapy.

● Liver surgery or chemoembolization over the past 4 months.

● Liver radiation or total body radiation therapy over the past 4 months.

● Study drug with a drug half-life of <5 or within 4 weeks.

● Currently (over the last 4 weeks) or continuously receiving aminobisphosphonate treatment.

● Impaired wound healing due to diabetes.

● Serious mental illness, alcohol dependence or illegal drug use.

● Are reluctant to comply with research protocols and reporting requirements.

● Cardiovascular, cerebrovascular (stroke), immunological (excluding viral infections, viral hepatitis or cirrhosis), endocrine or central nervous system diseases of clinical significance; current brain disease; variceal bleeding requiring hospitalization or transfusion within the past 4 months.

● Pregnancy, lactation or refusal to use barrier or chemical contraceptives throughout the test and follow-up interval.

LV-FDPS is a gene drug delivered to the liver by local administration from lentiviral vectors for the treatment of hepatitis B virus, hepatitis C virus, HIV or other hepatoviral infection-associated adjuvant aminobisphosphonate drug therapy

Phase I clinical trials will test the safety and feasibility of using ultrasound guided cannulae to deliver LV-FDPS to virally infected liver. It is reasonable to predict that this study will successfully treat liver infections. The study was an open label, 4x3 dose escalation (4 dose range, up to 3 subjects per dose) to determine the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with chronic viral liver disease and resistant to chemotherapy.

LV-FDPS is a genetic therapy aimed at reducing the expression of farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that LV-FDPS modified tumor cells induce human γ δ T cells, including the cytotoxic ability against virus infected cells. Previous experimental studies also showed the possibility of a positive interaction of LV-FDPS with specific aminobisphosphonate drugs, which can be prescribed during infectious diseases. To this endStudy, subjects will receive dose escalating amounts of LV-FDPS with continuous standard therapeutic dose, according to physician's recommendations and subject preferences, using

(pamidronate) which is a salt of,

(zoledronic acid) or

(risedronate).

Subjects identified as having a liver virus infection (including hepatitis b, c, HIV or other viruses) will initiate aminobisphosphonate therapy for 45 days prior to re-screening to meet the recruitment criteria for LV-FDPS for the treatment of infectious diseases. Eligible subjects recruit the next available LV-FDPS administration category. A maximum of 3 subjects were enrolled per dose group. The LV-FDPS dose is the number of transduction units of LV-FDPS delivered in a single bolus via an intrahepatic cannula, and the volume does not exceed 25mL as described in the product release standard. Based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al, phase I clinical trial of thymidine kinase-based Gene therapy for advanced hepatocellular carcinoma (a phase I clinical trial of thymidine kinase-based Gene therapy in advanced hepatocellular carcinoma), 2010, cancer Gene ther.17 837-43), the minimum dose was 1×10^{9} Transduce the unit and increment by 10-fold to the next dose $1X10^{10}$ Transduction unit, next dose $1X10^{11}$ Transduction unit, and maximum dose of $1X10^{12}$ A transduction unit. Subjects were enrolled, treated and evaluated for 3 months. All safety assessments were completed for each group prior to enrollment and treatment of subjects at the next higher dose level. Recruitment and dose escalation continued until the maximum tolerated dose was reached or the study was terminated.

Cannulation was performed through the left subclavian artery until the catheter tip was at the appropriate hepatic artery junction. The cannula was guided by ultrasound examination as described (Lin et al, clinical efficacy of cisplatin, mitomycin C, interleukin and 5-Fluorouracil arterial perfusion chemotherapy on terminal parenchymal carcinoma that cannot be surgically removed (Clinical effects of intra-acute infusion chemotherapy with cispain, mitomycin C, leucovor and 5-fluoroouracil for unresectable advanced hepatocellular carcinoma), 2004, J.Chin.Med.Assoc.67.602-10.

Measurement of primary outcome

Safety: systematic and local area adverse events were ranked according to CTCAS and coded according to MedRA. Prior to dose escalation, adverse event data will be assessed for all subjects within a single dose range. The final safety assessment contained data for all dose ranges.

Measurement of secondary outcome

● Local administration and subsequent biopsy or autopsy to obtain post-tissue LV-FDPS lesion distribution and retention.

● Objective Response Rate (ORR) measured as a Sustained Viral Response (SVR) in the organ or in the whole body within 3 months after treatment.

● Levels of LV-FDPS in the blood 10 min, 30 min, 1 h and 1 day after local injection.

● Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT within 3 months after treatment.

● Patients survived beyond the historical control (no LV-FDPS) without history in the interim analysis.

Admission standard

● Over the age of 18 years, both male and female are included.

● Diagnosis is confirmed histologically or cytologically or based on currently accepted clinical criteria for chronic viral liver disease, and resection, transplantation or other potentially curative therapy is not appropriate at the time of screening.

● The treating physician determines that the lesion is suitable for local area targeted delivery.

● Kamofsky expressed as 60-80% of the ECOG value.

● The expected life is more than or equal to 12 weeks.

● Hematopoietic function: WBC is more than or equal to 2,500/mm ^{3} ; ANC≥1000/mm ^{3} (ii) a Blood red eggWhite is more than or equal to 8g/dL; the platelet count is more than or equal to 50,000/mm ^{3} (ii) a Coagulation INR is less than or equal to 1.3.

● AST and ALT <5 times ULN; ALPS <5 times ULN. Bilirubin is less than or equal to 1.5 times ULV; creatine is less than or equal to 1.5 times ULN and eGFR is more than or equal to 50.

● Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.

● The attending physician considers renal, cardiovascular and respiratory functions to be adequate.

● And (3) immune function: the circulating V gamma 9V delta 2+ T cells are more than or equal to 30/mm ^{3} (ii) a Has no immunodeficiency.

● Serological and viral RNA tests are negative for HIV.

● Written informed consent.

Exclusion criteria

● Chronic viral diseases suitable for excision, transplantation or other potentially curative therapy.

● Liver surgery or chemoembolization within the past 4 months.

● Liver radiation or total body radiation therapy over the past 4 months.

● Study drug with a half-life of <5 drugs or within 4 weeks.

● Impaired wound healing due to diabetes.

● Severe psychiatric illness, alcohol dependence or illegal drug use.

● Are reluctant to comply with research protocols and reporting requirements.

● Cardiovascular, cerebrovascular (stroke), immunological (excluding viral infections, viral hepatitis or cirrhosis), endocrine or central nervous system diseases of clinical significance; current brain disease; variceal bleeding requiring hospitalization or transfusion within the past 4 months.

● Pregnancy, lactation or refusal to use barrier or chemical contraceptives throughout the test and follow-up interval.

Sequence of

The following sequences are mentioned herein:

SEQ ID NO:	描述	序列
1	FDPS shRNA 序列#1	GTCCTGGAGTACAATGCCATTCTCGAGAATG GCATTGTACTCCAGGACTTTTT
2	FDPS shRNA 序列#2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGT GCTGAACGAAATCCTGCTTTTT
3	FDPS shRNA 序列#2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGT GCTGAACGAAATCCTGCTTTTT
4	FDPS shRNA 序列#4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTT TCTCAGCCTCCTTCTGCTTTTT
5	miR30 FDPS	AAGGTATATTGCTGTTGACAGTGAGCGACAC

	序列#1	TTTCTCAGCCTCCTTCTGCGTGAAGCCACAG ATGGCAGAAGGAGGCTGAGAAAGTGCTGCC TACTGCCTCGGACTTCAAGGGGCT
6	miR30 FDPS 序列#2	AAGGTATATTGCTGTTGACAGTGAGCGACAC TTTCTCAGCCTCCTTCTGCGTGAAGCCACAG ATGGCAGAAGGGCTGAGAAAGTGCTGCCTAC TGCCTCGGACTTCAAGGGGCT
7	miR30 FDPS 序列#3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCT CCTTCTGCGTGAAGCCACAGATGGCAGAAGG AGGCTGAGAAAGTTGCCTACTGCCTCGGA
8	miR155 FDPS 序列#1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGAC TTTCTCAGCCTCCTTCTGCTTTTGGCCACTGA CTGAGCAGAAGGGCTGAGAAAGTCAGGACA CAAGGCCTGTTACTAGCACTCA
9	miR21 FDPS 序列#1	CATCTCCATGGCTGTACCACCTTGTCGGGAC TTTCTCAGCCTCCTTCTGCCTGTTGAATCTCA TGGCAGAAGGAGGCGAGAAAGTCTGACATTT TGGTATCTTTCATCTGACCA
10	miR185 FDPS 序列#1	GGGCCTGGCTCGAGCAGGGGGCGAGGGATA CTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCC CGCAGAAGGAGGCTGAGAAAGTCCTTCCCTC CCAATGACCGCGTCTTCGTCG
11	劳氏肉瘤病毒(RSV)启动子	GTAGTCTTATGCAATACTCTTGTAGTCTTGCA ACATGGTAACGATGAGTTAGCAACATGCCTT ACAAGGAGAGAAAAAGCACCGTGCATGCCGA

		TTGGTGGAAGTAAGGTGGTACGATCGTGCCT TATTAGGAAGGCAACAGACGGGTCTGACATG GATTGGACGAACCACTGAATTGCCGCATTGC AGAGATATTGTATTTAAGTGCCTAGCTCGAT ACAATAAACG
12	5’长末端重复(LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTG GGAGCTCTCTGGCTAACTAGGGAACCCACTG CTTAAGCCTCAATAAAGCTTGCCTTGAGTGC TTCAAGTAGTGTGTGCCCGTCTGTTGTGTGA CTCTGGTAACTAGAGATCCCTCAGACCCTTT TAGTCAGTGTGGAAAATCTCTAGCA
13	Psi 包装信号	TACGCCAAAAATTTTGACTAGCGGAGGCTAG AAGGAGAGAG
14	Rev 响应元件(RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCA GCAGGAAGCACTATGGGCGCAGCCTCAATGA CGCTGACGGTACAGGCCAGACAATTATTGTC TGGTATAGTGCAGCAGCAGAACAATTTGCTG AGGGCTATTGAGGCGCAACAGCATCTGTTGC

		AACTCACAGTCTGGGGCATCAAGCAGCTCCA GGCAAGAATCCTGGCTGTGGAAAGATACCTA AAGGATCAACAGCTCC
15	中心聚嘌呤区(cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTAC AGTGCAGGGGAAAGAATAGTAGACATAATAG CAACAGACATACAAACTAAAGAATTACAAAA ACAAATTACAAAATTCAAAATTTTA
16	聚合酶 III shRNA 启动子; H1 启动子	GAACGCTGACGTCATCAACCCGCTCCAAGGA ATCGCGGGCCCAGTGTCACTAGGCGGGAAC ACCCAGCGCGCGTGCGCCCTGGCAGGAAGA TGGCTGTGAGGGACAGGGGAGTGGCGCCCT GCAATATTTGCATGTCGCTATGTGTTCTGGG AAATCACCATAAACGTGAAATGTCTTTGGAT TTGGGAATCTTATAAGTTCTGTATGAGACCA CTT
17	长 WPRE 序列	AATCAACCTCTGATTACAAAATTTGTGAAAG ATTGACTGGTATTCTTAACTATGTTGCTCCTT TTACGCTATGTGGATACGCTGCTTTAATGCC TTTGTATCATGCTATTGCTTCCCGTATGGCTT TCATTTTCTCCTCCTTGTATAAATCCTGGTTG CTGTCTCTTTATGAGGAGTTGTGGCCCGTTG TCAGGCAACGTGGCGTGGTGTGCACTGTGTT TGCTGACGCAACCCCCACTGGTTGGGGCATT GCCACCACCTGTCAGCTCCTTTCCGGGACTT TCGCTTTCCCCCTCCCTATTGCCACGGCGGA ACTCATCGCCGCCTGCCTTGCCCGCTGCTGG ACAGGGGCTCGGCTGTTGGGCACTGACAATT CCGTGGTGTTGTCGGGGAAATCATCGTCCTT TCCTTGGCTGCTCGCCTGTGTTGCCACCTGG

		ATTCTGCGCGGGACGTCCTTCTGCTACGTCC CTTCGGCCCTCAATCCAGCGGACCTTCCTTC CCGCGGCCTGCTGCCGGCTCTGCGGCCTCTT CCGCGTCTTCGCCTTCGCCCTCAGACGAGTC GGATCTCCCTTTGGGCCGCCTCCCCGCCT
18	3’ δLTR	TGGAAGGGCTAATTCACTCCCAACGAAGATA AGATCTGCTTTTTGCTTGTACTGGGTCTCTCT GGTTAGACCAGATCTGAGCCTGGGAGCTCTC TGGCTAACTAGGGAACCCACTGCTTAAGCCT CAATAAAGCTTGCCTTGAGTGCTTCAAGTAG TGTGTGCCCGTCTGTTGTGTGACTCTGGTAA CTAGAGATCCCTCAGACCCTTTTAGTCAGTG TGGAAAATCTCTAGCAGTAGTAGTTCATGTC A
19	辅助/Rev；鸡β肌动蛋白(CAG)启动子；	GCTATTACCATGGGTCGAGGTGAGCCCCACG TTCTGCTTCACTCTCCCCATCTCCCCCCCCTC CCCACCCCCAATTTTGTATTTATTTATTTTTT

	转录	AATTATTTTGTGCAGCGATGGGGGCGGGGG GGGGGGGGGCGCGCGCCAGGCGGGGCGGG GCGGGGCGAGGGGCGGGGCGGGGCGAGGC GGAGAGGTGCGGCGGCAGCCAATCAGAGCG GCGCGCTCCGAAAGTTTCCTTTTATGGCGAG GCGGCGGCGGCGGCGGCCCTATAAAAAGCG AAGCGCGCGGCGGGCG
20	辅助/Rev; HIV Gag; 病毒衣壳	ATGGGTGCGAGAGCGTCAGTATTAAGCGGG GGAGAATTAGATCGATGGGAAAAAATTCGGT TAAGGCCAGGGGGAAAGAAAAAATATAAATT AAAACATATAGTATGGGCAAGCAGGGAGCTA GAACGATTCGCAGTTAATCCTGGCCTGTTAG AAACATCAGAAGGCTGTAGACAAATACTGGG ACAGCTACAACCATCCCTTCAGACAGGATCA GAAGAACTTAGATCATTATATAATACAGTAG CAACCCTCTATTGTGTGCATCAAAGGATAGA GATAAAAGACACCAAGGAAGCTTTAGACAAG ATAGAGGAAGAGCAAAACAAAAGTAAGAAAA AAGCACAGCAAGCAGCAGCTGACACAGGACA CAGCAATCAGGTCAGCCAAAATTACCCTATA GTGCAGAACATCCAGGGGCAAATGGTACATC AGGCCATATCACCTAGAACTTTAAATGCATG GGTAAAAGTAGTAGAAGAGAAGGCTTTCAGC CCAGAAGTGATACCCATGTTTTCAGCATTAT CAGAAGGAGCCACCCCACAAGATTTAAACAC CATGCTAAACACAGTGGGGGGACATCAAGCA GCCATGCAAATGTTAAAAGAGACCATCAATG AGGAAGCTGCAGAATGGGATAGAGTGCATCC AGTGCATGCAGGGCCTATTGCACCAGGCCAG ATGAGAGAACCAAGGGGAAGTGACATAGCA

		GGAACTACTAGTACCCTTCAGGAACAAATAG **GATGGATGACACATAATCCACCTATCCCAGT** **AGGAGAAATCTATAAAAGATGGATAATCCTG** **GGATTAAATAAAATAGTAAGAATGTATAGCC** **CTACCAGCATTCTGGACATAAGACAAGGACC** **AAAGGAACCCTTTAGAGACTATGTAGACCGA** **TTCTATAAAACTCTAAGAGCCGAGCAAGCTT** **CACAAGAGGTAAAAAATTGGATGACAGAAAC** **CTTGTTGGTCCAAAATGCGAACCCAGATTGT** **AAGACTATTTTAAAAGCATTGGGACCAGGAG** **CGACACTAGAAGAAATGATGACAGCATGTCA** **GGGAGTGGGGGGACCCGGCCATAAAGCAAG** **AGTTTTGGCTGAAGCAATGAGCCAAGTAACA** **AATCCAGCTACCATAATGATACAGAAAGGCA** **ATTTTAGGAACCAAAGAAAGACTGTTAAGTG** **TTTCAATTGTGGCAAAGAAGGGCACATAGCC** **AAAAATTGCAGGGCCCCTAGGAAAAAGGGCT**

		GTTGGAAATGTGGAAAGGAAGGACACCAAAT GAAAGATTGTACTGAGAGACAGGCTAATTTT TTAGGGAAGATCTGGCCTTCCCACAAGGGAA GGCCAGGGAATTTTCTTCAGAGCAGACCAGA GCCAACAGCCCCACCAGAAGAGAGCTTCAGG TTTGGGGAAGAGACAACAACTCCCTCTCAGA AGCAGGAGCCGATAGACAAGGAACTGTATCC TTTAGCTTCCCTCAGATCACTCTTTGGCAGC GACCCCTCGTCACAATAA
21	辅助/Rev; HIV Pol; 蛋白酶和逆转录酶	ATGAATTTGCCAGGAAGATGGAAACCAAAAA TGATAGGGGGAATTGGAGGTTTTATCAAAGT AGGACAGTATGATCAGATACTCATAGAAATC TGCGGACATAAAGCTATAGGTACAGTATTAG TAGGACCTACACCTGTCAACATAATTGGAAG AAATCTGTTGACTCAGATTGGCTGCACTTTA AATTTTCCCATTAGTCCTATTGAGACTGTACC AGTAAAATTAAAGCCAGGAATGGATGGCCCA AAAGTTAAACAATGGCCATTGACAGAAGAAA AAATAAAAGCATTAGTAGAAATTTGTACAGA AATGGAAAAGGAAGGAAAAATTTCAAAAATT GGGCCTGAAAATCCATACAATACTCCAGTAT TTGCCATAAAGAAAAAAGACAGTACTAAATG GAGAAAATTAGTAGATTTCAGAGAACTTAAT AAGAGAACTCAAGATTTCTGGGAAGTTCAAT TAGGAATACCACATCCTGCAGGGTTAAAACA GAAAAAATCAGTAACAGTACTGGATGTGGGC GATGCATATTTTTCAGTTCCCTTAGATAAAGA CTTCAGGAAGTATACTGCATTTACCATACCT AGTATAAACAATGAGACACCAGGGATTAGAT ATCAGTACAATGTGCTTCCACAGGGATGGAA

		AGGATCACCAGCAATATTCCAGTGTAGCATG **ACAAAAATCTTAGAGCCTTTTAGAAAACAAA** **ATCCAGACATAGTCATCTATCAATACATGGA** **TGATTTGTATGTAGGATCTGACTTAGAAATA** **GGGCAGCATAGAACAAAAATAGAGGAACTGA** **GACAACATCTGTTGAGGTGGGGATTTACCAC** **ACCAGACAAAAAACATCAGAAAGAACCTCCA** **TTCCTTTGGATGGGTTATGAACTCCATCCTG** **ATAAATGGACAGTACAGCCTATAGTGCTGCC** **AGAAAAGGACAGCTGGACTGTCAATGACATA** **CAGAAATTAGTGGGAAAATTGAATTGGGCAA** **GTCAGATTTATGCAGGGATTAAAGTAAGGCA** **ATTATGTAAACTTCTTAGGGGAACCAAAGCA** **CTAACAGAAGTAGTACCACTAACAGAAGAAG** **CAGAGCTAGAACTGGCAGAAAACAGGGAGAT** **TCTAAAAGAACCGGTACATGGAGTGTATTAT** **GACCCATCAAAAGACTTAATAGCAGAAATAC**

		AGAAGCAGGGGCAAGGCCAATGGACATATCA AATTTATCAAGAGCCATTTAAAAATCTGAAAA CAGGAAAATATGCAAGAATGAAGGGTGCCCA CACTAATGATGTGAAACAATTAACAGAGGCA GTACAAAAAATAGCCACAGAAAGCATAGTAA TATGGGGAAAGACTCCTAAATTTAAATTACC CATACAAAAGGAAACATGGGAAGCATGGTGG ACAGAGTATTGGCAAGCCACCTGGATTCCTG AGTGGGAGTTTGTCAATACCCCTCCCTTAGT GAAGTTATGGTACCAGTTAGAGAAAGAACCC ATAATAGGAGCAGAAACTTTCTATGTAGATG GGGCAGCCAATAGGGAAACTAAATTAGGAAA AGCAGGATATGTAACTGACAGAGGAAGACAA AAAGTTGTCCCCCTAACGGACACAACAAATC AGAAGACTGAGTTACAAGCAATTCATCTAGC TTTGCAGGATTCGGGATTAGAAGTAAACATA GTGACAGACTCACAATATGCATTGGGAATCA TTCAAGCACAACCAGATAAGAGTGAATCAGA GTTAGTCAGTCAAATAATAGAGCAGTTAATA AAAAAGGAAAAAGTCTACCTGGCATGGGTAC CAGCACACAAAGGAATTGGAGGAAATGAACA AGTAGATGGGTTGGTCAGTGCTGGAATCAGG AAAGTACTA
22	辅助 Rev; HIV 整合酶; 病毒RNA的整合	TTTTTAGATGGAATAGATAAGGCCCAAGAAG AACATGAGAAATATCACAGTAATTGGAGAGC AATGGCTAGTGATTTTAACCTACCACCTGTA GTAGCAAAAGAAATAGTAGCCAGCTGTGATA AATGTCAGCTAAAAGGGGAAGCCATGCATGG ACAAGTAGACTGTAGCCCAGGAATATGGCAG CTAGATTGTACACATTTAGAAGGAAAAGTTA

		TCTTGGTAGCAGTTCATGTAGCCAGTGGATA **TATAGAAGCAGAAGTAATTCCAGCAGAGACA** **GGGCAAGAAACAGCATACTTCCTCTTAAAAT** **TAGCAGGAAGATGGCCAGTAAAAACAGTACA** **TACAGACAATGGCAGCAATTTCACCAGTACT** **ACAGTTAAGGCCGCCTGTTGGTGGGCGGGG** **ATCAAGCAGGAATTTGGCATTCCCTACAATC** **CCCAAAGTCAAGGAGTAATAGAATCTATGAA** **TAAAGAATTAAAGAAAATTATAGGACAGGTA** **AGAGATCAGGCTGAACATCTTAAGACAGCAG** **TACAAATGGCAGTATTCATCCACAATTTTAAA** **AGAAAAGGGGGGATTGGGGGGTACAGTGCA** **GGGGAAAGAATAGTAGACATAATAGCAACAG** **ACATACAAACTAAAGAATTACAAAAACAAAT** **TACAAAAATTCAAAATTTTCGGGTTTATTACA** **GGGACAGCAGAGATCCAGTTTGGAAAGGAC** **CAGCAAAGCTCCTCTGGAAAGGTGAAGGGG**

		CAGTAGTAATACAAGATAATAGTGACATAAA AGTAGTGCCAAGAAGAAAAGCAAAGATCATC AGGGATTATGGAAAACAGATGGCAGGTGATG ATTGTGTGGCAAGTAGACAGGATGAGGATTA A
23	辅助/Rev; HIV RRE; 结合 Rev 元件	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCA GCAGGAAGCACTATGGGCGCAGCGTCAATG ACGCTGACGGTACAGGCCAGACAATTATTGT CTGGTATAGTGCAGCAGCAGAACAATTTGCT GAGGGCTATTGAGGCGCAACAGCATCTGTTG CAACTCACAGTCTGGGGCATCAAGCAGCTCC AGGCAAGAATCCTGGCTGTGGAAAGATACCT AAAGGATCAACAGCTCCT
24	辅助/Rev; HIV Rev; 核输出和稳定化病毒 mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAA GAACTCCTCAAGGCAGTCAGACTCATCAAGT TTCTCTATCAAAGCAACCCACCTCCCAATCCC GAGGGGACCCGACAGGCCCGAAGGAATAGA AGAAGAAGGTGGAGAGAGAGACAGAGACAG ATCCATTCGATTAGTGAACGGATCCTTAGCA CTTATCTGGGACGATCTGCGGAGCCTGTGCC TCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTG GGACGCAGGGGGTGGGAAGCCCTCAAATAT TGGTGGAATCTCCTACAATATTGGAGTCAGG AGCTAAAGAATAG
25	包膜; CMV 启动子; 转录	ACATTGATTATTGACTAGTTATTAATAGTAAT CAATTACGGGGTCATTAGTTCATAGCCCATA TATGGAGTTCCGCGTTACATAACTTACGGTA AATGGCCCGCCTGGCTGACCGCCCAACGACC

		TCCCATAGTAACGCCAATAGGGACTTTCCAT TGACGTCAATGGGTGGAGTATTTACGGTAAA CTGCCCACTTGGCAGTACATCAAGTGTATCA TATGCCAAGTACGCCCCCTATTGACGTCAAT GACGGTAAATGGCCCGCCTGGCATTATGCCC AGTACATGACCTTATGGGACTTTCCTACTTG GCAGTACATCTACGTATTAGTCATCGCTATT ACCATGGTGATGCGGTTTTGGCAGTACATCA ATGGGCGTGGATAGCGGTTTGACTCACGGG GATTTCCAAGTCTCCACCCCATTGACGTCAA TGGGAGTTTGTTTTGGCACCAAAATCAACGG GACTTTCCAAAATGTCGTAACAACTCCGCCC CATTGACGCAAATGGGCGGTAGGCGTGTACG GTGGGAGGTCTATATAAGC
26	包膜；VSV-G;糖蛋白包膜-细胞进入	ATGAAGTGCCTTTTGTACTTAGCCTTTTTATT CATTGGGGTGAATTGCAAGTTCACCATAGTT TTTCCACACAACCAAAAAGGAAACTGGAAAA

		ATGTTCCTTCTAATTACCATTATTGCCCGTCA AGCTCAGATTTAAATTGGCATAATGACTTAA TAGGCACAGCCTTACAAGTCAAAATGCCCAA GAGTCACAAGGCTATTCAAGCAGACGGTTGG ATGTGTCATGCTTCCAAATGGGTCACTACTT GTGATTTCCGCTGGTATGGACCGAAGTATAT AACACATTCCATCCGATCCTTCACTCCATCTG TAGAACAATGCAAGGAAAGCATTGAACAAAC GAAACAAGGAACTTGGCTGAATCCAGGCTTC CCTCCTCAAAGTTGTGGATATGCAACTGTGA CGGATGCCGAAGCAGTGATTGTCCAGGTGAC TCCTCACCATGTGCTGGTTGATGAATACACA GGAGAATGGGTTGATTCACAGTTCATCAACG GAAAATGCAGCAATTACATATGCCCCACTGT CCATAACTCTACAACCTGGCATTCTGACTATA AGGTCAAAGGGCTATGTGATTCTAACCTCAT TTCCATGGACATCACCTTCTTCTCAGAGGAC GGAGAGCTATCATCCCTGGGAAAGGAGGGC ACAGGGTTCAGAAGTAACTACTTTGCTTATG AAACTGGAGGCAAGGCCTGCAAAATGCAATA CTGCAAGCATTGGGGAGTCAGACTCCCATCA GGTGTCTGGTTCGAGATGGCTGATAAGGATC TCTTTGCTGCAGCCAGATTCCCTGAATGCCC AGAAGGGTCAAGTATCTCTGCTCCATCTCAG ACCTCAGTGGATGTAAGTCTAATTCAGGACG TTGAGAGGATCTTGGATTATTCCCTCTGCCA AGAAACCTGGAGCAAAATCAGAGCGGGTCTT CCAATCTCTCCAGTGGATCTCAGCTATCTTG CTCCTAAAAACCCAGGAACCGGTCCTGCTTT CACCATAATCAATGGTACCCTAAAATACTTTG

		AGACCAGATACATCAGAGTCGATATTGCTGC TCCAATCCTCTCAAGAATGGTCGGAATGATC AGTGGAACTACCACAGAAAGGGAACTGTGG GATGACTGGGCACCATATGAAGACGTGGAAA TTGGACCCAATGGAGTTCTGAGGACCAGTTC AGGATATAAGTTTCCTTTATACATGATTGGA CATGGTATGTTGGACTCCGATCTTCATCTTA GCTCAAAGGCTCAGGTGTTCGAACATCCTCA CATTCAAGACGCTGCTTCGCAACTTCCTGAT GATGAGAGTTTATTTTTTGGTGATACTGGGC TATCCAAAAATCCAATCGAGCTTGTAGAAGG TTGGTTCAGTAGTTGGAAAAGCTCTATTGCC TCTTTTTTCTTTATCATAGGGTTAATCATTGG ACTATTCTTGGTTCTCCGAGTTGGTATCCATC TTTGCATTAAATTAAAGCACACCAAGAAAAG ACAGATTTATACAGACATAGAGATGA
27	辅　助　/Rev;	TAGTTATTAATAGTAATCAATTACGGGGTCA

	CMV 早期(CAG)增强子；增强转录	TTAGTTCATAGCCCATATATGGAGTTCCGCG TTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACG TCAATAATGACGTATGTTCCCATAGTAACGC CAATAGGGACTTTCCATTGACGTCAATGGGT GGACTATTTACGGTAAACTGCCCACTTGGCA GTACATCAAGTGTATCATATGCCAAGTACGC CCCCTATTGACGTCAATGACGGTAAATGGCC CGCCTGGCATTATGCCCAGTACATGACCTTA TGGGACTTTCCTACTTGGCAGTACATCTACG TATTAGTCATC
28	辅助/Rev；鸡β肌动蛋白内含子；增强基因表达	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCC CGCTCCGCGCCGCCTCGCGCCGCCCGCCCCG GCTCTGACTGACCGCGTTACTCCCACAGGTG AGCGGGCGGGACGGCCCTTCTCCTCCGGGC TGTAATTAGCGCTTGGTTTAATGACGGCTCG TTTCTTTTCTGTGGCTGCGTGAAAGCCTTAA AGGGCTCCGGGAGGGCCCTTTGTGCGGGGG GGAGCGGCTCGGGGGGTGCGTGCGTGTGTG TGTGCGTGGGGAGCGCCGCGTGCGGCCCGC GCTGCCCGGCGGCTGTGAGCGCTGCGGGCG CGGCGCGGGGCTTTGTGCGCTCCGCGTGTG CGCGAGGGGAGCGCGGCCGGGGGCGGTGCC CCGCGGTGCGGGGGGGCTGCGAGGGGAACA AAGGCTGCGTGCGGGGTGTGTGCGTGGGGG GGTGAGCAGGGGGTGTGGGCGCGGCGGTCG GGCTGTAACCCCCCCCTGCACCCCCCTCCCC GAGTTGCTGAGCACGGCCCGGCTTCGGGTG CGGGGCTCCGTGCGGGGCGTGGCGCGGGGC TCGCCGTGCCGGGCGGGGGGTGGCGGCAGG

		TGGGGGTGCCGGGCGGGGCGGGGCCGCCTC GGGCCGGGGAGGGCTCGGGGGAGGGGCGC GGCGGCCCCGGAGCGCCGGCGGCTGTCGAG GCGCGGCGAGCCGCAGCCATTGCCTTTTATG GTAATCGTGCGAGAGGGCGCAGGGACTTCCT TTGTCCCAAATCTGGCGGAGCCGAAATCTGG GAGGCGCCGCCGCACCCCCTCTAGCGGGCG CGGGCGAAGCGGTGCGGCGCCGGCAGGAAG GAAATGGGCGGGGAGGGCCTTCGTGCGTCG CCGCGCCGCCGTCCCCTTCTCCATCTCCAGC CTCGGGGCTGCCGCAGGGGGACGGCTGCCT TCGGGGGGGACGGGGCAGGGCGGGGTTCGG CTTCTGGCGTGTGACCGGCGG
29	辅助/Rev；兔β球蛋白聚 A；RNA 稳定性	AGATCTTTTTCCCTCTGCCAAAAATTATGGG GACATCATGAAGCCCCTTGAGCATCTGACTT CTGGCTAATAAAGGAAATTTATTTTCATTGCA ATAGTGTGTTGGAATTTTTTGTGTCTCTCACT

		CGGAAGGACATATGGGAGGGCAAATCATTTA AAACATCAGAATGAGTATTTGGTTTAGAGTT TGGCAACATATGCCATATGCTGGCTGCCATG AACAAAGGTGGCTATAAAGAGGTCATCAGTA TATGAAACAGCCCCCTGCTGTCCATTCCTTA TTCCATAGAAAAGCCTTGACTTGAGGTTAGA TTTTTTTTATATTTTGTTTTGTGTTATTTTTTT CTTTAACATCCCTAAAATTTTCCTTACATGTT TTACTAGCCAGATTTTTCCTCCTCTCCTGACT ACTCCCAGTCATAGCTGTCCCTCTTCTCTTAT GAAGATC
30	包膜; β 球蛋白内含子；增强基因表达	GTGAGTTTGGGGACCCTTGATTGTTCTTTCT TTTTCGCTATTGTAAAATTCATGTTATATGGA GGGGGCAAAGTTTTCAGGGTGTTGTTTAGAA TGGGAAGATGTCCCTTGTATCACCATGGACC CTCATGATAATTTTGTTTCTTTCACTTTCTAC TCTGTTGACAACCATTGTCTCCTCTTATTTTC TTTTCATTTTCTGTAACTTTTTCGTTAAACTT TAGCTTGCATTTGTAACGAATTTTTAAATTCA CTTTTGTTTATTTGTCAGATTGTAAGTACTTT CTCTAATCACTTTTTTTTCAAGGCAATCAGGG TATATTATATTGTACTTCAGCACAGTTTTAGA GAACAATTGTTATAATTAAATGATAAGGTAG AATATTTCTGCATATAAATTCTGGCTGGCGT GGAAATATTCTTATTGGTAGAAACAACTACA CCCTGGTCATCATCCTGCCTTTCTCTTTATGG TTACAATGATATACACTGTTTGAGATGAGGA TAAAATACTCTGAGTCCAAACCGGGCCCCTC TGCTAACCATGTTCATGCCTTCTTCTCTTTCC TACAG

31	包膜; 兔 β 球蛋白聚 A; RNA 稳定性	AGATCTTTTTCCCTCTGCCAAAAATTATGGG GACATCATGAAGCCCCTTGAGCATCTGACTT CTGGCTAATAAAGGAAATTTATTTTCATTGCA ATAGTGTGTTGGAATTTTTTGTGTCTCTCACT CGGAAGGACATATGGGAGGGCAAATCATTTA AAACATCAGAATGAGTATTTGGTTTAGAGTT TGGCAACATATGCCCATATGCTGGCTGCCAT GAACAAAGGTTGGCTATAAAGAGGTCATCAG TATATGAAACAGCCCCCTGCTGTCCATTCCT TATTCCATAGAAAAGCCTTGACTTGAGGTTA GATTTTTTTTATATTTTGTTTTGTGTTATTTTT TTCTTTAACATCCCTAAAATTTTCCTTACATG TTTTACTAGCCAGATTTTTCCTCCTCTCCTGA CTACTCCCAGTCATAGCTGTCCCTCTTCTCTT ATGGAGATC
32	引物	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
33	引物	CCATACAATGAATGGACACTAGGCGGCCGCA

		CGAAT
34	Gag, Pol, 整合酶片段	GAATTCATGAATTTGCCAGGAAGATGGAAAC CAAAAATGATAGGGGGAATTGGAGGTTTTAT CAAAGTAAGACAGTATGATCAGATACTCATA GAAATCTGCGGACATAAAGCTATAGGTACAG TATTAGTAGGACCTACACCTGTCAACATAAT TGGAAGAAATCTGTTGACTCAGATTGGCTGC ACTTTAAATTTTCCCATTAGTCCTATTGAGAC TGTACCAGTAAAATTAAAGCCAGGAATGGAT GGCCCAAAAGTTAAACAATGGCCATTGACAG AAGAAAAAATAAAAGCATTAGTAGAAATTTG TACAGAAATGGAAAAGGAAGGAAAAATTTCA AAAATTGGGCCTGAAAATCCATACAATACTC CAGTATTTGCCATAAAGAAAAAAGACAGTAC TAAATGGAGAAAATTAGTAGATTTCAGAGAA CTTAATAAGAGAACTCAAGATTTCTGGGAAG TTCAATTAGGAATACCACATCCTGCAGGGTT AAAACAGAAAAAATCAGTAACAGTACTGGAT GTGGGCGATGCATATTTTTCAGTTCCCTTAG ATAAAGACTTCAGGAAGTATACTGCATTTAC CATACCTAGTATAAACAATGAGACACCAGGG ATTAGATATCAGTACAATGTGCTTCCACAGG GATGGAAAGGATCACCAGCAATATTCCAGTG TAGCATGACAAAAATCTTAGAGCCTTTTAGA AAACAAAATCCAGACATAGTCATCTATCAAT ACATGGATGATTTGTATGTAGGATCTGACTT AGAAATAGGGCAGCATAGAACAAAAATAGAG GAACTGAGACAACATCTGTTGAGGTGGGGAT TTACCACACCAGACAAAAAACATCAGAAAGA ACCTCCATTCCTTTGGATGGGTTATGAACTC

		CATCCTGATAAATGGACAGTACAGCCTATAG TGCTGCCAGAAAAGGACAGCTGGACTGTCAA TGACATACAGAAATTAGTGGGAAAATTGAAT TGGGCAAGTCAGATTTATGCAGGGATTAAAG TAAGGCAATTATGTAAACTTCTTAGGGGAAC CAAAGCACTAACAGAAGTAGTACCACTAACA GAAGAAGCAGAGCTAGAACTGGCAGAAAACA GGGAGATTCTAAAAGAACCGGTACATGGAGT GTATTATGACCCATCAAAAGACTTAATAGCA GAAATACAGAAGCAGGGGCAAGGCCAATGG ACATATCAAATTTATCAAGAGCCATTTAAAAA TCTGAAAACAGGAAAGTATGCAAGAATGAAG GGTGCCCACACTAATGATGTGAAACAATTAA CAGAGGCAGTACAAAAAATAGCCACAGAAAG CATAGTAATATGGGGAAAGACTCCTAAATTT AAATTACCCATACAAAAGGAAACATGGGAAG CATGGTGGACAGAGTATTGGCAAGCCACCTG

		GATTCCTGAGTGGGAGTTTGTCAATACCCCT CCCTTAGTGAAGTTATGGTACCAGTTAGAGA AAGAACCCATAATAGGAGCAGAAACTTTCTA TGTAGATGGGGCAGCCAATAGGGAAACTAAA TTAGGAAAAGCAGGATATGTAACTGACAGAG GAAGACAAAAAGTTGTCCCCCTAACGGACAC AACAAATCAGAAGACTGAGTTACAAGCAATT CATCTAGCTTTGCAGGATTCGGGATTAGAAG TAAACATAGTGACAGACTCACAATATGCATT GGGAATCATTCAAGCACAACCAGATAAGAGT GAATCAGAGTTAGTCAGTCAAATAATAGAGC AGTTAATAAAAAAGGAAAAAGTCTACCTGGC ATGGGTACCAGCACACAAAGGAATTGGAGGA AATGAACAAGTAGATAAATTGGTCAGTGCTG GAATCAGGAAAGTACTATTTTTAGATGGAAT AGATAAGGCCCAAGAAGAACATGAGAAATAT CACAGTAATTGGAGAGCAATGGCTAGTGATT TTAACCTACCACCTGTAGTAGCAAAAGAAAT AGTAGCCAGCTGTGATAAATGTCAGCTAAAA GGGGAAGCCATGCATGGACAAGTAGACTGTA GCCCAGGAATATGGCAGCTAGATTGTACACA TTTAGAAGGAAAAGTTATCTTGGTAGCAGTT CATGTAGCCAGTGGATATATAGAAGCAGAAG TAATTCCAGCAGAGACAGGGCAAGAAACAGC ATACTTCCTCTTAAAATTAGCAGGAAGATGG CCAGTAAAAACAGTACATACAGACAATGGCA GCAATTTCACCAGTACTACAGTTAAGGCCGC CTGTTGGTGGGCGGGGATCAAGCAGGAATTT GGCATTCCCTACAATCCCCAAAGTCAAGGAG TAATAGAATCTATGAATAAAGAATTAAAGAA

		AATTATAGGACAGGTAAGAGATCAGGCTGAA CATCTTAAGACAGCAGTACAAATGGCAGTAT TCATCCACAATTTTAAAAGAAAAGGGGGGAT TGGGGGGTACAGTGCAGGGGAAAGAATAGT AGACATAATAGCAACAGACATACAAACTAAA GAATTACAAAAACAAATTACAAAAATTCAAA ATTTTCGGGTTTATTACAGGGACAGCAGAGA TCCAGTTTGGAAAGGACCAGCAAAGCTCCTC TGGAAAGGTGAAGGGGCAGTAGTAATACAA GATAATAGTGACATAAAAGTAGTGCCAAGAA GAAAAGCAAAGATCATCAGGGATTATGGAAA ACAGATGGCAGGTGATGATTGTGTGGCAAGT AGACAGGATGAGGATTAA
35	含 Rev 的 DNA 片段，RRE 和兔 β 球蛋白聚 A	TCTAGAATGGCAGGAAGAAGCGGAGACAGC GACGAAGAGCTCATCAGAACAGTCAGACTCA TCAAGCTTCTCTATCAAAGCAACCCACCTCC CAATCCCGAGGGGACCCGACAGGCCCGAAG

		GAATAGAAGAAGAAGGTGGAGAGAGACA GAGACAGATCCATTCGATTAGTGAACGGATC CTTGGCACTTATCTGGGACGATCTGCGGAGC CTGTGCCTCTTCAGCTACCACCGCTTGAGAG ACTTACTCTTGATTGTAACGAGGATTGTGGA ACTTCTGGGACGCAGGGGGTGGGAAGCCCT CAAATATTGGTGGAATCTCCTACAATATTGG AGTCAGGAGCTAAAGAATAGAGGAGCTTTGT TCCTTGGGTTCTTGGGAGCAGCAGGAAGCAC TATGGGCGCAGCGTCAATGACGCTGACGGTA CAGGCCAGACAATTATTGTCTGGTATAGTGC AGCAGCAGAACAATTTGCTGAGGGCTATTGA GGCGCAACAGCATCTGTTGCAACTCACAGTC TGGGGCATCAAGCAGCTCCAGGCAAGAATCC TGGCTGTGGAAAGATACCTAAAGGATCAACA GCTCCTAGATCTTTTTCCCTCTGCCAAAAATT ATGGGGACATCATGAAGCCCCTTGAGCATCT GACTTCTGGCTAATAAAGGAAATTTATTTTCA TTGCAATAGTGTGTTGGAATTTTTTGTGTCTC TCACTCGGAAGGACATATGGGAGGGCAAATC ATTTAAAACATCAGAATGAGTATTTGGTTTA GAGTTTGGCAACATATGCCATATGCTGGCTG CCATGAACAAAGGTGGCTATAAAGAGGTCAT CAGTATATGAAACAGCCCCCTGCTGTCCATT CCTTATTCCATAGAAAAGCCTTGACTTGAGG TTAGATTTTTTTTATATTTTGTTTTGTGTTATT TTTTTCTTTAACATCCCTAAAATTTTCCTTAC ATGTTTTACTAGCCAGATTTTTCCTCCTCTCC TGACTACTCCCAGTCATAGCTGTCCCTCTTCT CTTATGAAGATCCCTCGACCTGCAGCCCAAG

		CTTGGCGTAATCATGGTCATAGCTGTTTCCT GTGTGAAATTGTTATCCGCTCACAATTCCAC ACAACATACGAGCCGGAAGCATAAAGTGTAA AGCCTGGGGTGCCTAATGAGTGAGCTAACTC ACATTAATTGCGTTGCGCTCACTGCCCGCTT TCCAGTCGGGAAACCTGTCGTGCCAGCGGAT CCGCATCTCAATTAGTCAGCAACCATAGTCC CGCCCCTAACTCCGCCCATCCCGCCCCTAAC TCCGCCCAGTTCCGCCCATTCTCCGCCCCAT GGCTGACTAATTTTTTTTATTTATGCAGAGGC CGAGGCCGCCTCGGCCTCTGAGCTATTCCAG AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAG GCTTTTGCAAAAAGCTAACTTGTTTATTGCA GCTTATAATGGTTACAAATAAAGCAATAGCA TCACAAATTTCACAAATAAAGCATTTTTTTCA CTGCATTCTAGTTGTGGTTTGTCCAAACTCAT CAATGTATCTTATCAGCGGCCGCCCCGGG

36	含 CAG 增强子/启动子/内含子序列的 DNA 片段	ACGCGTTAGTTATTAATAGTAATCAATTACG GGGTCATTAGTTCATAGCCCATATATGGAGT TCCGCGTTACATAACTTACGGTAAATGGCCC GCCTGGCTGACCGCCCAACGACCCCCGCCCA TTGACGTCAATAATGACGTATGTTCCCATAG TAACGCCAATAGGGACTTTCCATTGACGTCA ATGGGTGGACTATTTACGGTAAACTGCCCAC TTGGCAGTACATCAAGTGTATCATATGCCAA GTACGCCCCCTATTGACGTCAATGACGGTAA ATGGCCCGCCTGGCATTATGCCCAGTACATG ACCTTATGGGACTTTCCTACTTGGCAGTACA TCTACGTATTAGTCATCGCTATTACCATGGG TCGAGGTGAGCCCCACGTTCTGCTTCACTCT CCCCATCTCCCCCCCCTCCCCACCCCCAATTT TGTATTTATTTATTTTTTAATTATTTTGTGCA GCGATGGGGGCGGGGGGGGGGGGGGCGCG CGCCAGGCGGGGCGGGGCGGGGCGAGGGG CGGGGCGGGGCGAGGCGGAGAGGTGCGGCG GCAGCCAATCAGAGCGGCGCGCTCCGAAAGT TTCCTTTTATGGCGAGGCGGCGGCGGCGGC GGCCCTATAAAAAGCGAAGCGCGCGGCGGG CGGGAGTCGCTGCGTTGCCTTCGCCCCGTGC CCCGCTCCGCGCCGCCTCGCGCCGCCCGCCC CGGCTCTGACTGACCGCGTTACTCCCACAGG TGAGCGGGCGGGACGGCCCTTCTCCTCCGG GCTGTAATTAGCGCTTGGTTTAATGACGGCT CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTT AAAGGGCTCCGGGAGGGCCCTTTGTGCGGG GGGGAGCGGCTCGGGGGGTGCGTGCGTGTG TGTGTGCGTGGGGAGCGCCGCGTGCGGCCC

		GCGCTGCCCGGCGGCTGTGAGCGCTGCGGG CGCGGCGCGGGGCTTTGTGCGCTCCGCGTG TGCGCGAGGGGAGCGCGGCCGGGGGCGGTG CCCCGCGGTGCGGGGGGGCTGCGAGGGGAA CAAAGGCTGCGTGCGGGGTGTGTGCGTGGG GGGGTGAGCAGGGGGTGTGGGCGCGGCGGT CGGGCTGTAACCCCCCCCTGCACCCCCCTCC CCGAGTTGCTGAGCACGGCCCGGCTTCGGGT GCGGGGCTCCGTGCGGGGCGTGGCGCGGGG CTCGCCGTGCCGGGCGGGGGGTGGCGGCAG GTGGGGGTGCCGGGCGGGGCGGGGCCGCCT CGGGCCGGGGAGGGCTCGGGGGAGGGGCGC GGCGGCCCCGGAGCGCCGGCGGCTGTCGAG GCGCGGCGAGCCGCAGCCATTGCCTTTTATG GTAATCGTGCGAGAGGGCGCAGGGACTTCCT TTGTCCCAAATCTGGCGGAGCCGAAATCTGG GAGGCGCCGCCGCACCCCCTCTAGCGGGCG

		CGGGCGAAGCGGTGCGGCGCCGGCAGGAAG GAAATGGGCGGGGAGGGCCTTCGTGCGTCG CCGCGCCGCCGTCCCCTTCTCCATCTCCAGC CTCGGGGCTGCCGCAGGGGGACGGCTGCCT TCGGGGGGGACGGGGCAGGGCGGGGTTCGG CTTCTGGCGTGTGACCGGCGGGAATTC
37	含 VSV-G 的 DNA 片段	GAATTCATGAAGTGCCTTTTGTACTTAGCCTT TTTATTCATTGGGGTGAATTGCAAGTTCACC ATAGTTTTTCCACACAACCAAAAAGGAAACT GGAAAAATGTTCCTTCTAATTACCATTATTGC CCGTCAAGCTCAGATTTAAATTGGCATAATG ACTTAATAGGCACAGCCTTACAAGTCAAAAT GCCCAAGAGTCACAAGGCTATTCAAGCAGAC GGTTGGATGTGTCATGCTTCCAAATGGGTCA CTACTTGTGATTTCCGCTGGTATGGACCGAA GTATATAACACATTCCATCCGATCCTTCACTC CATCTGTAGAACAATGCAAGGAAAGCATTGA ACAAACGAAACAAGGAACTTGGCTGAATCCA GGCTTCCCTCCTCAAAGTTGTGGATATGCAA CTGTGACGGATGCCGAAGCAGTGATTGTCCA GGTGACTCCTCACCATGTGCTGGTTGATGAA TACACAGGAGAATGGGTTGATTCACAGTTCA TCAACGGAAAATGCAGCAATTACATATGCCC CACTGTCCATAACTCTACAACCTGGCATTCT GACTATAAGGTCAAAGGGCTATGTGATTCTA ACCTCATTTCCATGGACATCACCTTCTTCTCA GAGGACGGAGAGCTATCATCCCTGGGAAAG GAGGGCACAGGGTTCAGAAGTAACTACTTTG CTTATGAAACTGGAGGCAAGGCCTGCAAAAT GCAATACTGCAAGCATTGGGGAGTCAGACTC

		CCATCAGGTGTCTGGTTCGAGATGGCTGATA **AGGATCTCTTTGCTGCAGCCAGATTCCCTGA** **ATGCCCAGAAGGGTCAAGTATCTCTGCTCCA** **TCTCAGACCTCAGTGGATGTAAGTCTAATTC** **AGGACGTTGAGAGGATCTTGGATTATTCCCT** **CTGCCAAGAAACCTGGAGCAAAATCAGAGCG** **GGTCTTCCAATCTCTCCAGTGGATCTCAGCT** **ATCTTGCTCCTAAAAACCCAGGAACCGGTCC** **TGCTTTCACCATAATCAATGGTACCCTAAAAT** **ACTTTGAGACCAGATACATCAGAGTCGATAT** **TGCTGCTCCAATCCTCTCAAGAATGGTCGGA** **ATGATCAGTGGAACTACCACAGAAAGGGAAC** **TGTGGGATGACTGGGCACCATATGAAGACGT** **GGAAATTGGACCCAATGGAGTTCTGAGGACC** **AGTTCAGGATATAAGTTTCCTTTATACATGAT** **TGGACATGGTATGTTGGACTCCGATCTTCAT** **CTTAGCTCAAAGGCTCAGGTGTTCGAACATC**

		CTCACATTCAAGACGCTGCTTCGCAACTTCC TGATGATGAGAGTTTATTTTTTGGTGATACT GGGCTATCCAAAAATCCAATCGAGCTTGTAG AAGGTTGGTTCAGTAGTTGGAAAAGCTCTAT TGCCTCTTTTTTCTTTATCATAGGGTTAATCA TTGGACTATTCTTGGTTCTCCGAGTTGGTAT CCATCTTTGCATTAAATTAAAGCACACCAAG AAAAGACAGATTTATACAGACATAGAGATGA GAATTC
38	Rev; RSV 启动子; 转录	ATGGCAGGAAGAAGCGGAGACAGCGACGAA GAACTCCTCAAGGCAGTCAGACTCATCAAGT TTCTCTATCAAAGCAACCCACCTCCCAATCCC GAGGGGACCCGACAGGCCCGAAGGAATAGA AGAAGAAGGTGGAGAGAGAGACAGAGACAG ATCCATTCGATTAGTGAACGGATCCTTAGCA CTTATCTGGGACGATCTGCGGAGCCTGTGCC TCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTG GGACGCAGGGGGTGGGAAGCCCTCAAATAT TGGTGGAATCTCCTACAATATTGGAGTCAGG AGCTAAAGAATAG
39	Rev; HIV Rev; 核输出和稳定化病毒 mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAA GAACTCCTCAAGGCAGTCAGACTCATCAAGT TTCTCTATCAAAGCAACCCACCTCCCAATCCC GAGGGGACCCGACAGGCCCGAAGGAATAGA AGAAGAAGGTGGAGAGAGAGACAGAGACAG ATCCATTCGATTAGTGAACGGATCCTTAGCA CTTATCTGGGACGATCTGCGGAGCCTGTGCC TCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTG

		GGACGCAGGGGGTGGGAAGCCCTCAAATAT TGGTGGAATCTCCTACAATATTGGAGTCAGG AGCTAAAGAATAG
40	RSV 启动子和 HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGT ATCTGAGGGGACTAGGGTGTGTTTAGGCGAA AAGCGGGGCTTCGGTTGTACGCGGTTAGGA GTCCCCTCAGGATATAGTAGTTTCGCTTTTG CATAGGGAGGGGGAAATGTAGTCTTATGCAA TACACTTGTAGTCTTGCAACATGGTAACGAT GAGTTAGCAACATGCCTTACAAGGAGAGAAA AAGCACCGTGCATGCCGATTGGTGGAAGTAA GGTGGTACGATCGTGCCTTATTAGGAAGGCA ACAGACAGGTCTGACATGGATTGGACGAACC ACTGAATTCCGCATTGCAGAGATAATTGTAT TTAAGTGCCTAGCTCGATACAATAAACGCCA TTTGACCATTCACCACATTGGTGTGCACCTC CAAGCTCGAGCTCGTTTAGTGAACCGTCAGA

		TCGCCTGGAGACGCCATCCACGCTGTTTTGA CCTCCATAGAAGACACCGGGACCGATCCAGC CTCCCCTCGAAGCTAGCGATTAGGCATCTCC TATGGCAGGAAGAAGCGGAGACAGCGACGA AGAACTCCTCAAGGCAGTCAGACTCATCAAG TTTCTCTATCAAAGCAACCCACCTCCCAATCC CGAGGGGACCCGACAGGCCCGAAGGAATAG AAGAAGAAGGTGGAGAGAGAGACAGAGACA GATCCATTCGATTAGTGAACGGATCCTTAGC ACTTATCTGGGACGATCTGCGGAGCCTGTGC CTCTTCAGCTACCACCGCTTGAGAGACTTAC TCTTGATTGTAACGAGGATTGTGGAACTTCT GGGACGCAGGGGGTGGGAAGCCCTCAAATA TTGGTGGAATCTCCTACAATATTGGAGTCAG GAGCTAAAGAATAGTCTAGA
41	延伸因子-1α(EF1-α)启动子	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAAC TGGGAAAGTGATGTCGTGTACTGGCTCCGCCTT TTTCCCGAGGGTGGGGGAGAACCGTATATAAGT GCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC GGGTTTGCCGCCAGAACACAGGTAAGTGCCGTG TGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTT ATGGCCCTTGCGTGCCTTGAATTACTTCCACGC CCCTGGCTGCAGTACGTGATTCTTGATCCCGAG CTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGG CCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTT GAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCC GCGTGCGAATCTGGTGGCACCTTCGCGCCTGTC TCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAA TTTTTGATGACCTGCTGCGACGCTTTTTTTCTGG CAAGATAGTCTTGTAAATGCGGGCCAAGATCTG

		CACACTGGTATTTCGGTTTTTGGGGCCGCGGGC GGCGACGGGGCCCGTGCGTCCCAGCGCACATGT TCGGCGAGGCGGGGCCTGCGAGCGCGGCCACC GAGAATCGGACGGGGGTAGTCTCAAGCTGGCC GGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGT GTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCC GGTCGGCACCAGTTGCGTGAGCGGAAAGATGG CCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAA TGGAGGACGCGGCGCTCGGGAGAGCGGGCGGG TGAGTCACCCACACAAAGGAAAAGGGCCTTTCC GTCCTCAGCCGTCGCTTCATGTGACTCCACGGA GTACCGGGCGCCGTCCAGGCACCTCGATTAGTT CTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGG GGGGAGGGGTTTTATGCGATGGAGTTTCCCCAC ACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCT TGGCACTTGATGTAATTCTCCTTGGAATTTGCCC TTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCC

		TCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTT CAGGTGTCGTGA
42	启动子; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCC TGGGTTTGCGCAGGGACGCGGCTGCTCTGGGCG TGGTTCCGGGAAACGCAGCGGCGCCGACCCTGG GTCTCGCACATTCTTCACGTCCGTTCGCAGCGTC ACCCGGATCTTCGCCGCTACCCTTGTGGGCCCC CCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGG GAAGGTTCCTTGCGGTTCGCGGCGTGCCGGACG TGACAAACGGAAGCCGCACGTCTCACTAGTACC CTCGCAGACGGACAGCGCCAGGGAGCAATGGC AGCGCGCCGACCGCGATGGGCTGTGGCCAATA GCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGC GGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTG TGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGC GCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCG CACGTCGGCAGTCGGCTCCCTCGTTGACCGAAT CACCGACCTCTCTCCCCAG
43	启动子; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCC CCTCCTCACGGCGAGCGCTGCCACGTCAGACGA AGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCG GACGCTCAGGACAGCGGCCCGCTGCTCATAAGA CTCGGCCTTAGAACCCCAGTATCAGCAGAAGGA CATTTTAGGACGGGACTTGGGTGACTCTAGGGC ACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGA GGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGA GGGATCTCCGTGGGGCGGTGAACGCCGATGATT ATATAAGGACGCGCCGGGTGTGGCACAGCTAGT TCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTT GTTTGTGGATCGCTGTGATCGTCACTTGGTGAG

		TTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTG GCCGCCGGGCCGCTCGGTGGGACGGAAGCGTG TGGAGAGACCGCCAAGGGCTGTAGTCTGGGTCC GCGAGCAAGGTTGCCCTGAACTGGGGGTTGGG GGGAGCGCACAAAATGGCGGCTGTTCCCGAGTC TTGAATGGAAGACGCTTGTAAGGCGGGCTGTGA GGTCGTTGAAACAAGGTGGGGGGCATGGTGGG CGGCAAGAACCCAAGGTCTTGAGGCCTTCGCTA ATGCGGGAAAGCTCTTATTCGGGTGAGATGGGC TGGGGCACCATCTGGGGACCCTGACGTGAAGTT TGTCACTGACTGGAGAACTCGGGTTTGTCGTCT GGTTGCGGGGGCGGCAGTTATGCGGTGCCGTTG GGCAGTGCACCCGTACCTTTGGGAGCGCGCGCC TCGTCGTGTCGTGACGTCACCCGTTCTGTTGGCT TATAATGCAGGGTGGGGCCACCTGCCGGTAGGT GTGCGGTAGGCTTTTCTCCGTCGCAGGACGCAG GGTTCGGGCCTAGGGTAGGCTCTCCTGAATCGA

		CAGGCGCCGGACCTCTGGTGAGGGGAGGGATA AGTGAGGCGTCAGTTTCTTTGGTCGGTTTTATGT ACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTT GAACTATGCGCTCGGGGTTGGCGAGTGTGTTTT GTGAAGTTTTTTAGGCACCTTTTGAAATGTAATC ATTTGGGTCAATATGTAATTTTCAGTGTTAGACT AGTAAA
44	聚 A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAG CAATAGCATCACAAATTTCACAAATAAAGCATT TTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAA CTCATCAATGTATCTTATCA
45	聚 A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTT TGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAA GGTGCCACTCCCACTGTCCTTTCCTAATAAAAT GAGGAAATTGCATCGCATTGTCTGAGTAGGTGT CATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTGGGAAGACAATAGCAG GCATGCTGGGGATGCGGTGGGCTCTATGG
46	包膜; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGT AGCCTAATAATAGTTCGGGCAGGGTTTGACGAC CCCCGCAAGGCTATCGCATTAGTACAAAAACAA CATGGTAAACCATGCGAATGCAGCGGAGGGCA GGTATCCGAGGCCCCACCGAACTCCATCCAACA GGTAACTTGCCCAGGCAAGACGGCCTACTTAAT GACCAACCAAAAATGGAAATGCAGAGTCACTC CAAAAAATCTCACCCCTAGCGGGGGAGAACTCC AGAACTGCCCCTGTAACACTTTCCAGGACTCGA TGCACAGTTCTTGTTATACTGAATACCGGCAAT GCAGGGCGAATAATAAGACATACTACACGGCC

		ACCTTGCTTAAAATACGGTCTGGGAGCCTCAAC GAGGTACAGATATTACAAAACCCCAATCAGCTC CTACAGTCCCCTTGTAGGGGCTCTATAAATCAG CCCGTTTGCTGGAGTGCCACAGCCCCCATCCAT ATCTCCGATGGTGGAGGACCCCTCGATACTAAG AGAGTGTGGACAGTCCAAAAAAGGCTAGAACA AATTCATAAGGCTATGCATCCTGAACTTCAATA CCACCCCTTAGCCCTGCCCAAAGTCAGAGATGA CCTTAGCCTTGATGCACGGACTTTTGATATCCTG AATACCACTTTTAGGTTACTCCAGATGTCCAATT TTAGCCTTGCCCAAGATTGTTGGCTCTGTTTAAA ACTAGGTACCCCTACCCCTCTTGCGATACCCAC TCCCTCTTTAACCTACTCCCTAGCAGACTCCCTA GCGAATGCCTCCTGTCAGATTATACCTCCCCTCT TGGTTCAACCGATGCAGTTCTCCAACTCGTCCT GTTTATCTTCCCCTTTCATTAACGATACGGAACA AATAGACTTAGGTGCAGTCACCTTTACTAACTG CACCTCTGTAGCCAATGTCAGTAGTCCTTTATGT

		GCCCTAAACGGGTCAGTCTTCCTCTGTGGAAAT AACATGGCATACACCTATTTACCCCAAAACTGG ACAGGACTTTGCGTCCAAGCCTCCCTCCTCCCC GACATTGACATCATCCCGGGGGATGAGCCAGTC CCCATTCCTGCCATTGATCATTATATACATAGAC CTAAACGAGCTGTACAGTTCATCCCTTTACTAG CTGGACTGGGAATCACCGCAGCATTCACCACCG GAGCTACAGGCCTAGGTGTCTCCGTCACCCAGT ATACAAAATTATCCCATCAGTTAATATCTGATG TCCAAGTCTTATCCGGTACCATACAAGATTTAC AAGACCAGGTAGACTCGTTAGCTGAAGTAGTTC TCCAAAATAGGAGGGGACTGGACCTACTAACG GCAGAACAAGGAGGAATTTGTTTAGCCTTACAA GAAAAATGCTGTTTTTATGCTAACAAGTCAGGA ATTGTGAGAAACAAAATAAGAACCCTACAAGA AGAATTACAAAAACGCAGGGAAAGCCTGGCAT CCAACCCTCTCTGGACCGGGCTGCAGGGCTTTC TTCCGTACCTCCTACCTCTCCTGGGACCCCTACT CACCCTCCTACTCATACTAACCATTGGGCCATG CGTTTTCAATCGATTGGTCCAATTTGTTAAAGAC AGGATCTCAGTGGTCCAGGCTCTGGTTTTGACT CAGCAATATCACCAGCTAAAACCCATAGAGTAC GAGCCATGA
47	**包膜; GALV**	**ATGCTTCTCACCTCAAGCCCGCACCACCTTC GGCACCAGATGAGTCCTGGGAGCTGGAAAA GACTGATCATCCTCTTAAGCTGCGTATTCGG AGACGGCAAAACGAGTCTGCAGAATAAGAAC CCCCACCAGCCTGTGACCCTCACCTGGCAGG TACTGTCCCAAACTGGGGACGTTGTCTGGGA CAAAAAGGCAGTCCAGCCCCTTTGGACTTGG**

		TGGCCCTCTCTTACACCTGATGTATGTGCCC TGGCGGCCGGTCTTGAGTCCTGGGATATCCC GGGATCCGATGTATCGTCCTCTAAAAGAGTT AGACCTCCTGATTCAGACTATACTGCCGCTT ATAAGCAAATCACCTGGGGAGCCATAGGGTG CAGCTACCCTCGGGCTAGGACCAGGATGGCA AATTCCCCCTTCTACGTGTGTCCCCGAGCTG GCCGAACCCATTCAGAAGCTAGGAGGTGTGG GGGGCTAGAATCCCTATACTGTAAAGAATGG AGTTGTGAGACCACGGGTACCGTTTATTGGC AACCCAAGTCCTCATGGGACCTCATAACTGT AAAATGGGACCAAAATGTGAAATGGGAGCAA AAATTTCAAAAGTGTGAACAAACCGGCTGGT GTAACCCCCTCAAGATAGACTTCACAGAAAA AGGAAAACTCTCCAGAGATTGGATAACGGAA AAAACCTGGGAATTAAGGTTCTATGTATATG GACACCCAGGCATACAGTTGACTATCCGCTT

		AGAGGTCACTAACATGCCGGTTGTGGCAGTG GGCCCAGACCCTGTCCTTGCGGAACAGGGAC CTCCTAGCAAGCCCCTCACTCTCCCTCTCTCC CCACGGAAAGCGCCGCCCACCCCTCTACCCC CGGCGGCTAGTGAGCAAACCCCTGCGGTGC ATGGAGAAACTGTTACCCTAAACTCTCCGCC TCCCACCAGTGGCGACCGACTCTTTGGCCTT GTGCAGGGGGCCTTCCTAACCTTGAATGCTA CCAACCCAGGGGCCACTAAGTCTTGCTGGCT CTGTTTGGGCATGAGCCCCCCTTATTATGAA GGGATAGCCTCTTCAGGAGAGGTCGCTTATA CCTCCAACCATACCCGATGCCACTGGGGGGC CCAAGGAAAGCTTACCCTCACTGAGGTCTCC GGACTCGGGTCATGCATAGGGAAGGTGCCTC TTACCCATCAACATCTTTGCAACCAGACCTTA CCCATCAATTCCTCTAAAAACCATCAGTATCT GCTCCCCTCAAACCATAGCTGGTGGGCCTGC AGCACTGGCCTCACCCCCTGCCTCTCCACCT CAGTTTTTAATCAGTCTAAAGACTTCTGTGTC CAGGTCCAGCTGATCCCCCGCATCTATTACC ATTCTGAAGAAACCTTGTTACAAGCCTATGA CAAATCACCCCCCAGGTTTAAAAGAGAGCCT GCCTCACTTACCCTAGCTGTCTTCCTGGGGT TAGGGATTGCGGCAGGTATAGGTACTGGCTC AACCGCCCTAATTAAAGGGCCCATAGACCTC CAGCAAGGCCTAACCAGCCTCCAAATCGCCA TTGACGCTGACCTCCGGGCCCTTCAGGACTC AATCAGCAAGCTAGAGGACTCACTGACTTCC CTATCTGAGGTAGTACTCCAAAATAGGAGAG GCCTTGACTTACTATTCCTTAAAGAAGGAGG

		CCTCTGCGCGGCCCTAAAAGAAGAGTGCTGT TTTTATGTAGACCACTCAGGTGCAGTACGAG ACTCCATGAAAAAACTTAAAGAAAGACTAGA TAAAAGACAGTTAGAGCGCCAGAAAAACCAA AACTGGTATGAAGGGTGGTTCAATAACTCCC CTTGGTTTACTACCCTACTATCAACCATCGCT GGGCCCCTATTGCTCCTCCTTTTGTTACTCAC TCTTGGGCCCTGCATCATCAATAAATTAATC CAATTCATCAATGATAGGATAAGTGCAGTCA AAATTTTAGTCCTTAGACAGAAATATCAGAC CCTAGATAACGAGGAAAACCTTTAA
48	包膜; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCT TCTGGGTTTTTCGTTGTGTTTCGGGAAGTTC CCCATTTACACGATACCAGACGAACTTGGTC CCTGGAGCCCTATTGACATACACCATCTCAG CTGTCCAAATAACCTGGTTGTGGAGGATGAA GGATGTACCAACCTGTCCGAGTTCTCCTACA

		TGGAACTCAAAGTGGGATACATCTCAGCCAT CAAAGTGAACGGGTTCACTTGCACAGGTGTT GTGACAGAGGCAGAGACCTACACCAACTTTG TTGGTTATGTCACAACCACATTCAAGAGAAA GCATTTCCGCCCCACCCCAGACGCATGTAGA GCCGCGTATAACTGGAAGATGGCCGGTGACC CCAGATATGAAGAGTCCCTACACAATCCATA CCCCGACTACCACTGGCTTCGAACTGTAAGA ACCACCAAAGAGTCCCTCATTATCATATCCC CAAGTGTGACAGATTTGGACCCATATGACAA ATCCCTTCACTCAAGGGTCTTCCCTGGCGGA AAGTGCTCAGGAATAACGGTGTCCTCTACCT ACTGCTCAACTAACCATGATTACACCATTTG GATGCCCGAGAATCCGAGACCAAGGACACCT TGTGACATTTTTACCAATAGCAGAGGGAAGA GAGCATCCAACGGGAACAAGACTTGCGGCTT TGTGGATGAAAGAGGCCTGTATAAGTCTCTA AAAGGAGCATGCAGGCTCAAGTTATGTGGAG TTCTTGGACTTAGACTTATGGATGGAACATG GGTCGCGATGCAAACATCAGATGAGACCAAA TGGTGCCCTCCAGATCAGTTGGTGAATTTGC ACGACTTTCGCTCAGACGAGATCGAGCATCT CGTTGTGGAGGAGTTAGTTAAGAAAAGAGAG GAATGTCTGGATGCATTAGAGTCCATCATGA CCACCAAGTCAGTAAGTTTCAGACGTCTCAG TCACCTGAGAAAACTTGTCCCAGGGTTTGGA AAAGCATATACCATATTCAACAAAACCTTGAT GGAGGCTGATGCTCACTACAAGTCAGTCCGG ACCTGGAATGAGATCATCCCCTCAAAAGGGT GTTTGAAAGTTGGAGGAAGGTGCCATCCTCA

		TGTGAACGGGGTGTTTTTCAATGGTATAATA TTAGGGCCTGACGACCATGTCCTAATCCCAG AGATGCAATCATCCCTCCTCCAGCAACATAT GGAGTTGTTGGAATCTTCAGTTATCCCCCTG ATGCACCCCCTGGCAGACCCTTCTACAGTTT TCAAAGAAGGTGATGAGGCTGAGGATTTTGT TGAAGTTCACCTCCCCGATGTGTACAAACAG ATCTCAGGGGTTGACCTGGGTCTCCCGAACT GGGGAAAGTATGTATTGATGACTGCAGGGGC CATGATTGGCCTGGTGTTGATATTTTCCCTA ATGACATGGTGCAGAGTTGGTATCCATCTTT GCATTAAATTAAAGCACACCAAGAAAAGACA GATTTATACAGACATAGAGATGAACCGACTT GGAAAGTAA
49	包膜; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTC TGCCTCACATCATCGATGAGGTGATCAACAT TGTCATTATTGTGCTTATCGTGATCACGGGT

		ATCAAGGCTGTCTACAATTTTGCCACCTGTG GGATATTCGCATTGATCAGTTTCCTACTTCTG GCTGGCAGGTCCTGTGGCATGTACGGTCTTA AGGGACCCGACATTTACAAAGGAGTTTACCA ATTTAAGTCAGTGGAGTTTGATATGTCACAT CTGAACCTGACCATGCCCAACGCATGTTCAG CCAACAACTCCCACCATTACATCAGTATGGG GACTTCTGGACTAGAATTGACCTTCACCAAT GATTCCATCATCAGTCACAACTTTTGCAATCT GACCTCTGCCTTCAACAAAAAGACCTTTGAC CACACACTCATGAGTATAGTTTCGAGCCTAC ACCTCAGTATCAGAGGGAACTCCAACTATAA GGCAGTATCCTGCGACTTCAACAATGGCATA ACCATCCAATACAACTTGACATTCTCAGATC GACAAAGTGCTCAGAGCCAGTGTAGAACCTT CAGAGGTAGAGTCCTAGATATGTTTAGAACT GCCTTCGGGGGGAAATACATGAGGAGTGGC TGGGGCTGGACAGGCTCAGATGGCAAGACC ACCTGGTGTAGCCAGACGAGTTACCAATACC TGATTATACAAAATAGAACCTGGGAAAACCA CTGCACATATGCAGGTCCTTTTGGGATGTCC AGGATTCTCCTTTCCCAAGAGAAGACTAAGT TCTTCACTAGGAGACTAGCGGGCACATTCAC CTGGACTTTGTCAGACTCTTCAGGGGTGGAG AATCCAGGTGGTTATTGCCTGACCAAATGGA TGATTCTTGCTGCAGAGCTTAAGTGTTTCGG GAACACAGCAGTTGCGAAATGCAATGTAAAT CATGATGCCGAATTCTGTGACATGCTGCGAC TAATTGACTACAACAAGGCTGCTTTGAGTAA GTTCAAAGAGGACGTAGAATCTGCCTTGCAC

		TTATTCAAAACAACAGTGAATTCTTTGATTTC AGATCAACTACTGATGAGGAACCACTTGAGA GATCTGATGGGGGTGCCATATTGCAATTACT CAAAGTTTTGGTACCTAGAACATGCAAAGAC CGGCGAAACTAGTGTCCCCAAGTGCTGGCTT GTCACCAATGGTTCTTACTTAAATGAGACCC ACTTCAGTGATCAAATCGAACAGGAAGCCGA TAACATGATTACAGAGATGTTGAGGAAGGAT TACATAAAGAGGCAGGGGAGTACCCCCCTAG CATTGATGGACCTTCTGATGTTTTCCACATCT GCATATCTAGTCAGCATCTTCCTGCACCTTG TCAAAATACCAACACACAGGCACATAAAAGG TGGCTCATGTCCAAAGCCACACCGATTAACC AACAAAGGAATTTGTAGTTGTGGTGCATTTA AGGTGCCTGGTGTAAAAACCGTCTGGAAAAG ACGCTGA
50	包膜; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTG

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		AAAGTTTATTATTGGCAACTGGGATGAAGAA **CGTTCCCGAACCTTCCAAAAAAAGGAAAAAA** **AGAGGCCTGTTTGGCGCTATAGCAGGGTTTA** **TTGAAAATGGTTGGGAAGGTCTGGTCGACGG** **GTGGTACGGTTTCAGGCATCAGAATGCACAA** **GGAGAAGGAACTGCAGCAGACTACAAAAGCA** **CCCAATCGGCAATTGATCAGATAACCGGAAA** **GTTAAATAGACTCATTGAGAAAACCAACCAG** **CAATTTGAGCTAATAGATAATGAATTCACTG** **AGGTGGAAAAGCAGATTGGCAATTTAATTAA** **CTGGACCAAAGACTCCATCACAGAAGTATGG** **TCTTACAATGCTGAACTTCTTGTGGCAATGG** **AAAACCAGCACACTATTGATTTGGCTGATTC** **AGAGATGAACAAGCTGTATGAGCGAGTGAG** **GAAACAATTAAGGGAAAATGCTGAAGAGGAT** **GGCACTGGTTGCTTTGAAATTTTTCATAAAT** **GTGACGATGATTGTATGGCTAGTATAAGGAA**

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51	包膜; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGG CTACTAGACCATACCTAGCACATTGCGCCGA TTGCGGGGACGGGTACTTCTGCTATAGCCCA GTTGCTATCGAGGAGATCCGAGATGAGGCGT CTGATGGCATGCTTAAGATCCAAGTCTCCGC CCAAATAGGTCTGGACAAGGCAGGCACCCAC GCCCACACGAAGCTCCGATATATGGCTGGTC ATGATGTTCAGGAATCTAAGAGAGATTCCTT GAGGGTGTACACGTCCGCAGCGTGCTCCATA CATGGGACGATGGGACACTTCATCGTCGCAC ACTGTCCACCAGGCGACTACCTCAAGGTTTC GTTCGAGGACGCAGATTCGCACGTGAAGGCA TGTAAGGTCCAATACAAGCACAATCCATTGC CGGTGGGTAGAGAGAAGTTCGTGGTTAGACC ACACTTTGGCGTAGAGCTGCCATGCACCTCA TACCAGCTGACAACGGCTCCCACCGACGAGG AGATTGACATGCATACACCGCCAGATATACC GGATCGCACCCTGCTATCACAGACGGCGGGC AACGTCAAAATAACAGCAGGCGGCAGGACTA TCAGGTACAACTGTACCTGCGGCCGTGACAA CGTAGGCACTACCAGTACTGACAAGACCATC AACACATGCAAGATTGACCAATGCCATGCTG CCGTCACCAGCCATGACAAATGGCAATTTAC

		CTCTCCATTTGTTCCCAGGGCTGATCAGACA GCTAGGAAAGGCAAGGTACACGTTCCGTTCC CTCTGACTAACGTCACCTGCCGAGTGCCGTT GGCTCGAGCGCCGGATGCCACCTATGGTAAG AAGGAGGTGACCCTGAGATTACACCCAGATC ATCCGACGCTCTTCTCCTATAGGAGTTTAGG AGCCGAACCGCACCCGTACGAGGAATGGGTT GACAAGTTCTCTGAGCGCATCATCCCAGTGA CGGAAGAAGGGATTGAGTACCAGTGGGGCA ACAACCCGCCGGTCTGCCTGTGGGCGCAACT GACGACCGAGGGCAAACCCCATGGCTGGCC ACATGAAATCATTCAGTACTATTATGGACTAT ACCCCGCCGCCACTATTGCCGCAGTATCCGG GGCGAGTCTGATGGCCCTCCTAACTCTGGCG GCCACATGCTGCATGCTGGCCACCGCGAGGA GAAAGTGCCTAACACCGTACGCCCTGACGCC AGGAGCGGTGGTACCGTTGACACTGGGGCT

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		AGCCGAACCGCACCCGTACGAGGAATGGGTT GACAAGTTCTCTGAGCGCATCATCCCAGTGA CGGAAGAAGGGATTGAGTACCAGTGGGGCA ACAACCCGCCGGTCTGCCTGTGGGCGCAACT GACGACCGAGGGCAAACCCCATGGCTGGCC ACATGAAATCATTCAGTACTATTATGGACTAT ACCCCGCCGCCACTATTGCCGCAGTATCCGG GGCGAGTCTGATGGCCCTCCTAACTCTGGCG GCCACATGCTGCATGCTGGCCACCGCGAGGA GAAAGTGCCTAACACCGTACGCCCTGACGCC AGGAGCGGTGGTACCGTTGACACTGGGGCT GCTTTGCTGCGCACCGAGGGCGAATGCA
53	**包膜；埃博拉**	**ATGGGTGTTACAGGAATATTGCAGTTACCTC GTGATCGATTCAAGAGGACATCATTCTTTCT TTGGGTAATTATCCTTTTCCAAAGAACATTTT CCATCCCACTTGGAGTCATCCACAATAGCAC ATTACAGGTTAGTGATGTCGACAAACTGGTT**

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		GTCGCATCTGACAACCCTTGCCACAATCTCC ACGAGTCCTCAACCCCCCACAACCAAACCAG GTCCGGACAACAGCACCCACAATACACCCGT GTATAAACTTGACATCTCTGAGGCAACTCAA GTTGAACAACATCACCGCAGAACAGACAACG ACAGCACAGCCTCCGACACTCCCCCCGCCAC GACCGCAGCCGGACCCCTAAAAGCAGAGAAC ACCAACACGAGCAAGGGTACCGACCTCCTGG ACCCCCGCCACCACAACAAGTCCCCAAAACCA CAGCGAGACCGCTGGCAACAACAACACTCAT CACCAAGATACCGGAGAAGAGAGTGCCAGCA GCGGGAAGCTAGGCTTAATTACCAATACTAT TGCTGGAGTCGCAGGACTGATCACAGGCGG GAGGAGAGCTCGAAGAGAAGCAATTGTCAAT GCTCAACCCAAATGCAACCCTAATTTACATTA CTGGACTACTCAGGATGAAGGTGCTGCAATC GGACTGGCCTGGATACCATATTTCGGGCCAG

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54	聚合酶 III shRNA 启动子; U6 启动子	TTTCCCATGATTCCTTCATATTTGCATATACG ATACAAGGCTGTTAGAGAGATAATTGGAATT AATTTGACTGTAAACACAAAGATATTAGTAC AAAATACGTGACGTAGAAAGTAATAATTTCT TGGGTAGTTTGCAGTTTTAAAATTATGTTTTA AAATGGACTATCATATGCTTACCGTAACTTG AAAGTATTTCGATTTCTTGGCTTTATATATCT TGTGGAAAGGACGAAAC
55	聚合酶 III shRNA 启动子; 7SK 启动子	CTGCAGTATTTAGCATGCCCCACCCATCTGC AAGGCATTCTGGATAGTGTCAAAACAGCCGG AAATCAAGTCCGTTTATCTCAAACTTTAGCAT TTTGGGAATAAATGATATTTGCTATGCTGGT TAAATTAGATTTTAGTTAAATTTCCTGCTGAA GCTCTAGTACGATAAGCAACTTGACCTAAGT GTAAAGTTGAGATTTCCTTCAGGTTTATATA GCTTGTGCGCCGCCTGGCTACCTC

56	FDPS 靶标序列#1	GTCCTGGAGTACAATGCCATT
57	FDPS 靶标序列#2	GCAGGATTTCGTTCAGCACTT
58	FDPS 靶标序列#3	GCCATGTACATGGCAGGAATT
59	FDPS 靶标序列#4	GCAGAAGGAGGCTGAGAAAGT
60	非靶向序列	GCCGCTTTGTAGGATAGAGCTCGAGCTCTAT CCTACAAAGCGGCTTTTT
61	正向引物	AGGAATTGATGGCGAGAAGG
62	反向引物	CCCAAAGAGGTCAAGGTAATCA
63	正向引物	AGCGCGGCTACAGCTTCA
64	反向引物	GGCGACGTAGCACAGCTTCT
65	左反向末端重复(左 ITR)	CCTGCAGGCAGCTGCGCGCTCGCTCGCTCAC TGAGGCCGCCCGGGCGTCGGGCGACCTTTG GTCGCCCGGCCTCAGTGAGCGAGCGAGCGC GCAGAGAGGGAGTGGCCAACTCCATCACTAG GGGTTCCT
66	右反向末端重复(右 ITR)	GAGCGGCCGCAGGAACCCCTAGTGATGGAG TTGGCCACTCCCTCTCTGCGCGCTCGCTCGC TCACTGAGGCCGGGCGACCAAAGGTCGCCC GACGCCCGGGCTTTGCCCGGGCGGCCTCAG TGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

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

序列表

<110> 美国基因技术国际有限公司（American Gene Technologies International Inc.）

<120> 用于活化γ-δ T细胞的方法和组合物

<130> 70612.00316

<140> WO PCT/US2017/013399
<141> 2017-01-13

<150> US 62/279,474
<151> 2016-01-15

<160> 67

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agaacaattt 120
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agtggcgccc 120
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gcttcccgta 120
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tcggccctca 480
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ccactgctta 120
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agtaggagaa 780
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agagttttgg ctgaagcaat gagccaagta acaaatccag ctaccataat
gatacagaaa 1140
ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa
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cccaccagaa 1380
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taa
1503

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aggtacagta 120
ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca
gattggctgc 180
actttaaatt ttcccattag tcctattgag actgtaccag taaaattaaa
gccaggaatg 240
gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc
attagtagaa 300
atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga
aaatccatac 360
aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa
attagtagat 420
ttcagagaac ttaataagag aactcaagat ttctgggaag ttcaattagg
aataccacat 480
cctgcagggt taaaacagaa aaaatcagta acagtactgg atgtgggcga
tgcatatttt 540
tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc
tagtataaac 600
aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg
gaaaggatca 660
ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa
acaaaaatcca 720
gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt

agaaataggg 780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg
atttaccaca 840
ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga
actccatcct 900
gataaatgga cagtacagcc tatagtgctg ccagaaaagg acagctggac
tgtcaatgac 960
atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg
gattaaagta 1020
aggcaattat gtaaacttct taggggaacc aaagcactaa cagaagtagt
accactaaca 1080
gaagaagcag agctagaact ggcagaaaac agggagattc taaaagaacc
ggtacatgga 1140
gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg
gcaaggccaa 1200
tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa

atatgcaaga 1260
atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca
aaaaatagcc 1320
acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat
acaaaaggaa 1380
acatgggaag catggtggac agagtattgg caagccacct ggattcctga
gtgggagttt 1440
gtcaataccc ctcccttagt gaagttatgg taccagttag agaaagaacc
cataatagga 1500
gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg
aaaagcagga 1560
tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac
aaatcagaag 1620
actgagttac aagcaattca tctagctttg caggattcgg gattagaagt
aaacatagtg 1680
acagactcac aatatgcatt gggaatcatt caagcacaac cagataagag
tgaatcagag 1740
ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct
ggcatgggta 1800
ccagcacaca aaggaattgg aggaaatgaa caagtagatg ggttggtcag
tgctggaatc 1860
aggaaagtac ta
1872

<210> 22
<211> 867

<212> DNA
<213> 人工序列

<220>
<223> 辅助 Rev- HIV整合酶-病毒RNA的整合

<400> 22
ttttagatg gaatagataa ggcccaagaa gaacatgaga aatatcacag
taattggaga 60
gcaatggcta gtgattttaa cctaccacct gtagtagcaa aagaaatagt
agccagctgt 120
gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag
cccaggaata 180
tggcagctag attgtacaca tttagaagga aaagttatct tggtagcagt
tcatgtagcc 240
agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac
agcatacttc 300

ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa
tggcagcaat 360
ttcaccagta ctacagttaa ggccgcctgt tggtgggcgg ggatcaagca
ggaatttggc 420
attccctaca atccccaaag tcaaggagta atagaatcta tgaataaaga
attaaagaaa 480
attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca
aatggcagta 540
ttcatccaca attttaaaag aaaaggggggg attgggggggt acagtgcagg
ggaaagaata 600
gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat
tacaaaaatt 660
caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg
accagcaaag 720
ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgacat
aaaagtagtg 780
ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg
tgatgattgt 840
gtggcaagta gacaggatga ggattaa
867

<210> 23
<211> 234
<212> DNA
<213> 人工序列

<220>
<223> 辅助/Rev- HIV RRE-结合Rev元件

<400> 23
aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg
cagcgtcaat 60
gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc
agaacaattt 120
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg
gcatcaagca 180
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct
234

<210> 24
<211> 351
<212> DNA
<213> 人工序列

<220>
<223> 辅助/Rev- HIV Rev-核输出和稳定化病毒mRNA

<400> 24
atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag
actcatcaag 60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc
ccgaaggaat 120
agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga
acggatcctt 180
agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc
gcttgagaga 240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggt
gggaagccct 300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g
351

<210> 25
<211> 577
<212> DNA
<213> 人工序列

<220>
<223> 包膜- CMV启动子-转录

<400> 25
acattgatta ttgactagtt attaatagta atcaattacg gggtcattag

ttcatagccc 60
atatatggag ttccgcgtta cataacttac ggtaaatggc ccgcctggct
gaccgcccaa 120
cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc
caatagggac 180
tttccattga cgtcaatggg tggagtattt acggtaaact gcccacttgg
cagtacatca 240
agtgtatcat atgccaagta cgccccctat tgacgtcaat gacggtaaat
ggcccgcctg 300
gcattatgcc cagtacatga ccttatggga ctttcctact tggcagtaca
tctacgtatt 360
agtcatcgct attaccatgg tgatgcggtt ttggcagtac atcaatgggc
gtggatagcg 420
gtttgactca cggggatttc caagtctcca ccccattgac gtcaatggga
gtttgttttg 480

gcaccaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat
tgacgcaaat 540
gggcggtagg cgtgtacggt gggaggtcta tataagc
577

<210> 26
<211> 1519
<212> DNA
<213> 人工序列

<220>
<223> 包膜-VSV-G-糖蛋白包膜-细胞进入

<400> 26
atgaagtgcc ttttgtactt agcctttta ttcattgggg tgaattgcaa
gttcaccata 60
gtttttccac acaaccaaaa aggaaactgg aaaaatgttc cttctaatta
ccattattgc 120
ccgtcaagct cagatttaaa ttggcataat gacttaatag gcacagcctt
acaagtcaaa 180
atgcccaaga gtcacaaggc tattcaagca gacggttgga tgtgtcatgc
ttccaaatgg 240
gtcactactt gtgatttccg ctggtatgga ccgaagtata taacacattc
catccgatcc 300
ttcactccat ctgtagaaca atgcaaggaa agcattgaac aaacgaaaca
aggaacttgg 360

ctgaatccag gcttccctcc tcaaagttgt ggatatgcaa ctgtgacgga
tgccgaagca 420
gtgattgtcc aggtgactcc tcaccatgtg ctggttgatg aatacacagg
agaatgggtt 480
gattcacagt tcatcaacgg aaaatgcagc aattacatat gccccactgt
ccataactct 540
acaacctggc attctgacta taaggtcaaa gggctatgtg attctaacct
catttccatg 600
gacatcacct tcttctcaga ggacggagag ctatcatccc tgggaaagga
gggcacaggg 660
ttcagaagta actactttgc ttatgaaact ggaggcaagg cctgcaaaat
gcaatactgc 720
aagcattggg gagtcagact cccatcaggt gtctggttcg agatggctga
taaggatctc 780
tttgctgcag ccagattccc tgaatgccca gaagggtcaa gtatctctgc
tccatctcag 840
acctcagtgg atgtaagtct aattcaggac gttgagagga tcttggatta

ttccctctgc 900
caagaaacct ggagcaaaat cagagcgggt cttccaatct ctccagtgga
tctcagctat 960
cttgctccta aaaacccagg aaccggtcct gctttcacca taatcaatgg
taccctaaaa 1020
tactttgaga ccagatacat cagagtcgat attgctgctc caatcctctc
aagaatggtc 1080
ggaatgatca gtggaactac cacagaaagg gaactgtggg atgactgggc
accatatgaa 1140
gacgtggaaa ttggacccaa tggagttctg aggaccagtt caggatataa
gtttccttta 1200
tacatgattg gacatggtat gttggactcc gatcttcatc ttagctcaaa
ggctcaggtg 1260
ttcgaacatc ctcacattca agacgctgct tcgcaacttc ctgatgatga
gagtttattt 1320
tttggtgata ctgggctatc caaaaaatcca atcgagcttg tagaaggttg
gttcagtagt 1380
tggaaaagct ctattgcctc ttttttcttt atcatagggt taatcattgg
actattcttg 1440
gttctccgag ttggtatcca tctttgcatt aaattaaagc acaccaagaa
aagacagatt 1500
tatacagaca tagagatga
1519

<210> 27
<211> 352

<212> DNA
<213> 人工序列

<220>
<223> 辅助/Rev- CMV 早期(CAG)增强子-增强转录

<400> 27
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata
tggagttccg 60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc
cccgcccatt 120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc
attgacgtca 180
atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt
atcatatgcc 240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt
atgcccagta 300

catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tc
352

<210> 28
<211> 960
<212> DNA
<213> 人工序列

<220>
<223> 辅助/Rev-鸡β肌动蛋白内含子-增强基因表达

<400> 28
ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg
ccgcccgccc 60
cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc
ttctcctccg 120
ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct
gcgtgaaagc 180
cttaaagggc tccgggaggg ccctttgtgc gggggggagc ggctcggggg
gtgcgtgcgt 240
gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg
tgagcgctgc 300
gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg
gccgggggcg 360
gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg
gtgtgtgcgt 420
gggggggtga gcagggggtg tgggcgcggc ggtcgggctg taacccccccc

ctgcaccccc 480
ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg
gggcgtggcg 540
cggggctcgc cgtgccgggc gggggggtggc ggcaggtggg ggtgccgggc
ggggcggggc 600
cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg ccccggagcg
ccggcggctg 660
tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga
gggcgcaggg 720
acttcctttg tcccaaatct ggcggagccg aaatctggga ggcgccgccg
caccccctct 780
agcgggcgcg ggcgaagcgg tgcggcgccg gcaggaagga aatgggcggg
gagggccttc 840
gtgcgtcgcc gcgccgccgt ccccttctcc atctccagcc tcggggctgc
cgcaggggga 900
cggctgcctt cgggggggac ggggcagggc ggggttcggc ttctggcgtg

tgaccggcgg 960

<210> 29
<211> 448
<212> DNA
<213> 人工序列

<220>
<223> 辅助/Rev-兔β球蛋白聚A- RNA稳定性

<400> 29
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagcccctg
agcatctgac 60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt
ttttgtgtct 120
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg
agtatttggt 180
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg
ctataaagag 240
gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga
aaagccttga 300
cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta
acatccctaa 360
aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta
ctcccagtca 420
tagctgtccc tcttctctta tgaagatc

448

<210> 30
<211> 573
<212> DNA
<213> 人工序列

<220>
<223> 包膜-β球蛋白内含子-增强基因表达

<400> 30
gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt
catgttatat 60
ggaggggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt
gtatcaccat 120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc
attgtctcct 180

cttattttct tttcattttc tgtaactttt tcgttaaact ttagcttgca
tttgtaacga 240
attttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta
atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agttttagag
aacaattgtt 360
ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg
tggaaatatt 420
cttattggta gaaacaacta cacctggtc atcatcctgc ctttctcttt
atggttacaa 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg
cccctctgct 540
aaccatgttc atgccttctt ctctttccta cag
573

<210> 31
<211> 450
<212> DNA
<213> 人工序列

<220>
<223> 包膜-兔β球蛋白聚A- RNA稳定性

<400> 31
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg
agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt
ttttgtgtct 120
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg
agtatttggt 180
ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaaggtt
ggctataaag 240
aggtcatcag tatatgaaac agccccctgc tgtccattcc ttattccata
gaaaagcctt 300
gacttgaggt tagatttttt ttatattttg ttttgtgtta ttttttttctt
taacatccct 360
aaaatttttcc ttacatgttt tactagccag atttttcctc ctctcctgac
tactcccagt 420
catagctgtc cctcttctct tatggagatc
450

<210> 32
<211> 31

<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 32
taagcagaat tcatgaattt gccaggaaga t
31

<210> 33
<211> 36
<212> DNA
<213> 人工序列

<220>
<223> 引物

<400> 33
ccatacaatg aatggacact aggcggccgc acgaat
36

<210> 34
<211> 2745
<212> DNA
<213> 人工序列

<220>
<223> Gag, Pol,整合酶片段

<400> 34
gaattcatga atttgccagg aagatggaaa ccaaaaatga tagggggaat
tggaggtttt 60
atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa
agctataggt 120
acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt
gactcagatt 180
ggctgcactt taaattttcc cattagtcct attgagactg taccagtaaa
attaaagcca 240
ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat
aaaagcatta 300
gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg
gcctgaaaat 360

ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg
gagaaaatta 420
gtagatttca gagaacttaa taagagaact caagatttct gggaagttca
attaggaata 480
ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt
gggcgatgca 540
tatttttcag ttcccttaga taaagacttc aggaagtata ctgcatttac
catacctagt 600
ataaacaatg agacaccagg gattagatat cagtacaatg tgcttccaca
gggatggaaa 660
ggatcaccag caatattcca gtgtagcatg acaaaaatct tagagccttt
tagaaaacaa 720

aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggatc
tgacttagaa 780
atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag
gtggggattt 840
accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg
ttatgaactc 900
catcctgata aatggacagt acagcctata gtgctgccag aaaaggacag
ctggactgtc 960
aatgacatac agaaattagt gggaaaattg aattgggcaa gtcagattta
tgcagggatt 1020
aaagtaaggc aattatgtaa acttcttagg ggaaccaaag cactaacaga
agtagtacca 1080
ctaacagaag aagcagagct agaactggca gaaaacaggg agattctaaa

agaaccggta 1140
catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa
gcaggggcaa 1200
ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac
aggaaagtat 1260
gcaagaatga agggtgccca cactaatgat gtgaaacaat taacagaggc
agtacaaaaa 1320
atagccacag aaagcatagt aatatgggga aagactccta aatttaaatt
acccatacaa 1380
aaggaaacat gggaagcatg gtggacagag tattggcaag ccacctggat
tcctgagtgg 1440
gagtttgtca ataccctcc cttagtgaag ttatggtacc agttagagaa
agaacccata 1500
ataggagcag aaactttcta tgtagatggg gcagccaata gggaaactaa
attaggaaaa 1560
gcaggatatg taactgacag aggaagacaa aaagttgtcc ccctaacgga
cacaacaaat 1620

cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt
agaagtaaac 1680
atagtgacag actcacaata tgcattggga atcattcaag cacaaccaga
taagagtgaa 1740
tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt
ctacctggca 1800
tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt
ggtcagtgct 1860
ggaatcagga aagtactatt tttagatgga atagataagg cccaagaaga
acatgagaaa 1920
tatcacagta attggagagc aatggctagt gattttaacc taccacctgt
agtagcaaaa 1980
gaaatagtag ccagctgtga taaatgtcag ctaaaagggg aagccatgca
tggacaagta 2040
gactgtagcc caggaatatg gcagctagat tgtacacatt tagaaggaaa
agttatcttg 2100
gtagcagttc atgtagccag tggatatata gaagcagaag taattccagc
agagacaggg 2160
caagaaacag catacttcct cttaaaatta gcaggaagat ggccagtaaa
aacagtacat 2220
acagacaatg gcagcaattt caccagtact acagttaagg ccgcctgttg
gtgggcgggg 2280
atcaagcagg aatttggcat tccctacaat ccccaaagtc aaggagtaat
agaatctatg 2340
aataaagaat taaagaaaat tataggacag gtaagagatc aggctgaaca
tcttaagaca 2400

gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat
tgggggggtac 2460
agtgcagggg aaagaatagt agacataata gcaacagaca tacaaactaa
agaattacaa 2520
aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag
agatccagtt 2580
tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat
acaagataat 2640
agtgacataa aagtagtgcc aagaagaaaa gcaaagatca tcagggatta
tggaaaacag 2700
atggcaggtg atgattgtgt ggcaagtaga caggatgagg attaa
2745

<210> 35
<211> 1586
<212> DNA
<213> 人工序列

<220>
<223> 含Rev, RRE和兔β球蛋白聚A的DNA片段

<400> 35
tctagaatgg caggaagaag cggagacagc gacgaagagc tcatcagaac
agtcagactc 60
atcaagcttc tctatcaaag caacccacct cccaatcccg aggggacccg
acaggcccga 120
aggaatagaa gaagaaggtg gagagagaga cagagacaga tccattcgat
tagtgaacgg 180
atccttggca cttatctggg acgatctgcg gagcctgtgc ctcttcagct
accaccgctt 240
gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca
gggggtggga 300
agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa
agaatagagg 360
agctttgttc cttgggttct tgggagcagc aggaagcact atgggcgcag
cgtcaatgac 420
gctgacggta caggccagac aattattgtc tggtatagtg cagcagcaga
acaatttgct 480
gagggctatt gaggcgcaac agcatctgtt gcaactcaca gtctggggca
tcaagcagct 540
ccaggcaaga atcctggctg tggaaagata cctaaaggat caacagctcc
tagatctttt 600
tccctctgcc aaaaattatg gggacatcat gaagcccctt gagcatctga
cttctggcta 660

ataaaggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgtc
tctcactcgg 720
aaggacatat gggagggcaa atcatttaaa acatcagaat gagtatttgg
tttagagttt 780
ggcaacatat gccatatgct ggctgccatg aacaaaggtg gctataaaga
ggtcatcagt 840
atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg
acttgaggtt 900
agatttttt tatattttgt tttgtgttat ttttttcttt aacatcccta
aaattttcct 960
tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc
atagctgtcc 1020
ctcttctctt atgaagatcc ctcgacctgc agcccaagct tggcgtaatc
atggtcatag 1080
ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg
agccggaagc 1140

ataaagtgta aagcctgggg tgcctaatga gtgagctaac tcacattaat
tgcgttgcgc 1200
tcactgcccg ctttccagtc gggaaacctg tcgtgccagc ggatccgcat
ctcaattagt 1260
cagcaaccat agtcccgccc ctaactccgc ccatcccgcc cctaactccg
cccagttccg 1320
cccattctcc gccccatggc tgactaattt tttttattta tgcagaggcc
gaggccgcct 1380
cggcctctga gctattccag aagtagtgag gaggcttttt tggaggccta
ggcttttgca 1440
aaaagctaac ttgtttattg cagcttataa tggttacaaa taaagcaata
gcatcacaaa 1500
tttcacaaat aaagcatttt tttcactgca ttctagttgt ggtttgtcca
aactcatcaa 1560
tgtatcttat cagcggccgc cccggg
1586

<210> 36
<211> 1614
<212> DNA
<213> 人工序列

<220>
<223> 含CAG增强子/启动子/内含子序列的DNA片段

<400> 36

acgcgttagt tattaatagt aatcaattac ggggtcatta gttcatagcc
catatatgga 60
gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca
acgacccccg 120
cccattgacg tcaataatga cgtatgttcc catagtaacg ccaatagga
ctttccattg 180
acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc
aagtgtatca 240
tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct
ggcattatgc 300
ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat
tagtcatcgc 360
tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc
tccccccccct 420
ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg
atgggggcgg 480
gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg

cggggcgagg 540
cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc
ttttatggcg 600
aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg
agtcgctgcg 660
ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg
gctctgactg 720
accgcgttac tcccacaggt gagcgggcgg gacggccctt ctcctccggg
ctgtaattag 780
cgcttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct
taaagggctc 840
cgggagggcc ctttgtgcgg gggggagcgg ctcgggggggt gcgtgcgtgt
gtgtgtgcgt 900
ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgctgcgg
gcgcggcgcg 960
gggctttgtg cgctccgcgt gtgcgcgagg ggagcgcggc cgggggcggt
gccccgcggt 1020
gcgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg
gggggtgagc 1080
agggggtgtg ggcgcggcgg tcgggctgta acccccccct gcacccccct
ccccgagttg 1140
ctgagcacgg cccggcttcg ggtgcggggc tccgtgcggg gcgtggcgcg
gggctcgccg 1200
tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg
cctcgggccg 1260
gggagggctc gggggagggg cgcggcggcc ccggagcgcc ggcggctgtc
gaggcgcggc 1320
gagccgcagc cattgccttt tatggtaatc gtgcgagagg gcgcagggac

ttcctttgtc 1380
ccaaatctgg cggagccgaa atctgggagg cgccgccgca ccccctctag
cgggcgcggg 1440
cgaagcggtg cggcgccggc aggaaggaaa tgggcgggga gggccttcgt
gcgtcgccgc 1500
gccgccgtcc ccttctccat ctccagcctc ggggctgccg cagggggacg
gctgccttcg 1560
gggggggacgg ggcagggcgg ggttcggctt ctggcgtgtg accggcggga attc
1614

<210> 37
<211> 1531
<212> DNA
<213> 人工序列

<220>
<223> 含VSV-G的DNA片段

<400> 37
gaattcatga agtgcctttt gtacttagcc tttttattca ttggggtgaa
ttgcaagttc 60
accatagttt ttccacacaa ccaaaaagga aactggaaaa atgttccttc
taattaccat 120
tattgcccgt caagctcaga tttaaattgg cataatgact taataggcac
agccttacaa 180
gtcaaaatgc ccaagagtca caaggctatt caagcagacg gttggatgtg
tcatgcttcc 240
aaatgggtca ctacttgtga tttccgctgg tatggaccga agtatataac
acattccatc 300
cgatccttca ctccatctgt agaacaatgc aaggaaagca ttgaacaaac
gaaacaagga 360
acttggctga atccaggctt ccctcctcaa agttgtggat atgcaactgt
gacggatgcc 420
gaagcagtga ttgtccaggt gactcctcac catgtgctgg ttgatgaata
cacaggagaa 480
tgggttgatt cacagttcat caacggaaaa tgcagcaatt acatatgccc
cactgtccat 540
aactctacaa cctggcattc tgactataag gtcaaagggc tatgtgattc
taacctcatt 600
tccatggaca tcaccttctt ctcagaggac ggagagctat catccctggg
aaaggagggc 660

acagggttca gaagtaacta ctttgcttat gaaactggag gcaaggcctg
caaaatgcaa 720
tactgcaagc attggggagt cagactccca tcaggtgtct ggttcgagat
ggctgataag 780
gatctctttg ctgcagccag attccctgaa tgcccagaag ggtcaagtat
ctctgctcca 840
tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt
ggattattcc 900
ctctgccaag aaacctggag caaaatcaga gcgggtcttc caatctctcc
agtggatctc 960
agctatcttg ctcctaaaaa cccaggaacc ggtcctgctt tcaccataat
caatggtacc 1020
ctaaaatact ttgagaccag atacatcaga gtcgatattg ctgctccaat
cctctcaaga 1080
atggtcggaa tgatcagtgg aactaccaca gaaagggaac tgtgggatga
ctgggcacca 1140
tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg

atataagttt 1200
cctttataca tgattggaca tggtatgttg gactccgatc ttcatcttag
ctcaaaggct 1260
caggtgttcg aacatcctca cattcaagac gctgcttcgc aacttcctga
tgatgagagt 1320
ttatttttg gtgatactgg gctatccaaa aatccaatcg agcttgtaga
aggttggttc 1380
agtagttgga aaagctctat tgcctctttt ttctttatca tagggttaat
cattggacta 1440
ttcttggttc tccgagttgg tatccatctt tgcattaaat taaagcacac
caagaaaaga 1500
cagatttata cagacataga gatgagaatt c
1531

<210> 38
<211> 351
<212> DNA
<213> 人工序列

<220>
<223> Rev- RSV启动子-转录

<400> 38
atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag
actcatcaag 60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc
ccgaaggaat 120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga
acggatcctt 180
agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc
gcttgagaga 240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggt
gggaagccct 300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g
351

<210> 39
<211> 351
<212> DNA
<213> 人工序列

<220>
<223> Rev- HIV Rev-核输出和稳定化病毒mRNA

<400> 39
atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag
actcatcaag 60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc
ccgaaggaat 120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga
acggatcctt 180
agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc
gcttgagaga 240
cttactcttg attgtaacga ggattgtgga acttctggga cgcaggggtg
gggaagccct 300
caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g
351

<210> 40
<211> 884
<212> DNA
<213> 人工序列

<220>
<223> RSV启动子和HIV Rev

<400> 40
caattgcgat gtacgggcca gatatacgcg tatctgaggg gactagggtg
tgtttaggcg 60
aaaagcgggg cttcggttgt acgcggttag gagtcccctc aggatatagt

agtttcgctt 120
ttgcataggg aggggaaat gtagtcttat gcaatacact tgtagtcttg
caacatggta 180
acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg
ccgattggtg 240
gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct
gacatggatt 300
ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct
agctcgatac 360
aataaacgcc atttgaccat tcaccacatt ggtgtgcacc tccaagctcg
agctcgttta 420
gtgaaccgtc agatcgcctg gagacgccat ccacgctgtt ttgacctcca
tagaagacac 480
cgggaccgat ccagcctccc ctcgaagcta gcgattaggc atctcctatg
gcaggaagaa 540

gcggagacag cgacgaagaa ctcctcaagg cagtcagact catcaagttt
ctctatcaaa 600
gcaacccacc tcccaatccc gaggggaccc gacaggcccg aaggaataga
agaagaaggt 660
ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc
acttatctgg 720
gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt
actcttgatt 780
gtaacgagga ttgtggaact tctgggacgc agggggtggg aagccctcaa
atattggtgg 840
aatctcctac aatattggag tcaggagcta aagaatagtc taga
884

<210> 41
<211> 1104
<212> DNA
<213> 人工序列

<220>
<223> 延伸因子-1 α (EF1-α)启动子

<400> 41
ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg
tactggctcc 60
gccttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc
gtgaacgttc 120

ttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt
tcccgcgggc 180
ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg
cccctggctg 240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag
ttcgaggcct 300
tgcgcttaag gagccccttc gcctcgtgct tgagttgagg cctggcctgg
gcgctggggc 360
cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg ctgctttcga
taagtctcta 420
gccatttaaa atttttgatg acctgctgcg acgcttttt tctggcaaga
tagtcttgta 480
aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg
cggcgacggg 540
gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg
gccaccgaga 600
atcggacggg ggtagtctca agctggccgg cctgctctgg tgcctggcct

cgcgccgccg 660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc
gtgagcggaa 720
agatggccgc ttcccggccc tgctgcaggg agctcaaaat ggaggacgcg
gcgctcggga 780
gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct ttccgtcctc
agccgtcgct 840
tcatgtgact ccacggagta ccgggcgccg tccaggcacc tcgattagtt
ctcgagcttt 900
tggagtacgt cgtctttagg ttgggggggag gggttttatg cgatggagtt
tccccacact 960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc
cttggaattt 1020
gccctttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt
tcaaagtttt 1080
tttcttccat ttcaggtgtc gtga
1104

<210> 42
<211> 511
<212> DNA
<213> 人工序列

<220>
<223> 启动子- PGK

<400> 42
ggggttgggg ttgcgccttt tccaaggcag ccctgggttt gcgcagggac
gcggctgctc 60
tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgcacat
tcttcacgtc 120
cgttcgcagc gtcacccgga tcttcgccgc taccttgtg ggcccccggg
cgacgcttcc 180
tgctccgccc ctaagtcggg aaggttcctt gcggttcgcg gcgtgccgga
cgtgacaaac 240
ggaagccgca cgtctcacta gtaccctcgc agacggacag cgccagggag
caatggcagc 300
gcgccgaccg cgatgggctg tggccaatag cggctgctca gcagggcgcg
ccgagagcag 360
cggccgggaa ggggcggtgc gggaggcggg gtgtggggcg gtagtgtggg
ccctgttcct 420
gcccgcgcgg tgttccgcat tctgcaagcc tccggagcgc acgtcggcag
tcggctccct 480

cgttgaccga atcaccgacc tctctcccca g
511

<210> 43
<211> 1162
<212> DNA
<213> 人工序列

<220>
<223> 启动子- UbC

<400> 43
gcgccgggtt ttggcgcctc ccgcgggcgc ccccctcctc acggcgagcg
ctgccacgtc 60
agacgaaggg cgcaggagcg ttcctgatcc ttccgcccgg acgctcagga
cagcggcccg 120
ctgctcataa gactcggcct tagaacccca gtatcagcag aaggacattt
taggacggga 180
cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg
aggaaaagta 240
gtcccttctc ggcgattctg cggagggatc tccgtggggc ggtgaacgcc
gatgattata 300
taaggacgcg ccgggtgtgg cacagctagt tccgtcgcag ccgggatttg
ggtcgcggtt 360
cttgtttgtg gatcgctgtg atcgtcactt ggtgagttgc gggctgctgg
gctggccggg 420

gctttcgtgg ccgccgggcc gctcggtggg acggaagcgt gtggagagac
cgccaagggc 480
tgtagtctgg gtccgcgagc aaggttgccc tgaactgggg gttgggggga
gcgcacaaaa 540
tggcggctgt tcccgagtct tgaatggaag acgcttgtaa ggcgggctgt
gaggtcgttg 600
aaacaaggtg gggggcatgg tgggcggcaa gaacccaagg tcttgaggcc
ttcgctaatg 660
cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc
ctgacgtgaa 720
gtttgtcact gactggagaa ctcgggtttg tcgtctggtt gcgggggcgg
cagttatgcg 780
gtgccgttgg gcagtgcacc cgtacctttg ggagcgcgcg cctcgtcgtg
tcgtgacgtc 840
acccgttctg ttggcttata atgcagggtg gggccacctg ccggtaggtg
tgcggtaggc 900
ttttctccgt cgcaggacgc agggttcggg cctagggtag gctctcctga

atcgacaggc 960
gccggacctc tggtgagggg agggataagt gaggcgtcag tttctttggt
cggttttatg 1020
tacctatctt cttaagtagc tgaagctccg gttttgaact atgcgctcgg
ggttggcgag 1080
tgtgttttgt gaagtttttt aggcaccttt tgaaatgtaa tcatttgggt
caatatgtaa 1140
ttttcagtgt tagactagta aa
1162

<210> 44
<211> 120
<212> DNA
<213> 人工序列

<220>
<223> 聚 A- SV40

<400> 44
gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt
tcacaaataa 60
agcatttttt tcactgcatt ctagttgtgg tttgtccaaa ctcatcaatg
tatcttatca 120

<210> 45
<211> 227

<212> DNA
<213> 人工序列

<220>
<223> 聚 A- bGH

<400> 45
gactgtgcct tctagttgcc agccatctgt tgtttgcccc tcccccgtgc
cttccttgac 60
cctggaaggt gccactccca ctgtcctttc ctaataaaat gaggaaattg
catcgcattg 120
tctgagtagg tgtcattcta ttctgggggg tggggtgggg caggacagca
agggggagga 180
ttgggaagac aatagcaggc atgctgggga tgcggtgggc tctatgg
227

<210> 46

<211> 1695
<212> DNA
<213> 人工序列

<220>
<223> 包膜- RD114

<400> 46
atgaaactcc caacaggaat ggtcatttta tgtagcctaa taatagttcg
ggcagggttt 60
gacgaccccc gcaaggctat cgcattagta caaaaacaac atggtaaacc
atgcgaatgc 120
agcggagggc aggtatccga ggccccaccg aactccatcc aacaggtaac
ttgcccaggc 180
aagacggcct acttaatgac caaccaaaaa tggaaatgca gagtcactcc
aaaaaatctc 240
acccctagcg ggggagaact ccagaactgc ccctgtaaca ctttccagga
ctcgatgcac 300
agttcttgtt atactgaata ccggcaatgc agggcgaata ataagacata
ctacacggcc 360
accttgctta aaatacggtc tgggagcctc aacgaggtac agatattaca
aaaccccaat 420
cagctcctac agtcccttg taggggctct ataaatcagc ccgtttgctg
gagtgccaca 480
gcccccatcc atatctccga tggtggagga cccctcgata ctaagagagt
gtggacagtc 540

caaaaaaggc tagaacaaat tcataaggct atgcatcctg aacttcaata
ccacccctta 600
gccctgccca aagtcagaga tgaccttagc cttgatgcac ggacttttga
tatcctgaat 660
accactttta ggttactcca gatgtccaat tttagccttg cccaagattg
ttggctctgt 720
ttaaaactag gtacccctac ccctcttgcg atacccactc cctctttaac
ctactcccta 780
gcagactccc tagcgaatgc ctcctgtcag attatacctc ccctcttggt
tcaaccgatg 840
cagttctcca actcgtcctg tttatcttcc cctttcatta acgatacgga
acaaatagac 900
ttaggtgcag tcacctttac taactgcacc tctgtagcca atgtcagtag
tcctttatgt 960
gccctaaacg ggtcagtctt cctctgtgga aataacatgg catacaccta
tttaccccaa 1020
aactggacag gactttgcgt ccaagcctcc ctcctccccg acattgacat

catcccgggg 1080
gatgagccag tccccattcc tgccattgat cattatatac atagacctaa
acgagctgta 1140
cagttcatcc ctttactagc tggactggga atcaccgcag cattcaccac
cggagctaca 1200
ggcctaggtg tctccgtcac ccagtataca aaattatccc atcagttaat
atctgatgtc 1260
caagtcttat ccggtaccat acaagattta caagaccagg tagactcgtt
agctgaagta 1320
gttctccaaa ataggagggg actggaccta ctaacggcag aacaaggagg
aatttgttta 1380
gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag
aaacaaaata 1440
agaaccctac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa
ccctctctgg 1500
accgggctgc agggctttct tccgtacctc ctacctctcc tgggacccct
actcaccctc 1560
ctactcatac taaccattgg gccatgcgtt ttcaatcgat tggtccaatt
tgttaaagac 1620
aggatctcag tggtccaggc tctggttttg actcagcaat atcaccagct
aaaacccata 1680
gagtacgagc catga
1695

<210> 47
<211> 2013

<212> DNA
<213> 人工序列

<220>
<223> 包膜- GALV

<400> 47
atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtcctgg
gagctggaaa 60
agactgatca tcctcttaag ctgcgtattc ggagacggca aaacgagtct
gcagaataag 120
aacccccacc agcctgtgac cctcacctgg caggtactgt cccaaactgg
ggacgttgtc 180
tgggacaaaa aggcagtcca gccccctttgg acttggtggc cctctcttac
acctgatgta 240
tgtgccctgg cggccggtct tgagtcctgg gatatcccgg gatccgatgt
atcgtcctct 300

aaaagagtta gacctcctga ttcagactat actgccgctt ataagcaaat
cacctgggga 360
gccatagggt gcagctaccc tcgggctagg accaggatgg caaattcccc
cttctacgtg 420
tgtccccgag ctggccgaac ccattcagaa gctaggaggt gtgggggggct
agaatcccta 480
tactgtaaag aatggagttg tgagaccacg ggtaccgttt attggcaacc
caagtcctca 540
tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa
atttcaaaag 600
tgtgaacaaa ccggctggtg taacccccctc aagatagact tcacagaaaa
aggaaaactc 660
tccagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata
tggacaccca 720
ggcatacagt tgactatccg cttagaggtc actaacatgc cggttgtggc
agtgggccca 780
gaccctgtcc ttgcggaaca gggacctcct agcaagcccc tcactctccc
tctctcccca 840
cggaaagcgc cgcccacccc tctacccccg gcggctagtg agcaaacccc
tgcggtgcat 900
ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact
ctttggcctt 960
gtgcaggggg ccttcctaac cttgaatgct accaacccag gggccactaa
gtcttgctgg 1020
ctctgtttgg gcatgagccc cccttattat gaagggatag cctcttcagg
agaggtcgct 1080

tatacctcca accatacccg atgccactgg ggggcccaag gaaagcttac
cctcactgag 1140
gtctccggac tcgggtcatg catagggaag gtgcctctta cccatcaaca
tctttgcaac 1200
cagaccttac ccatcaattc ctctaaaaac catcagtatc tgctcccctc
aaaccatagc 1260
tggtgggcct gcagcactgg cctcaccccc tgcctctcca cctcagtttt
taatcagtct 1320
aaagacttct gtgtccaggt ccagctgatc ccccgcatct attaccattc
tgaagaaacc 1380
ttgttacaag cctatgacaa atcacccccc aggtttaaaa gagagcctgc
ctcacttacc 1440
ctagctgtct tcctggggtt agggattgcg gcaggtatag gtactggctc
aaccgcccta 1500
attaaagggc ccatagacct ccagcaaggc ctaaccagcc tccaaatcgc
cattgacgct 1560
gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac

ttccctatct 1620
gaggtagtac tccaaaatag gagaggcctt gacttactat tccttaaaga
aggaggcctc 1680
tgcgcggccc taaaagaaga gtgctgtttt tatgtagacc actcaggtgc
agtacgagac 1740
tccatgaaaa aacttaaaga aagactagat aaaagacagt tagagcgcca
gaaaaaccaa 1800
aactggtatg aagggtggtt caataactcc ccttggttta ctaccctact
atcaaccatc 1860
gctgggcccc tattgctcct ccttttgtta ctcactcttg ggccctgcat
catcaataaa 1920
ttaatccaat tcatcaatga taggataagt gcagtcaaaa ttttagtcct
tagacagaaa 1980
tatcagaccc tagataacga ggaaaacctt taa
2013

<210> 48
<211> 1530
<212> DNA
<213> 人工序列

<220>
<223> 包膜- FUG

<400> 48
atggttccgc aggttctttt gtttgtactc cttctgggtt tttcgttgtg

tttcgggaag 60

ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga
catacaccat 120

ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct
gtccgagttc 180

tcctacatgg aactcaaagt gggatacatc tcagccatca aagtgaacgg
gttcacttgc 240

acaggtgttg tgacagaggc agagacctac accaactttg ttggttatgt
cacaaccaca 300

ttcaagagaa agcatttccg ccccacccca gacgcatgta gagccgcgta
taactggaag 360

atggccggtg accccagata tgaagagtcc ctacacaatc catacccccga
ctaccactgg 420

cttcgaactg taagaaccac caaagagtcc ctcattatca tatccccaag
tgtgacagat 480

ttggacccat atgacaaatc ccttcactca agggtcttcc ctggcggaaa
gtgctcagga 540

ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg
gatgcccgag 600
aatccgagac caaggacacc ttgtgacatt tttaccaata gcagagggaa
gagagcatcc 660
aacgggaaca agacttgcgg ctttgtggat gaaagaggcc tgtataagtc
tctaaaagga 720
gcatgcaggc tcaagttatg tggagttctt ggacttagac ttatggatgg
aacatgggtc 780
gcgatgcaaa catcagatga gaccaaatgg tgccctccag atcagttggt
gaatttgcac 840
gactttcgct cagacgagat cgagcatctc gttgtggagg agttagttaa
gaaaagagag 900
gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt
cagacgtctc 960
agtcacctga gaaaacttgt cccagggttt ggaaaagcat ataccatatt
caacaaaacc 1020
ttgatggagg ctgatgctca ctacaagtca gtccggacct ggaatgagat
catcccctca 1080
aaagggtgtt tgaaagttgg aggaaggtgc catcctcatg tgaacggggt
gtttttcaat 1140
ggtataatat tagggcctga cgaccatgtc ctaatcccag agatgcaatc
atccctcctc 1200
cagcaacata tggagttgtt ggaatcttca gttatccccc tgatgcaccc
cctggcagac 1260
ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt
tcacctcccc 1320

gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg
aaagtatgta 1380
ttgatgactg caggggccat gattggcctg gtgttgatat tttccctaat
gacatggtgc 1440
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gatttataca 1500
gacatagaga tgaaccgact tggaaagtaa
1530

<210> 49
<211> 1497
<212> DNA
<213> 人工序列

<220>
<223> 包膜- LCMV

<400> 49
atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatcgatga
ggtgatcaac 60
attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa
ttttgccacc 120
tgtgggatat tcgcattgat cagtttccta cttctggctg gcaggtcctg
tggcatgtac 180
ggtcttaagg gacccgacat ttacaaagga gtttaccaat ttaagtcagt
ggagtttgat 240
atgtcacatc tgaacctgac catgcccaac gcatgttcag ccaacaactc
ccaccattac 300
atcagtatgg ggacttctgg actagaattg accttcacca atgattccat
catcagtcac 360
aacttttgca atctgacctc tgccttcaac aaaaagacct ttgaccacac
actcatgagt 420
atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc
agtatcctgc 480
gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg
acaaagtgct 540
cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac
tgccttcggg 600
gggaaataca tgaggagtgg ctggggctgg acaggctcag atggcaagac
cacctggtgt 660
agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa
ccactgcaca 720
tatgcaggtc cttttgggat gtccaggatt ctcctttccc aagagaagac

taagttcttc 780

actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg
ggtggagaat 840

ccaggtggtt attgcctgac caaatggatg attcttgctg cagagcttaa
gtgtttcggg 900

aacacagcag ttgcgaaatg caatgtaaat catgatgccg aattctgtga
catgctgcga 960

ctaattgact acaacaaggc tgctttgagt aagttcaaag aggacgtaga
atctgccttg 1020

cacttattca aaacaacagt gaattctttg atttcagatc aactactgat
gaggaaccac 1080

ttgagagatc tgatgggggt gccatattgc aattactcaa agttttggta
cctagaacat 1140

gcaaagaccg gcgaaactag tgtccccaag tgctggcttg tcaccaatgg
ttcttactta 1200

aatgagaccc acttcagtga tcaaatcgaa caggaagccg ataacatgat
tacagagatg 1260

ttgaggaagg attacataaa gaggcagggg agtacccccc tagcattgat
ggaccttctg 1320
atgttttcca catctgcata tctagtcagc atcttcctgc accttgtcaa
aataccaaca 1380
cacaggcaca taaaaggtgg ctcatgtcca aagccacacc gattaaccaa
caaaggaatt 1440
tgtagttgtg gtgcatttaa ggtgcctggt gtaaaaaccg tctggaaaag
acgctga 1497

<210> 50
<211> 1692
<212> DNA
<213> 人工序列

<220>
<223> 包膜- FPV

<400> 50
atgaacactc aaatcctggt tttcgccctt gtggcagtca tccccacaaa
tgcagacaaa 60
atttgtcttg gacatcatgc tgtatcaaat ggcaccaaag taaacacact
cactgagaga 120
ggagtagaag ttgtcaatgc aacggaaaca gtggagcgga caaacatccc
caaaatttgc 180
tcaaaaggga aaagaaccac tgatcttggc caatgcggac tgttagggac
cattaccgga 240

ccacctcaat gcgaccaatt tctagaattt tcagctgatc taataatcga
gagacgagaa 300
ggaaatgatg tttgttaccc ggggaagttt gttaatgaag aggcattgcg
acaaatcctc 360
agaggatcag gtgggattga caaagaaaca atgggattca catatagtgg
aataaggacc 420
aacggaacaa ctagtgcatg tagaagatca gggtcttcat tctatgcaga
aatggagtgg 480
ctcctgtcaa atacagacaa tgctgctttc ccacaaatga caaaatcata
caaaaacaca 540
aggagagaat cagctctgat agtctgggga atccaccatt caggatcaac
caccgaacag 600
accaaactat atgggagtgg aaataaactg ataacagtcg ggagttccaa
atatcatcaa 660
tcttttgtgc cgagtccagg aacacgaccg cagataaatg gccagtccgg
acggattgat 720
tttcattggt tgatcttgga tcccaatgat acagttactt ttagtttcaa

tggggctttc 780
atagctccaa atcgtgccag cttcttgagg ggaaagtcca tggggatcca
gagcgatgtg 840
caggttgatg ccaattgcga aggggaatgc taccacagtg gagggactat
aacaagcaga 900
ttgcctttc aaaacatcaa tagcagagca gttggcaaat gcccaagata
tgtaaaacag 960
gaaagtttat tattggcaac tgggatgaag aacgttcccg aaccttccaa
aaaaaggaaa 1020
aaaagaggcc tgtttggcgc tatagcaggg tttattgaaa atggttggga
aggtctggtc 1080
gacgggtggt acggtttcag gcatcagaat gcacaaggag aaggaactgc
agcagactac 1140
aaaagcaccc aatcggcaat tgatcagata accggaaagt taaatagact
cattgagaaa 1200
accaaccagc aatttgagct aatagataat gaattcactg aggtggaaaa
gcagattggc 1260
aatttaatta actggaccaa agactccatc acagaagtat ggtcttacaa
tgctgaactt 1320
cttgtggcaa tggaaaacca gcacactatt gatttggctg attcagagat
gaacaagctg 1380
tatgagcgag tgaggaaaca attaagggaa aatgctgaag aggatggcac
tggttgcttt 1440
gaaatttttc ataaatgtga cgatgattgt atggctagta taaggaacaa
tacttatgat 1500
cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgaccc

agtcaaattg 1560
agtagtggct acaaagatgt gatactttgg tttagcttcg gggcatcatg
cttttgctt 1620
cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaaacat
gcggtgcact 1680
atttgtatat aa
1692

<210> 51
<211> 1266
<212> DNA
<213> 人工序列

<220>
<223> 包膜- RRV

<400> 51

agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc
acattgcgcc 60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat
ccgagatgag 120
gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga
caaggcaggc 180
acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga
atctaagaga 240
gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat
gggacacttc 300
atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga
cgcagattcg 360
cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg
tagagagaag 420
ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca
gctgacaacg 480
gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga
tcgcaccctg 540
ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat
caggtacaac 600
tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat
caacacatgc 660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt
tacctctcca 720
tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc
gttccctctg 780

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta
tggtaagaag 840
gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag
gagtttagga 900
gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat
catcccagtg 960
acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct
gtgggcgcaa 1020
ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta
ctattatgga 1080
ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc
cctcctaact 1140
ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac
accgtacgcc 1200
ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc
accgagggcg 1260
aatgca

1266

<210> 52
<211> 1266
<212> DNA
<213> 人工序列

<220>
<223> 包膜- MLV 10A1

<400> 52
agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc
acattgcgcc 60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat
ccgagatgag 120
gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga
caaggcaggc 180
acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga
atctaagaga 240
gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat
gggacacttc 300
atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga
cgcagattcg 360
cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg
tagagagaag 420
ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca

gctgacaacg 480
gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga
tcgcaccctg 540
ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat
caggtacaac 600
tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat
caacacatgc 660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt
tacctctcca 720
tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc
gttccctctg 780
actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta
tggtaagaag 840
gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag
gagtttagga 900
gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat
catcccagtg 960

acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct
gtgggcgcaa 1020
ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta
ctattatgga 1080
ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc
cctcctaact 1140
ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac
accgtacgcc 1200
ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc
accgagggcg 1260
aatgca
1266

<210> 53
<211> 2030
<212> DNA
<213> 人工序列

<220>
<223> 包膜- 埃博拉

<400> 53
atgggtgtta caggaatatt gcagttacct cgtgatcgat tcaagaggac
atcattcttt 60
ctttgggtaa ttatcctttt ccaaagaaca ttttccatcc cacttggagt
catccacaat 120

agcacattac aggttagtga tgtcgacaaa ctggtttgcc gtgacaaact
gtcatccaca 180
aatcaattga gatcagttgg actgaatctc gaagggaatg gagtggcaac
tgacgtgcca 240
tctgcaacta aaagatgggg cttcaggtcc ggtgtcccac caaaggtggt
caattatgaa 300
gctggtgaat gggctgaaaa ctgctacaat cttgaaatca aaaaacctga
cgggagtgag 360
tgtctaccag cagcgccaga cgggattcgg ggcttccccc ggtgccggta
tgtgcacaaa 420
gtatcaggaa cgggaccgtg tgccggagac tttgccttcc acaaagaggg
tgctttcttc 480
ctgtatgacc gacttgcttc cacagttatc taccgaggaa cgactttcgc
tgaaggtgtc 540
gttgcatttc tgatactgcc ccaagctaag aaggacttct tcagctcaca
ccccttgaga 600
gagccggtca atgcaacgga ggacccgtct agtggctact attctaccac

aattagatat 660
caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga
caatttgacc 720
tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa
tgagacaata 780
tatacaagtg ggaaaaggag caataccacg ggaaaactaa tttggaaggt
caaccccgaa 840
attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaaacctc
actagaaaaa 900
ttcgcagtga agagttgtct ttcacagctg tatcaaacag agccaaaaac
atcagtggtc 960
agagtccggc gcgaacttct tccgacccag ggaccaacac aacaactgaa
gaccacaaaa 1020
tcatggcttc agaaaattcc tctgcaatgg ttcaagtgca cagtcaagga
agggaagctg 1080
cagtgtcgca tctgacaacc cttgccacaa tctccacgag tcctcaaccc
cccacaacca 1140
aaccaggtcc ggacaacagc acccacaata cacccgtgta taaacttgac
atctctgagg 1200
caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc
tccgacactc 1260
cccccgccac gaccgcagcc ggaccccctaa aagcagagaa caccaacacg
agcaagggta 1320
ccgacctcct ggaccccgcc accacaacaa gtccccaaaa ccacagcgag
accgctggca 1380
acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg

aagctaggct 1440
taattaccaa tactattgct ggagtcgcag gactgatcac aggcgggagg
agagctcgaa 1500
gagaagcaat tgtcaatgct caacccaaat gcaaccctaa tttacattac
tggactactc 1560
aggatgaagg tgctgcaatc ggactggcct ggataccata tttcgggcca
gcagccgagg 1620
gaatttacat agaggggctg atgcacaatc aagatggttt aatctgtggg
ttgagacagc 1680
tggccaacga gacgactcaa gctcttcaac tgttcctgag agccacaacc
gagctacgca 1740
ccttttcaat cctcaaccgt aaggcaattg atttcttgct gcagcgatgg
ggcggcacat 1800
gccacatttt gggaccggac tgctgtatcg aaccacatga ttggaccaag
aacataacag 1860
acaaaattga tcagattatt catgattttg ttgataaaac ccttccggac
cagggggaca 1920

atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga
gttacaggcg 1980
ttataattgc agttatcgct ttattctgta tatgcaaatt tgtcttttag
2030

<210> 54
<211> 237
<212> DNA
<213> 人工序列

<220>
<223> 聚合酶III shRNA启动子- U6启动子

<400> 54
tttcccatga ttccttcata tttgcatata cgatacaagg ctgttagaga
gataattgga 60
attaatttga ctgtaaacac aaagatatta gtacaaaata cgtgacgtag
aaagtaataa 120
tttcttgggt agtttgcagt tttaaaatta tgttttaaaa tggactatca
tatgcttacc 180
gtaacttgaa agtatttcga tttcttggct ttatatatct tgtggaaagg
acgaaac 237

<210> 55
<211> 243
<212> DNA

<213> 人工序列

<220>
<223> 聚合酶III shRNA启动子- 7SK启动子

<400> 55
ctgcagtatt tagcatgccc cacccatctg caaggcattc tggatagtgt
caaaacagcc 60
ggaaatcaag tccgtttatc tcaaacttta gcattttggg aataaatgat
atttgctatg 120
ctggttaaat tagattttag ttaaatttcc tgctgaagct ctagtacgat
aagcaacttg 180
acctaagtgt aaagttgaga tttccttcag gtttatatag cttgtgcgcc
gcctggctac 240
ctc
243

<210> 56
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> FDPS靶序列#1

<400> 56
gtcctggagt acaatgccat t
21

<210> 57
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> FDPS靶序列#2

<400> 57
gcaggatttc gttcagcact t

<210> 58
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> FDPS靶序列#3

<400> 58

gccatgtaca tggcaggaat t
21

<210> 59
<211> 21
<212> DNA
<213> 人工序列

<220>
<223> FDPS靶序列#4

<400> 59
gcagaaggag gctgagaaag t
21

<210> 60
<211> 49
<212> DNA
<213> 人工序列

<220>
<223> 非靶向序列

<400> 60
gccgctttgt aggatagagc tcgagctcta tcctacaaag cggcttttt
49

<210> 61
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> 正向引物

<400> 61

aggaattgat ggcgagaagg
20

<210> 62
<211> 22
<212> DNA
<213> 人工序列

<220>
<223> 反向引物

<400> 62
cccaaagagg tcaaggtaat ca
22

<210> 63
<211> 18

<212> DNA
<213> 人工序列

<220>
<223> 正向引物

<400> 63
agcgcggcta cagcttca
18

<210> 64
<211> 20
<212> DNA
<213> 人工序列

<220>
<223> 反向引物

<400> 64
ggcgacgtag cacagcttct
20

<210> 65
<211> 130
<212> DNA
<213> 人工序列

<220>
<223> 左反向末端重复(左 ITR)

<400> 65
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg
ggcgaccttt 60
ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa
ctccatcact 120
aggggttcct
130

<210> 66
<211> 151
<212> DNA
<213> 人工序列

<220>
<223> 右反向末端重复(右 ITR)

<400> 66
gagcggccgc aggaacccct agtgatggag ttggccactc cctctctgcg
cgctcgctcg 60
ctcactgagg ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg
ggcggcctca 120
gtgagcgagc gagcgcgcag ctgcctgcag g
151

<210> 67
<211> 1227
<212> DNA
<213> 人工序列

<220>
<223> 无REV的辅助质粒

<400> 67
tctagaagga gctttgttcc ttgggttctt gggagcagca ggaagcacta
tgggcgcagc 60
gtcaatgacg ctgacggtac aggccagaca attattgtct ggtatagtgc
agcagcagaa 120
caatttgctg agggctattg aggcgcaaca gcatctgttg caactcacag
tctggggcat 180

caagcagctc caggcaagaa tcctggctgt ggaaagatac ctaaaggatc
aacagctcct 240
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg
agcatctgac 300
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt
ttttgtgtct 360
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg
agtatttggt 420
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg
ctataaagag 480
gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga
aaagccttga 540
cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta
acatccctaa 600
aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta
ctcccagtca 660
tagctgtccc tcttctctta tgaagatccc tcgacctgca gcccaagctt

ggcgtaatca 720
tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca
caacatacga 780
gccggaagca taaagtgtaa agcctggggt gcctaatgag tgagctaact
cacattaatt 840
gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagcg
gatccgcatc 900
tcaattagtc agcaaccata gtcccgcccc taactccgcc catcccgccc
ctaactccgc 960
ccagttccgc ccattctccg ccccatggct gactaatttt ttttatttat
gcagaggccg 1020
aggccgcctc ggcctctgag ctattccaga agtagtgagg aggcttttt
ggaggcctag 1080
gcttttgcaa aaagctaact tgtttattgc agcttataat ggttacaaat
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gtttgtccaa 1200
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1227

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US20120027725A1 *	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple

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WO2012145624A2 *	2011-04-21	2012-10-26	University Of Massachusetts	Raav-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
US20130090371A1 *	2010-04-20	2013-04-11	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-adrenergic receptor degradation
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WO1993024632A1	1992-05-22	1993-12-09	Dana Farber Cancer Institute	Hybrid siv/hiv-1 viral vectors and monkey model for aids
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
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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

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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
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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
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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
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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
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WO2007133674A2	2006-05-12	2007-11-22	Lentigen Corporation	Lentiviral vector compositions, methods and

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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
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US8673477B2	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
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CA3018281C	2007-09-28	2022-02-22	Anthrogenesis Corporation	Tumor suppression using human placental perfusate and human placenta-derived intermediate natural killer cells
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
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WO2010022195A2	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system

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US20120114618A1	2009-03-26	2012-05-10	The Regents Of The University Of California	Mesenchymal Stem Cells Producing Inhibitory RNA for Disease Modification
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EP2419113B1	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
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EP3329772B1	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
CN101805750B *	2009-12-29	2011-11-30	浙江大学	Construction and application of farnesyl pyrophosphoric acid synthetase RNA (Ribonucleic Acid) interference recombinant lentivirus vector
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AU2013273483A1	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
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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
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KR20160011645A	2013-06-03	2016-02-01	떼라벡띠스	LENTIVIRAL VECTORS CONTAINING AN MHC CLASS I, MHC CLASS II OR β2 MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE
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WO2015042308A2	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors
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CN107405357B	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
WO2016069716A1	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds
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
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US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells

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JP7173548B2	2016-06-08	2022-11-16	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
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
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
BR112019017839A2 *	2017-02-28	2020-04-14	Univ Pennsylvania	clade f vector of adeno-associated virus (aav) and its use
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
DE112019000348B4	2018-01-05	2021-10-28	Nantbio, Inc.	REPROGRAMMED T-CELLS SIMILAR NK-CELLS
WO2020011247A1	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
KR20220068954A	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression
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US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
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EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
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KR20200051011A *	2017-09-08	2020-05-12	제너레이션 바이오 컴퍼니	Modified closed-terminated DNA (CEDNA)
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CN109363955B *	2018-11-14	2021-09-21	广州玮弘祺生物科技有限公司	Solid styling spray for hair and preparation method thereof
CN109456993A *	2018-11-28	2019-03-12	上海安民生物技术有限公司	The albumin expression vectors of the promoter containing CAG
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US11534450B2	2022-12-27	Methods and compositions for the activation of gamma-delta T-cells
US11242527B1	2022-02-08	Combination vectors and methods for treating cancer
US20200181645A1	2020-06-11	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells

Priority And Related Applications

Child Applications (2)

Application	Priority date	Filing date	Relation	Title
CN202211395043.5A	2016-01-15	2017-01-13	Division	Methods and compositions for activating gamma-delta T cells
CN202010396594.8A	2016-01-15	2017-01-13	Division	Methods and compositions for activating gamma-T cells

Priority Applications (2)

Application	Priority date	Filing date	Title
CN202211395043.5A	2016-01-15	2017-01-13	Methods and compositions for activating gamma-delta T cells
CN202010396594.8A	2016-01-15	2017-01-13	Methods and compositions for activating gamma-T cells

Applications Claiming Priority (3)

Application	Filing date	Title
US201662279474P	2016-01-15	

US62/279,474	2016-01-15	
PCT/US2017/013399	2017-01-13	Methods and compositons for the activation of gamma-delta t-cells

Legal Events

Date	Code	Title	Description
2018-11-23	PB01	Publication	
2018-11-23	PB01	Publication	
2019-02-22	SE01	Entry into force of request for substantive examination	
2019-02-22	SE01	Entry into force of request for substantive examination	
2022-11-25	GR01	Patent grant	
2022-11-25	GR01	Patent grant	

Concepts

machine-extracted

Download Filter table

Name	Image	Sections	Count	Query match
mixture		title,claims,abstract,description	52	0.000
gamma-delta t lymphocyte		title,claims,abstract,description	22	0.000
activating effect		title,claims,abstract,description	16	0.000
method		title,abstract,description	60	0.000
Neoplasm		claims,abstract,description	74	0.000
immunotherapy		claims,abstract,description	9	0.000
cell		claims,description	271	0.000
vector		claims,description	135	0.000
Farnesyl pyrophosphate synthase		claims,description	127	0.000
Geranyltranstransferase		claims,description	126	0.000
Small hairpin RNA		claims,description	95	0.000

small Interfering RNA	claims,description	95	0.000
cancer	claims,description	50	0.000
zoledronic acid	claims,description	43	0.000
zoledronic acid	claims,description	43	0.000
particle	claims,description	38	0.000
treatment	claims,description	38	0.000
hepatocellular carcinoma	claims,description	36	0.000
Enzymes	claims,description	32	0.000
Enzymes	claims,description	32	0.000
hepatocellular carcinoma	claims,description	32	0.000
viral vector	claims,description	31	0.000
packaging method and process	claims,description	28	0.000
pathway	claims,description	25	0.000
drug	claims,description	23	0.000
DL-mevalonic acid	claims,description	22	0.000
diseases, disorders, signs and symptoms	claims,description	22	0.000
infectious disease	claims,description	22	0.000
(R)-mevalonate	claims,description	21	0.000
pharmaceutical composition	claims,description	20	0.000
manufacturing process	claims,description	18	0.000
inhibitory effect	claims,description	17	0.000
Envelope protein	claims,description	15	0.000
Protein X	claims,description	15	0.000
leukemia	claims,description	14	0.000
MicroRNAs	claims,description	11	0.000

increasing effect	claims,description	11	0.000
microRNA	claims,description	11	0.000
Prostate cancer	claims,description	10	0.000
Prostatic Neoplasms	claims,description	10	0.000
B-cell lymphoma	claims,description	8	0.000
Squamous Cell Carcinoma of Head and Neck	claims,description	8	0.000
Acute myeloid leukaemia	claims,description	7	0.000
Carcinoma	claims,description	7	0.000
non-small cell lung carcinoma	claims,description	6	0.000
Tumor Necrosis Factor	claims,description	5	0.000
melanoma	claims,description	5	0.000
mucosa	claims,description	5	0.000
Anaplastic large-cell lymphoma	claims,description	4	0.000
Angiosarcoma	claims,description	4	0.000
Astrocytoma	claims,description	4	0.000
B-cell chronic lymphocytic leukemia	claims,description	4	0.000
BCR-ABL1 positive chronic myelogenous leukemia	claims,description	4	0.000
Bladder cancer	claims,description	4	0.000
Breast cancer	claims,description	4	0.000
Breast neoplasm	claims,description	4	0.000
Burkitt lymphomas	claims,description	4	0.000
Cervix carcinoma	claims,description	4	0.000
Chronic myeloid leukaemia	claims,description	4	0.000
Colon cancer	claims,description	4	0.000
Colorectal Neoplasms	claims,description	4	0.000

Endometrial cancer	claims,description	4	0.000
Endometrial neoplasm	claims,description	4	0.000
Ewing Sarcoma	claims,description	4	0.000
Fibrosarcoma	claims,description	4	0.000
Glial tumor	claims,description	4	0.000
Glioma	claims,description	4	0.000
Hemangiosarcoma	claims,description	4	0.000
Hodgkin disease	claims,description	4	0.000
Hodgkin lymphoma	claims,description	4	0.000
Hodgkins lymphoma	claims,description	4	0.000
Kaposi sarcoma	claims,description	4	0.000
Large-Cell Anaplastic Lymphoma	claims,description	4	0.000
Laryngeal squamous cell carcinoma	claims,description	4	0.000
Leiomyosarcoma	claims,description	4	0.000
Lipoma	claims,description	4	0.000
Lung neoplasm malignant	claims,description	4	0.000
Lymphocytic Chronic B-Cell Leukemia	claims,description	4	0.000
Mantle-Cell Lymphoma	claims,description	4	0.000
Medulloblastoma	claims,description	4	0.000
Mesothelioma	claims,description	4	0.000
Multiple myelomas	claims,description	4	0.000
Myelodysplastic syndrome	claims,description	4	0.000
Myelogenous Chronic BCR-ABL Positive Leukemia	claims,description	4	0.000
Myeloid Acute Leukemia	claims,description	4	0.000
Myxofibrosarcoma	claims,description	4	0.000

Nasopharyngeal cancer	claims,description	4	0.000
Neuroblastoma	claims,description	4	0.000
Neurofibroma	claims,description	4	0.000
Non-Hodgkin lymphomas	claims,description	4	0.000
Oesophageal carcinoma	claims,description	4	0.000
Oesophageal squamous cell carcinoma	claims,description	4	0.000
Ovarian cancer	claims,description	4	0.000
Ovarian neoplasm	claims,description	4	0.000
Pancreatic neoplasm	claims,description	4	0.000
Plasma cell myeloma	claims,description	4	0.000
Renal cell carcinoma	claims,description	4	0.000
Retinoblastoma	claims,description	4	0.000
Squamous cell carcinoma of the esophagus	claims,description	4	0.000
Stomach Neoplasms	claims,description	4	0.000
Testicular Neoplasms	claims,description	4	0.000
Thyroid neoplasm	claims,description	4	0.000
Urinary Bladder Neoplasms	claims,description	4	0.000
Uterine Cervical Neoplasms	claims,description	4	0.000
Wilms tumor	claims,description	4	0.000
cervical cancer	claims,description	4	0.000
chronic lymphocytic leukemia	claims,description	4	0.000
esophageal squamous cell carcinoma	claims,description	4	0.000
gastric cancer	claims,description	4	0.000
gastrin-producing neuroendocrine tumor	claims,description	4	0.000
gastrinoma	claims,description	4	0.000

glioblastoma	claims,description	4	0.000
head and neck cancer	claims,description	4	0.000
head and neck neoplasm	claims,description	4	0.000
head and neck squamous cell carcinoma	claims,description	4	0.000
hepatoblastoma	claims,description	4	0.000
liposarcoma	claims,description	4	0.000
lung cancer	claims,description	4	0.000
lung neoplasm	claims,description	4	0.000
lymphoid tissue	claims,description	4	0.000
malignant pancreatic neoplasm	claims,description	4	0.000
neoplasm of testis	claims,description	4	0.000
osteosarcoma	claims,description	4	0.000
pancreatic cancer	claims,description	4	0.000
pancreatic carcinoma	claims,description	4	0.000
pheochromocytoma	claims,description	4	0.000
rhabdomyosarcoma	claims,description	4	0.000
secretion	claims,description	4	0.000
small cell lung carcinoma	claims,description	4	0.000
stomach cancer	claims,description	4	0.000
testicular cancer	claims,description	4	0.000
thyroid cancer	claims,description	4	0.000
tumor of salivary gland	claims,description	4	0.000
urinary bladder cancer	claims,description	4	0.000
transitional cell carcinoma	claims,description	3	0.000
Nasopharyngeal Carcinoma	claims,description	2	0.000

endocytic effect	claims,description	2	0.000
nasopharynx carcinoma	claims,description	2	0.000
schwann cell	claims,description	2	0.000
Syncytin-1	claims	4	0.000
Carcinoma of esophagus	claims	3	0.000
Lymphocytic leukaemia	claims	3	0.000
esophageal carcinoma	claims	3	0.000
laryngeal carcinoma	claims	3	0.000
lymphoid leukemia	claims	3	0.000
Nasopharyngeal Neoplasms	claims	2	0.000
disorder	claims	2	0.000
neurilemmoma	claims	2	0.000
schwannoma	claims	2	0.000
tumor necrosis factor	claims	2	0.000
gene therapy	abstract,description	17	0.000
Communicable disease	abstract,description	16	0.000

Show all concepts from the description section