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(54) **METHODS AND COMPOSITIONS FOR THE
ACTIVATION OF GAMMA-DELTA T-CELLS**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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This patent is subject to a terminal dis-
claimer.

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(57) **ABSTRACT**

The present invention relates generally to methods and
compositions for gene therapy and immunotherapy that
activate gamma delta T-cells, and in particular, can be used
in the treatment of various cancers and infectious diseases.

20 Claims, 15 Drawing Sheets

Specification includes a Sequence Listing.

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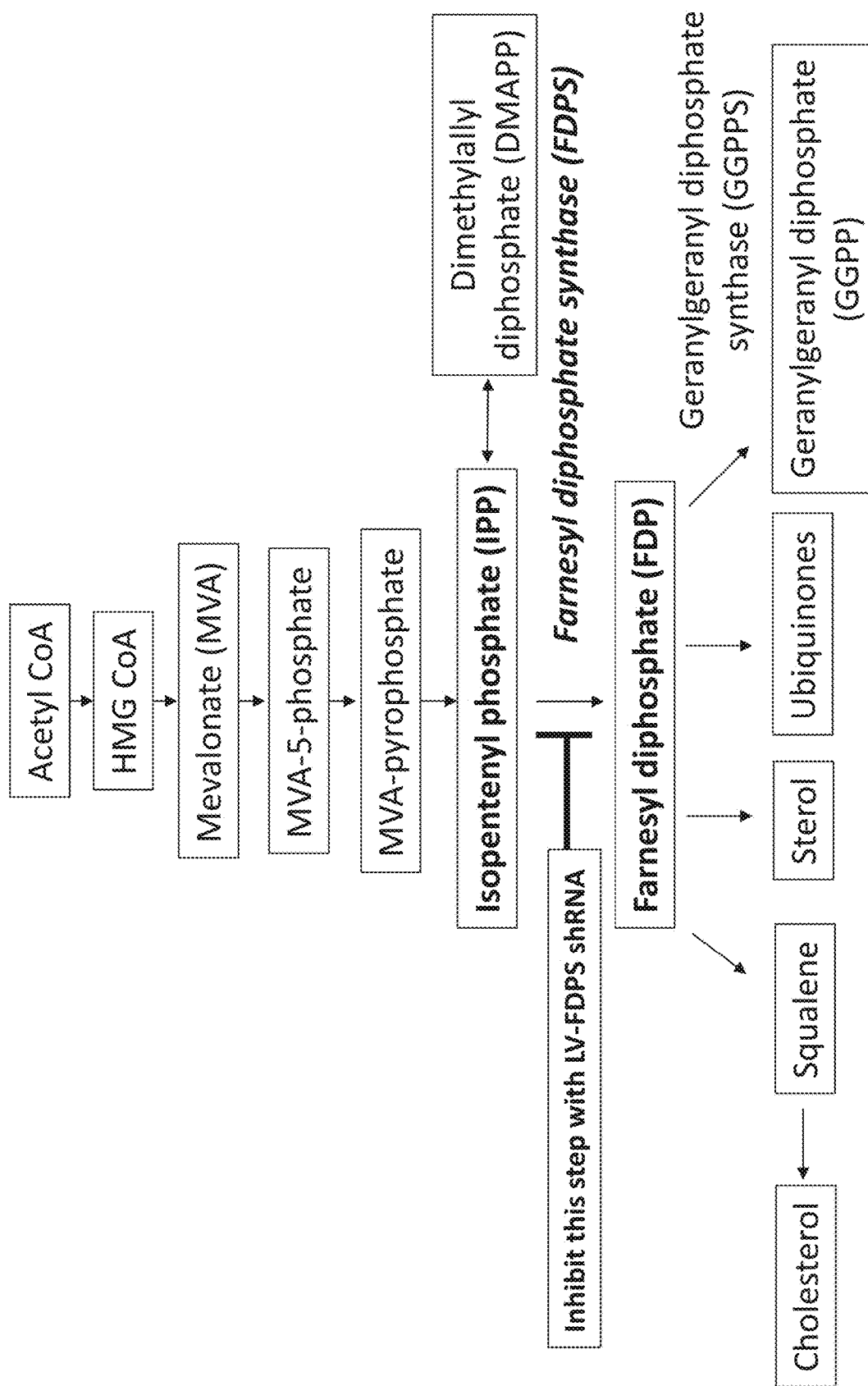


Figure 1

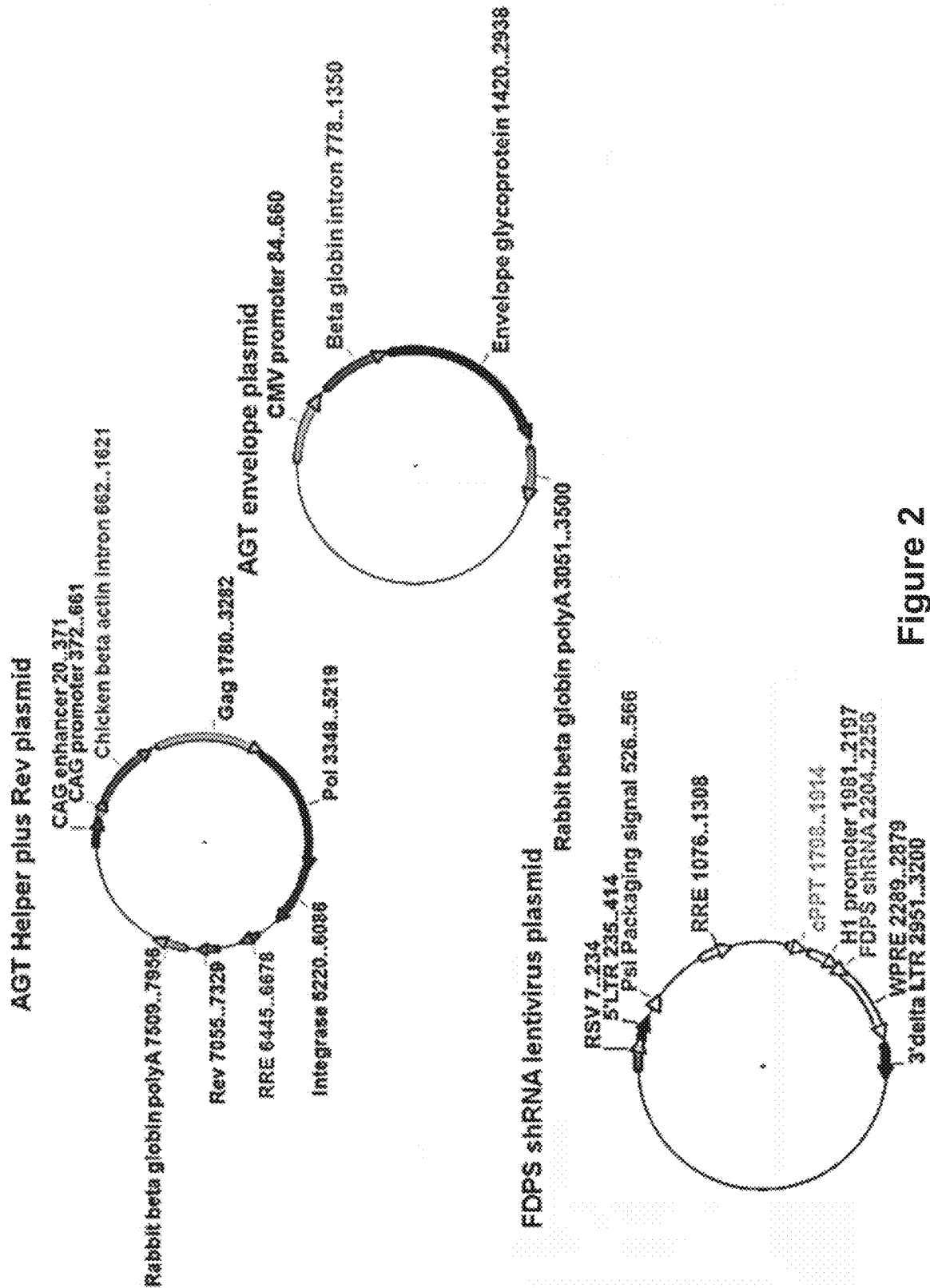


Figure 2

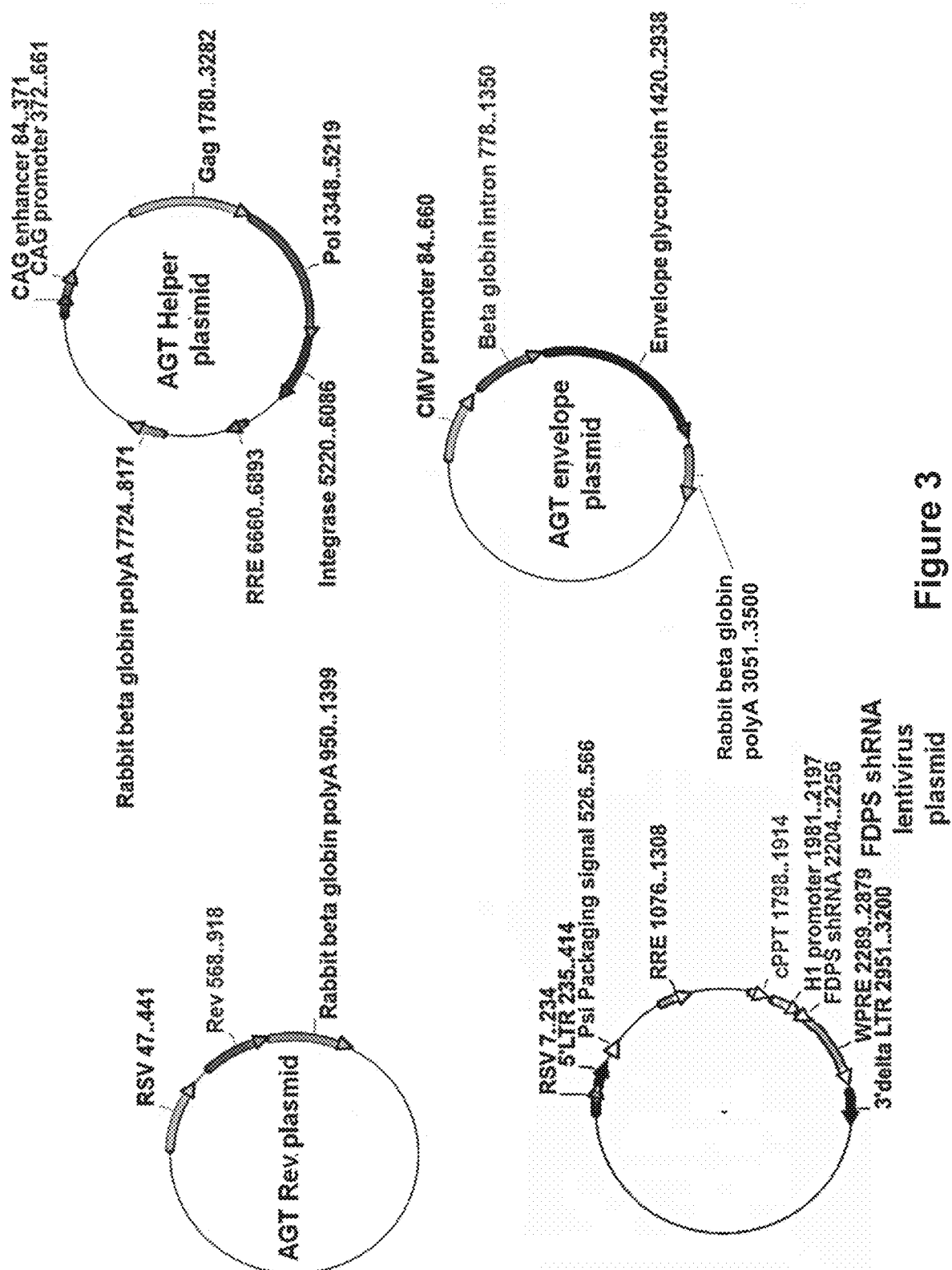


Figure 3

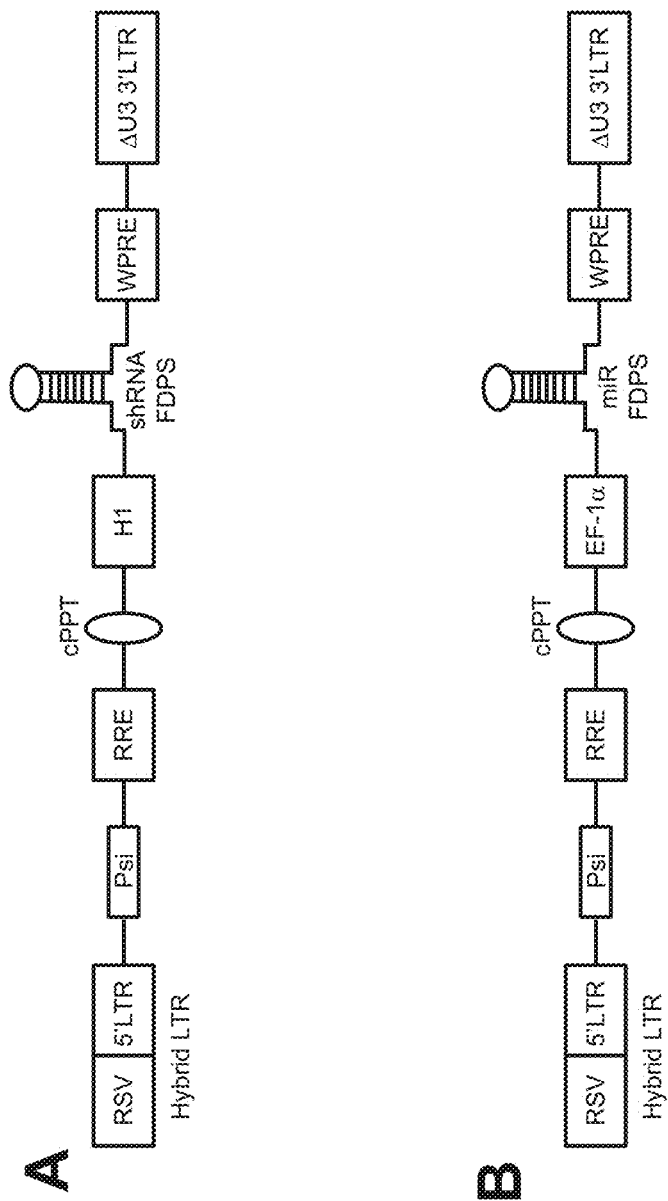


Figure 4

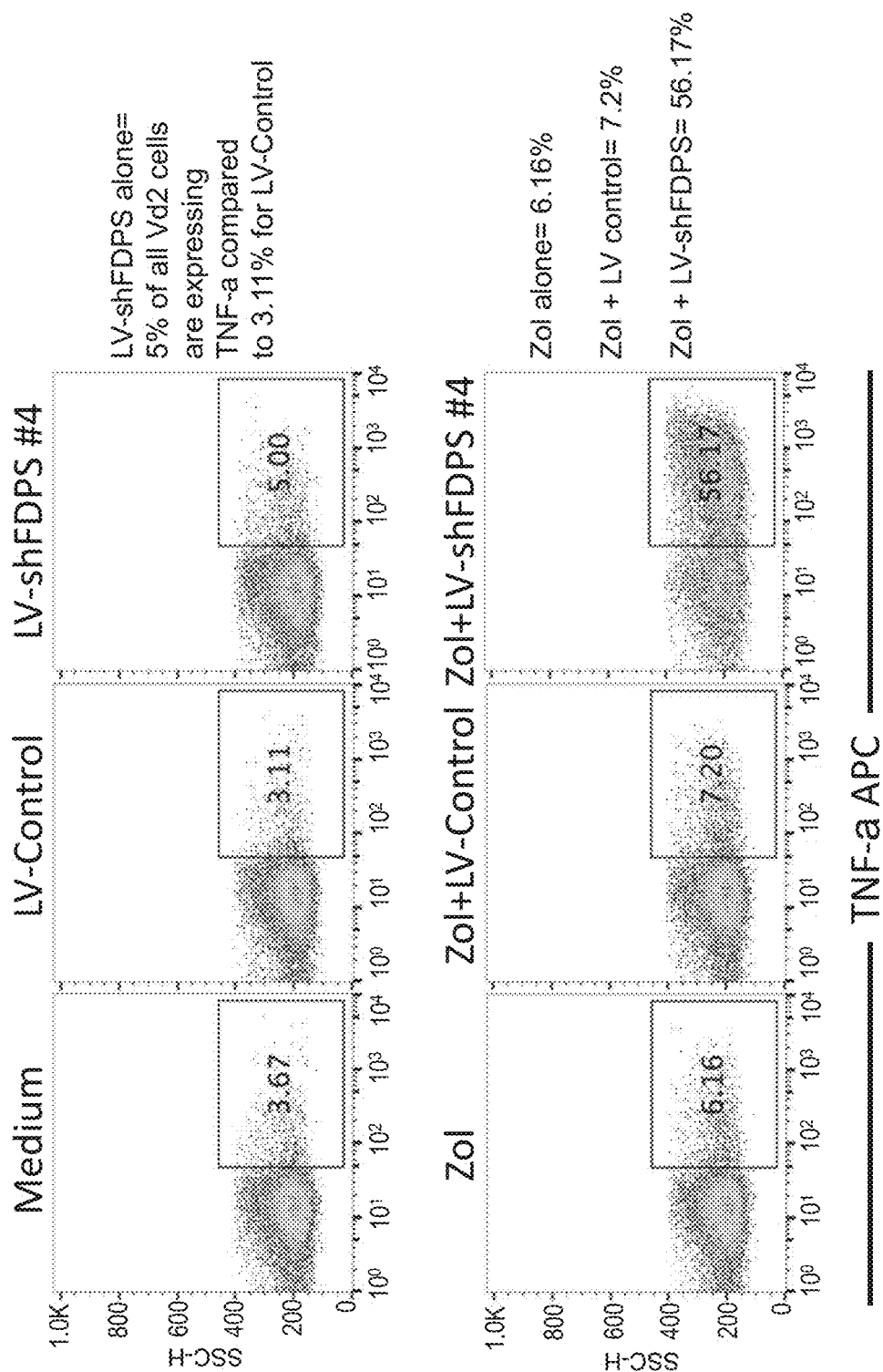


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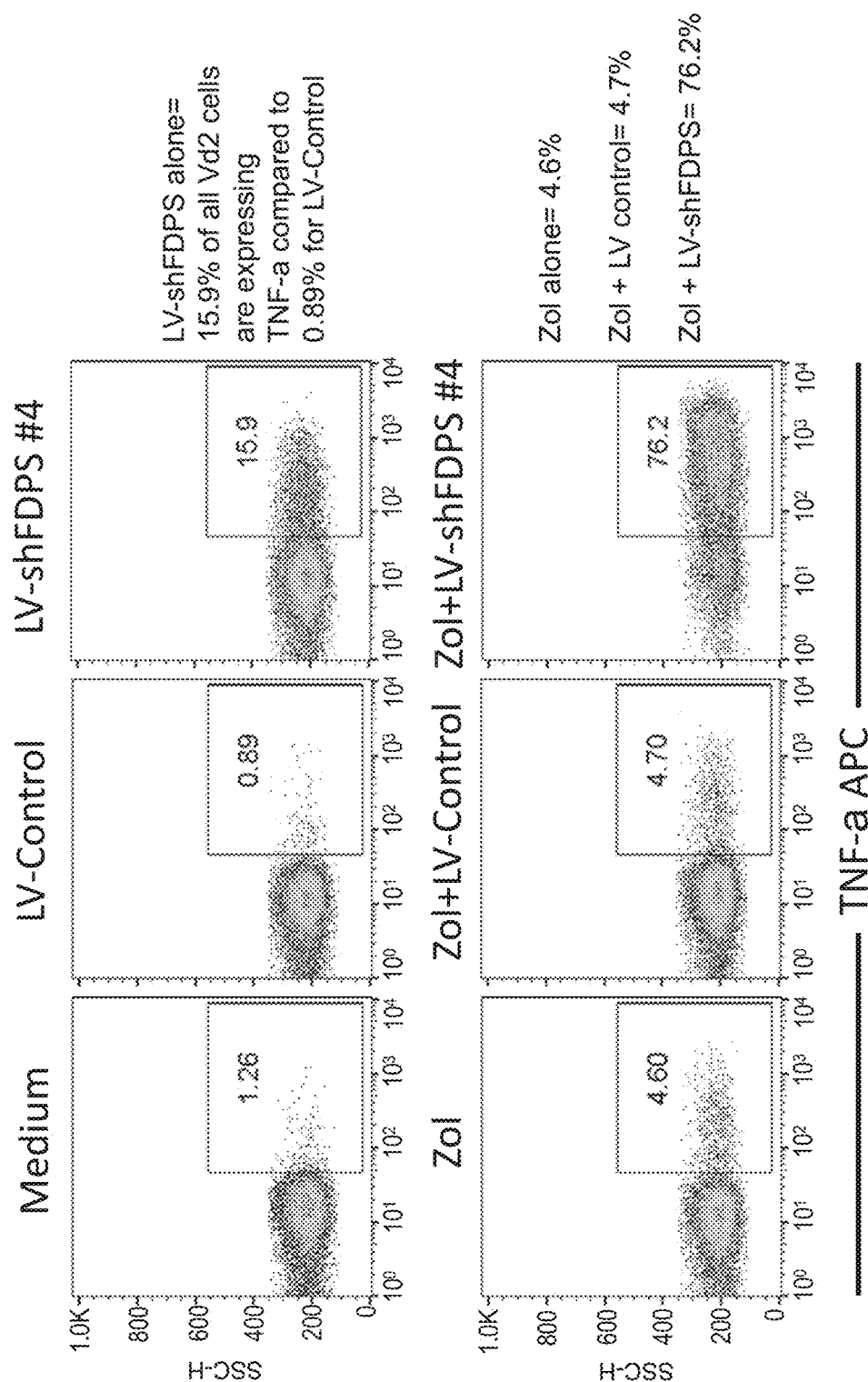


Figure 6

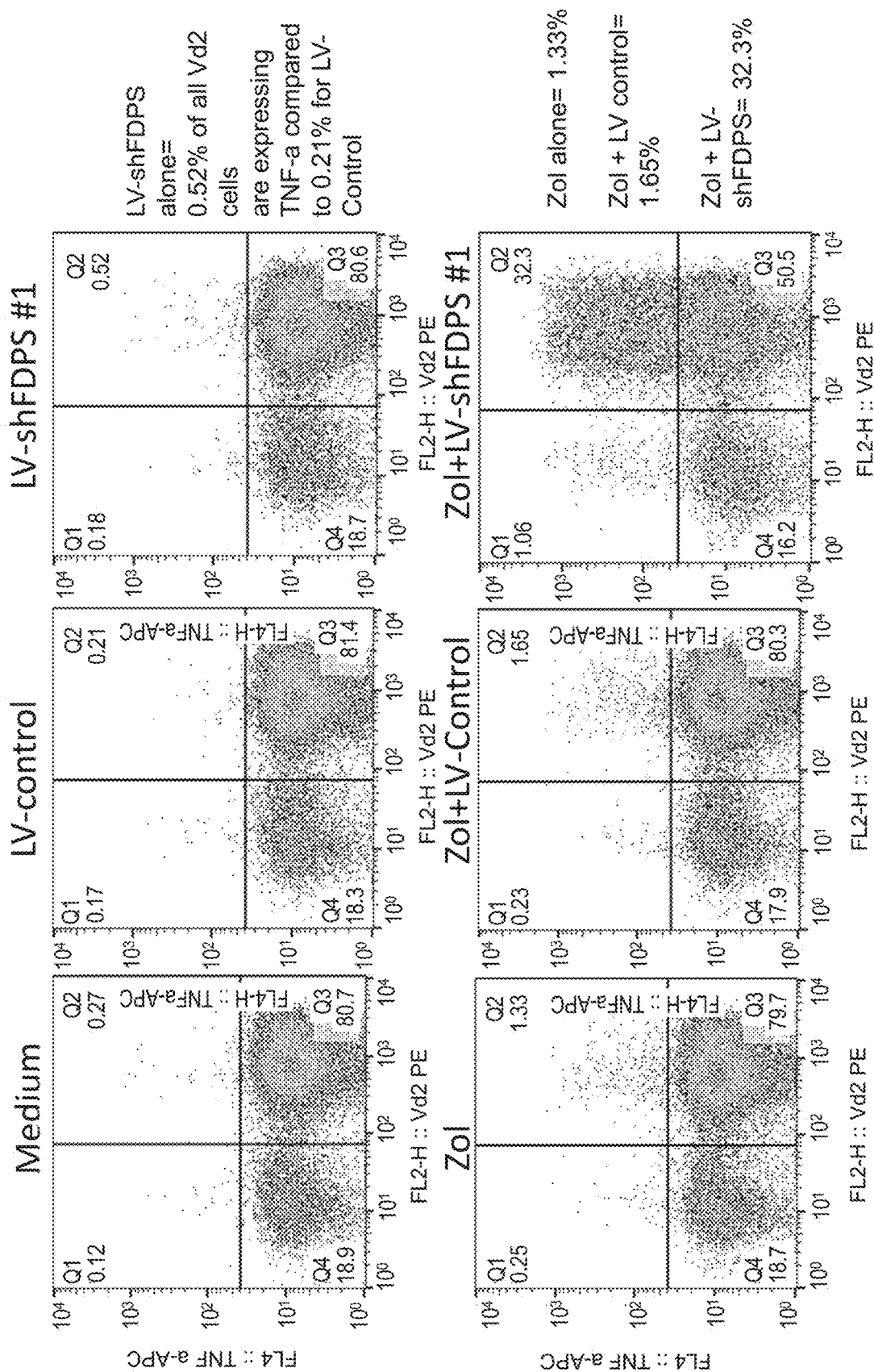


Figure 7

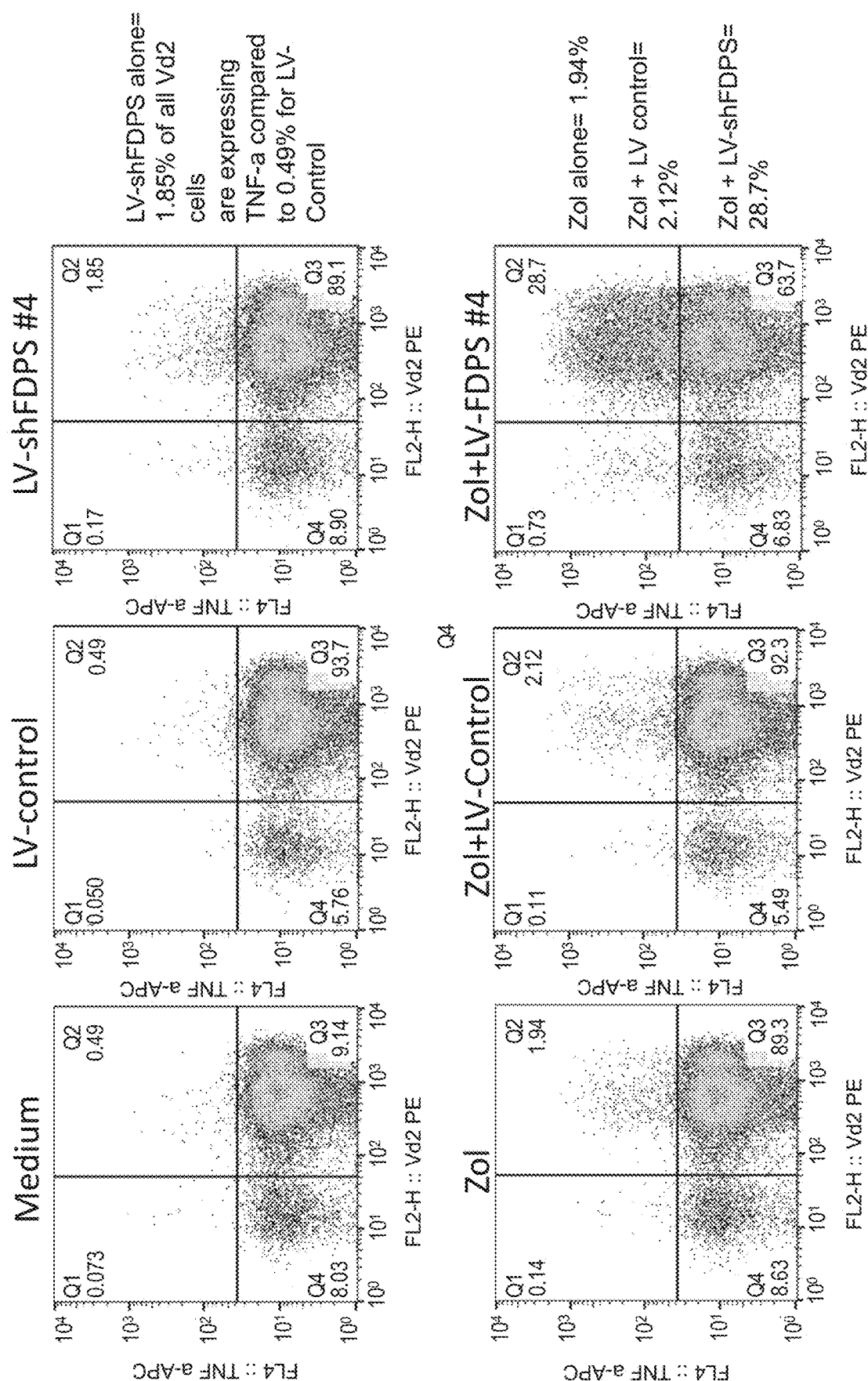


Figure 8

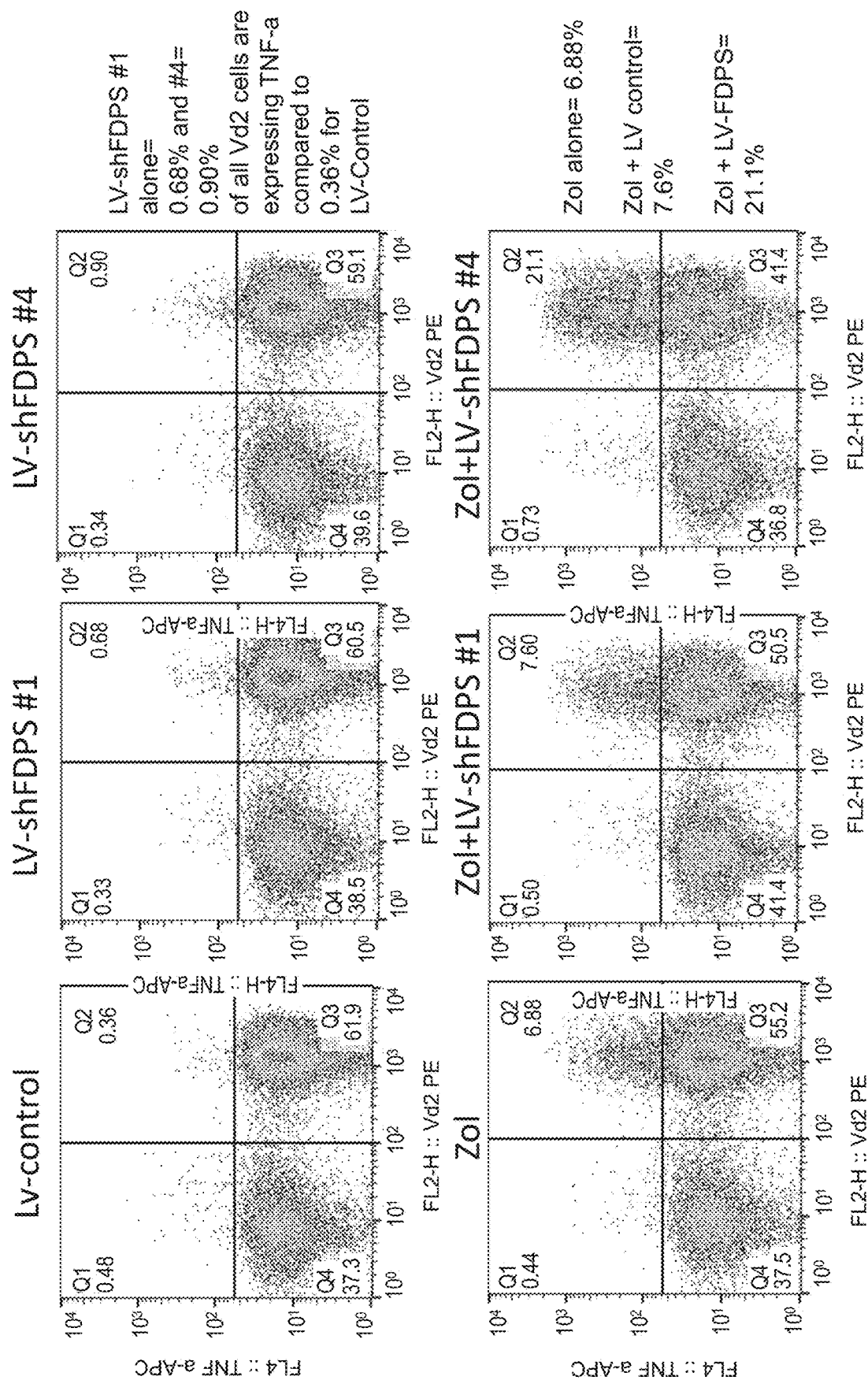


Figure 9

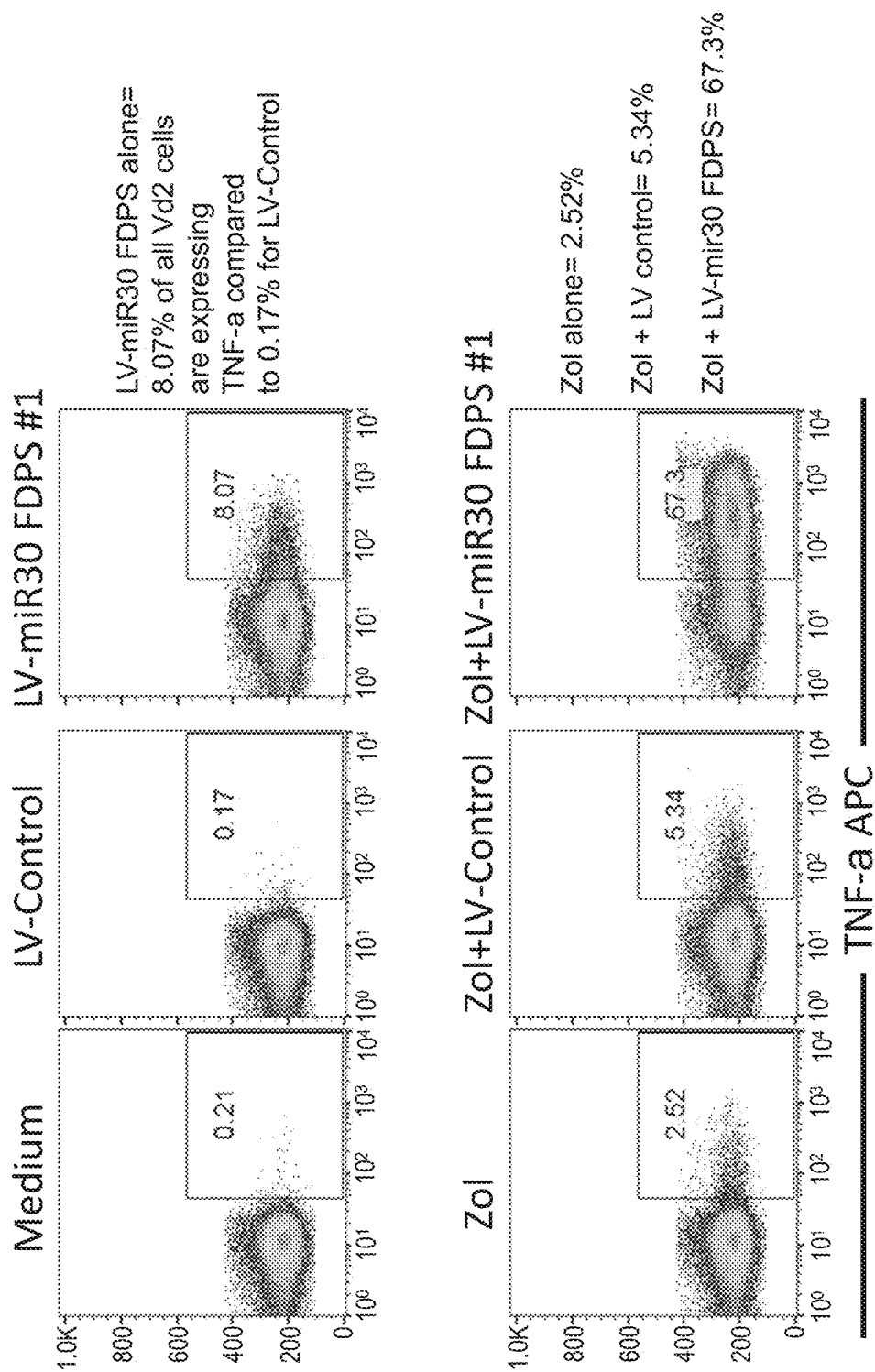


Figure 10

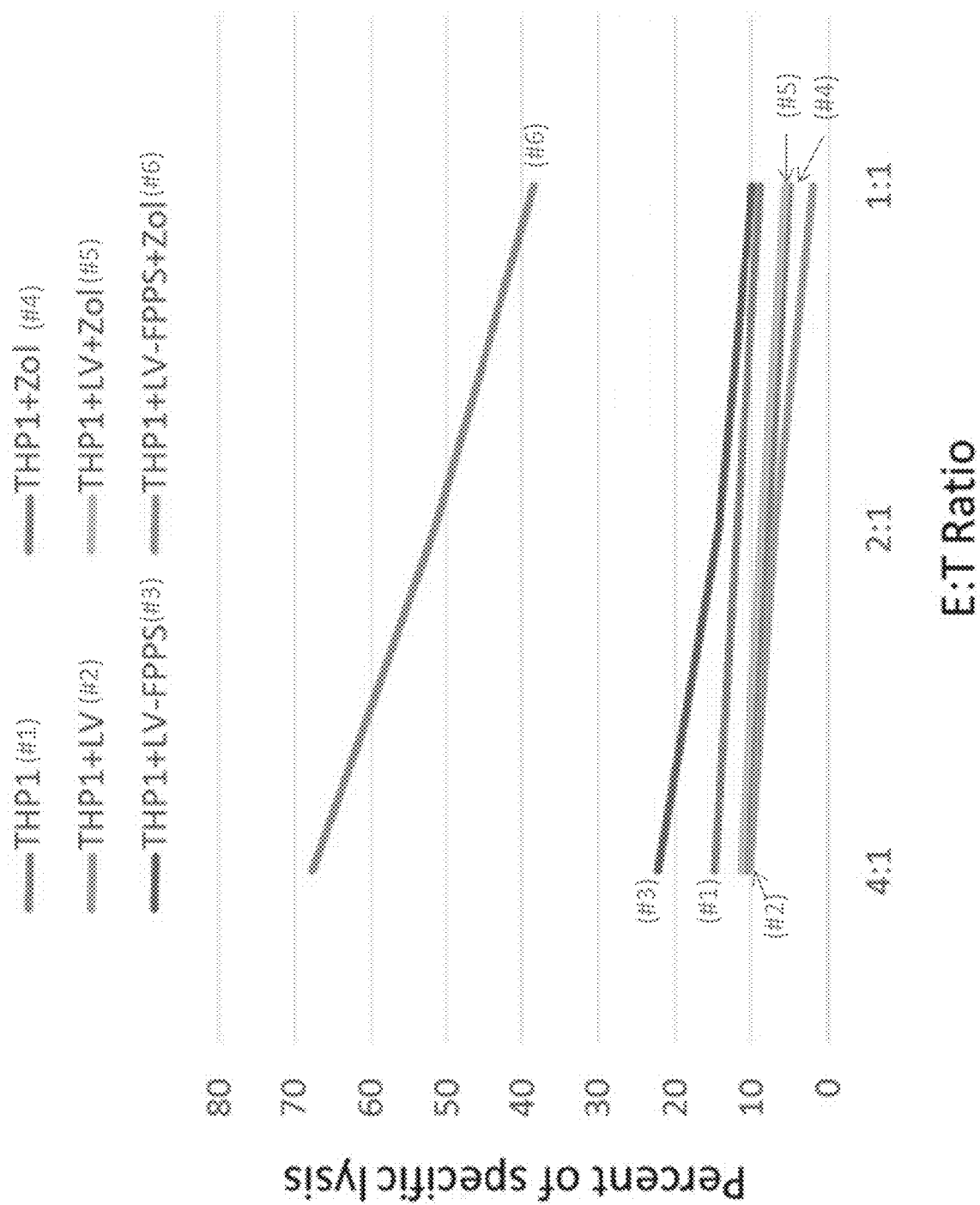


Figure 11

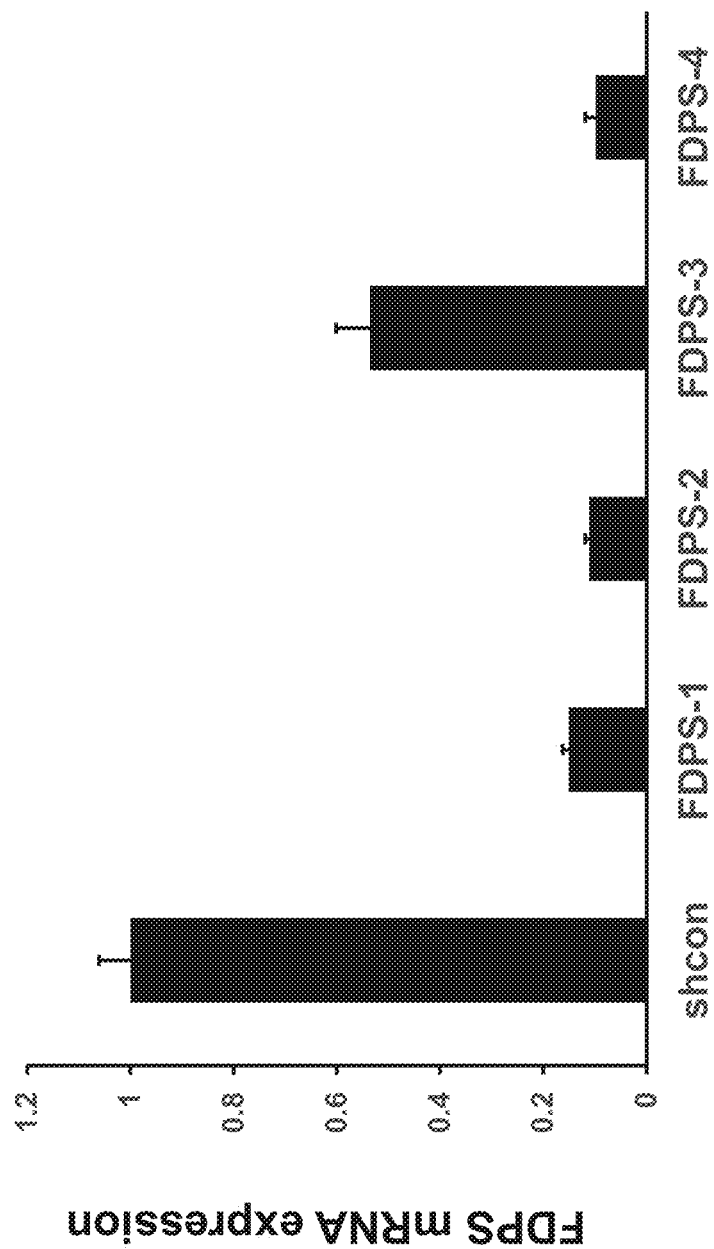


Figure 12

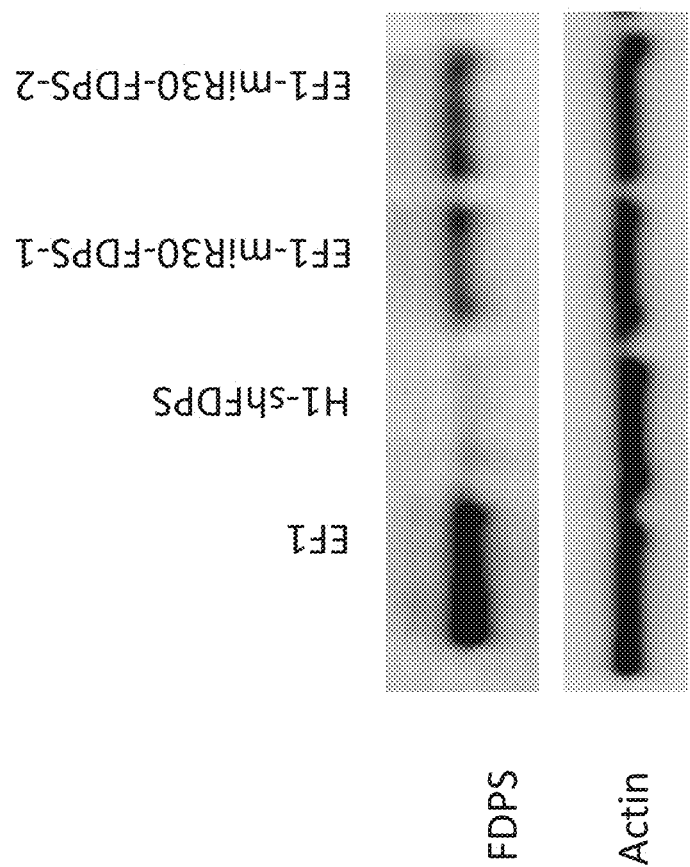


Figure 13

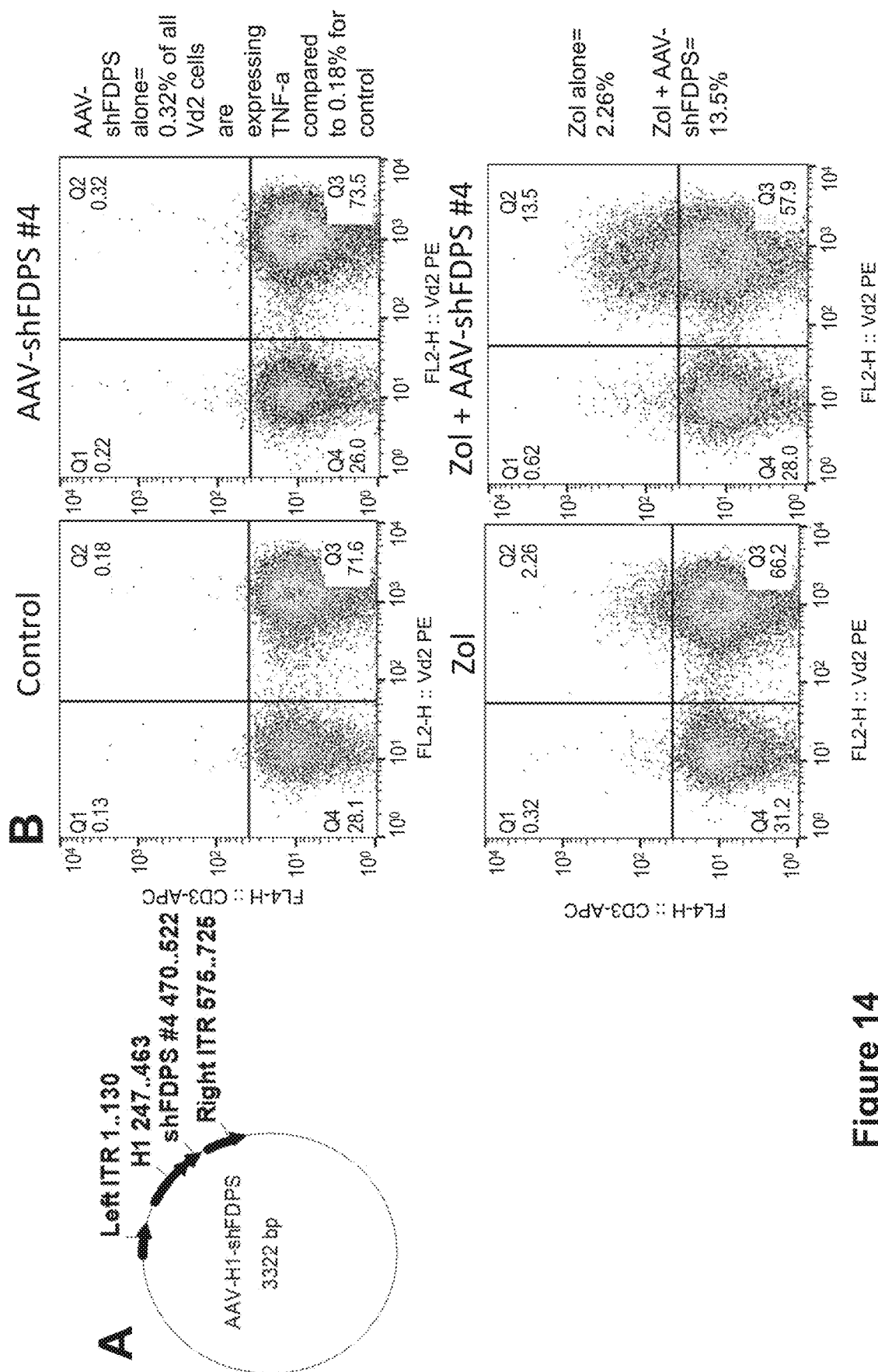


Figure 14

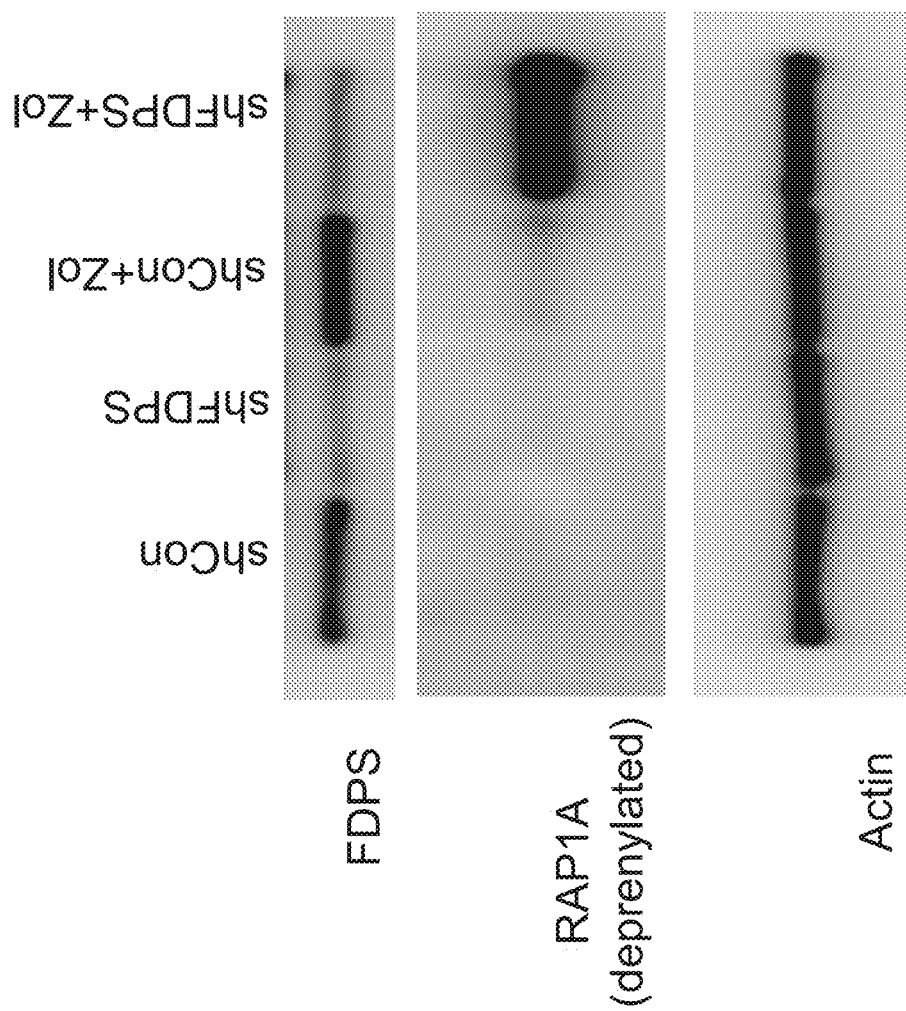


Figure 15

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**METHODS AND COMPOSITIONS FOR THE
ACTIVATION OF GAMMA-DELTA T-CELLS****CROSS-REFERENCE TO RELATED
APPLICATIONS**

This application is a continuation of U.S. patent application Ser. No. 16/530,908 filed on Aug. 2, 2019 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS", which is a continuation of U.S. patent application Ser. No. 16/132,247 filed on Sep. 14, 2018 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS," which is a continuation of U.S. patent application Ser. No. 15/904,131 filed on Feb. 23, 2018 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS", which is a continuation in part of U.S. patent application Ser. No. 15/652,080 filed on Jul. 17, 2017 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS", which is a continuation of International Application No. PCT/US17/13399 filed on Jan. 13, 2017 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS", which claims priority to U.S. Provisional Patent Application No. 62/279,474 filed on Jan. 15, 2016 entitled "METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS", the disclosures of which are incorporated herein by reference.

SEQUENCE LISTING

A Sequence Listing is enclosed with this application and is incorporated herein by reference. The text file of the Sequence Listing is named 7061203137_SL.txt and the file size is 61 kilobytes.

FIELD OF THE INVENTION

The present disclosure relates generally to the fields of gene therapy and immunotherapy, specifically in relation to increased activation and effector cell function of gamma delta ("GD") T cells.

BACKGROUND

Human T cells are distinguished on the basis of T cell receptor structure. The major populations, including CD4+ and CD8+ subsets, express a receptor composed of alpha and beta chains. A smaller subset expresses T cell receptor made from gamma and delta chains. Gamma delta ("GD") T cells make up 3-10% of circulating lymphocytes, and a Vδ2+ subset makes up 75% of GD T cells in blood. Vδ2+ cells recognize non-peptide epitopes and do not require antigen presentation by major histocompatibility complexes ("MHC") or human leukocyte antigen ("HLA"). The majority of Vδ2+ T cells also express a Vγ9 chain and are stimulated by exposure to 5-carbon pyrophosphate compounds that are intermediates in mevalonate and non-mevalonate sterol/isoprenoid synthesis pathways. The response to isopentenyl pyrophosphate (5-carbon) is universal among healthy human beings.

Another subset of GD T cells, Vδ+, make up a much smaller percentage of the T cells circulating in the blood, but Vδ+1 cells are commonly found in the epithelial mucosa and the skin.

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In general, GD T cells have several functions, including killing tumor cells and pathogen-infected cells. Stimulation through their unique T cell receptor ("TCRs") composed of two glycoprotein chains, γ and δ, improves the capacity for cellular cytotoxicity, cytokine secretion and other effector functions. The TCRs of GD T cells have unique specificities and the cells themselves occur in high clonal frequencies, thus allowing rapid innate-like responses to tumors and pathogens.

Bisphosphonate drugs and other inhibitors of farnesyl diphosphate synthase ("FDPS"), which are downstream from isopentenyl pyrophosphate ("IPP") in the mevalonate pathway (see, for e.g., FIG. 1), have been used to treat various diseases, including cancers, specifically those involving bone metastasis. Bisphosphonate drugs include, for example, Zometa® (Novartis) and Fosamax® (Merck).

Certain bisphosphonates have also been investigated for stimulation of GD T cells. This may be because when FDPS is inhibited in myeloid cells, IPP begins to accumulate and geranylgeranyl pyrophosphate ("GGPP"), a downstream product of FDPS that suppresses activation of the inflammasome pathway, is reduced. The reduction in GGPP removes an inhibitor of the caspase-dependent inflammasome pathway and allows secretion of mature cytokines including interleukin-beta and interleukin-18, the latter being especially important for gamma delta T cell activation.

Thus, when FDPS is blocked, the increased IPP and decreased GGPP combine to activate Vδ2+ T cells. Vδ2+ cells activated by IPP or bisphosphonates will proliferate rapidly, express a number of cytokines and chemokines, and can function to cytotoxically destroy tumor cells or cells infected with pathogenic microorganisms.

However, bisphosphonates are associated with inflammation and osteonecrosis, as well as having poor bioavailability due to their chemistry. Likewise, IPP has a very short half-life and is difficult to synthesize. Both types of compounds require systemic administration in an individual. Accordingly, both bisphosphonates in general, and IPP specifically, leave a great deal to be desired for therapeutic purposes involving activation of GD T cells.

SUMMARY OF THE INVENTION

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

In another aspect, a method of treating cancer in a subject is provided. The method includes administering to the subject a therapeutically-effective amount of a viral delivery system that encodes at least one genetic element. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of an enzyme involved in

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the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell activates the GD T cell, to thereby treat the cancer. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA. In further embodiments, the target cell is also contacted with a bisphosphonate drug. In embodiments, the bisphosphonate drug is zoledronic acid.

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

In another aspect, the at least one encoded genetic element includes a shRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with GTCCTG-GAGTACAATGCCATTCTCGAGAATGGCAT-TGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGAT-TTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAA ATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAA TTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGGCTGA GAAAGTCTCGA-GACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In a preferred embodiment, the shRNA includes GTCCTGGAGTACAATGCCATTCTCGAG AATGGCAT-TGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGAT-TTCGTTCA GCACTTCTCGAGAAGTGCT-GAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCA TGTACATGGCAGGAATTCTCGAGAATTCCTGC-CATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGGCTGAGAAAGTCTCGA-GACTTTCTCAGCCTCCTT CTGCTTTTT (SEQ ID NO: 4).

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with AAGGTATATTGCTGTTGACAGT-GAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGC CACAGATGGCAGAAGGAGGCT-GAGAAAGTGCTGCTACTGCTCGGACTTCAAGGG GCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGT-GAGCGACACT TTCTCAGCCTCCTTCTGCGT-GAAGCCACAGATGGCAGAAGGGCT-GAGAAAGTGCTGC CTACTGCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTGTTGACAGTG AGCGACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAGGAGGCTGAG AAAGTTGCC-TACTGCCTCGGA (SEQ ID NO: 7); CCTG-GAGGCTTGCTGAAG GCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTT GGCCACTGACTGAGCAGAAGGG CTGAGAAAGTCAGGACACAAGGCCTGT-TACTAGCACTCA (SEQ ID NO: 8); CATCTC-CATGGCTGTAC-

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CACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTG TTGAA TCT- CATGGCAGAAGGAGGCGAGAAAGTCTGACAT-TTTGGTATCTTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGGA-TACTTTCT CAGCCTCCTTCTGCTGGTCCCCCTCCCCGCAGAAGG AGGCTGAGAAAGTCCTTCCCTC CCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). In a preferred embodiment, the microRNA includes AAGGTAT-ATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCT CCTTCTGCGTGAAGCCACA-GATGGCAGAAGGAGGCTGAGAAAGTGTGCC-TACTGC CTCGGACTTCAAGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGT GAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAGGGCTGA GAAAGTGTGCC-TACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTG TTGACAGT-GAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAGG AGGCTGAGAAAGTTGCC-TACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCT TGCT-GAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCT TTTTGGCCACTGACTGAG CAGAAGGGCT-GAGAAAGTCAGGACACAAGGCCTGT-TACTAGCACTCA (SEQ ID NO: 8); CATCTC-CATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCT CCTT CTGCCTGTTGAATCT-CATGGCAGAAGGAGGCGAGAAAGTCTGACAT-TTTGGTATCTT TCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGG ATACTTTCTCAGCCTCCTTCTGCTGGTCCCCCTCCCC GCAGAAGGAGGCTGAGAAAGT CCTTCCCTCC-CAATGACCGCGTCTTCGTCG (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 includes a small RNA capable of inhibiting 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 includes a microRNA or a shRNA.

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

In embodiments, the viral vector is comprised of any vector that can effectively transduce the small RNA into a target cell. In embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated virus vector.

In another aspect, the viral vector includes a second encoded genetic element. In embodiments, the second genetic element includes at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from the group consisting of: IL-18, TNF- α , interferon- γ , IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least

one chemokine is a CC chemokine or a CXC chemokine. In further embodiments, 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, at least one envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes. When the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, a lentiviral particle is produced by the packaging cell. In embodiments, the lentiviral particle is capable of infecting a targeting cell, and inhibiting an enzyme involved in the mevalonate pathway within the target cell. In embodiments, the enzyme involved in the mevalonate pathway is FDPS. In embodiments, the lentiviral vector system includes a first helper plasmid for expressing the gag and pol genes, and a second helper plasmid for expressing the rev gene. In embodiments, the envelope protein is preferably optimized for infecting a target cell. In embodiments, the target cell is a cancer cell. In other embodiments, the target cell is a cell that is infected with an infectious agent.

In another aspect a pharmaceutical combination is disclosed which includes a bisphosphonate compound; and a lentiviral particle produced by a packaging cell and capable of infecting a target cell. The lentiviral particle comprises an envelope protein capable of infecting the target cell, and: at least one encoded shRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded shRNA comprises a sequence having at least 80% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4; or at least one encoded microRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded microRNA comprises a sequence having at least 80% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10, wherein the pharmaceutical combination is at least one of fixed and non-fixed. In embodiments, the at least one encoded shRNA comprises a sequence having at least 85% or at least 90% or at least 95% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4; or the at least one encoded microRNA comprises a sequence having at least 85% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the at least one encoded shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4 or the at least one encoded microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the pharmaceutical composition comprises a fixed combination. In embodiments, the pharmaceutical composition comprises a non-fixed combination. In embodiments, the bisphosphonate drug comprises zoledronic acid. In embodiments, the bisphosphonate drug and the lentiviral particle are present in synergistically effective amounts. In embodiments, the target cell is one or more cancer cells that are present in a cancer selected from one or more of a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof. In embodiments, the target cell is one or more cancer cells that are present in a hepatocellular carcinoma. In embodiments, the target cell is capable of activating a gamma delta T cell following infection with the lentiviral particle. In embodiments, the enzyme is FDPS.

In another aspect, a method of treating a cancer in a subject using an immunotherapy-based composition is disclosed. The method includes administering a therapeutically-effective amount of a bisphosphonate drug to the subject; and administering a therapeutically-effective amount of the immunotherapy-based composition to the subject, wherein the immunotherapy-based composition comprises a lentiviral particle. The lentiviral particle comprises an envelope protein capable of infecting one or more cancer cells, and at least one encoded shRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded shRNA comprises a sequence having at least 80% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4 or at least one encoded microRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded microRNA comprises a sequence having at least 80% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the at least one encoded shRNA comprises a sequence having at least 85% or at least 90% or at least 95% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4, or at least one encoded microRNA comprises a sequence having at least 85% or at least 90% or at least 95% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the at least one encoded shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In embodiments, the at least one encoded microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the one or more cancer cells are present in a cancer selected from one or more of a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof. In embodiments, the bisphosphonate drug comprises zoledronic acid. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in a fixed combination. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in a non-fixed combination. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered simultaneously. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered sequentially. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in synergistically effective amounts. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered at a synergistically effective time interval. In embodiments, the one or more cancer cells are capable of activating a gamma delta T cell resident in the subject following infection of the one or more cancer cells with the immunotherapy-based composition. In embodiments, activating the gamma delta T cell comprises increasing tumor necrosis factor (TNF)- α expression by the gamma delta T cell. In embodiments, activating the gamma delta T cell comprises increasing expression and/or secretion of cytokines, chemokines, and/or cell death ligands including but not limited to FasL and TRAIL. In embodiments, the enzyme of the mevalonate pathway is farnesyl diphosphate synthase (FDPS).

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

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

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

FIG. 5 depicts data demonstrating activation of Vδ2+ T cells THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 6 depicts data demonstrating activation of Vδ2+ T cells by THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 7 depicts data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #1 (SEQ ID NO: 1), as described herein.

FIG. 8 depicts data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. 9 depicts data demonstrating activation of Vδ2+ T cells by HepG2 carcinoma cells with a lentivirus 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 Vδ2+ T cells by THP-1 leukemia cells with a lentivirus expressing miR30 FDPS #1 (SEQ ID NO: 5), as described herein.

FIG. 11 depicts data demonstrating the percent of specific lysis versus an E:T ratio for a variety of experimental conditions, as described herein.

FIG. 12 depicts data demonstrating lentiviral-delivered shRNA-based RNA interference targeting the human FDPS gene.

FIG. 13 depicts data demonstrating lentiviral-delivered miR-based RNA interference targeting the human FDPS gene.

FIG. 14 depicts data demonstrating activation of Vδ2+ T cells by HepG2 carcinoma cells with an adeno-associated virus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

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

DETAILED DESCRIPTION

Overview of Disclosure

The present disclosure relates to gene therapy constructs and delivery of the same to cells, resulting in suppression of Farnesyl diphosphate synthase ("FDPS"), which is necessary to convert isopentenyl phosphate (IPP) to farnesyl diphosphate (FDP), as shown, for example, in FIG. 1. In embodiments, one or more viral vectors are provided with microRNAs or short hairpin RNAs (shRNA) that target FDPS, thereby reducing expression levels of this enzyme. The viral vectors include lentiviral vectors and AAV vectors. A consequence of modulating expression of FDPS is to increase the accumulation of IPP, which is a stimulator of GD T cell proliferation and differentiation. Accordingly, the constructs provided herein are used to activate GD T cells, and are used to treat cancers and infectious diseases.

Definitions and Interpretation

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those

of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g.: Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

As used in the description and the appended claims, the singular forms "a", "an" and "the" are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, "and/or" refers to and encompasses 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 designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by 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" also includes the exact value "X" in addition to minor increments of "X" such as "X+0.1" or "X-0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

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

As used herein, the terms "administration of" or "administering" refer to providing an active agent to a subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

As used herein, the terms "bisphosphonates" and "bisphosphonate drugs" refer to therapeutic agents of various embodiments, and encompass any of aminobisphosphonates, diphosphonates, biphosphonic acids, and diphosphonic acids, as well as pharmaceutically acceptable salts and derivatives thereof. The use of a specific nomenclature in

referring to bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated.

As used herein, the terms “co-administration” or “combined administration” or “combined use” or “combination therapy” or the like as utilized herein refer to administration of a therapeutic vector or a lentiviral particle and a bisphosphonate drug or a therapeutic vector or a lentiviral particle and an antibody or a therapeutic vector or a lentiviral particle and a bisphosphonate drug and an antibody to a single subject in need thereof (e.g., a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration and/or at the same time.

As used herein, the term “fixed combination,” refers to two or more active ingredients or components, including any of their respective compositions, formulations or drug forms, e.g., a therapeutic vector or a lentiviral particle and a bisphosphonate drug or any combination of these, that are administered essentially in combination to a patient, for example essentially simultaneously, in the form of a single entity or dosage or combined entities or dosages, e.g., in one tablet or in one capsule or in combined tablets or capsules or combined liquid forms.

As used herein, the term “non-fixed combination,” refers to two or more active ingredients or components, including any of their respective compositions, formulations or drug forms, e.g., a therapeutic vector or a lentiviral particle and a bisphosphonate drug or any combination of these, that are administered in combination to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the active components in the patient. The non-fixed combination can be dosed independently of each other or by use of different fixed combinations e.g., simultaneously or at different time points. The active components may be administered as separate pharmaceutical dosage forms or pharmaceutical formulations that may be, for example, sold independently of each other, with or without label instructions concerning the possibility of a combined use. Such instructions may be provided in the package equipment, e.g., leaflet or the like, or in other information, e.g., provided to physicians and medical staff. A non-fixed combination, its respective active ingredients or components, including any of their respective compositions, formulations or drug forms, or the parts thereof, can be administered simultaneously or chronologically staggered, e.g., at different time points and with equal or different time intervals for any part of the administration. Such time intervals may be chosen such that the effect on the treated disease, when treated in combination, is more effective than would be obtained by use of only any one of the active components.

As used herein, the terms “combination,” “in combination” and “combination therapies,” may refer generally to any or both of the “fixed combination” and “non-fixed combination” definitions and embodiments described above.

As used herein, the transitional term “comprising,” when used to define compositions and methods, means that the compositions and methods include the recited elements, but does not exclude others. As used herein, “consisting essentially of,” when used to define compositions and methods, means that the composition and methods include additional elements, but only if those additional elements do not materially affect the basic and novel characteristics of the composition or methods. As used herein, “consisting of,” when used to define compositions and methods, means that the compositions and methods exclude more than trace

elements of other ingredients for compositions and substantial method steps. Embodiments defined by each of these transitional terms are within the scope of this disclosure. For example, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

As used herein, the terms “expression,” “expressed,” or “encodes” refer to a process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

As used herein, 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.

As used herein, the term “gamma delta T cell” may also be referred to herein as a $\gamma\delta$ T cell, or further as a GD T cell. The term “gamma delta T cell activation” refers to any measurable biological phenomenon associated with a gamma delta T cell that is representative of such T cell being activated. Non-limiting examples of such a biological phenomenon include an increase of cytokine production, changes in the qualitative or quantitative composition of cell surface proteins, an increase in T cell proliferation, and/or an increase in T cell effector function, such killing or a target cell or assisting another effector cell to kill a target cell.

As used herein, the terms “individual,” “subject,” and “patient” are used interchangeably herein, and refer to any individual mammal subject, e.g., bovine, canine, feline, equine, and/or human.

As used herein, the term “miRNA” refers to a microRNA, and also may be referred to herein as “miR”.

The term “packaging cell line” refers to any cell line that can be used to express a lentiviral particle.

As used herein, the term “homology” refers to the percentage number of amino acids, nucleic acids, or analogs thereof, that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., 1984, Nucleic Acids Research 12, 387-395). In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

As used herein, the term “sequence identity,” which also may appear in the non-limiting context of “a sequence 50% identical to,” and “having at least 80%, or at least 85%, or at least 90%, or at least 95% identity with” a given sequence, as similar phrasings, as used herein, refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a “percentage of sequence identity” may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence

identity. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, Wis., USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., Nucl. Acids Res. 25:3389, 1997.

As used here, the term "percent identity," which may be used interchangeably with the term "sequence identity", in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the "percent identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

Suitable algorithms for determining percent sequence identity include the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information web site.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a

PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and BLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, word length=12 to obtain nucleotide sequences homologous to the nucleic acid molecules provided in the disclosure. BLAST protein searches can be performed with the XBLAST program, score=50, word-length=3 to obtain amino acid sequences homologous to the protein molecules of the disclosure. 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 of the respective programs (e.g., BLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

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

As used herein, the term "pharmaceutically acceptable carrier" refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see, e.g., Berge et al. (1977) *J Pharm Sci* 66:1-19).

As used herein, the term "pharmaceutically acceptable salt" refers to derivatives of compounds or other active ingredients, wherein the parent compound or active ingredient is modified by converting an existing acid or base moiety to its salt form. Non-limiting examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; alkali metal, alkaline metal, ammonium, and mono-, di-, tri-, or tetra-C1-C30-alkyl-substituted ammonium; and the like. The pharmaceutically acceptable salts of various embodiments include the conventional non-toxic salts of the compound or active ingredient formed, for example, from nontoxic inorganic or organic acids. Suitable organic acids are, e.g., carboxylic acids or sulfonic acids, such as acetic acid, succinic acid, fumaric acid or methane-sulfonic acid. The pharmaceutically acceptable salts herein can be synthesized from the parent compound or active ingredient which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and Journal of Pharmaceutical Science, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

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

As used herein, the term "small RNA" refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). In embodiments, "small RNA" are capable of inhibiting or knocking-down gene expression of a target gene, generally through pathways that result in the inhibitions or destruction of the target gene mRNA.

As used herein, the term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present disclosure, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient's condition or its severity, and the age, weight, etc., of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, e.g., the route of administration, the condition of the subject, as well as other factors understood by those in the art.

As used herein, the term "therapeutic vector" includes, without limitation, reference to a lentiviral vector or an AAV vector.

As used herein, the terms "treatment" and "treating" refer to the intended targeting of a disease state and combatting of it, i.e., ameliorating or preventing the disease state. A particular treatment thus will depend on the disease state to be targeted and the current or future state of medicinal therapies and therapeutic approaches. A treatment may have associated toxicities.

Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

Description of Aspects of the Disclosure

In one aspect, a method of activating a GDT cell is provided. The method includes infecting, in the presence of the GD T cell, a target cell with a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme is FDPS. In embodiments, when the enzyme is inhibited in the target cell, the target cell activates the GD T 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 includes a microRNA or a shRNA.

In embodiments, the at least one encoded genetic element includes a 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 with GTCCTGGAGTACAATGC-CATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGAT-TTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAA

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ATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTA-CATGGCAGGAATTCTCGAGAA TTCCTGCCATGTA-CATGGCTTTTT (SEQ ID NO: 3); or GCAGAAAGGAGGCTGA GAAAGTCTCGA-GACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In a preferred embodiment, the shRNA includes GTCCTGGAGTACAATGCCATTCTCGAG AATGGCAT-TGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGAT-TTCGTTCA GCACTTCTCGAGAAGTGCT-GAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCA TGTACATGGCAGGAATTCTCGAGAATTCTGCTGC-CATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAAGGAGGCTGAGAAAGTCTCGA-GACTTTCTCAGCCTCCTT CTGCTTTTT (SEQ ID NO: 4).

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with AAGGTATAT-TGCTGTTGACAGT-GAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAAGGAGGCT-GAGAAAGTGCTGCGCTACTGCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTGTTGACAGTG-AGCGACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAAGGAGGCTGAG AAAGTTGCC-TACTGCCTCGGA (SEQ ID NO: 7); CCTG-GAGGCTTGCTGAAG GCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTT GGCCACTGACTGAGCAGAAAGGG CTGAGAAAGTCAGGACACAAGGCCTGT-TACTAGCACTCA (SEQ ID NO: 8); CATCTC-CATGGCTGTAC-CACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTG TTGAA TCT-CATGGCAGAAAGGAGGCGAGAAAGTCTGACAT-TTTGGTATCTTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGGA-TACTTTCT CAGCCTCCTTCTGCTGGTCCCCCTCCCCGAGAAGG AGGCTGAGAAAGTCCTTCCCTC CCAATGACCGCGTCTTCGTGCG (SEQ ID NO: 10). In a preferred embodiment, the microRNA includes AAGGTAT-ATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCT CTTCTGCGTGAAGCCACA-GATGGCAGAAAGGAGGCTGAGAAAGTGCTGCC-TACTGC CTCGGACTTCAAGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGT GAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAAGGAGGCTGA GAAAGTGCTGCC-TACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTG TTGACAGT-GAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGC-CACAGATGGCAGAAAGG AGGCTGAGAAAGTTGCC-TACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCT TGCT-GAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTGCTTTTGGCCACTGACTGAG CAGAAGGGCT-GAGAAAGTCAGGACACAAGGCCTGT-TACTAGCACTCA (SEQ ID NO: 8); CATCTC-

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CATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCT
CCTT CTGCCTGTTGAATCT-
CATGGCAGAAGGAGGCGAGAAAGTCTGACAT-
TTTGGTATCTT TCATCTGACCA (SEQ ID NO: 9); or
GGGCCTGGCTCGAGCAGGGGGCGAGGG
ATACTTTCTCAGCCTCCTTCTGCTGGTCCCCCTCCCC
GCAGAAGGAGGCTGAGAAAGT CCTTCCCTCC-
CAATGACCGCTCTTCGTCG (SEQ ID NO: 10).

In another aspect, the target cell is also contacted with a bisphosphonate drug. In a preferred embodiment, the bisphosphonate drug is zoledronic acid. The bisphosphonate drug may be a pharmaceutically acceptable salt, hydrate or a solvate thereof.

In another aspect, a method of treating cancer in a subject is provided. The method includes 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 includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell activates the GD T cell, to thereby treat the cancer. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In another aspect, a method of treating an infectious disease in a subject is provided. The method includes 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 includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cell that is infected with an infectious agent and is in the presence of a GD T cell, the infected cell activates the GD T cell, to thereby treat the infected cell, and the infectious disease. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In embodiments, the at least one encoded genetic element includes a 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 with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In a preferred embodiment, the shRNA includes SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In other embodiments, the at least one encoded genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In a preferred embodiment, the microRNA includes SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; 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 includes a small RNA capable of inhibiting 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 includes a microRNA or a shRNA.

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In another aspect, the at least one encoded genetic element includes a 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 with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In a preferred embodiment, the shRNA includes 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 includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In a preferred embodiment, the microRNA includes SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10.

In embodiments, the viral vector includes any vector that can effectively transduce the small RNA. In 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 includes a second encoded genetic element. In embodiments, the second genetic element includes at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from the group consisting of: IL-18, TNF- α , interferon- γ , IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least one chemokine is a CC chemokine, CXC chemokine, a CX3 chemokine or a XC chemokine. In a further embodiment, the at least one chemokine is the CC chemokine, RANTES.

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

In another aspect a pharmaceutical combination is disclosed which includes a bisphosphonate compound; and a lentiviral particle produced by a packaging cell and capable of infecting a target cell. The lentiviral particle comprises an envelope protein capable of infecting the target cell, and: at least one encoded shRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded shRNA comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98% or at least 99% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4; or at least

one encoded microRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded microRNA comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98% or at least 99% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10, wherein the pharmaceutical combination is at least one of fixed and non-fixed. In embodiments, the at least one encoded shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4 or the at least one encoded microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the pharmaceutical composition comprises a fixed combination. In embodiments, the pharmaceutical composition comprises a non-fixed combination. In embodiments, the bisphosphonate drug comprises zoledronic acid. In embodiments, the bisphosphonate drug and the lentiviral particle are present in synergistically effective amounts. In embodiments, the target cell is one or more cancer cells that are present in a cancer selected from one or more of a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof. In embodiments, the target cell is one or more cancer cells that are present in a hepatocellular carcinoma. In embodiments, the target cell is capable of activating a gamma delta T cell following infection with the lentiviral particle. In embodiments, the enzyme is FDPS.

In another aspect, a method of treating a cancer in a subject using an immunotherapy-based composition is disclosed. The method includes administering a therapeutically-effective amount of a bisphosphonate drug to the subject; and administering a therapeutically-effective amount of the immunotherapy-based composition to the subject, wherein the immunotherapy-based composition comprises a lentiviral particle. The lentiviral particle comprises an envelope protein capable of infecting one or more cancer cells, and at least one encoded shRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded shRNA comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98% or at least 99% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4 or at least one encoded microRNA capable of inhibiting production of an enzyme of the mevalonate pathway, wherein the at least one encoded microRNA comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98% or at least 99% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodiments, the at least one encoded shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In embodiments, the at least one encoded microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In embodi-

ments, the one or more cancer cells are present in a cancer selected from one or more of a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof. In embodiments, the bisphosphonate drug comprises zoledronic acid. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in a fixed combination. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in a non-fixed combination. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered simultaneously. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered sequentially. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered in synergistically effective amounts. In embodiments, the bisphosphonate drug and the immunotherapy-based composition are administered at a synergistically effective time interval. In embodiments, the one or more cancer cells are capable of activating a gamma delta T cell resident in the subject following infection of the one or more cancer cells with the immunotherapy-based composition. In embodiments, activating the gamma delta T cell comprises increasing tumor necrosis factor (TNF)- α expression by the gamma delta T cell. In embodiments, the enzyme of the mevalonate pathway is farnesyl diphosphate synthase (FDPS).
Cancer

The compositions and methods provided herein are used to treat cancer. A cell, tissue, or target may be a cancer cell, a cancerous tissue, harbor cancerous tissue, or be a subject or patient diagnosed or at risk of developing a disease or condition. In certain aspects, a cell may be an epithelial, an endothelial, a mesothelial, a glial, a stromal, or a mucosal cell. The cancer cell population can include, but is not limited to a brain, a neuronal, a blood, an endometrial, a meninges, an esophageal, a lung, a cardiovascular, a liver, a lymphoid, a breast, a bone, a connective tissue, a fat, a retinal, a thyroid, a glandular, an adrenal, a pancreatic, a stomach, an intestinal, a kidney, a bladder, a colon, a prostate, a uterine, an ovarian, a cervical, a testicular, a splenic, a skin, a smooth muscle, a cardiac muscle, or a striated muscle cell, and can also include a cancer cell population from any of the foregoing, and can be associated with one or more of carcinomas, sarcomas, myelomas, leukemias, lymphomas, mixed types or mixtures of the foregoing. In still a further aspect cancer includes, but is not limited to astrocytoma, acute myeloid leukemia, anaplastic large cell lymphoma, acute lymphoblastic leukemia, angiosarcoma, B-cell lymphoma, Burkitt's lymphoma, breast carcinoma, bladder carcinoma, carcinoma of the head and neck, cervical carcinoma, chronic lymphoblastic leukemia, chronic myeloid leukemia, colorectal carcinoma, endometrial carcinoma, esophageal squamous cell carcinoma, Ewing's sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, gastric carcinoma, hepatoblastoma, hepatocellular carcinoma, Kaposi's sarcoma, Hodgkin lymphoma, laryngeal squamous cell carcinoma, larynx carcinoma, 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 lymphoma, non-Hodgkin lymphoma, lung carcinoma, non-small cell lung carcinoma, ovarian carcinoma, esophageal carcinoma, osteosarcoma, pancreatic carcinoma, pheochromocytoma, prostate carcinoma, renal cell carcinoma, retino-

blastoma, rhabdomyosarcoma, salivary gland tumor, schwannoma, small cell lung cancer, squamous cell carcinoma of the head and neck, testicular tumor, thyroid carcinoma, urothelial carcinoma, and Wilms tumor.

The compositions and methods provided herein are also used to treat NSCLC (non-small cell lung cancer), pediatric malignancies, cervical and other tumors caused or promoted by human papilloma virus (HPV), melanoma, Barrett's esophagus (pre-malignant syndrome), adrenal and skin cancers and auto immune, neoplastic cutaneous diseases.

Infectious Diseases

The compositions and methods disclosed herein can be used to treat infectious diseases. The term "infectious disease" includes any disease that is caused by an infectious agent. An "infectious agent" includes any exogenous pathogen including, without limitation, bacteria, fungi, viruses, mycoplasma, and parasites. Infectious agents that may be treated with compositions provided for in this disclosure include any art-recognized infectious organisms that cause pathogenesis in an animal, including such organisms as bacteria that are 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, herpesviruses 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 may be treated with the compositions and methods of the disclosure include fungi that grow as molds or are yeast-like, including, for example, fungi that cause diseases such as ringworm, histoplasmosis, blastomycosis, aspergillosis, cryptococcosis, sporotrichosis, coccidioidomycosis, paracoccidio-omycosis, and candidiasis. Compositions and methods provided for herein may be utilized to treat parasitic infections including, but not limited to, infections caused by somatic tapeworms, blood flukes, tissue roundworms, ameba, and *Plasmodium*, *Trypanosoma*, *Leishmania*, and *Toxoplasma* species.

Methods of GD T Cell Activation

Provided herein are compositions and methods for activating GD T cells in an individual, as well as methods for treating tumors and infectious diseases. For instance, in embodiments, the compositions and methods provided herein can be used in methods to treat all known cancers because activated GD T cells comprise a natural mechanism for immune surveillance of tumors (See for e.g.: Pauza et al. 2014 *Frontiers in Immunol.* 5:687). Likewise, in embodiments, the compositions and methods provided herein can be used to treat infectious diseases, including but not limited to flavivirus, influenza virus, human retrovirus, mycobacteria, plasmodia and a variety of other viral, fungal and bacterial infections. (See for e.g.: Pauza and Cairo, 2015 *Cell Immunol.* 296(1).

In general, a vector system is administered to an individual to transfect or transduce a target cell population with the disclosed constructs for decreasing expression of FDPS and, in other embodiments, increasing expression of chemokines or cytokines. Administration and transfection/transduction can occur in vivo or ex vivo, with the transfected cells later administered back into the subject in the latter scenario.

Administration of the disclosed vectors and transfection or transduction of the disclosed constructs into a subject's cells result in decreased expression of FDPS, increased expression of cytokines or chemokines, accumulation of IPP and in many cases, reduced growth rates for genetically

modified tumor cells. All of these features work together to activate and co-localize GD T cells to the site of a tumor or infection.

The disclosed methods can also increase the capacity of NK cells to recognize and destroy tumor cells and/or infected cells. Crosstalk between GD T cells and NK cells is an important aspect of regulating the immune and inflammatory responses. Further, GD T cells are known to trigger dendritic cell maturation, recruit B cells and macrophages, and participate in a variety of cytolytic activities, such as secretion of interferon- γ and TNF- α .

In embodiments, the disclosed compositions and methods provided herein comprise a form of gene therapy for activating GD T cells at the site of tumor or infectious disease pathology. In an aspect, the compositions and methods provided herein activate GD T cells and support their proliferation, differentiation, and functional capacities by promoting the production of specific cytokines needed for cytolytic activity capable of killing cancer cells or treating infectious diseases.

In embodiments the gene therapy sequences (e.g., FDPS shRNAs) are carried by therapeutic vectors, including but not limited to viral vectors such as lentiviruses or adeno-associated viruses, although other viral vectors can 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 formulations. For instance, a protein-nucleic acid complex can comprise nucleic acids of interest in a complex with cationic peptides such as lysine and arginine. Lipid-nucleic acids complexes can comprise lipid emulsions, micelles, liposomes, and/or mixtures of neutral and cationic lipids such as DOTMA, DOSPA, DOTAP, and DMRIE.

In embodiments, therapeutic vectors 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 a vector the constructs may be identical, or they may be different. For instance, the constructs may vary in terms of their promoters, the presence or absence of integrating elements, and/or their sequences. In some embodiments, a therapeutic vector will comprise at least one construct that encodes a small RNA capable of knocking down the expression of FDPS. In embodiments, the therapeutic vector will also encode a specific cytokine(s) and/or chemokine(s), 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 may encode both small RNAs 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, viral vectors may introduce nucleic acid constructs that become integrated into the host chromosome. Alternately, transient delivery vectors may be used to prevent chromosomal integration and limit the lifespan of gene therapy constructs.

In embodiments, the disclosed constructs and vectors comprise short hairpin RNA ("shRNA"), micro RNA ("miRNA"), or siRNA 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, which control steroid and isoprenoid synthesis, isopentenyl pyrophosphate ("IPP") levels are elevated. Elevation and accumulation of IPP is a

known mechanism for increasing GD T cells activation. Further, down regulation of these pyrophosphate synthase genes removes an important negative regulator of inflammation. In addition, the disclosed constructs may be regulated by specific promoters that are capable of producing interleukin-1 beta and/or interleukin-18 and/or interferon-gamma required for GD T cell differentiation and acquisition of all effector cell function. Desirable effector cell functions include the capacity for direct cytotoxic cell killing of tumors and/or infected cells, secretion of beneficial cytokines and/or chemokines, increased expression of NK receptors required to recognize cancerous or infected cells, and increased expression of Fc receptors needed to bind targeting antibodies in order to co-localize GD T cells with cancerous or infected cell targets.

In embodiments, the disclosed constructs are regulated by specific promoters that are capable of producing interleukin-2 and/or interleukin-15 to sustain GD T cell proliferation. In addition, the disclosed constructs may be regulated by specific promoters that are capable of producing interleukin-1 beta and/or interleukin-18 and/or interferon-gamma required for GD T cell differentiation and acquisition of all effector cell function. Desirable effector cell functions include the capacity for direct cytotoxic cell killing of tumors and/or infected cells, secretion of beneficial cytokines and/or chemokines, increased expression of NK receptors required to recognize cancerous or infected cells, and increased expression of Fc receptors needed to bind targeting antibodies in order to co-localize GD T cells with cancerous or infected cell targets.

In embodiments, the disclosed methods activate GD T cells, resulting in the indirect effect of increasing the capacity for NK cells to attack and destroy cancerous cells, tumors, or infected cells. The activation of NK cells requires GD T cells that are stimulated to proliferate and differentiate, and to express 4-1BBL costimulatory ligand needed to engage the 4-1BB costimulatory receptor on NK cells. This form of crosstalk is known as an important mechanism for activating NK cells and is achieved here through the action of the disclosed methods and compositions.

In another aspect, crosstalk between GD T cells and NK cells is an important mechanism for eliminating inflammatory dendritic cells that accumulate in diseased tissues. Alone, neither GD T cells nor NK cells are capable of destroying dendritic cells, but once the aforementioned crosstalk interactions have occurred, NK cells are altered to become cytotoxic against inflammatory dendritic cells. This immuno-regulatory mechanism depends on strong activation and proliferation of GD T cells.

In embodiments, the disclosed methods for activation of GD T cells further comprise a step of suppressing pathologic inflammatory responses that may include cellular proliferation leading to atherosclerosis, chronic immune activation that stimulates tumor growth, autoimmune diseases including psoriasis and other presentations in the epidermis, inflammatory diseases of the central nervous system, and arthritis and other diseases of unregulated immune responses.

In embodiments, therapeutic vectors are administered in combination with bisphosphonate drugs. In various embodiments, such combinations achieve synergistic, positive or heightened activation of gamma delta T cells. Such positive activation may allow alternate, modified or reduced doses of bisphosphonates and may decrease adverse reactions to bisphosphonates including acute inflammatory responses and chronic diseases. Combinations of therapeutic vectors with bisphosphonates may be together or separate, with or without instructions for combined use or to combination products. The therapeutic vectors and/or bisphosphonates may be administered entirely separately and may be formulated in entirely distinct pharmaceutical dosage forms. The therapeutic vectors and/or bisphosphonates may be sold independently of each other, with or without label instructions concerning the possibility of a combined use. Such instructions also may be provided in the package equipment, e.g., leaflet or the like, or in other information, e.g., provided

to physicians and medical staff (e.g., oral communications, communications in writing or the like). Such labels or other instructions can refer to either a fixed combination in one dosage unit form, or a non-fixed combination as a kit of parts for the combined administration where the therapeutic vector may be administered independently of the bisphosphonate drug, at the same time, or separately within time intervals. In various embodiments, the combination exhibits a cooperative or joint effect, or a decrease in toxicity or complications of treatment. In one embodiment the effect of the combination is synergistic. A synergistic effect is achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together, albeit subject to potential variances in timing as detailed herein.

The combinations herein may be manufactured and/or formulated by the same or different manufacturers. The active ingredients may be brought together into a combination therapy: (i) prior to release of the combination product to physicians (e.g., in the case of a kit comprising the compound of the disclosure and the other therapeutic agent); (ii) by the treating physician (or under the guidance of a physician) shortly before administration; (iii) in the actual patient, e.g., during sequential administration of the active ingredients disclosed herein.

In embodiments, a therapeutically effective amount of each of the combinations may be administered simultaneously or sequentially and in any order, and the components may be administered together or separate. For example, the method of treating a proliferative disease according to the disclosure may comprise (i) administration of a first agent such as a therapeutic vector that forms part of a lentiviral particle, and (ii) administration of a second agent such as a bisphosphonate drug in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in cooperative, jointly effective, and/or synergistically effective, amounts, e.g., in daily or intermittent dosages corresponding to the amounts described herein. The combinations may be administered separately at different times during the course of therapy or concurrently in divided or single drug forms. Furthermore, the term "administering" also encompasses the use of a pro-drug of a combination partner that converts in vivo to the combination partner as such. The instant disclosure is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

In embodiments, agents (i) and (ii) can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, in semi-solid media such as agarose or gelatin, or via a buccal

or nasal spray formulation, and/or in solid media such as granules or powders including inert excipients. For example, a therapeutic vector and/or bisphosphonate drug may be administered intravenously. Further, agents (i) and (ii) can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, a solution, an emulsion, and a suspension. For example, a bisphosphonate drug may be formulated into a tablet and administered orally.

A combination therapy according to the disclosure can besides or in addition be administered especially for cancer therapy in combination with chemotherapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above. In embodiments, a combination therapy can also include immune adjuvants (e.g., Toll-like receptor ligands), immune stimulating toxins, or stimulatory protozoans or stimulatory bacilli (e.g., bacille Calmette-Guerin), cancer therapeutic drugs, cell-based therapies (gamma delta T cell or other cell types known to be in use or under evaluation for tumor therapy and may also include natural or genetically-engineered cells and cells cultured under) ionizing radiation or surgery. Other possible treatments are therapy to maintain the patient's status after tumor regression, or even chemo-preventive therapy, for example in patients at risk.

Constructs for GD T Cell Activation

Inhibition of FDPS results in IPP accumulation, resulting in activation of Vδ2+ GD T cells and expression of IL-18, which is also important in activating GD T cells. Inhibition of farnesyl transferase results in decreased prenylation of proteins. The disclosed constructs can be transfected or transduced into specific target cells, like tumor cells or infected cells, where they can express RNA sequences (i.e., siRNA, shRNA or microRNA) that will inhibit translation of FDPS as well as encode and express cytotoxic cytokines or chemokines.

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

Expression of cytokines and chemokines, like those listed above, will result in localized cytotoxic destruction of tumor cells or cells infected with pathogenic organisms. Accordingly, expression of such constructs by a tumor cell or an infected cell will result in the unwanted cells assisting in its own destruction.

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

Decreased expression of FDPS can be achieved via shRNA, microRNA, siRNA, or other means known in the art. For instance, shRNAs according to SEQ ID NOS: 1, 2, 3, or 4, or variants thereof can be used in the disclosed

constructs and methods, although this example is not limiting. The coding regions for RNAs to decrease expression of FDPS and FT and the coding regions of cytokine and chemokines may be in the same construct or on different constructs.

The classical approach for the production of recombinant polypeptides or gene regulatory molecules including small RNA is the use of stable expression constructs. These constructs are based upon chromosomal integration of a transduced expression plasmid (or at least a portion thereof) into the genome of the host cell, short-duration plasmid transfection, or non-integrating viral vectors also with limited half-life. The sites of gene integration are generally random, and the number and ratio of genes integrating at any particular site are often unpredictable; likewise, non-integrating plasmids or viral vectors also generate nuclear DNA but these species usually lack sequences required for DNA replication and continuous maintenance. Thus, constructs that rely on chromosomal integration result in permanent maintenance of the recombinant gene that may exceed the therapeutic interval.

An alternative to stable expression constructs for gene expression are transient expression constructs. The expression of the latter gene expression construct is based on non-integrated plasmids, and hence the expression is typically lost as the cell undergoes division or the plasmid vectors are destroyed by endogenous nucleases.

The disclosed constructs are preferably episomal constructs that are transiently expressed. Episomal constructs are degraded or diluted over time such that they do not make permanent changes to a subject's genome, nor are they incorporated into the chromosome of a target cell. The process of episomal replication typically incorporates both host cell replication machinery and viral trans-acting factors.

Avoiding chromosomal integration reduces certain barriers to in vivo gene delivery. However, even integration-defective constructs can have a background frequency of integration, and any DNA molecule can find rare homologs to recombine with host sequences; but these rates of integration are exceptionally rare and generally not clinically significant.

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 longer. Any combination of these time periods can also be used in the methods of the invention, e.g., 1 month and 1 week, or 3 months and 2 weeks.

However, in some embodiments, the constructs comprise integrating elements that depend on a retroviral integrase gene, such that the construct becomes integrated into the subject's chromosome. Retrotransposition and transposition are additional examples of mechanisms whereby mobile genetic elements become integrated or inserted into the chromosome. Plasmids may become integrated into the chromosome by recombination, and gene editing technologies including CRISPR and TALEN utilize guide RNA sequences and alter chromosomal loci by gene conversion mechanisms.

Constructs may comprise specific promoters for expressing cytokines involved in the maintenance of 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, ATCG-middle promoters, ATCG-less promoters, AT-less promoters, CG-less promoters, AT-spike promoters, and CG-spike promoters. See Gagnic and Ionescu-Tirgoviste, *Eukaryotic genomes may exhibit up to 10 generic classes of gene promoters*, BMC GENOMICS 13:512 (2012).

Therapeutic Vectors

The construct can be delivered via known transfection and/or transduction vectors, including but not limited to lentiviral vectors, gamma-retroviruses, adeno-associated virus, poxvirus, herpesvirus vectors, protein and/or lipid complexes, liposomes, micelles, and the like.

Viral vectors can be preferentially targeted to cell types that are useful for the disclosed methods (i.e., tumor cells or myeloid cells). Viral vectors can be used to transduce genes into target cells owing to specific virus envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for the transfer of genes into many different cell types including whole embryos, fertilized eggs, isolated tissue samples, tissue targets in situ, and cultured cell lines. The ability to introduce and express foreign genes in a cell is useful for the study of gene expression, and the elucidation of cell lineages as well as providing the potential for therapeutic interventions such as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy. Viral components from viruses like Papovaviridae (e.g. bovine papillomavirus or BPV) or Herpesviridae (e.g. Epstein Barr Virus or EBV) or Hepadnaviridae (e.g. Hepatitis B Virus or HBV) or pox vectors including vaccinia may be used in the disclosed vectors.

Lentiviral vectors are a preferred type of vector for the disclosed compositions and methods, although the disclosure is not specifically limited to lentiviral vectors. Lentivirus is a genus of viruses that can deliver a significant amount of viral nucleic acid into a host cell. Lentiviruses are characterized as having a unique ability to infect/transduce non-dividing cells, and following transduction, lentiviruses integrate their nucleic acid into the host cell's chromosomes.

Infectious lentiviruses have three main genes coding for the virulence proteins gag, pol, and env, and two regulatory genes including tat and rev. Depending on the specific serotype and virus, there may be additional accessory genes that code for proteins involved in regulation, synthesis, and/or processing viral nucleic acids and other replicative functions.

Moreover, lentiviruses contain long terminal repeat (LTR) regions, which may be approximately 600 nt long. LTRs may be segmented into U3, R, and U5 regions. LTRs can mediate integration of retroviral DNA into the host chromosome via the action of integrase. Alternatively, without functioning integrase, the LTRs may be used to circularize the viral nucleic acid.

Viral proteins involved in early stages of lentivirus replication include reverse transcriptase and integrase. Reverse transcriptase is the virally encoded, RNA-dependent DNA polymerase. The enzyme uses a viral RNA genome as a template for the synthesis of a complementary DNA copy. Reverse transcriptase also has RNaseH activity for destruction of the RNA-template. Integrase binds both the viral cDNA generated by reverse transcriptase and the host DNA. Integrase processes the LTR before inserting the viral genome into the host DNA. Tat acts as a trans-activator

during transcription to enhance initiation and elongation. The rev responsive element acts post-transcriptionally, regulating mRNA splicing and transport to the cytoplasm.

Viral vectors, in general, comprise glycoproteins and the various glycoproteins may provide specific affinities. For instance, VSV-G peptides can increase transfection into myeloid cells. Alternatively, viral vectors can also have targeting moieties, such as antibodies, attached to their shell peptides. Targeting antibodies can be specific for antigens that are overexpressed on a tumor, for instance, like 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 particular populations of cells. For example, poxvirus vectors target to macrophages and dendritic cells.

Lentiviral Vector System

A lentiviral virion (particle) is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). There is at least one vector containing a nucleic acid sequence encoding the lentiviral pol proteins necessary for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol proteins are expressed by multiple vectors. There is also a vector containing a nucleic acid sequence encoding the lentiviral gag proteins necessary for forming a viral capsid operably linked to a promoter. In an embodiment, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence. In another embodiment, the gag nucleic acid is on a separate vector from all the pol nucleic acid sequences that encode pol proteins.

Numerous modifications can be made to the vectors, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions.

The gag, pol and env vector(s) do not contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

The vector(s) forming the particle preferably do not contain a nucleic acid sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope protein.

In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelopes one can "infect" virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment such as that of the influenza virus, VSV-G, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia. Virus such as MLV-E, MLV-A and GALV. These latter envelopes are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the desired host cell. For example, targeting specific receptors such as a dopamine receptor can be used for brain delivery. Another target can be vascular endothelium. These cells can be targeted using a filovirus envelope. For example, the GP

of Ebola, which by post-transcriptional modification become the GP, and GP₂ glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Pat. No. 5,654, 195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

As detailed herein, a lentiviral vector system typically includes at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (e.g., 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). Accordingly, both 3-vector and 4-vector systems can be used to produce a lentivirus 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/17 HEK cell line. When the therapeutic vector, the envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, a lentiviral particle is ultimately produced.

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

In another aspect, and as detailed in FIG. 2, the lentiviral vector, which is also referred to herein as a therapeutic vector, can include 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 NOS: 1, 2, 3, 4), Woodchuck Post-Transcriptional Regulatory Element (WPPE) (SEQ ID NO: 17), and 3' Delta LTR (SEQ ID NO: 18). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, and as detailed herein, a helper plasmid has been designed to include the following elements: 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 plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, and as detailed herein, an envelope plasmid has been designed to include the following elements being 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 variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, the plasmids used for lentiviral packaging can be modified with similar elements and the intron sequences could potentially be removed without loss of vector function. For example, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Of note, lentiviral packaging systems can be acquired commercially (e.g., Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, Md.), and can also be designed as described herein. Moreover, it is within the skill of a person skilled in the art to substitute or modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the production efficiency of a lentiviral particle.

Doses and Dosage Forms

The disclosed vectors allow for short, medium, or long-term expression of genes or sequences of interest and episomal maintenance of the disclosed vectors. Accordingly, dosing regimens may vary based upon the condition being treated and the method of administration.

In one embodiment, transduction vectors may be administered to a subject in need in varying doses. Specifically, a subject may be administered about $\geq 10^6$ infectious doses (where 1 dose is needed on average to transduce 1 target cell). More specifically, a subject may be administered about $\geq 10^7$, about $\geq 10^8$, about $\geq 10^9$, or about $\geq 10^{10}$ infectious doses, or any number of doses in-between these values. Upper limits of transduction vector dosing will be determined for each disease indication and will depend on toxicity/safety profiles for each individual product or product lot.

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

In one embodiment, the disclosed vectors are administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprising the disclosed vectors can be formulated in a wide variety of dosage forms, including but not limited to nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various solubilizing agents, disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition comprising a vector can also be formulated for injection, insufflation, infusion, or intradermal exposure. For

instance, an injectable formulation may comprise the disclosed vectors in an aqueous or non-aqueous solution at a suitable pH and tonicity.

The disclosed vectors may be administered to a subject via direct injection into a tumor site or at a site of infection. In some embodiments, the vectors can be administered systemically. In some embodiments, the vectors can be administered via guided cannulation to tissues immediately surrounding the sites of tumor or infection.

The disclosed vector compositions can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, in semi-solid media such as agarose or gelatin, or via a buccal or nasal spray formulation.

Further, the disclosed vector compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, a solution, an emulsion, and a suspension. Further, the composition may be a controlled release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the composition may be a transdermal delivery system.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In some embodiments, the solid dosage form can include one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form can be immediate release, or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

In a further embodiment, the pharmaceutical composition comprising a vector can be formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated as a nasal dosage form. Such dosage forms of the present invention comprise solution, suspension, and gel compositions for nasal delivery.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In some embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In particular embodiments, the composition comprising vectors can be formulated to be suitable for administration to a pediatric patient.

In some embodiment, the pharmaceutical composition can be formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emul-

sions, non-aqueous solutions or suppositories. In some embodiments, the solutions or suspensions can include propylene glycol, polyethylene glycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate.

The dosage of the pharmaceutical composition can vary depending on the patient's weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

In some embodiments, the treatment of cancer is accomplished by guided direct injection of the disclosed vector constructs into tumors, using needle, or intravascular cannulation. In some embodiments, the disclosed vectors are administered into the cerebrospinal fluid, blood or lymphatic circulation by venous or arterial cannulation or injection, intradermal delivery, intramuscular delivery or injection into a draining organ near the site of disease.

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

EXAMPLES

Example 1: Development of a Lentiviral Vector System

A lentiviral vector system was developed as summarized in FIG. 4 (circularized form). Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, Va.) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid. The transfection of 293T/17 HEK cells, which produced functional viral particles, employed the reagent Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by anion-exchange chromatography. The concentration of lentiviral particles can be expressed in terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per cell by quantitative PCR, or by infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

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

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

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

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Synthesis of a 2-Vector Lentiviral Packaging System including Helper (plus Rev) and Envelope Plasmids.

Materials and Methods:

Construction of the helper plasmid: The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTC ATGAATTTGCCAG-GAAGAT-3') (SEQ ID NO: 32) and reverse primer was (5'-CCATACAAT-GAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 33).

The sequence for the Gag, Pol, Integrase fragment was as follows:

(SEQ ID NO: 34)

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAATGATAGGGGGAAT
TGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT
GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC
ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCC
CATTAGTCTCTATTGAGACTGTACCAGTAAATTAAGCCAGGAATGGATG
GCCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATT
GTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAATTTCAAAATTTGG
GCCTGAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACA
GTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAATTAAAGAGAACT
CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA
ACAGAAAAAATCAGTAACAGTACTGGATGTGGCGCATATTTTTCAG
TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT
ATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA
GGGATGGAAGGATCACCAGCAATATTCAGTGTAGCATGACAAAAATCT
TAGAGCCTTTTAGAAAAAATCCAGACATAGTCATCTATCAATACATG
GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA
AATAGAGGAACTGAGACACATCTGTTGAGGTGGGATTACACACCCAG
ACAAAAAATCAGAAAGAACCTCCATTCTTTGGATGGGTTATGAATC
CATCTGATAAATGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAG
CTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAAATGGGCAA
GTCAGATTTATGACAGGATTAAAGTAAGCAATTATGTAACCTCTTAGG
GGAACCAAGCACTAACAGAAGTAGTACCATAACAGAAGAAGCAGAGCT
AGAACTGGCAGAAAACAGGAGATTCTAAAGAACCGGTACATGGAGTGT
ATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAA
GGCCAATGGACATATCAAAATTTATCAAGAGCCATTTAAAAATCTGAAAC
AGGAAAGTATGCAAGAATGAAGGGTGCCCACTAATGATGTGAAACAAT
TAACAGAGGCAGTACAAAAATAGCCACAGAAAGCATAGTAATATGGGGA
AAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATG
GTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCA

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ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCATA
ATAGGAGCAGAACTTTCTATGTAGATGGGCGAGCCAATAGGGAACTAA
ATTAGGAAAAGCAGGATATGTAAGTACAGAGGAAAGACAAAAGTTGTCC
CCCTAACGGACACACAAATCAGAAGACTGAGTTACAAGCAATTCATCTA
GCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATA
TGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAG
TCAGTCAAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA
TGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATT
GGTCAGTGTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGG
CCCAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT
GATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA
TAAATGTCAGCTAAAAGGGGAGCCATGCATGGACAAGTAGACTGTAGCC
CAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTG
GTAGCAGTTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATCCAGC
AGAGACAGGGCAAGAAACAGCATACTTCTCTTAAATTAGCAGGAAGAT
GGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTCACCAGTACT
ACAGTTAAGGCCGCTGTGGTGGCGGGGATCAAGCAGGAATTTGGCAT
TCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAA
TAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA
GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGAT
TGGGGGTACAGTGCAGGGGAAAGAAATAGTAGACATAATAGCAACAGACA
TACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGG
GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT
CCTCTGGAAGGTGAAGGGCAGTAGTAATACAAGATAATAGTGACATAA
AAGTAGTGCCAAGAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAG
ATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA

Next, a DNA fragment containing the Rev, RRE, and rabbit beta globin poly A sequence with XbaI and XmaI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites. The DNA sequence was as follows:

(SEQ ID NO: 35)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAGAGCTCATCAGAAC
AGTCAGACTCATCAAGCTTCTCTATCAAGCAACCCACCTCCCAATCCCCG
AGGGGACCCGACAGGCCGAAGGAATAGAAGAAGAGGTGGAGAGAGAGA
CAGAGACAGATCCATTTCGATTAGTGAACGGATCCTTGGCACTTATCTGGG
ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCAACGCTTGAGAGACTTA
CTCTTGATTGTAACGAGGATTGTGGAACCTCTGGGACGCGGGGGTGGGA
AGCCCTCAAATATTGGTGAATCTCCTACAATATTGGAGTCAGGAGCTAA
AGAATAGAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACT
ATGGGCGCAGCGTCAATGACGCTGACGGTACAGCCAGACAATTATTGTC

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TGGTATAGTGCAGCAGCAGAAACAATTGCTGAGGGCTATTGAGGCGCAAC
 AGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGA
 ATCCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT
 TCCCTCTGCCAAAAATTATGGGACATCATGAAGCCCTTGAGCATCTGA
 CTTCTGGCTAATAAGGAAATTTATTTTCATTGCAATAGTGTGTGGAAT
 TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGCAAAATCATTTAAA
 ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCT
 GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTATCAGTATATGAAACA
 GCCCCCTGCTGTCATTCTTATTTCCATAGAAAAGCCTTGACTTGAGGTT
 AGATTTTTTTTATATTTTGTGTTTGTGTTATTTTTTTCTTTAACATCCCTA
 AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCCTGACT
 ACTCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGC
 AGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTG
 TTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTA
 AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTCGCG
 TCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCAT
 CTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCC
 CCTAACTCCGCCCAGTTCCGCCCATCTCCGCCCATGGCTGACTAATTT
 TTTTATTATGACAGAGCCGAGGCGCCTCGGCCTCTGAGCTATTCCAG
 AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAAC
 TTGTTTATTGACAGCTTATAATGGTTACAATAAAGCAATAGCATCACAAA
 TTTCACAAATAAGCATTTTTTTTCACTGCATTCTAGTTGTGTTTGTCCA
 AACTCATCAATGTATCTTATCAGCGGCCGCCCGGG

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

(SEQ ID NO: 36)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCC
 CATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC
 TGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC
 CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT
 TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT
 ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC
 CCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTAT
 TAGTCATCGCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTTCAC
 TCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTGTATTATTATTATT
 TTTAATTATTTTGTGACGCGATGGGGGCGGGGGGGGGGGGCGCGCGCC
 AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG

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CGGCGGCAGCCAATCAGAGCGGCGCTCCGAAAGTTTCCTTTTATGGCG
 AGGCGGCGGGCGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGG
 AGTCGCTGCGTTGCCCTTCGCCCCGTGCCCGCTCCGCGCGCCCTCGCGCC
 GCGCGCCCCGCTCTGACTGACCGCTTACTCCCACAGGTGAGCGGGCGG
 GACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCT
 CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCC
 CTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCCTGTGTGTGCGCT
 GGGGAGCGCCGCTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGG
 GCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGGAGGGGAGCGCGGC
 CGGGGGCGGTGCCCGCGGTGCGGGGGGTGCGAGGGGAACAAAGGCTG
 CGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGCGG
 TCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGG
 CCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGGGGGCTCGCCG
 TGCCGGGCGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG
 CCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCC
 GGCGGCTGTGAGGCGCGGCGAGCGCAGCCATTGCTTTTATGGTAATC
 GTGCGAGAGGGCGCAGGGACTTCTTTTGTCCCAATCTGGCGGAGCCGAA
 ATCTGGGAGGCGCGCCCGCACCCCCCTCTAGCGGGCGCGGCGAAGCGGTG
 CGGCGCGCGCAGGAAGGAAATGGGCGGGGAGGGCTTCGTGCGTGCCTGC
 GCCGCCGTCCCCCTCTCCATCTCCAGCCTCGGGGTGCCGAGGGGGACG
 GCTGCTTCGGGGGGGACGGGCGAGGGCGGGTTCGGCTTCTGCGCTGTG
 ACCGGCGGGAATTC

Construction of the VSV-G Envelope Plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was synthesized by MWG Operon with flanking EcoRI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and the correct orientation was determined by sequencing using a CMV specific primer. The DNA sequence was as follows:

(SEQ ID NO: 37)

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTATTTCATTGGGGTGA
 ATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAGGAACTGGAA
 AAATGTTCTCTTAATTACCATATTGCCCCGTCAAGCTCAGATTTAAAT
 TGGCATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCAAGA
 GTCACAAGGCTATTCAAGCAGACGTTGGATGTGTATGCTTCCAAATG
 GGTCACTACTTGTGATTTCGCTGGTATGGACCAAGTATATAACACAT
 TCCATCCGATCCTTCACTCCATCTGTAGAACAAATGCAAGGAAAGCATTG
 AACAAACGAAACAAGGAACCTTGGCTGAATCCAGGCTTCCCTCCTCAAAG
 TTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCAGGTG
 ACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAAATGGGTTGATT
 CACAGTTTCATCAACGAAATGCAGCAATTACATATGCCCCACTGTCCA
 TAATCTACAACCTGGCATTCTGACTATAAGGTCAAGGGCTATGTGAT

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-continued

TCTAACCTCATTTCATGGACATCACCTTCTCTCAGAGGACGGAGAGC
TATCATCCCTGGGAAAGGAGGGCACAGGGTTCAGAAGTAATACTTTGC
TTATGAACTGGAGGCAAGGCCTGCAAATGCAATACTGCAAGCATTGG
GGAGTCAGACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATAAGGATC
TCTTTGCTGCAGCCAGATTCCCTGAATGCCAGAGGGTCAAGTATCTC
TGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAG
AGGATCTTGGATTATTCCCTCTGCCAAGAACTGGAGCAAAATCAGAG
CGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAA
CCCAGGAACCGGTCTGCTTTACCATAATCAATGGTACCTAAAATAC
TTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAA
GAATGGTCGGAATGATCAGTGGAACTACCACAGAAAGGGAAGTGTGGGA
TGACTGGGCACCATATGAAGACGTGGAATTTGGACCAATGGAGTTCTG
AGGACCAGTTCAGGATATAAGTTTCTTTATACATGATTGGACATGGTA
TGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTGGAACA
TCCTCACATTCAAGACGCTGCTTCGCAACTTCTGATGATGAGAGTTTA
TTTTTTGGTGATACTGGGCTATCCAAAATCCAATCGAGCTTGTAAGAAG
GTTGGTTCAGTAGTTGGAAGCTCTATTGCCTCTTTTTCTTTATCAT
AGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTT
TGCATTAAATTAAAGCACACCAAGAAAAGACAGATTATACAGACATAG
AGATGAGAATTC

A 4-vector system (i.e., a 3-vector lentiviral packaging system) has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in FIG. 3. Briefly, and with reference to FIG. 3, the top-most vector is a helper plasmid, which, in this case, does not include Rev. The vector second from the top is a separate Rev plasmid. The vector second from the bottom is the envelope plasmid. The bottom-most vector is the previously described therapeutic vector.

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

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

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

Synthesis of a 3-Vector Lentiviral Packaging System including Helper, Rev, and Envelope Plasmids.

Materials and Methods:

Construction of the Helper Plasmid without Rev:

The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by MWG Operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

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(SEQ ID NO: 65)

TCTAGAAGGAGCTTGTTCCTTGGGTCTTGGGAGCAGCAGGAAGCACTA
TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT
GGTATAGTGCAGCAGCAGAACAATTGTCTGAGGGCTATTGAGGCGCAACA
GCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA
TCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTT
CCCTCTGCCAAAATTATGGGGACATCATGAAGCCCTTGAGCATCTGAC
TTCTGGCTAATAAAGGAAATTATTTTCATTGCAATAGTGTGTGGAATT
TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAAATCATTAAAA
CATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTG
GCTGCCATGAACAAAGGTGGCTATAAAGAGGTATCAGTATATGAAACAG
CCCCCTGCTGTCCATTCTTATTCATAGAAAAGCCTTGACTTGAGGTTA
GATTTTTTTTATATTTTGTGTTTATTTTTCTTTAACATCCCTAA
AATTTCTTACATGTTTACTAGCCAGATTTTCTCTCTCTGACTA
CTCCAGTCATAGCTGTCCCTCTCTCTTATGAAGATCCCTCGACCTGCA
GCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGT
TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT
CACTGCCCGCTTTCCAGTCGGGAAACCTGTGTCGAGCGGATCCGCATC
TCAATTAGTCAGCAACCATAGTCCCGCCCTAATCCGCCCATCCGCCCC
CTAATCCGCCCAGTTCGCCCATCTCCGCCCATGGCTGACTAATTTT
TTTTATTTATGCAGAGGCGGAGGCCCTCGGCCCTCTGAGCTATTCCAGA
AGTAGTGAGGAGGCTTTTTTGGAGGCTAGGCTTTTGCAAAAAGCTAACT
TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCAGAAAT
TTCACAAATAAAGCATTTTTTCTAGTGCATCTAGTTGTGGTTTGTCCAA
ACTCATCAATGTATCTTATCACCCTGGG

Construction of the Rev Plasmid:

The RSV promoter and HIV Rev sequence was synthesized as a single DNA fragment by MWG Operon with flanking MfeI and XbaI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA sequence was as follows:

(SEQ ID NO: 38)

CAATTGCGATGTACGGCCAGATATACGCGTATCTGAGGGACTAGGGTGT
GTTTAGGCGAAAAGCGGGCTTCGGTTGTACGCGTTAGGAGTCCCCCTCAG
GATATAGTAGTTTCGCTTTTGCATAGGGAGGGGAAATGTAGTCTTATGCA
ATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTA
CAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAAGTAAGTGGTAC
GATCGTGCCTTATTAGGAAGCAACAGACAGGCTGTGACATGGATTGGACGA
ACCACTGAATTCGCATTCGAGAGATAATTGTATTAAAGTGCCTAGCTCGA
TACAATAAACGCCATTTGACCATTACCACATGGTGTGCACCTCCAAGCT
CGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGT

-continued

TTTGACCTCCATAGAACACCGGACCGATCCAGCCTCCCTCGAAGCTA
 GCGATTAGGCATCTCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC
 TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAGCAACCCACCTC
 CCAATCCCAGGGGACCCGACAGGCCGAAGGAATAGAAGAAGAGGTGGA
 GAGAGAGACAGAGACAGATCCATTGCGATTAGTGAACGGATCCTTAGCACTT
 ATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGA
 GACTTACTCTTGATTGTAACGAGGATTGTGGAATTCTGGGACGCAGGGGG
 TGGGAAGCCCTCAAATATTGGTGAATCTCCTACAATATTGGAGTCAGGAG
 CTAAGAATAGTCTAGA

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

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 39), phosphoglycerate kinase (PGK) (SEQ ID NO: 40), and ubiquitin C (UbC) (SEQ ID NO: 41) can replace the CMV (SEQ ID NO: 25) or CAG promoter (SEQ ID NO: 19). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 42) and bGH poly A (SEQ ID NO: 43) can replace the rabbit beta globin poly A (SEQ ID NO: 29). These sequences can also be further varied by addition, substitution, deletion or mutation.

HIV Gag, Pol, and Integrase sequences: The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 20); HIV Pol (SEQ ID NO: 21); and HIV Int (SEQ ID NO: 22) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 44), gibbon ape leukemia virus (GALV) (SEQ ID NO: 45), Rabies (FUG) (SEQ ID NO: 46), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 47), influenza A fowl plague virus (FPV) (SEQ ID NO: 48), Ross River alphavirus (RRV) (SEQ ID NO: 49), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 50), or Ebola virus (EboV) (SEQ ID NO: 51). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3' delta LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3' delta LTR. Sequences cor-

responding with the above elements are identified in the sequence listings portion herein.

Example 2: Development of a Lentiviral Vector that Expresses FDPS

The purpose of this Example was to develop an FDPS lentivirus vector.

Inhibitory RNA Design: The sequence of Homo sapiens Farnesyl diphosphate synthase (FDPS) (NM_002004.3) mRNA was used to search for potential siRNA or shRNA candidates to knockdown FDPS levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from GPP Web Portal hosted by the Broad Institute (<http://portals.broadinstitute.org/gpp/public/>) or the BLOCK-iT RNAi Designer from Thermo Scientific (<https://rnaidesigner.thermofisher.com/rnaexpress/>). Individual selected shRNA sequences were inserted into a lentiviral vector immediately 3 prime to a RNA polymerase III promoter such as H1 (SEQ ID NO: 16), U6 (SEQ ID NO: 52), or 7SK (SEQ ID NO: 53) to regulate shRNA expression. These lentivirus shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the EF-1alpha or CMV RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

Vector Construction: For FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo Scientific. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Thermo Scientific DNA mini prep kit. Insertion of shRNA sequences in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Using the following target sequences, exemplary shRNA sequences were determined to knock-down FDPS:

(FDPS target sequence #1; SEQ ID NO: 54)
 GTCCTGGAGTACAATGCCATT;

(FDPS shRNA sequence #1; SEQ ID NO: 1)
 GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACT
 TTTT;

-continued

(FDPS target sequence #2; SEQ ID NO: 55)
GCAGGATTCGTTTCAGCACTT;

(FDPS shRNA sequence #2; SEQ ID NO: 2) 5
GCAGGATTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCT
TTTT;

(FDPS target sequence #3; SEQ ID NO: 56)
GCCATGTACATGGCAGGAATT;

(FDPS shRNA sequence #3; SEQ ID NO: 3) 10
GCCATGTACATGGCAGGAATTCTCGAGAATCTCCATGTACATGGCT
TTTT;

(FDPS target sequence #4; SEQ ID NO: 57) 15
GCAGAAGGAGGCTGAGAAAGT;
and

(FDPS shRNA sequence #4; SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCT
TTTT.

shRNA sequences were then assembled into a synthetic microRNA (miR) under control of the EF-1 alpha promoter. Briefly, a miR hairpin sequences, such as miR30, miR21, or miR185 as detailed below, was obtained from mirbase.org. The 19-22 mer shRNA target sequence was used to construct the synthetic miR sequence. The miR sequence was arranged as an anti-sense-target-sequence-hairpin loop sequence (specific for each microRNA)—sense target sequence.

The following miR sequences were developed:

(miR30 FDPS sequence #1; SEQ ID NO: 5)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTG
CGTGAAGCCACAGATGGCAGAGGAGGCTGAGAAAGTGCTGCCTACTGC
CTCGACTTCAAGGGGCT

(miR30 FDPS sequence #2; SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTG
CGTGAAGCCACAGATGGCAGAGGAGGCTGAGAAAGTGCTGCCTACTGCCT
CGACTTCAAGGGGCT

(miR30 FDPS sequence #3; SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCAC
AGATGGCAGAGGAGGCTGAGAAAGTTGCTACTGCCTCGGA

(miR155 FDPS sequence #1; SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGATGCTGACTTTCTCAGCCTCCTTCTG
CTTTTGCCACTGACTGAGCAGAGGAGGCTGAGAAAGTCAGGACACAAGG
CCTGTTACTAGCACTCA

(miR21 FDPS sequence #1; SEQ ID NO: 9)
CATCTCCATGGCTGTACACAGTTGTCGGGACTTTCTCAGCCTCCTTCTG
CCTGTTGAATCTCATGGCAGAGGAGGCGAGAAAGTCGACATTTGGT
ATCTTTTCATCTGACCA

(miR185 FDPS sequence #1; SEQ ID NO: 10)
GGGCCTGGCTCGAGCAGGGGCGAGGGATACTTTCTCAGCCTCCTTCTG
CTGGTCCCTCCCGCAGAGGAGGCTGAGAAAGTCCTCCCTCCCAAT
GACCGCGTCTTCGTCG

Example 3—Knock-Down of FDPS for 3 Days in THP1 Monocytic Leukemia by shRNA #4

This Example illustrates that knock-down of FDPS in THP1 monocytic leukemia cells by lentiviral (LV)—expressing FDPS shRNA #4 stimulates TNF- α expression in gamma delta T cells, as shown in FIG. 5.

THP1 cells (1×10^5 cells) were transduced with LV-control or LV-FDPS shRNA #4 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

co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 3.1% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 stimulated 5%. With zoledronic acid treatment, LV-control stimulated 7.2% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 stimulated 56.2%.

Example 4—Knock-Down of FDPS for 14 Days in THP1 Leukemia Cells by shRNA #4

This Example illustrates that Knock-down of FDPS for 14 days in THP1 leukemia cells by lentiviral (LV)—expressing FDPS shRNA #4 stimulates TNF- α expression in GD T cells, as shown in FIG. 6.

THP1 cells (1×10^5 cells) were transduced with LV-control or LV-FDPS shRNA #4 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.9% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 15.9%. With zoledronic acid treatment, LV-control stimulated 4.7% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 76.2%.

Example 5—Knock-Down of FDPS for 3 Days in PC3 Prostate Carcinoma Cells by shRNA #1

This Example illustrates that knock-down of FDPS for 3 days in PC3 prostate carcinoma cells by lentiviral (LV)—expressing FDPS shRNA #1 stimulates TNF- α expression in GD T cells, as shown in FIG. 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #1 stimulated 0.5%. With zoledronic acid treatment, LV-

control stimulated 1.7% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) stimulated 32.2%.

Example 6—Knock-Down of FDPS for 3 Days in
PC3 Prostate Carcinoma Cells by shRNA #4

This Example illustrates that Knock-down of FDPS for 3 days in PC3 prostate carcinoma cells by lentiviral (LV)—expressing FDPS shRNA #4 stimulates TNF- α expression in GD T cells, as shown in FIG. 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.5% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 1.9%. With zoledronic acid treatment, LV-control stimulated 2.1% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 stimulated 28.7%.

Example 7—Knock-Down of FDPS for 3 Days in
HepG2 Liver Carcinoma Cells by shRNA #1 and
#4

This Example illustrates that Knock-down of FDPS for 3 days in HepG2 liver carcinoma cells by lentiviral (LV)—expressing FDPS shRNA #1 (SEQ ID NO: 1) and shRNA #4 (SEQ ID NO: 4) 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.4% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) and #4 (SEQ ID NO: 4) stimulated 0.7% and 0.9%, respectively. With zoledronic acid treatment, LV-control stimulated 6.9% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #1 and #4 stimulated 7.6% and 21.1%, respectively.

Example 8—Knock-Down of FDPS for 3 Days in
THP1 Leukemia by MicroRNA-30

This Example illustrates that Knock-down of FDPS for 3 days in THP1 leukemia cells by lentiviral (LV)—expressing FDPS-targeted synthetic microRNA-30 stimulates TNF- α expression in gamma delta T cells, as shown in FIG. 10.

THP1 cells (1×10^5 cells) were transduced with LV-control or LV-miR30 FDPS #1 (SEQ ID NO: 5) 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF- α expressing V γ 9V δ 2 T cells and LV-miR30 FDPS stimulated 8.1%. With zoledronic acid treatment, LV-control stimulated 5.3% of TNF- α expressing V γ 9V δ 2 T cells and LV-miR30 FDPS #1 (SEQ ID NO: 5) stimulated 67.3%.

Example 9: E:T Ratios Resulting from Mixture of
THP-1 Cells, Cultured Human GD T Cells, and/or
Zometa (Zol)

This Example demonstrates results from mixing treated THP-1 monocytoid tumor cells with cultured human GD T cells, as shown in FIG. 11.

The monocytoid cell line THP-1 was treated with control lentivirus vector (LV), LV suppressing farnesyl diphosphate synthase gene expression (LV-FDPS), zoledronic acid (Zol) or combinations. The legend, as shown in FIG. 11, was: lentiviral control vectors (LV-Control), lentiviral vectors expressing microRNA to down regulate FDPS (LV-FPPS), Zometa (Zol), Zometa plus lentiviral control (Zol+LV-Control), or Zometa plus lentiviral vectors expressing microRNA to down regulate FPPS (Zol+LV-FPPS).

Human GD T cells were cultured from an anonymous donor and added to treated THP-1 cells in 4:1, 2:1 or 1:1 ratios (GD T:THP-1) for 4 hours. Cell killing was measured by a fluorescence assay. When THP-1 cells were treated with a combination of LV-FDPS and Zol, cytotoxic T cell killing by GD T cells was increased greatly compared to either treatment alone. When LV-FDPS treatment alone was compared to Zol treatment alone, the LV-FDPS lead to greater killing but was >3-fold below tumor cell killing after combination treatment. The combined LV-FDPS plus Zol treatment caused nearly 70% tumor cell killing with 4:1 ratio; this was more than 3-fold higher than the second best treatment (LV-FDPS alone).

Example 10—Lentiviral-Delivered shRNA-Based
RNA Interference Targeting the Human Farnesyl
Diphosphate Synthase (FDPS) Gene

HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing the H1 promoter and either a 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 containing the H1 promoter and either a

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non-targeting sequence (5'-GCCGCTTTGTAGGA-TAGAGCTCGAGCTCTATCTACAAAGCGGCTTTTT-3') (SEQ ID NO: 58) or one of four different FDPS shRNA sequences GTCCTGGAGTACAATGCCATTCTCGAGAATGGCAT-TGTACTCCAGGACTTTTT (FDPS shRNA sequence #1; SEQ ID NO: 1); GCAGGATTCGTTTCAGCACTTCTCGAGAAGTGCT-GAACGAAATCCTGCTTTTT (FDPS shRNA sequence #2; SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAAT-TCTTCCATGTACATGGCTTTTT (FDPS shRNA sequence #3; SEQ ID NO: 3); and GCAGAAGGAGGCTGAGAAAGTCTCGA-GACTTTCTCAGCCTCCTTCTGCTTTTT (FDPS shRNA sequence #4; SEQ ID NO: 4) were produced in 293 T cells.

HepG2 human hepatocellular carcinoma cells were then infected with lentiviral vectors to determine the efficacy of FDPS knock-down. After 48 hours, RNA was extracted from the cells using the RNeasy RNA isolation kit (Qiagen) and converted to cDNA with the SuperScript VILO cDNA synthesis kit (Thermo Scientific). Expression of FDPS cDNA was determined by quantitative PCR on an Applied Biosystems StepOne qPCR machine using a SYBR Green PCR mix (Thermo Scientific) and FDPS primers (Forward primer: 5'-AGGAATTGATGGCGAGAAGG-3' (SEQ ID NO: 59) and Reverse primer: 5'-CCCAAAGAGGT-CAAGGTAATCA-3' (SEQ ID NO: 60)). FDPS expression was normalized to actin levels for each sample using the actin primers (Forward primer: 5'-AGCGCGGCTA-CAGCTTCA-3' (SEQ ID NO: 61) and Reverse primer: 5'-GGCGACGTAGCACAGCTTCT-3' (SEQ ID NO: 62)). The relative FDPS RNA expression of the shCon sample is set at 100%. There was an 85% (FDPS sequence #1), 89% (FDPS sequence #2), 46% (FDPS sequence #3), and 98% (FDPS sequence #4) decrease in FDPS expression.

Example 11—Lentiviral-Delivered miR-based RNA Interference Targeting the Human Farnesyl Diphosphate Synthase (FDPS) Gene

As shown in FIG. 13, HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the H1 promoter (SEQ ID NO: 16) the FDPS shRNA #4 (SEQ ID NO: 4) sequence or the EF-1 α promoter (SEQ ID NO: 40) and miR30-based FDPS sequences. After 48 hours, cells were lysed and an immunoblot was performed using an anti-FDPS (Thermo Scientific) and an anti-actin (Sigma) antibody as a protein loading control.

More specifically, HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the H1 promoter (SEQ ID NO: 16) and the FDPS shRNA sequence

GCAGAAGGAGGCTGAGAAAGTCTCGA-GACTTTCTCAGCCTCCTTCTGCTTTTT (FDPS shRNA sequence #4; SEQ ID NO: 4) or the EF-1 α promoter (SEQ ID NO: 39) and miR30-based FDPS sequences AAGGTATATTGCTGTTGACAGT-GAGCGACACTTTCTCAGCCTCCTTCTGCGT-GAAGC CACAGATGGCAGAAGGAGGCT-GAGAAAGTGCTGCCTACTGCCTCGGACTTCAAG GG GCT (miR30 FDPS sequence #1; SEQ ID NO: 5) and AAGGTATATTGCTGTTGACAGT-GAGCGACACTTTCTCAGCCTCCTTCTGCGT-GAAGC CACAGATGGCAGAAGGGCT-

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GAGAAAGTGCTGCCTACTGCCTCGGACTTCAAG GGGC T (miR30 FDPS sequence #2; SEQ ID NO: 6).

After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified with the Bio-Rad protein assay reagent. Protein samples at 50 micrograms were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific and transferred to PVDF membranes (EMD Millipore). An immunoblot was performed using an anti-FDPS (Thermo Scientific) and an anti-actin (Sigma) antibody as a protein loading control. Antibodies were bound with HRP-conjugated secondary antibodies and detected with a Licor c-DiGit Blot scanner using the Immobilon Western ECL reagent (EMD Millipore). The densitometry of the immunoblot bands were quantified with the NIH image software. The LV control with the EF-1 promoter was set at 100%. There was a 68% (LV-shFDPS #4), 43% (LV-miR FDPS #1), and 38% (LV-miR FDPS #3) reduction of FDPS protein expression.

Example 12—Knock-Down of FDPS for 3 Days in HepG2 Liver Carcinoma Cells by Adeno-Associated Virus (AAV)—Expressing FDPS shRNA #4

This Example illustrates that knock-down of FDPS for 3 days in HepG2 liver carcinoma cells by adeno-associated virus (AAV)—expressing FDPS shRNA #4 (SEQ ID NO: 4) 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 co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms (FIG. 14, Panel B).

AAV Vector Construction. FDPS shRNA sequence #4 (SEQ ID NO: 4) was inserted into the pAAV plasmid (Cell Biolabs). FDPS oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The pAAV was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested pAAV plasmid was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo Scientific. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Thermo Scientific DNA

mini prep kit. Insertion of shRNA sequences in the pAAV plasmid was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. An exemplary AAV vector with a H1 promoter (SEQ ID NO: 16), shFDPS sequence (e.g., SEQ ID NO: 4), Left Inverted Terminal Repeat (Left ITR; SEQ ID NO: 63), and Right Inverted Terminal Repeat (Right ITR; SEQ ID NO: 64) can be found in FIG. 14, Panel A).

Production of AAV particles. The AAV-FDPS shRNA plasmid was combined with the plasmids pAAV-RC2 (Cell Biolabs) and pHelper (Cell Biolabs). The pAAV-RC2 plasmid contains the Rep and AAV2 capsid genes and pHelper contains the adenovirus E2A, E4, and VA genes. To produce AAV particles, these plasmids were transfected in the ratio 1:1:1 (pAAV-shFDPS:pAAV-RC2:pHelper) into 293T cells. For transfection of cells in 150 mm dishes (BD Falcon), 10 micrograms of each plasmid were added together in 1 ml of DMEM. In another tube, 60 microliters of the transfection reagent PEI (1 microgram/ml) (Polysciences) was added to 1 ml of DMEM. The two tubes were mixed together and allowed to incubate for 15 minutes. Then the transfection mixture was added to cells and the cells were collected after 3 days. The 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. Cell debris were then pelleted by centrifugation at 4 degrees Celsius for 15 minutes at 12,000 rpm. The supernatant was collected and then added to target cells.

Example 13—Decreased RAP1 Prenylation in the 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 synergize to inhibit farnesyl diphosphate production.

FDPS is an enzyme in the isoprenoid synthesis pathway that catalyzes the production of farnesyl diphosphate. Inhibiting the enzyme activity of FDPS by zoledronic acid or reduced protein expression by shRNA-mediated knock-down will result in reduced farnesyl diphosphate levels. Farnesylation of cellular proteins requires farnesyl diphosphate. RAP1A is a protein that is modified by farnesylation, which can be used as a biomarker for levels of cellular farnesyl diphosphate. An antibody that specifically recognizes reduced RAP1A farnesylation was used to measure FDPS activity after transduction with LV-shFDPS alone or in combination with zoledronic acid. HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing FDPS shRNA sequence #4. For the zoledronic acid treated cells, zoledronic acid (Sigma) was added for the last 24 hours. After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified with the Bio-Rad protein assay reagent. Protein samples at 50 micrograms were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific and transferred to PVDF membranes (EMD Millipore). As shown in FIG. 15, an immunoblot was performed using an anti-FDPS (Thermo Scientific), anti-RAP1A (Santa Cruz), and an anti-actin (Sigma) antibody as a protein loading control. Antibodies were bound with HRP-conjugated secondary antibodies and detected with a Licor c-DiGit Blot scanner using the Immobilon Western ECL reagent (EMD Millipore). An increase in the RAP1A band intensity correlates with reduced farnesylation.

RAP1A defarnesylation occurred only in the cells transduced with LV-shFDPS and treated with zoledronic acid.

Example 14—Treatment of a Subject with Cancer

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to the Site of Late Stage, Non-Resectable Hepatocellular Carcinoma

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound guided cannulation of the liver in patients without concomitant radiotherapy or chemotherapy. It is rationally predicted that this study will result in the successful treatment of HCC. The study is an open label, 4x3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with Stage III/IV non-resectable HCC.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce the anti-tumor activity of human gamma delta T cells, including the capacity for tumor killing by cellular cytotoxicity.

Subjects with target lesions ≥ 1 cm in longest diameter (measured by helical CT) and ≤ 4.9 cm maximum diameter and meeting inclusion and exclusion criteria detailed below, are enrolled into the next available dosing category. A maximum of 3 subjects are recruited for each dosage group. The dose is number of transducing units of LV-FDPS as described in the product release criteria, delivered via intra-hepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is 1×10^9 transducing units and escalation is 10-fold to a next dose of 1×10^{10} transducing units, the next dose is 1×10^{11} transducing units, and a maximum dose of 1×10^{12} transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovorin and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) in target and measurable non-local lesions (if present) by physical analysis, medical imaging or biopsy during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of hepatocellular carcinoma of parenchyma cell origin that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Target lesion must represent measurable disease with a unidimensional longest diameter of ≥ 1.0 cm by computed tomography; the maximum longest diameter is ≤ 5.0 cm.

Karnofsky performance score 60-80% of ECOG values.

Life expectancy ≥ 12 weeks.

Hematopoietic function: WBC $\geq 2,500/\text{mm}^3$; ANC $\geq 1000/\text{mm}^3$; Hemoglobin ≥ 8 g/dL; Platelet count $\geq 50,000/\text{mm}^3$; Coagulation INR ≤ 1.3 .

AST and ALT < 5 times ULN; ALPS < 5 time ULN. Bilirubin ≤ 1.5 times ULV; Creatine ≤ 1.5 times ULN and eGFR ≥ 50 .

Thyroid function: Total T3 or free T3, total T4 or free T4 and THCAE Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells $\geq 30/\text{mm}^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Target lesion contiguous with, encompasses or infiltrates blood vessel.

Primary HCC amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

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

Current or within past 4 weeks receipt of bisphosphonate therapy

Investigational agents within 4 weeks or < 5 drug half-lives.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

Bisphosphonate treatment within past 4 months.

Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine

or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

History of HIV or acquired immune deficiency syndrome.

Current or prior treatment with antiretroviral medications.

Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to the Site of Late Stage, Non-Resectable Hepatocellular Carcinoma—Adjunct Administration of Bisphosphonate

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound guided cannulation of the liver in patients with concomitant bisphosphonate chemotherapy. It is rationally predicted that this study will result in the successful treatment of HCC. The study is an open label, 4x3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with Stage III/IV non-resectable HCC.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce the anti-tumor activity of human gamma delta T cells, including the capacity for tumor killing by cellular cytotoxicity. Prior experimental studies also showed the potential for positive interactions of LV-FDPS and specific bisphosphonate drugs that may be prescribed in primary or metastatic diseases. For this study, subjects will receive dose escalating amounts of LV-FDPS with continuous standard of care dosing with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference.

Subjects with target lesions ≥ 1 cm in longest diameter (measured by helical CT) and ≤ 4.9 cm maximum diameter and meeting inclusion and exclusion criteria detailed below, are enrolled and started on bisphosphonate therapy. 30 days later size of the target lesion is re-evaluated to ensure subjects still meet starting criteria for LV-FDPS. Subjects without objective clinical response on bisphosphonate are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group and all continue on bisphosphonate for the study duration unless otherwise advised by the attending physician. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is 1×10^9 transducing units and escalation is 10-fold to a next dose of 1×10^{10} transducing units, the next dose is 1×10^{11} transducing units, and a maximum dose of 1×10^{12} transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for

unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) in target and measurable non-local lesions (if present) by physical analysis, medical imaging or biopsy during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of hepatocellular carcinoma of parenchyma cell origin that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Target lesion must represent measurable disease with a unidimensional longest diameter of ≥ 1.0 cm by computed tomography; the maximum longest diameter is ≤ 5.0 cm.

Karnofsky performance score 60-80% of ECOG values. Life expectancy ≥ 12 weeks.

Hematopoietic function: WBC $\geq 2,500/\text{mm}^3$; ANC $\geq 1000/\text{mm}^3$; Hemoglobin ≥ 8 g/dL; Platelet count $\geq 50,000/\text{mm}^3$; Coagulation INR ≤ 1.3 .

AST and ALT < 5 times ULN; ALPS < 5 time ULN. Bilirubin ≤ 1.5 times ULN; Creatine ≤ 1.5 times ULN and eGFR ≥ 50 .

Thyroid function: Total T3 or free T3, total T4 or free T4 and THCAE Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells $\geq 30/\text{mm}^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Intolerant to or unwilling to continue bisphosphonate adjunct therapy.

Objective clinical response after bisphosphonate therapy. Target lesion contiguous with, encompasses or infiltrates blood vessel.

Primary HCC amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

Chemotherapy excluding bisphosphonate, within 4 weeks or any use of nitrosourea, mitomycin C or cisplatin.

Investigational agents within 4 weeks or < 5 drug half-lives.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

History of HIV or acquired immune deficiency syndrome. Current or prior treatment with antiretroviral medications. Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

Example 15—Treatment of a Subject with Chronic Viral Disease(s) of the Liver LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to Liver for the Treatment of Hepatitis B Virus, Hepatitis C Virus, HIV or Other Viral Infection of the Liver

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to virally infected liver using ultrasound guided cannulation. It is rationally predicted that this study will result in the successful treatment of infections of the liver. The study is an open label, 4x3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with chronic viral disease of the liver that is resistant to chemotherapy.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce human gamma delta T cells, including a capacity for cellular cytotoxicity against virally-infected cells.

Subjects with confirmed viral infection of the liver including hepatitis B virus, hepatitis C virus, HIV or other viruses are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is 1×10^9 transducing units and escalation is 10-fold to a next dose of 1×10^{10} transducing units, the next dose is 1×10^{11} transducing units, and a maximum dose of 1×10^{12} transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al.,

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Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) measured as a Sustained Viral Response (SVR) within the organ or systemically during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of chronic viral infection of the liver that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Karnofsky performance score 60-80% of ECOG values.

Life expectancy ≥ 12 weeks.

Hematopoietic function: WBC $\geq 2,500/\text{mm}^3$; ANC $\geq 1000/\text{mm}^3$; Hemoglobin ≥ 8 g/dL; Platelet count $\geq 50,000/\text{mm}^3$; Coagulation INR ≤ 1.3 .

AST and ALT < 5 times ULN; ALPS < 5 time ULN. Bilirubin ≤ 1.5 times ULV; Creatine ≤ 1.5 times ULN and eGFR ≥ 50 .

Thyroid function: Total T3 or free T3, total T4 or free T4 and THCAE Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells $\geq 30/\text{mm}^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Chronic viral disease amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

Investigational agents within 4 weeks or < 5 drug half-lives.

Current (within past 4 weeks) or ongoing receipt of bisphosphonate therapy.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

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Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to Liver for the Treatment of Hepatitis B Virus, Hepatitis C Virus, HIV or other Viral Infection of the Liver—Concomitant Adjunct Bisphosphonate Therapy

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to virally infected liver using ultrasound guided cannulation. It is rationally predicted that this study will result in the successful treatment of infections of the liver. The study is an open label, 4x3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with chronic viral disease of the liver that is resistant to chemotherapy.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce human gamma delta T cells, including a capacity for cellular cytotoxicity against virally-infected cells. Prior experimental studies also showed the potential for positive interactions of LV-FDPS and specific bisphosphonate drugs that may be prescribed during infectious disease. For this study, subjects will receive dose escalating amounts of LV-FDPS with continuous standard of care dosing with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference.

Subjects with confirmed viral infection of the liver including hepatitis B virus, hepatitis C virus, HIV or other viruses will initiate bisphosphonate therapy for 45 days before re-screening to meet enrollment criteria for LV-FDPS treatment of infectious disease. Eligible subjects are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is 1×10^9 transducing units and escalation is 10-fold to a next dose of 1×10^{10} transducing units, the next dose is 1×10^{11} transducing units, and a maximum dose of 1×10^{12} transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

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Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) measured as a Sustained Viral Response (SVR) within the organ or systemically during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of chronic viral infection of the liver that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Karnofsky performance score 60-80% of ECOG values.

Life expectancy ≥ 12 weeks.

Hematopoietic function: WBC $\geq 2,500/\text{mm}^3$; ANC $\geq 1000/\text{mm}^3$; Hemoglobin ≥ 8 g/dL; Platelet count $\geq 50,000/\text{mm}^3$; Coagulation INR ≤ 1.3 .

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AST and ALT < 5 times ULN; ALPS < 5 time ULN. Bilirubin < 1.5 times ULV; Creatine ≤ 1.5 times ULN and eGFR ≥ 50 .

Thyroid function: Total T3 or free T3, total T4 or free T4 and $\text{THC} \leq \text{CTCAE}$ Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells $\geq 30/\text{mm}^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Chronic viral disease amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

Investigational agents within 4 weeks or < 5 drug half-lives.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval

Sequences

The following sequences are referred to herein:

SEQ ID NO:	Description	Sequence
1	FDPS shRNA sequence #1	GTCCTGGAGTACAAATGCCATTCTCGAGAATGGCATTGTA CTCCAGGACTTTTT
2	FDPS shRNA sequence #2	GCAGGATTTTCGTTTCAGCACTTCTCGAGAAGTGCTGAACG AAATCCTGCTTTTT
3	FDPS shRNA sequence #3	GC CATGTACATGGCAGGAATTCTCGAGAATTCCTGCCAT GTACATGGCTTTTT
4	FDPS shRNA sequence #4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCC TCCTTCTGCTTTTT
5	miR30 FDPS sequence #1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAG CCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGGCT GAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
6	miR30 FDPS sequence #2	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAG CCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGGCTGA GAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
7	miR30 FDPS sequence #3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCG TGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGC CTACTGCCTCGGA
8	miR155 FDPS sequence #1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAG CCTCCTTCTGCTTTTGCCACTGACTGAGCAGAAGGGCT GAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA
9	miR21 FDPS sequence #1	CATCTCCATGGCTGTACCACTTGTGCGGACTTTCTCAGC CTCCTTCTGCGTGTGAATCTCATGGCAGAAGGAGGCGA GAAAGTCTGACATTTTGGTATCTTTCATCTGACCA

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SEQ ID NO:	Description	Sequence
10	miR185 FDPS sequence #1	GGGCCTGGCTCGAGCAGGGGCGAGGGATACTTTCTCAG CCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGA GAAAGTCCTTCCCTCCCAATGACCGCTCTTCGTCG
11	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTA ACGATGAGTTAGCAACATGCCCTTACAAGGAGAGAAAA GCACCGTGCATGCCGATTGGTGGAGTAAGGTGGTACGA TCGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATG GATTGGACGAACCACTGAATTGCCGCATTGCAGAGATAT TGTATTTAAGTGCCCTAGCTCGATACAATAACG
12	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCT CTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAA GCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCCGTCTGTT GTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTA GTCAGTGTGAAAATCTCTAGCA
13	Psi Packaging signal	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGA GAG
14	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAG CACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGC CAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAA TTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCA ACTCACAGTCTGGGCGATCAAGCAGCTCCAGGCAAGAAT CCTGGCTGTGAAAGATACCTAAAGGATCAACAGCTCC
15	Central polypurine tract (cPPT)	TTTTAAAGAAAAGGGGGGATTGGGGGGTACAGTGCCAG GGGAAAGAATAGTAGACATAATAGCAACAGACATACAA ACTAAAGAATTACAAAACAAATTACAAAATTCAAATT TTA
16	Polymerase III shRNA promoters; H1 promoter	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGG CCCAGTGTCACTAGGCGGGAACCCAGCGCGCTGCGC CCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGC GCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAAT CACCATAAACGTGAAATGTCTTTGGATTGGGAATCTTA TAAGTTCTGTATGAGACCACTT
17	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTG GTATTCTTAACATATGTTGCTCCTTTTACGCTATGTGGATA CGCTGCTTAAATGCCTTTGTATCATGCTATTGCTTCCCGT ATGGCTTTCATTTTCTCCTCCTTGATAAAATCCTGGTTGC TGTCTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAAC GTGGCGTGGTGTGCACTGTGTTGCTGACGCAACCCCA CTGGTTGGGGCATTGCCACCCTGTGAGTCTCCTTCCGG GACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACCTC ATCGCCGCTGCTTGCCTGCTGAGCAGGGGCTCGG CTGTTGGGCACTGACAATTCGTTGGTGTGTCGGGGAAA TCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCT GGATTCTGCGCGGAGCTCCTTCTGCTACGTCCCTTCGGC CCTCAATCCAGCGGACCTTCTTCCCGCGCTGCTGCC GGCTCTGCGGCCTCTTCCGCTCTTCGCTTCGCCCTCAG ACGAGTCGGATCTCCCTTGGGCCGCTCCCCGCT
18	3' delta LTR	TGGAAGGGCTAATTCACCTCCCAACGAAGATAAGATCTGC TTTTTGCTGTACTGGGTCTCTCTGGTTAGACCAGATCTG AGCCTGGGAGCTCTCTGGCTAACTAGGGAACCACTGCT TAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGT GTGTGCCCGCTCTGTTGTGACTCTGGTAACATAGAGATC CCTCAGACCCTTTTAGTCAGTGTGAAAATCTCTAGCAG TAGTAGTTCATGTCA
19	Helper/Rev; Chicken beta actin (CAG) promoter; Transcription	GCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTT CACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTG TATTTATTTATTTTAAATATTTTGTGTCAGCGATGGGGG CGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGG GGGGCGAGGGGCGGGGCGGGGCGAGGCGAGAGGTGC GGCGGCAGCCAATCAGAGCGCGCGCTCCGAAAGTTTCC TTTTATGGCGAGGCGGCGGCGGCGGCGCCCTATAAAAA CGAAGCGCGGGCGGGG
20	Helper/Rev; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAATT AGATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAA AGAAAAATATAAATTAAACATATAGTATGGGAAGC AGGGAGCTAGAACGATTGCGAGTTAATCCTGGCCTGTTA

SEQ ID NO:	Description	Sequence
		GAAACATCAGAAGGCTGTAGACAAATACTGGGACAGCT ACAACCATCCCTTCAGACAGGATCAGAAGAAGCTTAGATC ATTATATAATACAGTAGCAACCTCTATTGTGTGCATCA AAGGATAGAGATAAAAGACACCAAGGAAGCTTTAGACA AGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAGC ACAGCAAGCAGCAGCTGACACAGGACACAGCAATCAGG TCAGCCAAAATTACCTATAGTGCAGAACATCCAGGGGC AAATGGTACATCAGGCCATATCACCTAGAACTTTAAATG CATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCA GAAGTGATACCATGTTTTTCAGCATTATCAGAAGGAGCC ACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGG GGACATCAAGCAGCCATGCAAAATGTTAAAGAGACCAT CAATGAGGAAGCTGCAGAAATGGGATAGAGTGCATCCAG TGCATGCAGGGCTATTGACACAGGCCAGATGAGAGAA CCAAGGGGAAGTGACATAGCAGGAAGCTAGTACCCCTT CAGGAACAAATAGGATGGATGACATAAATCCACCTATC CCAGTAGGAGAAATCTATAAAGATGGATAATCCTGGG ATTAATAAAATAGTAGAATGTATAGCCCTACCAGCAT TCTGGACATAAGACAAGGACCAAGGAACCTTTAGAG ACTATGTAGACCGATTCTATAAACTCTAAGAGCCGAGC AAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAACC TTGTTGGTCCAAATGCGAACCAGATTGTAAGACTATT TTAAGGCATTTGGGACCAGGAGCGACACTAGAAGAAAT GATGACAGCATGTGAGGAGTGGGGGACCCGGCCATA AAGCAAGAGTTTTGGCTGAAGCAATGAGCCAGTAACA AATCCAGCTACCATAATGATACAGAAAGGCAATTTTAGG AACCAAGAAAGAGCTGTTAAGTGTTCATTTGTGGCAAA GAAGGGCACATAGCCAAAATTGCAGGGCCCTAGGAA AAAGGGCTGTTGGAATGTGGAAGGAAGGACACCAAA TGAAGAGTTGTACTGAGAGACAGGCTAATTTTTAGGGA AGATCTGGCTTCCCAAGGAAGGCCAGGGAATTTTC TTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAG AGCTTCAGGTTTGGGGAAGAGACAACAACTCCCTCTCAG AAGCAGGAGCCGATAGACAAGGAAGCTATCCTTTAGCT TCCCTCAGATCACTCTTTGGCAGCGACCCCTCGTCACAA TAA
21	Helper/Rev; HIV Pol; Protease and reverse transcriptase	ATGAATTTGCCAGGAAGATGGAACCAAAATGATAGG GGGAAATTGGAGGTTTTATCAAAGTAGGACAGTATGATCA GATACTCATAGAAATCTGCGGACATAAAGCTATAGGTAC AGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAG AAATCTGTTGACTCAGATTGGCTGCATTTAAATTTTCCC ATTAGTCTATTGAGACTGTACCAGTAAATTAAGCCA GGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGAC AGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAG AAATGGAAGGAAGGAAAAATTTCAAAATTTGGGCT GAAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAA AAAGACAGTACTAAATGGAGAAAATTAGTAGATTTTCA AGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCA ATTAGGAATACCACATCCTGCAGGGTTAAACAGAAAA AATCAGTAACAGTACTGGATGTGGGCGATGCATATTTT CAGTTCCTTAGATAAAGACTTCAGGAAGTATACTGCAT TTACCATACCTAGTATAAACAATGAGACACCAGGGATTA GATATCAGTACAAATGTGCTTCACAGGGATGGAAGGAT CACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAG AGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATC AATACATGGATGATTTGTATGTAGGATCTGACTTAGAAA TAGGGCAGCATAGAACAAAAATAGAGGAAGTGAACAA CATCTGTTGAGGTGGGATTTACCACACCAGACAAAAAA CATCAGAAAGAACCTCCATTCTTTGGATGGGTTATGAA CTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTG CCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAA ATTAGTGGGAAAAATGAATTTGGGCAAGTCAGATTTATGC AGGATTAAGTAAGGCAATTATGTAACTTCTTAGGGG AACCAAGCACTAACAGAAGTAGTACCCTAACAGAAG AAGCAGAGCTAGAAGTGGCAGAAAACAGGGAGATTCTA AAAGAACCGGTACATGGAGTGTATTATGACCCATCAAAA GACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCA ATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCT GAAAACAGGAAAAATATGCAAGAATGAAGGGTGCCCA CTAATGATGTGAAACAATTAAACAGAGGCAGTACAAAA ATAGCCACAGAAAGCATAGTAATATGGGGAAGACTCC TAAATTTAAATTACCATACAAAAGGAACATGGGAAG CATGGTGGACAGATATTGGCAAGCCACCTGGATTCTCTG AGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTAT GGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAA

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SEQ ID NO:	Description	Sequence
		ACTTCTATGTAGATGGGGCAGCCAATAGGGAACTAAA TTAGGAAAAGCAGGATATGTAACGTACAGAGGAAGACA AAAAGTTGTCCCCCTAACGGACACACAATCAGAAGA CTGAGTTACAAGCAATTCATCTAGCTTTCAGGATTCGG GATTAGAAGTAAACATAGTGACAGACTCACAAATATGCAT TGGGAATCATTCAAGCACAACAGATAAGAGTGAATCA GAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAA GGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAAG GAATTGGAGGAAATGAACAAGTAGATGGGTGGTCAGT GCTGGAATCAGGAAGTACTA
22	Helper Rev; HIV Integrase; Integration of viral RNA	TTTTGTAGTGAATAGATAAGGCCCAAGAAGACATGA GAAATATCACAGTAATTGGAGAGCAATGGCTAGTGATT TAACCTACCACTGTAGTAGCAAAAGAAATAGTAGCCAG CTGTGATAAATGTGAGCTAAAAGGGGAAGCCATGCATG GACAAGTAGACTGTAGCCAGGAATATGGCAGCTAGATT GTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTC ATGTAGCCAGTGGATATATAGAAGCAGAAGTAATCCAG CAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAT TAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGAC AATGGCAGCAATTTACCCAGTACTACAGTTAAGGCCGCC TGTTGGTGGCGGGGATCAAGCAGGAATTTGGCATTCCC TACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAAT AAAGAATTAAAGAAAATATAGGACAGGTAAGAGATCA GGCTGAACATCTTAAGACAGCAGTACAATGGCAGTATT CATCCACAATTTAAAAAGAAAAGGGGGGATTGGGGGGT ACAGTGCAGGGGAAAGAAATAGTAGACATAATAGCAACA GACATACAACTAAAGAATTACAAAAACAAATTACAA AATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGA TCCAGTTTGGAAAGGACCAGCAAGCTCCTCTGGAAAGG TGAAGGGCAGTAGTAATACAAGATAATAGTGACATAA AAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGAT TATGGAAACAGATGGCAGGTGATGATTGTGTGGCAAGT AGACAGGATGAGGATTAA
23	Helper/Rev; HIV RRE; Binds Rev element	AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAG CACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGC CAGACAATTATTGTCTGGTATAGTGACGACGAGAACAA TTTGTCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCA ACTCACAGTCTGGGCGCATCAAGCAGCTCAGGCAAGAAT CCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCT
24	Helper/Rev; HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCT CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAA CCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAA GGAATAGAAGAAGAAGGTGGAGAGAGACAGAGACA GATCCATTTCGATTAGTGAACGGATCCTTAGCACTTATCT GGGACGATCTGCGGAGCCTGTGCCTTTCAGCTACCAAC GCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGG AACTTCTGGGACGAGGGGTGGGAAGCCCTCAAATATT GGTGGAATCTCTACAATATTGGAGTCAGGAGCTAAAGA ATAG
25	Envelope; CMV promoter; Transcription	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTT ACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCC AACGACCCCGCCCATTGACGTCAATAATGACGTATGTT CCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAA TGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTA CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC GTCAATGACGGTAATAGGCCCGCTGGCATTATGCCAG TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCT ACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTG GCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACG GGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAG TTTGTTTGGCACCAAAATCAACGGGACTTTCAAAATG TCGTAACAACCTCCGCCCATTTGACGCAATGGGCGGTAG GCGGTACGGTGGGAGGTCTATATAAGC
26	Envelope; VSV- G; Glycoprotein envelope-cell entry	ATGAAGTGCCTTTTGTACTIONAGCCTTTTATTATTGGGG TGAATTGCAAGTTCAACATAGTTTTTCCACACAACCAAA AAGGAACTGGAAAAATGTTCTTCTAATTACCATTATT GCCCGTCAAGCTCAGATTAAATTTGGCATAATGACTTAA TAGGCACAGCCTTACAAGTCAAATGCCAAGAGTCACA AGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCA AATGGGTCACTACTTGTGATTTCGCTGGTATGGACCGA

SEQ ID NO:	Description	Sequence
		AGTATATAACACATTCCATCCGATCCTTCACTCCATCTGT AGAACAATGCAAGGAAAGCATTGAACAAACGAAACAAG GAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTG GATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCC AGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAG GAGAATGGGTTGATTACAGTTCATCAACGAAATGCA GCAATTACATATGCCCCACTGTCCATACTTACAACCT GGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTA ACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGACG GAGAGCTATCATCCTTGGGAAAGGAGGCCACAGGTTT AGAAGTAACACTTTGCTTATGAAACTGGAGGCAAGGCC TGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTC CCATCAGGTGTCTGGTTGAGATGGCTGATAAGGATCTC TTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTCA AGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGT CTAATTCAGGACGTTGAGAGGATCTTGGATTATCCCTCT GCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCA ATCTCTCCAGTGGATCTCAGCTATCTTGTCTCTAAAAAC CAGGAACCGGTCTGCTTTCACCATATCAATGGTACCC TAAATACTTTGAGACCAGATACATCAGAGTCGATATTG CTGCTCAATCCTCTCAAGAATGGTCGGAATGATCAGTG GAACTACCACAGAAAGGGAAGTGGGATGACTGGGCA CCATATGAAGACGTGGAATTTGGACCAATGGAGTTCTG AGGACCACTTCCAGATATAAGTTTCTTTATACATGATT GGACATGGTATGTTGGACTCCGATCTTCTTAGCTCA AAGGCTCAGGTGTTTCAACATCCTCACATTCAAGACGCT GCTTCGCAACTTCTGATGATGAGAGTTATTTTTTGGTG ATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAGAAG GTTGGTTCAGTAGTTGGAAAAGCTCTATGCGCTCTTTTTT CTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTC CGAGTTGGTATCCATCTTTCATTAAATTAAGCACACC AAGAAAAGACAGATTTATACAGACATAGAGATGA
27	Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription	TAGTTATTAATAGTAATCAATTACGGGTCATTAGTTCAT AGCCCATATATGGAGTTCGCGTTACATAACTTACGGTA AATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCA TTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA ATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTA CGGTAACCTGCCCCCTTGGCAGTACATCAAGTGTATCAT ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAA TGGCCCGCTGGCATTATGCCCAGTACATGACCTTATGG GACTTCTTACTTGGCAGTACATCTACGTATTAGTCATC
28	Helper/Rev; Chicken beta actin intron; Enhance gene expression	GGAGTCGCTGCGTTGCTTTCGCCCCGTGCCCGCTCCGC GCCGCTTCGCGCCGCGCCGCTCTGACTGACCGCG TTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCT CCGGGCTGTAATTAGCGCTTGGTTTAAAGCGCTCGTTT CTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGG AGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGTGC GTGCGTGTGTGTGTGCGTGGGGAGCGCCGCTGCGGCCC GCGCTGCCCGCGGCTGTGAGCGCTGCGGGCGCGCGC GGGGCTTTGTGCGCTCCGCTGTGCGGAGGGGAGCGCG GCCGGGGCGGTGCCCGCGGTGCGGGGGGCTGCGAG GGGAACAAAGCTGCGTGCGGGGTGTGTGCTGGGGG GTGAGCAGGGGGTGTGGGCGCGCGGTGCGGCTGTAAC CCCCCTGCACCCCTCCCGAGTTGCTGAGCACGGC CCGCTTCGGGTGCGGGCTCCGTGCGGGGCTGGCGCG GGGCTCGCGTGC CGGGCGGGGGTGGCGGCAAGTGGG GGTGCCGGGCGGGCGGGCCGCTCGGGCCGGGGAGG GCTCGGGGAGGGGCGCGCGGCCCCGGAGCGCCGGCG GCTGTGAGGCGCGCGGAGCGCAGCCATTGCTTTTAT GGTAATCGTGCGAGAGGGCGCAGGACTTCTTTGTCCC AAATCTGGCGAGCCGAAATCTGGGAGCGCCGCGCA CCCCCTTAGCGGGCGCGGGCAAGCGGTGCGGCGCG GCAGGAAGGAAATGGGCGGGGAGGGCTTCTGCGTCTG CCGCGCGCGCTCCCTTCTCCATCTCAGCCTCGGGGCT GCCGAGGGGACGGCTGCCTTCGGGGGGACGGGGCA GGGCGGGGTTGCGCTTCTGGCGTGTACCGGCGG
29	Helper/Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACATCAT GAAGCCCTTGAGCATCTGACTTCTGGCTAATAAGGAA ATTTATTTTATTGCAATAGTGTGTGGAAATTTTTGTGT CTCTCACTCGGAAGGACATATGGGAGGCAATCATTTA AAACATCAGAAATAGTATTTGGTTTAGAGTTTGGCAACA TATGCCATATGCTGGCTGCCATGAACAAAGTGGCTATA AAGAGGTCATCAGTATATGAACAGCCCCCTGCTGTCCA

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SEQ ID NO:	Description	Sequence
		TTCCTTATTCATAGAAAAGCCTTGACTTGAGGTTAGATT TTTTATATTTTGTGTTATTTTCTTTAACATCC CTAATAATTTCTTACATGTTTACTAGCCAGATTTTCC TCCTCTCTGACTACTCCAGTCATAGCTGTCCCTCTTCT CTTATGAAGATC
30	Envelope; Beta globin intron; Enhance gene expression	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTCGCTA TTGTAAAATTCATGTTATATGGAGGGGCAAGTTTCA GGGTGTGTTTAGAATGGGAAGATGTCCCTTGATCACC ATGGACCCTCATGATAATTTTGTTCCTTCACTTCTACT CTGTTGACAACCATTTGCTCCTCTTATTTCTTTTCATTTT CTGTAACTTTTTCGTTAACTTTAGCTTGCATTTGTAACG AATTTTAAATTCACCTTTGTTTATTGTCTAGATTGTAAG TACTTTCTCTAATCACTTTTTTTCAAGGCAATCAGGGTA TATTATATTGACTTCAGCACAGTTTAGAGAACAAATGT TATAATTAAATGATAAGGTAGAATTTCTGCATATAAA TTCTGGCTGGCGTGGAATATTCTTATTGGTAGAAACAA CTACACCCTGGTCATCCTGCCCTTCTCTTTATGGTTA CAATGATATACACTGTTGAGATGAGGATAAAATACTCT GAGTCAAACCGGGCCCTCTGCTAACCATGTTTCATGCC TTCTTCTCTTCTCTACAG
31	Envelope; Rabbit beta globin poly A; RNA stability	AGATCTTTTCCCTCTGCCAAAAATTATGGGGACATCAT GAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAA ATTTATTTTCATTGCAATAGTGTGTGGAAATTTTGTGT CTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTA AAACATCAGAAATGAGTATTTGGTTTAGAGTTTGGCAACA TATGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTA TAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTC CATTCTTATTCATAGAAAAGCCTTGACTTGAGGTTAG ATTTTATTTATATTTGTTTGTGTTATTTTCTTTAACA TCCCTAAAATTTCTTACATGTTTACTAGCCAGATTTT TCCTCTCTCTGACTACTCCAGTCATAGCTGTCCCTCT TCTCTTATGGAGATC
32	Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
33	Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
34	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAACCAAAAAAT GATAGGGGGAATTGGAGGTTTATCAAAGTAAGACAGT ATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTA TAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAA TTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAA ATTTTCCATTAGTCCCTATTGAGACTGTACAGTAAAAT AAAGCCAGGAATGGATGGCCCAAAGTTAAACAATGGC CATTGACAGAAGAAAAATAAAGCATTAGTAGAAAT TGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAAAT TGGGCTGAAAAATCCATACAATACTCCAGTATTGGCCAT AAAGAAAAAGACAGTACTAAATGGAGAAAATTAGTAG ATTTTCAAGAACTTAATAAGAGAACTCAAGATTTCTGGG AAGTTCATTAGGAATACCACATCTGCAGGGTTAAAC AGAAAAATCAGTAACAGTACTGGATGTGGCGATGCA TATTTTTCAGTTCCTTAGATAAAGACTTCAGGAAGTATA CTGCATTTACCATACTAGTATAAACAATGAGACACCAG GGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGA AAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAA ATCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTC ATCTATCAATACATGGATGATTTGTATGTAGGATCTGAC TTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAAC GAGACAAACATCTGTTGAGGTGGGGATTACACACCAGA CAAAAACATCAGAAAGAACCTCCATTCTTTGGATGGG TTATGAATCCATCCTGATAAATGGACAGTACAGCCTAT AGTGCTGCCAGAAAGGACAGCTGGACTGTCAATGACA TACAGAAATTAGTGGGAAAAATGAATTGGGCAAGTCAG ATTTATGCAGGGATTAAAGTAAGCAATTATGTAACTT CTAGGGGAACCAAAGCACTAACAGAAGTAGTACCCT AACAGAAGAAGCAGAGCTAGAAGTGGCAGAAAAACAGG AGATTCTAAAAGAACCAGTACATGGAGTGTATTATGACC CATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGG CAAGCCAATGGACATATCAAAATTTATCAAGAGCCATTT AAAAAATCTGAAAAAGGAAAGTATGCAAGAATGAAGGG TGCCCACTAATGATGTGAACAATTAACAGAGGCAGT ACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAA AGACTCTAAATTTAAATTACCCATACAAAAGGAACAT GGAAGCATGTTGGACAGAGTATTGGCAAGCCACCTGG

SEQ ID NO:	Description	Sequence
		ATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGA AGTTATGGTACCAGTTAGAGAAAGAACCCATAATAGGA GCAGAACTTTCTATGTAGATGGGGCAGCCAAATAGGGA AACTAAATTAGGAAAAGCAGGATATGTAAC TGACAGAG GAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAAT CAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAG GATTCTGGGATTAGAAGTAAACATAGTGACAGACTCACA ATATGCATTGGGAATCATTCAAGCACACCAGATAAGAG TGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAAT AAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCAC ACAAGGAATTGGAGGAAATGAACAAGTAGATAAATTG GTCAGTGCTGGAATCAGGAAAAGTACTATTTTAGATGGA ATAGATAAGGCCAAGAAGAACATGAGAAATATCACAG TAATTGGAGAGCAATGGCTAGTGATTTTAACTTACCACC TGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAAT GTCAGCTAAAGGGGAAGCCATGCATGGACAAAGTAGAC TGTAGCCAGGAATATGGCAGCTAGATTGTACACATTTA GAAGGAAAAGTTATCTTGGTAGCAGTTTCATGTAGCCAGT GGATATATAGAAGCAGAAAGTAATTCCAGCAGAGACAGG GCAAGAAACAGCATACTTCTCTTAAATTAGCAGGAAG ATGGCCAGTAAAAACAGTACATACAGACAATGGCAGCA ATTTCAACAGTACTACAGTTAAGGCCGCTGTGTGGTGGG CGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCC AAAGTCAAGGAGTAATAGAATCTATGAATAAAGAATTA AAGAAAATTATAGGACAGGTAAAGAGTCAGGCTGAACA TCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAA TTTTAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAG GGGAAAGAAATAGTAGACATAATAGCAACAGACATACAA ACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAA TTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTG GAAAGGACCAGCAAAGCTCCTCTGGAAGGTGAAGGGG CAGTAGTAATACAAGATAATAGTGACATAAAGTAGTG CCAAGAGAAAAGCAAAGATCATCAGGATTATGGAAA ACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGG ATGAGGATTAA
35	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGA GCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCA AAGCAACCCACCTCCCAATCCGAGGGGACCCGACAGG CCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACA GAGACAGATCCATTTCGATTAGTGAACGGATCCTTGGCAC TTATCTGGGACGATCTGCGGAGCCTGTGCCCTTTCAGCT ACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGA TTGTGGAACTTCTGGGACGCAAGGGGTGGGAAGCCCTCA AATATTGGTGAATCTCCTACAATATTGGAGTCAGGAGC TAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAG CAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTG ACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAG CAGCAGAACAAATTTGCTGAGGGCTATTGAGGCGCAACA GCATCTGTGCAACTCACAGTCTGGGGCATCAAGCAGCT CCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGG ATCAACAGCTCCTAGATCTTTTCCCTCTGCCAAAAATTA TGGGGACATCATGAAGCCCTTGAGCATCTGACTTCTGG CTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTG GAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG GGCAATCATTTAAACATCAGAATGAGTATTGGTTTA GAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAAC AAAGGTGGCTATAAAGAGGTATCAGTATATGAAACAG CCCCTGCTGTCCATTCCCTTATTCATAGAAAAGCCTTGA CTTGAGGTAGATTTTTTTTATATTTTGTGTTTATTT TTTTCTTAAACATCCCTAAAAATTTCTTACATGTTTACT AGCCAGATTTTCTCTCTCTCTGACTACTCCAGTCATA GCTGTCCCTCTCTCTTATGAAGATCCCTCGACCTGCAGC CCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGT GAAATTGTTATCCGCTCACAATCCACACAACATACGAG CCGGAAGCATAAAGTGTAAGCCTGGGGTGCCATATGA GTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCC GCTTTCAGTCGGGAAACCTGTGCTGCCAGCGGATCCGC ATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTC CGCCCATCCCGCCCTAACTCCGCCAGTTCGGCCCATTC TCCGCCCATGGCTGACTAATTTTTTTTATTTATGCAGAG GCCGAGGCCGCTCGGCTCTGAGCTATTCAGAAGTAG TGAGGAGGCTTTTTTGGAGGCTAGGCTTTTGCAAAAG CTAACCTGTTTATTCAGCTTATAATGGTTACAAATAAA

SEQ ID NO:	Description	Sequence
		GCAATAGCATCACAAATTCACAAATAAGCATTTTTTT CACTGCATTCTAGTTGTGGTTGTCCAACTCATCAATGT ATCTTATCAGCGGCCGCCCCGGG
36	DNA fragment containing the CAG enhancer/promoter/ intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATT AGTTCATAGCCCATATATGGAGTTCGCGTTACATAACT TACGGTAAATGGCCGCGCTGGCTGACCGCCCAACGACCC CCGCCATTGACGTCAATAATGACGTATGTTCCCATAGT AACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGA CTATTACGGTAACTGCCACTTGGCAGTACATCAAGT GTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA CGGTAAATGGCCCGCTGGCATTATGCCAGTACATGAC CTTATGGGACTTCTCTACTTGGCAGTACATCTACGTATTA GTCATCGCTATTACCATGGGTGAGGTGAGCCCCACGTT CTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCA ATTTGTATTATTATTATTTTAAATTATTTTGTGCAGCGAT GGGGGCGGGGGGGGGGGGGCGCGCCAGGCGGGG GGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGA GGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAA GTTTCCTTTTATGGCGAGGCGGCGGGCGGCGGCGCCCTA TAAAAGCGAAGCGCGCGGGCGGGCGGAGTCGTCGCT TGCCCTCGCCCCGTGCCCGCTCCGCGCGCCTCGCGCC GCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGG TGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATT AGCGCTTGGTTAATGACGGCTCGTTCTTTCTGTGGCT GCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTG CGGGGGGAGCGGGCTCGGGGGGTGCGTGCCTGTGTGTG TGCGTGGGAGCGCGCGCTGCGGCCGCGCTGCCCGGCG GCTGTGAGCGCTGCGGGCGCGGCGGGGCTTTGTGCGC TCCGCGTGTGCGCGAGGGGAGCGCGGCGGGGGCGGTG CCCCGCGTGCGGGGGGCTGCGAGGGGAACAAAGGCT GCGTGCGGGGTGTGCGTGGGGGGTGAGCAGGGGT GTGGGCGCGGGGTGCGGCTGTAACCCCCCTGCACCC CCCTCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGC GGGGCTCCGTGCGGGGCTGGCGCGGGGCTCGCCGTGCC GGGCGGGGGGTGGCGGAGGTGGGGGTGCCGGGCGGG CGGGGCGCCTCGGGCGGGGAGGCTCGGGGAGGGG CGCGGCGGCCCGGAGCGCGCGGGCTGTCGAGGCGCG GCGAGCGCAGCCATTGCTTTTATGGTAATCGTGCGAG AGGGCGCAGGACTTCTTTGTCCCAATCTGGCGGAGC CGAAATCTGGAGGCGCGCGCACCCCCCTAGCGGGC GCGGGCGAAGCGGTGCGCGCGCGCAGGAAGAAATGG GCGGGGAGGGCCTTCGTGCTGCGCGCGCGCGCTCCCC TTCTCCATCTCCAGCCTCGGGGCTGCGCAGGGGGACGG CTGCTTTCGGGGGACGGGACAGGGCGGGTTCGGCTT CTGCGTGTGACCGCGGGAATTC
37	DNA fragment containing VSV-G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTATTCA TTGGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACA ACCAAAAAGGAACTGGAAAAATGTTCTTCTAATTACC ATTATTGCCCGTCAAGCTCAGATTAAATTGGCATAATG ACTTAATAGGCACAGCCTTACAAGTCAAAATGCCAAGA GTCACAAGGCTATTCAAGCAGACGGTTGGATGTGTCTATG CTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGG ACCGAAGTATATAACACATTCCATCCGATCCTTCACTCC ATCTGTAGAACAATGCAAGGAAAGCATTGAACAAACGA AACAAGGAAGTGGCTGAATCCAGGCTTCCTCTCTCAA GTTGTGATATGCAACTGTGACGGATGCGAAGCAGTGA TTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAAT ACACAGGAGAATGGGTTGATTACAGTTTCATCAACGGAA AATGCAGCAATTACATATGCCCCACTGTCCATAACTCTA CAACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTG ATTCTAACCTCATTCCATGGACATCACCTTCTTCTCAGA GGACGGAGAGCTATCATCCCTGGGAAAGGAGGACAG GGTTCAGAAGTAAGTACTTTGCTTATGAACTGGAGGCA AGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTC AGACTCCCATCAGGTGTCTGGTTCGAGATGGCTGATAAG GATCTCTTTGCTGACGCAGATTCCCTGAATGCCAGAA GGGTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGAT GTAAGTCTAATTCAGGACGTTGAGAGGATCTTGATTAT TCCCTCTGCCAAGAACTGGAGCAAAATCAGAGCGGGT CTTCCAATCTCTCAGTGGATCTCAGCTATCTTGCTCCTA AAAACCCAGGAACCGGTCTGCTTTCACCATAATCAATG GTACCTTAAATACTTTGAGACCAGATACATCAGAGTCG ATATTGCTGCTCCAATCCTCAAGAATGGTCGGAATGA TCAGTGGAACTACCACAGAAAGGAACTGTGGGATGAC

SEQ ID NO:	Description	Sequence
		TGGGCACCATATGAAGACGTGGAAATTGGACCCAATGG AGTTCTGAGGACCAGTTTCAGGATATAAGTTTCCTTTATA CATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTT AGCTCAAAGGCTCAGGTGTTTCAACATCCTCACATTCAA GACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTATTTT TTGGTGATACTGGGCTATCCAAAATCCAATCGAGCTTG TAGAAGGTGGTTCAGTAGTTGGAAGCTCTATTGCCT CTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTT GGTTCCTCGAGTTGGTATCCATCTTGCATTAAATTAAAG CACACCAAGAAAAGACAGATTTATACAGACATAGAGAT GAGAATTC
38	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGG GGACTAGGGTGTGTTTAGGCGAAAAGCGGGCTTCGGTT GTACGCGTTAGGAGTCCCTCAGGATATAGTAGTTTCG CTTTTCATAGGGAGGGGAAATGTAGTCTTATGCAATA CACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAA CATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCG ATTGGTGAAGTAAGGTGGTACGATCGTGCTTATTAGG AAGGCAACAGACAGGTCTGACATGGATTGGACGAACCA CTGAATCCCGATTGCAGAGATAATTGTATTAAAGTGCC TAGCTCGATACAATAAACGCCATTGACCATTCACCACA TTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACC GTCAGATCGCTGGAGACGCCATCCACGCTGTTTGGACC TCCATAGAAGACACCGGACCGATCCAGCTCCCTCGA AGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCG GAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTC ATCAAGTTTCTCTATCAAAGCAACCCACTCCCAATCCC GAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAA GGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGT GAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAG CCTGTGCTCTTCAGCTACCACCGCTTGAGAGACTTACTC TTGATTGTAACGAGGATTGTGGAATCTTGGGACGCAAG GGTGGGAAGCCCTCAAATATTGGTGAATCTCCTACAA TATTGAGTCAGGAGCTAAAGAATAGTCTAGA
39	Elongation Factor- 1 alpha (EF1- alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTAAGTGGCTCCGCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCGGTGAAC GTTCTTTTTCGCAACGGTTTGGCCGACAGACAGGTA AGTGCCGTGTGTGGTTCCCGCGGCTGGCCTCTTTACG GGTATGGCCCTTGCCTGCTTGAATTACTTCCACGCCCC TGGCTGCAGTACGTGATCTTGATCCCGAGCTTCGGTT GGAAGTGGGTGGGAGATTGAGGCCTTGCCTTAAGG AGCCCCCTCGCTCGTCTGAGTTGAGGCTTGGCCTGG GCGCTGGGCGCGCGCTGCGAATCTGGTGGCACCTTCG CGCTGTCTCGCTGCTTTGATAAGTCTCTAGCCATTTAA AATTTTGTGATGACCTGCTGCGACGCTTTTTTCTGGCAAG ATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTA TTTGCGTTTTTGGGGCCGCGGGCGGCGACGGGGCCGTG CGTCCAGCGCACATGTTCGGCGAGGCGGGCTGCGAG CGCGCCACCGAGAATCGGACGGGGTAGTCTCAAGCT GGCGGCTGCTCTGGTGCCTGGCCTCGCGCCGCTGT ATCGCCCCGCTGGGCGGCAAGGCTGGCCCGCTCGGCA CCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGCCCT GCTGCAGGGAGCTCAAATGGAGGACGCGGCGCTCGGG AGAGCGGGCGGGTGAATCACCACACAAAGGAAAGGG CTTTTCGTCTCAGCCGTGCTTCATGTGACTCCACGGA GTACCGGGCGCGTCCAGGCACCTCGATTAGTTCTCGAG CTTTGGAGTACGTCGTCTTAGGTTGGGGGAGGGGTT TTATGCGATGGAGTTTCCCACTGAGTGGGTGGAGAC TGAAGTTAGGCCAGCTTGGCACTTGATGTAATCTCCTTG GAATTTGCCCTTTTGAATTTGGATCTTGGTTCATTCTCA AGCCTCAGACAGTGGTTCAAAGTTTTTCTTCCATTCTCA GGTGTCGTGA
40	Promoter; PGK	GGGGTTGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTT TGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAA ACGCAGCGGCGCCGACCTGGGTCTCGCACATTCTTCAC GTCCTTTCGACGCTCACCAGATCTTCGCGCTACCTT GTGGGCCCCCGGCGACGCTTCTGCTCGCCCCCTAAGT CGGGAAGGTTCTTGGGTTTCGCGGCTGCGGACGTGA CAAACGGAAGCCGACGCTCTCACTAGTACCTTCGAGAC GGACAGCGCCAGGAGCAATGGCAGCGCGCCGACCGCG ATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGC CGAGAGCAGCGCCGGGAAGGGCGGTGCGGAGGCGG

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SEQ ID NO:	Description	Sequence
		GGTGTGGGGCGGTAGTGTGGGCCCTGTTCTGCCCGCGC GGTGTTCGCATTCTGCAAGCCTCCGGAGCGCACGTCCG CAGTCGGCTCCCTCGTTGACCGAATCACCACCTCTCTCC CCAG
41	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCCT CACGGCGAGCGCTGCCACGTGACAGCAAGGGCGCAGGA GCGTTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGG CCCGCTGCTCATAAGACTCGGCCTTAGAACCCAGTATC AGCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTA GGGCACCTGGTTTTCTTCCAGAGAGCGGAACAGGCGAGG AAAAGTAGTCCCTTCTCGGCGATTCTCGGAGGGGATCTC CGTGGGGCGGTGAACGCCGATGATTATATAAGGACGCG CCGGGTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATT GGGTGCGGGTTCTTGTGTGGATCGCTGTGATCGTCACT TGGTGAGTTGCGGGCTGCTGGGCTGGCCGGGCTTTCGT GGCCGCCGGGCGCTCGGTGGGACGGAAGCGTGTGGAG AGACCGCCAAAGGGCTGTAGTCTGGGTCCGCGAGCAAGG TTGCCCTGAACTGGGGTTGGGGGAGCGCACAAATG GCGGCTGTCCCGAGTCTGAATGGAAGACGCTTGTAAAG GCGGGCTGTGAGGTGCTGAAACAAGGTGGGGGGCATG GTGGGCGCAAGAACCAGGTCTTGAAGCCTTCGCTAA TGCGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCA CCATCTGGGGACCTGACGTGAAGTTTGTCACTGACTGG AGAACTCGGGTTTGTCTGCTGTTGCGGGGCGGCAGTT ATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTTGGGAG CGCGCGCTCGTCTGCTGACGTACCCGTTCTGTTGG CTTATAATGCAGGGTGGGGCCACCTGCCGTTAGGTGTGC GGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTTCGGGCC TAGGGTAGGCTCTCCTGAATCGACAGCGCCGACCTCT GGTGAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTGGT CGGTTTTATGTACCTATCTTCTAAGTAGCTGAAGCTCCG GTTTTGAATATGCGCTCGGGGTGGCGAGTGTGTTTGT GAAGTTTTTTAGGCACCTTTTGAATGTAATCATTTGGGT CAATATGTAATTTTCAGTGTTAGACTAGTAAA
42	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG CATCACAAATTTACAAATAAAGCATTTTTTCACTGCAT TCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATC A
43	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTGCCCC TCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACTCCCA CTGTCTTTTCTAATAAAATGAGGAAATGTCATCGCATT GTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGG GGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGC AGGCATGCTGGGGATGCGGTGGGCTCTATGG
44	Envelope; RD 114	ATGAAACTCCCAACAGGAATGGTCATTTATGTAGCCTA ATAATAGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCT ATCGCATTAGTACAAAAACAATGGTAAACCATGCGA ATGCAGCGGAGGGCAGGTATCCGAGGCCCAACCGAACT CCATCCAACAGGTAACCTGCCCAGGCAAGACGGCCTACT TAATGACCAACAAAAATGGAATGCAGAGTCACTCCA AAAAATCTCACCCCTAGCGGGGAGAACTCCAGAACTG CCCCGTGAACACTTTCCAGGACTCGATGCACAGTTCTTGT TATACTGAATACCGGCAATGCAGGGCGAATAATAAGAC ATACTACACGGCCACCTTGCTTAAATACGGTCTGGGAG CCTCAACGAGGTACAGATATTACAAAACCCCAATCAGCT CCTACAGTCCCCTTGTAGGGGCTCTATAATCAGCCCCGT TTGCTGGAGTGCCACAGCCCCCATCCATATCTCCGATGG TGGAGGACCCCTCGATACTAAGAGAGTGTGGACAGTCCA AAAAGGCTAGAACAAATTATAAGGCTATGCATCCTGA ACTTCAATACCACCCCTTAGCCCTGCCCAAAGTCAGAGA TGACCTTAGCCTTGATGCACGGACTTTTGATATCCTGAAT ACCACTTTTAGGTTACTCCAGATGTCCAATTTTAGCCTTG CCCAAGATTGTTGGCTCTGTTTAAACTAGGTACCCCTA CCCCCTTTGCGATACCCACTCCCTCTTAACTACTCCCT AGCAGACTCCCTAGCGAATGCCTCCTGTGAGATTATACC TCCCCCTTGTTCAACCGATGCAGTTCTCCAACCTGTC TGTTTATCTTCCCCTTTCATTAACGATACGGAACAAATAG ACTTAGGTGCAGTCACCTTTACTAACTGCACCTCTGTAGC CAATGTGAGTAGTCTTTATGTGCCCTAAACGGGTGAGT CTTCCTCTGTGGAAATAACATGGCATAACCTATTTACCC CAAACTGGACAGGACTTTGCGTCCAAGCCTCCCTCCTC CCCGACATTGACATCATCCCGGGGATGAGCCAGTCCCC

SEQ ID NO:	Description	Sequence
		ATTCCTGCCATTGATCATTATATACATAGACCTAAACGA GCTGTACAGTTCATCCCTTTACTAGCTGGACTGGGAATC ACCGCAGCATTACACCCGGAGCTACAGGCCTAGGTGTC TCCGTACCCAGTATACAAAATTATCCCATCAGTTAATA TCTGATGTCCAAGTCTTATCCGGTACCATAACAAGATTAC AAGACCAGGTAGACTCGTTAGCTGAAGTAGTTCTCCAAA ATAGGAGGGGACTGGACCTACTAACGGCAGAACCAAGGA GGAATTTGTTTAGCCTTACAAGAAAAATGCTGTTTTTATG CTAACAAGTCAGGAATTGTGAGAAACAAAAAAGAACC CTACAAGAAGAATTACAAAACGCAGGGAAAGCCTGGC ATCCAACCCCTCTCTGGACCCGGGCTGCAGGGCTTTCTTCC GTACCTCCTACCTCTCTGGGACCCCTACTCACCTCCTA CTCATACTAACCATTGGGCATGCGTTTCAATCGATTGG TCCAATTTGTTAAAGACAGGATCTCAGTGGTCCAGGCTC TGGTTTTGACTCAGCAATATCACCAGCTAAAACCCATAG AGTACGAGCCATGA
45	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCCACCTTCGGCACCAG ATGAGTCCTGGGAGCTGGAAGAAAGACTGATCATCCTCTTA AGCTGCGTATTTCGGAGACGGCAAAACGAGTCTGCAGAA TAAGAACCCCCACAGCCTGTGACCCCTACCTGGCAGGT ACTGTCCAAACTGGGGACGTGTCTGGGACAAAAGGC AGTCCAGCCCCCTTGGACTTGGTGGCCCTCTCTTACACCT GATGTATGTGCCCTGGCGGCCGGTCTTGAGTCCTGGGAT ATCCCGGATCCGATGTATCGTCTCTAAAAGAGTTAGA CCTCCTGATTGAGACTATACTGCCGCTTATAAGCAAATC ACCTGGGAGCCATAGGGTGCAGCTACCCCTCGGGCTAGG ACCAGGATGGCAAATTCCCCCTTCTACGTGTGTCCCCGA GCTGGCCGAACCCATTTCAGAAGCTAGGAGGTGTGGGGG GCTAGAATCCCTATACTGTAAGAATGGAGTTGTGAGAC CACGGGTACCGTTTATTGGCAACCAAGTCTCATGGGA CCTCATAACTGTAAAATGGGACCAAAATGTGAAATGGG AGCAAAAATTTCAAAGTGTGAACAAACCGGCTGGTGT AACCCCTCAAGATAGACTTCACAGAAAAAGGAAAACT CTCCAGAGATTGGATAACGGAAAAAACCCTGGGAATTAA GGTTCATGTATATGGACACCCAGGCATACAGTTGACTA TCCGCTTAGAGGTCACTAACATGCGGTTGTGGCAGTGG GCCCAGACCCCTGTCTTGGGAACAGGGACTCTCTAGCA AGCCCTCTACTCTCCCTCTCTCCCCAGGAAAGCGCCGC CCACCCCTCTACCCCGGCGGTAGTGAGCAACCCCTG CGGTGCATGGAGAACTGTTACCCCTAAACTCTCCGCCTC CCACCAGTGGCGACCGACTCTTTGGCCTTGTGCAGGGGG CCTTCTAACCTTGAATGCTACCAACCCAGGGGCCACTA AGTCTTGCTGGCTCTGTTTGGGCATGAGCCCCCTTATTA TGAAGGGATAGCCTCTTCAGGAGAGGTCTGTTATACCTC CAACCATACCCGATGCCACTGGGGGGCCCAAGGAAAGC TTACCCCTCACTGAGGTCTCCGACTCGGGTCATGCATAG GGAAGGTGCCCTCTTACCCTCAACATCTTTGCAACCCAGA CCTTACCCTCAATTCCTCTAAAACCATCAGTATCTGCT CCCCTCAAACCATAGCTGGTGGGCCTGCAGCACTGGCCT CACCCCTGCTCTCTCCACTCAGTTTAAATCAGTCTAAA GACTTCTGTGTCCAGGTCCAGCTGATCCCCGCATCTATT ACCATTCTGAAGAAACCTTGTACAGCCTATGACAAAT CACCCCCCAGGTTTAAAGAGAGCTGCTCACTTACCC TAGCTGTCTTCTGGGGTTAGGGATTGCGGCAGGTATAG GTACTGGCTCAACGCGCCTAATTAAGGGCCCATAGACC TCCAGCAAGGCCTAACAGCCTCCAAATCGCCATTGACG CTGACCTCCGGGCCCTTCAGGACTCAATCAGCAAGCTAG AGGACTCACTGACTTCCCTATCTGAGGTAGTACTCCAAA ATAGGAGAGGCCTTGACTTACTATTCTTAAAGAAAGGAG GCCTCTGCGCGGCCCTAAAAGAAAGAGTGCTGTTTTATG TAGACCACTCAGGTGCAGTACGAGACTCCATGAAAAAA CTTAAAGAAAGACTAGATAAAAGACAGTTAGAGCGCCA GAAAAACCAAACTGGTATGAAGGTTGGTTCAATAACT CCCCTTGGTTTACTACCTACTATCAACCATCGCTGGGCC CCTATTGCTCCTCCTTTGTACTCACTCTTGGGCCCTGC ATCATCAATAAATTAATCCAATTCATCAATGATAGGATA AGTGCAGTCAAAATTTAGTCCTTAGACAGAAATATCAG ACCCTAGATAACGAGGAAAACCTTTAA
46	Envelope; FUG	ATGGTTCGCGAGGTTCTTTGTTTGTAATCCTTCTGGGTT TTTCTGTGTGTTTCGGGAAGTTCCCCATTTACACGATACC AGACGAACCTTGGTCCCTGGAGCCCTATTGACATACACCA TCTCAGCTGTCCAAATAACCTGGTTGTGGAGGATGAAGG ATGTACCAACCTGTCGAGTTCTCCTACATGGAACCTCAA AGTGGGATACATCTCAGCCATCAAAGTGAACGGGTTCCAC

SEQ ID NO:	Description	Sequence
		TTGCACAGGTGTTGTGACAGAGGCAGAGACCTACACCAA CTTTGTGGTTATGTCAACACCATTCAGAGAAAGCA TTTCGCCCCACCCAGACGCATGTAGAGCCGCGTATAA CTGGAAGATGGCCGGTGACCCAGATATGAAGAGTCCCT ACACAATCCATACCCCGACTACCACTGGCTTCGAACTGT AAGAACCACCAAGAGTCCCTCATTATCATATCCCCAAG TGTGACAGATTGGACCCATATGACAAATCCCTTCACTC AAGGGTCTTCCTGGCGAAAGTGCTCAGGAATAACGGT GTCCTCTACCTACTGCTCAACTAACCATGATTACACCATT TGGATGCCCGAGAATCCGAGACCAAGGACACCTTGTGAC ATTTTACCAATAGCAGAGGGAAGAGAGCATCCAACGG GAACAAGACTTGCGGCTTTGTGGATGAAAGAGGCCTGTA TAAGTCTCTAAAGGAGCATGCAAGCTCAAGTTATGTGG AGTTCTTGGACTTAGACTTATGGATGGAACATGGGTGCG GATGCAACATCAGATGAGACCAAATGGTGCCTCCAG ATCAGTTGGTGAATTTGCACGACTTCGCTCAGACGAGA TCGAGCATCTCGTTGTGGAGGAGTTAGTTAAGAAAAGAG AGGAATGTCTGGATGCATTAGAGCCATCATGACCACCA AGTCAGTAAGTTTCAGACGCTCTCAGTCACCTGAGAAAAC TTGTCCCAGGGTTTGGAAAAGCATATACCATATTCAACA AAACCTTGATGGAGGCTGATGCTCACTACAAGTCAGTCC GGACCTGGAATGAGATCATCCCTCAAAGGGTGTGTA AAGTTGGAGGAAGGTGCCATCCTCATGTGAACGGGTGT TTTTCAATGGTATAATATTAGGGCTGACGACCATGTCTT AATCCCAGAGATGCAATCATCCCTCCTCCAGCAACATAT GGAGTTGTGGAATCTTCAGTTATCCCCCTGATGCACCCC CTGGCAGACCTTCTACAGTTTTCAAAGAAGGTGATGAG GCTGAGGATTTTGTGAAGTTCACCTCCCCGATGTGTAC AAACAGATCTCAGGGTTGACCTGGGTCTCCCGAACTGG GGAAAGTATGTATTGATGACTGCAGGGGCCATGATTGGC CTGGTGTTGATATTTCCCTAATGACATGGTGCAGAGTTG GTATCCATCTTGCATTAAATTAAGCACACCAAGAAAA GACAGATTTATACAGACATAGAGATGAACCGACTTGA AAGTAA
47	Envelope; LCMV	ATGGGTGAGATTGTGACAATGTTTGAGGCTCTGCCTCAC ATCATCGATGAGGTGATCAACATTGTATTATGTGCTTA TCGTGATCACGGGTATCAAGGCTGTCTACAATTTGCCA CCTGTGGGATATTCGCATTGATCAGTTTCCTACTTCTGGC TGGCAGGTCCTGTGGCATGTACGGTCTTAAGGGACCCGA CATTTACAAAGGAGTTTACCAATTTAAGTCAGTGGAGTT TGATATGTCACATCTGAACCTGACCATGCCCAACGCATG TTCAGCCAACAACCTCCACCATTACATCAGTATGGGGAC TTCTGGACTAGAATTGACCTTCACCAATGATTCATCATC AGTCACAACCTTTGCAATCTGACCTCTGCCTTCAACAAA AAGACCTTTGACCACACACTCATGAGTATAGTTTCGAGC CTACACCTCAGTATCAGAGGGAACCTCAACTATAAGGCA GTATCCTGCGACTTCAACAATGGCATAAACCATCCAATAC AACTTGACATTCTCAGATCGACAAAGTGCTCAGAGCCAG TGTAAGACCTTCAGAGGTAGAGTCTAGATATGTTAGA ACTGCCCTTCGGGGGGAATACATGAGGAGTGGCTGGGG CTGGACAGGCTCAGATGGCAAGACCCTGGGTGAGCCA GACGAGTTACCAATACCTGATTATACAAAAAGAACCTG GGAAAACCACTGCACATATGCAGGTCTTTTGGGATGTC CAGGATTCCTTTTCCCAAGAGAAGACTAAGTTCTTCAC TAGGAGACTAGCGGGCATTACCTGGACTTTGTGAGA CTCTTCAGGGGTGAGAAATCCAGGTGGTTATTGCCTGAC CAAATGGATGATTCTGCTGCAGAGCTTAAGTGTTTCGG GAACACAGCAGTTGCGAAATGCAATGTAATCATGATGC CGAATTCTGTGACATGCTGCGACTAATTGACTACAACAA GGCTGCTTTGAGTAAGTCAAAGAGGACGTAGAATCTGC CTTGCACTTATTCAAAACAACAGTGAATTCTTTGATTTC GATCAACTACTGATGAGGAACCACTTGAGAGATCTGATG GGGGTGCCATATTGCAATTACTCAAAGTTTGGTACCTA GAACATGCAAGACCGGCGAAACTAGTGTCCCCAAGTG CTGGCTTGTCAACCAATGGTTCTTACTAAATGAGACCCA CTTCAGTGATCAAATCGAACAGGAAGCCGATAACATGAT TACAGAGATGTTGAGGAAGGATTACATAAAGAGGCAGG GGAGTACCCCTAGCATTGATGGACCTTCTGATGTTTC CACATCTGCATATCTAGTCAGCATCTTCCTGCACCTTGTC AAAATACCAACACACAGGCACATAAAAGGTGGCTCATG TCCAAAGCCACACCGATTAAACCAAAAGGAATTTGTAG TTGTGGTGCATTTAAGGTGCCTGGTGTAACCAACCGTCTG GAAAAGACGCTGA

SEQ ID NO:	Description	Sequence
48	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTTCGCCCTTGTGGCAGTC ATCCCCACAAATGCAGACAAAATTGTCTTGGACATCAT GCTGTATCAAATGGCACCAAAGTAAACACACTCACTGAG AGAGGAGTAGAAGTTGTCAATGCAACGGAAACAGTGGAA GCGGACAAACATCCCCAAAATTGTCTAAAAGGGAAAA GAACCACTGATCTTGGCCAATGCGGACTGTTAGGGACCA TTACCGGACCACCTCAATGCGACCAATTTCTAGAATTTTC AGCTGATCTAATAATCGAGAGACGAGAAGGAAATGATG TTTGTTACCCGGGGAAGTTTGTTAATGAAGAGGCATTGC GACAAATCCTCAGAGGATCAGGTGGGATTGACAAAGAA ACAATGGGATTTCATATAGTGGAAATAAGGACCACCGG AACAACTAGTGCATGTAGAAGATCAGGGTCTTCATTCTA TGCAGAAATGGAGTGGCTCCTGTCAAATACAGACAATGC TGCTTTCCACAAATGACAAAATCATACAAAACACAAAG GAGAGAATCAGCTCTGATAGTCTGGGGAATCCACCATT AGGATCAACCACCGAACAGACCAAACTATATGGGAGTG GAAATAAACTGATAACAGTCCGGGAGTTCAAATATCATC AATCTTTTGTGCCGAGTCCAGGAACACGACCGCAGATAA ATGGCCAGTCCGGACGGATTGATTTTCATTGGTTGATCTT GGATCCC AATGATACAGTTACTTTTAGTTTCAATGGGGC TTTCATAGCTCCAATCGTGCCAGCTTCTTGAGGGGAAA GTCCATGGGGATCCAGAGCGATGTGCAGGTTGATGCCAA TTGCGAAGGGGAATGTACCACAGTGGAGGGACTATAA CAAGCAGATTGCCTTTTCAAACATCAATAGCAGAGCAG TTGGCAAATGCCAAGATATGTAAAACAGGAAAGTTTAT TATTGGCAACTGGGATGAAGAACGTTCCCGAACCTTCCA AAAAAAGGAAAAAAGAGGCCTGTTGGCGCTATAGCA GGGTTTATGAAAATGGTTGGGAAGGTCTGGTCGACGGG TGGTACGGTTTCAGGCATCAGAATGCACAAGGAGAAGG AACTGCAGCAGACTACAAAAGCACCCAAATCGGCAATTG ATCAGATAACCGGAAAGTTAAATAGACTCATTGAGAAA ACCAACAGCAATTTGAGCTAATAGATAATGAATTCAC GAGGTGGAAGCAGATGTGCAATTTAATTAAGTGGACC AAAGACTCCATCACAGAAGTATGGTCTTACAATGCTGAA CTTCTTGTGGCAATGGAACACAGCAGCTATTGATTG GCTGATTCAGAGATGAACAAGCTGTATGAGCGAGTGAG GAAACAATTAAGGGAAAATGCTGAAGAGGATGGCACTG GTTGCTTTGAAATTTTTCATAAATGTGACGATGATTGTAT GGCTAGTATAAGGAACAATACTTATGATCACAGCAATA CAGAGAAGAAGCGATGCAAAATAGAATACAAATTGACC CAGTCAAATTGAGTAGTGGCTACAAAGATGTGATACTTT GGTTTAGCTTCGGGCGCATGCTTTTGTCTTCTGCCAT TGCAATGGGCTTGTTCATATGTGTGAAGAACGGAAA CATGCGGTGCACTATTTGTATATAA
49	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGA CCATACCTAGCACATTGCGCCGATTGCGGGGACGGGTAC TTCTGCTATAGCCAGTTGCTATCGAGGAGATCCGAGAT GAGGCGTCTGATGGCATGCTTAAGATCCAAGTCTCCGCC CAAATAGGTCTGGACAAGGCAGGCACCCACGCCACAC GAAGCTCCGATATATGGCTGGTCATGATGTTCAGGAATC TAAGAGAGATTCCTTGAGGGTGTACACGTCCGACGCGTG CTCCATACATGGGACGATGGGACACTTCATCGTCGCACA CTGTCCACAGGCGACTACTCAAGGTTTCGTTGAGGA CGCAGATTGCGACGTGAAGGCATGTAAGGTCCAATACAA GCACAATCCATTGCCGGTGGGTAGAGAGAAGTTCGTGGT TAGACCACACTTTGGCGTAGAGCTGCCATGCACCTCATA CCAGCTGACAACGGCTCCCAACGACGAGGAGATTGACAT GCATACACCGCCAGATATACCGGATCGCACCTTGCTATC ACAGACGGCGGGCAACGTCAAATAACAGCAGGCGGCA GGACTATCAGGTACAACGTACCTGCGGCGGTGACAACG TAGGCATACCACTACTGACAAGACCATCAACACATGCA AGATTGACCAATGCCATGCTGCCGTACCAGCCATGACA AATGGCAATTTACCTCTCCATTTGTTCCAGGGCTGATCA GACAGCTAGGAAAGGCAAGGTACACGTTCCGTTCCCTCT GACTAACGTACCTGCGGAGTGGCGTTGGCTCGAGCGCC GGATGCCACCTATGGTAAGAAGGAGGTGACCTGAGATT ACACCCAGATCATCCGACGCTCTTCTCTATAGGAGTTT AGGAGCGGAACCGCACCCGTACGAGGAATGGGTTGACA AGTTCTCTGAGCGCATCATCCAGTGACGGAAGAAGGGA TTGAGTACCAAGTGGGGCAACAACCCGCGGTCTGCCTGT GGGCGCAACTGACGACCGAGGGCAAAACCCATGGCTGG CCACATGAAATCATTAGTACTATTATGAGCTATACCCC GCCGCCACTATTGCCGAGTATCCGGGGCGAGTCTGATG GCCCTCCTAACTCTGGCGGCCACATGCTGCATGCTGGCC

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SEQ ID NO:	Description	Sequence
		ACCGCGAGGAGAAAGTGCCTAACACCGTACGCCCTGAC GCCAGGAGCGGTGGTACCGTTGACACTGGGGCTGCTTTG CTGCGCACCGAGGGCGAATGCA
50	Envelope; MLV 10A1	ATGGAAGTCCAGCGTTCTCAAAACCCCTTAAAGATAAG ATTAACCCGTGGAAGTCCCTTAATGGTCATGGGGGTCTAT TTAAGAGTAGGGATGGCAGAGAGCCCCATCAGGTCCTT AATGTAACCTGGAGAGTCACCAACCTGATGACTGGGCGT ACCGCCAATGCCACCTCCCTTTTAGGAAGTGTACAAGAT GCCTTCCCAAGATTATATTTTGATCTATGTGATCTGGTCG GAGAAGAGTGGGACCCCTTCAGACCAGGAACCATATGTC GGGTATGGCTGCAATAACCCGGAGGGAGAAAGCGGAC CCGGACTTTTGACTTTTACGTGTGCCCTGGGCATACCGTA AAATCGGGGTGTGGGGGGCCAAGAGAGGGCTACTGTGG TGAATGGGGTTGTGAAACACCGGACAGGCTTACTGGAA GCCCACATCATCATGGGACCTAATCTCCCTTAAGCGCGG TAACACCCCTGGGACACGGGATGCTCCAAATGGCTTG TGGCCCTGTGTACGACCTCTCCAAAGTATCCAATTCCTT CAAGGGGCTACTCGAGGGGGCAGATGCAACCTCTAGTC CTAGAATTCAGTATGCAGGAAAAAGGCTAATTGGGA CGGGCCCAATCGTGGGACTGAGACTGTACCGGACAG GAACAGATCCTATTACCATGTCTCTCCGTGACCGCCAGG TCCTCAATATAGGGCCCCGCATCCCCATGGGCCTAATC CCGTGATCACTGGTCAACTACCCCCCTCCGACCCGTGC AGATCAGGCTCCCGAGGCTCCTCAGCCTCCTCCTACAG GCGCAGCCTCTATAGTCCCTGAGACTGCCCCACCTTCTC AACAACTGGGACGGGAGACAGGCTGTAAACCTGGTA GAAGGAGCCTATCAGGCGCTTAACCTCACCAATCCCGAC AAGACCAAGAATGTTGGCTGTGCTTAGTGTCCGGACCT CCTTATTACGAAGGAGTAGCGGTGCTGGGCACTTATACC AATCATTCTACGCCCGGCCAGCTGTACGGCACTTCC CAACATAAGCTTACCCCTATCTGAAGTACAGGACAGGGC CTATGCATGGGAGCACTACCTAAACTCACCAGGCCTTA TGTAACACCACCCAAAGTGCCGGCTCAGGATCCTACTAC CTTGCAGCACCCGCTGGAACAATGTGGGCTGTAGCACT GGATTGACTCCCTGCTTGTCCACCACGATGCTCAATCTA ACCACAGACTATTGTGTATTAGTTGAGCTCTGGCCAGA ATAATTTACCACTCCCCGATTATATGTATGGTCAGCTTG AACAGCGTACCAATATAAGAGGGAGCCAGTATCGTTG ACCCTGGCCCTTCTGCTAGGAGGATTAAACATGGGAGGG ATTGCAGCTGGAATAGGGACGGGGACCACTGCCCTAATC AAAACCCAGCAGTTTGAGCAGCTTCACGCCGTATCCAG ACAGACCTCAACGAAGTCGAAAAATCAATTACCAACCTA GAAAAGTCACTGACCTCGTTGTCTGAAGTAGTCTTACAG AACCGAAGAGGCTTAGATTTGCTCTTCTAAAGAGGGGA GGTCTCTGCGCAGCCCTAAAAGAAGAATGTTGTTTTAT GCAGACCACACGGGACTAGTGAGAGACAGCATGGCCAA ACTAAGGGAAAGGCTTAATCAGAGACAAAACTATTTG AGTCAGGCCAAGGTTGGTTCAAGGGCAGTTTAATAGAT CCCCTGGTTTACCACCTTAATCTCCACCATCATGGGACC TCTAATAGTACTCTTACTGATCTTACTCTTTGGACCCGTG ATTCTCAATCGATTGGTCCAATTTGTAAAGACAGGATC TCAGTGGTCCAGGCTCTGGTTTGAAGTCAACAATATCAC CAGCTAAACCTATAGAGTACGAGCCATGA
51	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGA TTCAAGAGGACATCATTTCTTTGGGTAATTATCCTTT TCCAAAGAACATTTTCCATCCCACTTGGAGTCATCCACA ATAGCACATTACAGGTTAGTGATGTCGACAACTGGTTT GCCGTGACAACTGTCTCCACAAATCAATTGAGATCAG TTGGACTGAATCTCGAAGGGAATGGAGTGGCAACTGAC GTGCCATCTGCAACTAAAAGATGGGGCTTCAGGTCGGGT GTCCACCAAAGGTGGTCAATTATGAAGCTGGTGAATGG GCTGAAACTGTCTACAATCTTGAATCAAAAACCTGAC GGGAGTGAGTGCTACCAAGCAGCGCCAGACGGGATTG GGGCTTCCCCGGTGCCGGTATGTGCACAAAGTATCAGG AACGGGACCGTGTGCCGAGACTTTGCCTTCCACAAAGA GGGTGCTTTCTTCTGTATGACCGACTTGCTTCCACAGTT ATCTACCGAGGAACGACTTTCGCTGAAGGTGTCGTTGCA TTTCTGATACTGCCCAAGCTAAGAAGGACTTCTTCAGC TCACACCCCTTGAGAGAGCCGGTCAATGCAACGGAGGA CCCGTCTAGTGGCTACTATTCTACCACAATTAGATATCA AGCTACCGGTTTGGAAACCAATGAGACAGAGTATTGTT CGAGGTTGACAATTGACCTACGTCCAACCTGAATCAAG ATTCACACCACAGTTTCTGCTCCAGCTGAATGAGACAAT ATATACAAGTGGGAAAAGGAGCAATACCACGGGAAAC

SEQ ID NO:	Description	Sequence
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52	Polymerase III shRNA promoters; U6 promoter	TTTCCCATGATTCCTTCATATTTGCATATACGATACAAGG CTGTTAGAGAGATAAATTGGAATTAATTTGACTGTAAACA CAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAA TAATTTCTGGGTAGTTTGCAAGTTTAAATTAATGTTT AAATGGACTATCATATGCTTACCGTAACCTGAAAGTATT TCGATTTCTTGGCTTTATATATCTTGTGAAAGGACGAA AC
53	Polymerase III shRNA promoters; 7SK promoter	CTGCAGTATTTAGCATGCCCCACCATCTGCAAGGCATT CTGGATAGTGTCAAACAGCCGGAATCAAGTCCGTTTA TCTCAAACCTTTAGCATTTTGGGAATAAATGATATTTGCTA TGCTGGTTAAATTAGATTTTGTAAATTTCTGCTGAAG CTCTAGTACGATAAGCAACTTGACCTAAGTGTAAGTTG AGATTTCTTCAGGTTTATATAGCTTGTGCGCCGCTGGC TACCTC
54	FDPS target sequence #1	GTCCTGGAGTACAATGCCATT
55	FDPS target sequence #2	GCAGGATTTTCGTTACGACTT
56	FDPS target sequence #3	GCCATGTACATGGCAGGAATT
57	FDPS target sequence #4	GCAGAAGGAGCTGAGAAAGT
58	Non-targeting sequence	GCCGCTTTGTAGGATAGAGCTCGAGCTCTATCTACAAA GCGGCTTTT
59	Forward primer	AGGAATTGATGGCGAGAAGG
60	Reverse primer	CCCAAAGAGGTCAAGGTAATCA
61	Forward primer	AGCGCGGTACAGCTTCA
62	Reverse primer	GGCGACGTAGCACAGCTTCT
63	Left Inverted Terminal Repeat (Left ITR)	CCTGCAGGCAGCTGCGGCTCGCTCGCTCACTGAGGCCG CCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCTCAGTG AGCGAGCGAGCGCGCAGAGAGGAGTGCCCAACTCCAT CACTAGGGGTTCTT

SEQ ID NO:	Description	Sequence
64	Right Inverted Terminal Repeat (Right ITR)	GAGCGGCCGCAGGAACCCCTAGTGATGGAGTTGGCCACT CCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGA CCAAAGGTCGCCCAGCGCCGGGCTTTGCCCGGGCGGCC TCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG
65	RRE/rabbit poly A beta globin	TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGC AGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGG TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGC AGAACAATTTGCTGAGGGCTATTGAGCGCAACAGCATC TGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGG CAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA CAGCTCCTAGATCTTTTCCCTCTGCCAAAATTATGGGG ACATCATGAAGCCCCCTTGAGCATCTGACTTCTGGCTAAT AAAGGAAATTTATTTTCATTGCAATAGTGTGTGGAAATTT TTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAA TCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTT GGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGT GGCTATAAAGAGGTCATCAGTATATGAACAGCCCCCTG CTGTCCATTCTTATTTCCATAGAAAAGCCTTGACTTGAGG TTAGATTTTTTTTATATTTTGTGTGTATTTTTTCTTT AACATCCCTAAAATTTTCCCTACATGTTTACTAGCCAGA TTTTTCTCTCTCTCTGACTACTCCAGTCATAGCTGTCC CTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCT TGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTG TTATCCGCTCACAATTCACACACATACGAGCCGGAAG CATAAAGTGTAAGCCCTGGGGTGCCTAATGAGTGAGCTA ACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAG TCGGGAAACCTGTGCGTGCCAGCGGATCCGCATCTCAATT AGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCC CGCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCA TGGCTGACTAATTTTTTTTATTTATGCAAGGCCGAGGCC GCCTCGGCCTCTGAGCTATTCAGAAGTAGTGAGGAGGC TTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTT TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCAT CACAAATTTCAAATAAAGCATTTTTTCACTGCATTCT AGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCACC CGGG

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such

embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 65

<210> SEQ ID NO 1

<211> LENGTH: 53

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: FDPS shRNA sequence #1

<400> SEQUENCE: 1

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53

<210> SEQ ID NO 2

<211> LENGTH: 53

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: FDPS shRNA sequence #2

<400> SEQUENCE: 2

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53

<210> SEQ ID NO 3

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<211> LENGTH: 53
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: FDPS shRNA sequence #3

 <400> SEQUENCE: 3

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 <210> SEQ ID NO 4
 <211> LENGTH: 53
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: FDPS shRNA sequence #4

 <400> SEQUENCE: 4

 gcagaaggag gctgagaaag tctcgagact ttctcagcct ccttctgctt ttt 53

 <210> SEQ ID NO 5
 <211> LENGTH: 116
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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 <400> SEQUENCE: 5

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 agatggcaga aggaggctga gaaagtgtg cctactgcct cggacttcaa ggggct 116

 <210> SEQ ID NO 6
 <211> LENGTH: 114
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miR30 FDPS sequence #2

 <400> SEQUENCE: 6

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 agatggcaga agggtctgaga aagtgtgcc tactgcctcg gacttcaagg ggct 114

 <210> SEQ ID NO 7
 <211> LENGTH: 91
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miR30 FDPS sequence #3

 <400> SEQUENCE: 7

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 ggaggctgag aaagttgcct actgcctcgg a 91

 <210> SEQ ID NO 8
 <211> LENGTH: 115
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miR155 FDPS sequence #1

 <400> SEQUENCE: 8

 cctggaggct tgctgaaggc tgtatgtga ctttctcagc ctctttctgc ttttggccac 60
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<210> SEQ ID NO 9
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 FDPS sequence #1

<400> SEQUENCE: 9

catctccatg gctgtaccac cttgtcggga ctttctcagc ctccttctgc ctgttgaatc      60
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<210> SEQ ID NO 10
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR185 FDPS sequence #1

<400> SEQUENCE: 10

gggcctggct cgagcagggg gcgagggata ctttctcagc ctccttctgc tggccccctc      60
cccgcagaag gaggctgaga aagtccttcc ctcccaatga ccgcgtcttc gtcg          114

<210> SEQ ID NO 11
<211> LENGTH: 228
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rous Sarcoma virus (RSV) promoter

<400> SEQUENCE: 11

gtagtcttat gcaatactct ttagtcttg caacatggta acgatgagtt agcaacatgc      60
cttacaagga gagaaaaagc accgtgcatg ccgattggtg gaagtaaggt ggtacgatcg      120
tgccttatta ggaaggcaac agacgggtct gacatggatt ggacgaacca ctgaattgcc      180
gcattgcaga gatattgtat ttaagtgcct agctcgatac aataaacg          228

<210> SEQ ID NO 12
<211> LENGTH: 180
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5' Long terminal repeat (LTR)

<400> SEQUENCE: 12

ggctctctct gtttagaccag atctgagcct gggagctctc tggctaacta gggaaccac      60
tgcttaagcc tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt      120
gtgactctgg taactagaga tcctcagac ccttttagtc agtgtggaaa atctctagca      180

<210> SEQ ID NO 13
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Psi Packaging signal

<400> SEQUENCE: 13

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<210> SEQ ID NO 14
<211> LENGTH: 233
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Rev response element (RRE)

<400> SEQUENCE: 14

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gttgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca    180
gtccaggcca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcc          233
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<210> SEQ ID NO 15

<211> LENGTH: 118

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Central polypurine tract (cPPT)

<400> SEQUENCE: 15

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agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaattca aaatttta    118
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<210> SEQ ID NO 16

<211> LENGTH: 217

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Polymerase III shRNA promoters; H1 promoter

<400> SEQUENCE: 16

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gaacgctgac gtcacaaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa    60
caccagcgcg gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc    120
tgcaatatatt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg    180
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<210> SEQ ID NO 17

<211> LENGTH: 590

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Long WPRE sequence

<400> SEQUENCE: 17

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tggtcttcat tttctctcc ttgtataaat cctggttgct gtctctttat gaggagtgtg    180
ggcccggtgt caggcaacgt ggcgtggtgt gcaactgtgt tgctgacgca acccccactg    240
gttggggcat tgccaccacc tgctagctcc ttccgggac ttctgcttcc cccctcccta    300
ttgccacggc ggaactcacc gccgcctgcc ttgcccgctg ctggacaggg gctcggtgtg    360
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atccagcgga ccttccttcc cgcggcctgc tgccggtctc gcggcctctt ccgctcttc    540
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<210> SEQ ID NO 18

<211> LENGTH: 250

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: 3' delta LTR

<400> SEQUENCE: 18

tggaagggtc	aattcactcc	caacgaagat	aagatctgct	ttttgcttgt	actgggtctc	60
tctggttaga	ccagatctga	gcctgggagc	tctctggcta	actagggaac	ccactgctta	120
agcctcaata	aagcttgect	tgagtgettc	aagtagtggt	tgcccgctcg	ttgtgtgact	180
ctggtaacta	gagatccctc	agaccctttt	agtcagtgtg	gaaaatctct	agcagtagta	240
gttcattgtc						250

<210> SEQ ID NO 19

<211> LENGTH: 290

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper/Rev; Chicken beta actin (CAG) promoter;
 Transcription

<400> SEQUENCE: 19

gctattacca	tgggtcgagg	tgagccccc	gttctgcttc	actctcccca	tctccccccc	60
ctcccccccc	ccaattttgt	attttttat	tttttaatta	ttttgtgcag	cgatgggggc	120
gggggggggg	ggggcgcgcg	ccaggcgggg	cgggcggggg	cgagggggcg	ggcggggcga	180
ggcgggagag	tgcgggcgga	gccaatcaga	gcggcgcgct	ccgaaagtgt	cctttttatg	240
cgagggcgcg	gcggcgggcg	ccctataaaa	agcgaagcgc	gcggcgggcg		290

<210> SEQ ID NO 20

<211> LENGTH: 1503

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper/Rev; HIV Gag; Viral capsid

<400> SEQUENCE: 20

atgggtgcga	gagcgtcagt	attaagcggg	ggagaattag	atcgatggga	aaaaattcgg	60
ttaaggccag	ggggaaagaa	aaaatataaa	ttaaaacata	tagtatgggc	aagcaggagag	120
ctagaacgat	tcgcagttaa	tcttgccctg	ttagaaacat	cagaaggctg	tagacaaata	180
ctgggacagc	tacaaccatc	ccttcagaca	ggatcagaag	aacttagatc	attatataat	240
acagtagcaa	ccctctattg	tgtgcatcaa	aggatagaga	taaaagacac	caagggaagct	300
ttagacaaga	tagaggaaga	gcaaaacaaa	agtaagaaaa	aagcacagca	agcagcagct	360
gacacaggac	acagcaatca	ggtcagccaa	aattacccta	tagtgcagaa	catccagggg	420
caaatggtac	atcaggccat	atcacctaga	actttaaatg	catgggtaaa	agtagtagaa	480
gagaaggctt	tcagcccaga	agtgataccc	atgtttttcag	cattatcaga	aggagccacc	540
ccacaagatt	taaacaccat	gctaaacaca	gtgggggggac	atcaagcagc	catgcaaatg	600
ttaaaagaga	ccatcaatga	ggaagctgca	gaatgggata	gagtgcattc	agtgcatgca	660
gggcctattg	caccaggcca	gatgagagaa	ccaaggggaa	gtgacatagc	aggaactact	720
agtacccttc	aggaacaaat	aggatggatg	acacataatc	cacctatccc	agtaggagaa	780
atctataaaa	gatggataat	cctgggatta	aataaaatag	taagaatgta	tagccctacc	840
agcattctgg	acataagaca	aggaccaaag	gaacccttta	gagactatgt	agaccgattc	900
tataaaactc	taagagccga	gcaagcttca	caagaggtaa	aaaattggat	gacagaaacc	960
ttgttggtcc	aaaatgcgaa	cccagattgt	aagactattt	taaaagcatt	gggaccagga	1020

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gcgacactag aagaaatgat gacagcatgt cagggagtgg ggggacccgg ccataaagca	1080
agagtttttg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa	1140
ggcaatttta ggaaccaaag aaagactggt aagtgtttca attgtggcaa agaagggcac	1200
atagccaaaa attgcagggc ccctaggaaa aagggtctgt ggaaatgtgg aaaggaagga	1260
caccaaataa aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc	1320
cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa	1380
gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac	1440
aaggaaactgt atccttttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa	1500
taa	1503

<210> SEQ ID NO 21
 <211> LENGTH: 1872
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helper/Rev; HIV Pol; Protease and reverse transcriptase
 <400> SEQUENCE: 21

atgaatttgc caggaagatg gaaacccaaa atgatagggg gaattggagg ttttatcaaa	60
gtaggacagt atgatcagat actcatagaa atctgctggc ataaagctat aggtacagta	120
ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc	180
acttttaaatt 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
ttcagagaa ctaataagag aactcaagat ttctgggaag ttcaattagg aataccacat	480
cctgcagggt taaaacagaa aaaatcagta acagtactgg atgtggcgga tgcatatttt	540
tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac	600
aatgagacac cagggttagg atatcagtac aatgtgcttc cacagggatg gaaaggatca	660
ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca	720
gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg	780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgagggtggg atttaccaca	840
ccagacaaaa aacatcagaa agaacctcca ttccttttga tgggttatga actccatcct	900
gataaatgga cagtacagcc tatagtgtct ccagaaaagg acagctggac tgtcaatgac	960
atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta	1020
aggcaattat gtaaaacttct taggggaacc aaagcactaa cagaagtagt accactaaca	1080
gaagaagcag agctagaact ggcagaaaa caggagattc taaaagaacc ggtacatgga	1140
gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa	1200
tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga	1260
atgaaggggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc	1320
acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa	1380
acatgggaag catgggtggc agagtattgg caagccacct ggattcctga gtgggagttt	1440
gtcaataccc ctcccttagt gaagttagt taccagttag agaaagaacc cataatagga	1500

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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 gggtggtcag tgctggaatc 1860
aggaaagtac ta 1872
```

```
<210> SEQ ID NO 22
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper Rev; HIV Integrase; Integration of viral
RNA
```

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<400> SEQUENCE: 22
```

```
tttttagatg gaatatgataa ggcccaagaa gaacatgaga aatatcacag taattggaga 60
gcaatggcta gtgattttta cctaccacct gtagtagcaa aagaaatagt agccagctgt 120
gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag cccaggaata 180
tggcagctag attgtacaca tttagaagga aaagtatatc tggtagcagt tcatgtagcc 240
agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc 300
ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat 360
ttcaccagta ctacagttaa ggcgcctgt tggtagggcg ggatcaagca ggaatttggc 420
attccctaca atcccaaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa 480
attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta 540
ttcatccaca attttaaaag aaaagggggg attggggggg acagtgcagg ggaagaata 600
gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt 660
caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag 720
ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgcacat aaaagtagtg 780
ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt 840
gtggcaagta gacaggatga ggattaa 867
```

```
<210> SEQ ID NO 23
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev; HIV RRE; Binds Rev element
```

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<400> SEQUENCE: 23
```

```
aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat 60
gacgtgacg gtacaggcca gacaattatt gtctgggata gtgcagcagc agaacaattt 120
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca 180
gctccaggca agaactctgg ctgtggaaaag atacctaaag gatcaacagc tcct 234
```

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<210> SEQ ID NO 24
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:

<223> OTHER INFORMATION: Helper/Rev; HIV Rev; Nuclear export and stabilize viral mRNA

<400> SEQUENCE: 24

```

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

```

<210> SEQ ID NO 25

<211> LENGTH: 577

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; CMV promoter; Transcription

<400> SEQUENCE: 25

```

acattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcataagccc    60
atatatggag ttccgcgtta cataacttac ggtaaatggc ccgcctgggt gaccgcccaa    120
cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac    180
tttcattga cgtcaatggg tggagtattt acggtaaaact gcccaattgg cagtacatca    240
agtgtatcat atgccaagta cgcacctat tgacgtcaat gacggtaaat ggcccgctg    300
gcattatgcc cagtacatga ctttatggga ctttcctact tggcagtaca tctacgtatt    360
agtcacgct attaccatgg tgatgcggtt ttggcagtac atcaatgggc gtggatagcg    420
gtttgactca cggggatttc caagtctcca cccattgac gtcaatggga gtttgttttg    480
gcacaaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat tgacgcaaat    540
gggcggtagg cgtgtacggt gggaggtcta tataagc                                577

```

<210> SEQ ID NO 26

<211> LENGTH: 1519

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; VSV-G; Glycoprotein envelope-cell entry

<400> SEQUENCE: 26

```

atgaagtgcc ttttgtactt agccttttta ttcattgggg tgaattgcaa gtccaccata    60
gtttttccac acaacaaaaa aggaaactgg aaaaatgttc cttctaatta ccattattgc    120
ccgtcaagct cagatttaaa ttggcataat gacttaatag gcacagcctt acaagtcaaa    180
atgcccaaga gtcacaaggc tattcaagca gacggttgga tgtgtcatgc ttccaaatgg    240
gtcactactt gtgatttcog ctgggtatga ccgaagtata taacacattc catccgatcc    300
ttcactccat ctgtagaaca atgcaaggaa agcattgaac aaacgaaaca aggaacttgg    360
ctgaatccag gcttcctccc tcaaagtgtg ggatatgcaa ctgtgacgga tgccgaagca    420
gtgattgtcc aggtgactcc tcaccatgtg ctgggtgatg aatacacagg agaatggggt    480
gattcacagt tcataacgg aaaatgcagc aattacatat gcccactgt ccataactct    540
acaacctggc attctgacta taaggtaaaa gggctatgtg attctaacct catttccatg    600

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gacatcacct tcttctcaga ggacggagag ctatcatccc tgggaaagga gggcacaggg	660
ttcagaagta actactttgc ttatgaaact ggaggcaagg cctgcaaaat gcaatactgc	720
aagcattggg gagtcagact cccatcaggt gtctggttcg agatggctga taaggatctc	780
tttgctgcag ccagattccc tgaatgccc gaagggtaa gtatctctgc tccatctcag	840
acctcagtg atgtaagtct aattcaggac gttgagagga tcttgatta tccctctgc	900
caagaaacct ggagcaaaat cagagcgggt ctccaatct ctccagtga tctcagctat	960
cttgctccta aaaaccagg 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 gatcttcac ttagctcaaa ggctcaggtg	1260
ttcgaaacac ctcacattca agacgtgctc tcgcaacttc ctgatgatga gagtttattt	1320
tttggtgata ctgggctatc caaaaatcca atcgagcttg tagaaggttg gttcagtagt	1380
tggaaaagct ctattgcctc tttttcttt atcataggtt taatcattgg actattcttg	1440
gttctccgag ttggtatcca tctttgcatt aaattaaagc acaccaagaa aagacagatt	1500
tatacagaca tagagatga	1519

<210> SEQ ID NO 27

<211> LENGTH: 352

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription

<400> SEQUENCE: 27

tagttattaa tagtaatcaa ttacgggggc attagtctat agcccatata tggagttccg	60
cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc ccgcccatt	120
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca	180
atgggtggac tatttacggt aaactgccc cttggcagta catcaagtgt atcatatgcc	240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta	300
catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tc	352

<210> SEQ ID NO 28

<211> LENGTH: 960

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Helper/Rev; Chicken beta actin intron; Enhance gene expression

<400> SEQUENCE: 28

ggagtcgctg cgttgcttc gcccggtgcc ccgctccgcg ccgcctcgcg ccgcccgcc	60
cggtctgac tgaccgctt actccacag gtgagcgggc gggacggccc ttctcctccg	120
ggctgtaatt agcgttgggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc	180
cttaaagggc tccgggaggg cctttgtgc gggggggagc ggctcggggg gtgctgctgt	240
gtgtgtgtgc gtggggagcg ccgcgtgcgg ccgcgtgc cggcggctg tgagcgtgc	300
gggcgcggcg cggggctttg tgcgtccgc gtgtgcgcga ggggagcgcg gccggggcg	360
gtgccccgcg gtgcgggggg gctgcgaggg gaacaaagcg tgcgtgcggg gtgtgtgcgt	420

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gggggggtga gcaggggggtg tgggcgcggc ggtcgggctg taaccccccc ctgcaccccc 480
ctccccgagt tgctgagcac ggccccggtt cgggtgcggg gctccgtgcg gggcgtggcg 540
cggggctcgc cgtgccgggc ggggggtggc ggcaggtggg ggtgccgggc ggggcggggc 600
cgcctcgggc cggggagggc tggggggagg ggcgcggcgg ccccgagcg cggcggctg 660
tcgaggcgcg gcgagccgca gccattgctt tttatggtaa tcgtgcgaga gggcgcaggg 720
acttcctttg tcccaaattc ggccgagccg aaatctggga ggcgcgcggc cccccctct 780
agcgggcgcg ggcgaagcgg tcgggcgcgg gcaggaagga aatgggcggg gagggccttc 840
gtgcgtcgcc gcgcgcgcgt ccccttctcc atctccagcc tcggggctgc cgcaggggga 900
cggctgcctt cggggggggac ggggcagggc ggggttcggc ttctggcgtg tgaccggcgg 960

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<210> SEQ ID NO 29
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev; Rabbit beta globin poly A; RNA
      stability

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<400> SEQUENCE: 29

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agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120
ctcactcgga aggacatagtg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180
ttagagtttg gcaacatagtg ccatatgctg gctgccatga acaaaggtgg ctataaagag 240
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga 300
cttgaggtta gatttttttt atattttggt ttgtgttatt tttttcttta acatccctaa 360
aattttcctt acatgtttta ctagccagat ttttcctcct ctctgacta ctcccagtca 420
tagctgtccc tcttctctta tgaagatc 448

```

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<210> SEQ ID NO 30
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope; Beta globin intron; Enhance gene
      expression

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<400> SEQUENCE: 30

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```

gtgagtttgg ggacccttga ttgtctttc tttttcgcta ttgtaaaatt catgttatat 60
ggagggggca aagttttcag ggtgttggtt agaatgggaa gatgtccctt gtatcaccat 120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180
cttattttct tttcattttc tgtaactttt tcgttaaaact ttagcttgca tttgtaacga 240
atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt 300
tttcaaggca atcagggtat attatattgt acttcagcac agtttttagag aacaattggt 360
ataattaaat gataaggtag aatattttcg catataaatt ctggctggcg tggaaatatt 420
cttattggta gaaacaacta caccctggtc atcactctgc ctttctcttt atgggttaca 480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540
aaccatgttc atgccttctt ctcttttcta cag 573

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<210> SEQ ID NO 31

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<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope; Rabbit beta globin poly A; RNA
      stability

<400> SEQUENCE: 31
agatcttttt ccctctgcc aaaaattatgg ggacatcatg aagccccttg agcatctgac      60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttgggaatt ttttgtgtct      120
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt      180
ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaagggt ggctataaag      240
agggtcatcag tatatgaac agcccctgc tgtccattcc ttattccata gaaaagcctt      300
gacttgaggt tagatttttt ttatatattg ttttgtgtta tttttttcct taacatccct      360
aaaattttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt      420
catagctgtc cctcttctct tatggagatc      450

<210> SEQ ID NO 32
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32
taagcagaat tcataaattt gccaggaaga t      31

<210> SEQ ID NO 33
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33
ccatacaatg aatggacact aggcggccgc acgaat      36

<210> SEQ ID NO 34
<211> LENGTH: 2745
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag, Pol, Integrase fragment

<400> SEQUENCE: 34
gaattcatga atttgccagg aagatggaaa ccaaaaatga tagggggaat tggaggtttt      60
atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa agctataggt      120
acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt gactcagatt      180
ggctgcactt taaattttcc cattagtctt attgagactg taccagtaaa attaaagcca      240
ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat aaaagcatta      300
gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg gcctgaaaat      360
ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg gagaaaaatta      420
gtagatttca gagaacttaa taagagaact caagatttct gggaagtcca attaggaata      480
ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt gggcgatgca      540
tatttttcag ttcccttaga taaagacttc aggaagtata ctgcatttac catacctagt      600

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ataaacaatg agacaccagg gattagatat cagtacaatg tgcttcacac gggatggaaa	660
ggatcaccag caatatcca gtgtagcatg acaaaaatct tagagccttt tagaaaacaa	720
aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggac tgacttagaa	780
atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag gtggggattt	840
accacaccag acaaaaaaca tcagaagaa 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 attatgacc atcaaaagac ttaatagcag aaatacagaa gcaggggcaa	1200
ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac aggaaagtat	1260
gcaagaatga aggggtgccc cactaatgat gtgaacaat taacagaggc agtacaaaaa	1320
atagccacag aaagcatagt aatatgggga aagactccta aatttaaatt acccatacaa	1380
aaggaaacat ggggaagcatg gtggacagag tattggcaag ccacctggat tcctgagtgg	1440
gagtttgtca ataccctcc cttagtgaag ttatggtacc agttagagaa agaaccata	1500
ataggagcag aaactttcta tgtagatggg gcagccaata gggaaactaa attaggaaaa	1560
gcaggatatg taactgcag aggaagacaa aaagtgtgcc ccctaacgga cacaacaaat	1620
cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt agaagtaaac	1680
atagtgcag actcacaata tgcattggga atcattcaag cacaaccaga taagagtga	1740
tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt ctacctggca	1800
tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt ggtcagtgtc	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
caagaacag catacttctc cttaaaatta gcaggaagat ggccagtaaa aacagtacat	2220
acagacaatg gcagcaatct caccagtact acagttaagg ccgcctgttg gtgggcgggg	2280
atcaagcagg aatttggcat tccttacaat ccccaaagtc aaggagtaat agaacttatg	2340
aataaagaat taaagaaaat tataggacag gtaagagatc aggtcgaaca tcttaagaca	2400
gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat tgggggggtac	2460
agtgcagggg aaagaatagt agacataata gcaacagaca tacaactaa agaattacaa	2520
aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag agatccagtt	2580
tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat acaagataat	2640
agtgacataa aagtagtgcc aagaagaaaa gcaagatca tcagggatta tggaaaacag	2700
atggcagggtg atgatttgtg ggcaagtaga caggatgagg attaa	2745

<210> SEQ ID NO 35

<211> LENGTH: 1586

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA Fragment containing Rev, RRE and rabbit

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beta globin poly A

<400> SEQUENCE: 35

tctagaatgg caggaagaag cggagacagc gacgaagagc tcatcagaac agtcagactc	60
atcaagcttc tctatcaaag caaccacact cccaatcccg aggggacccg acaggcccga	120
aggaatagaa gaagaagggtg gagagagaga cagagacaga tccattcgat tagtgaacgg	180
atccttggca cttatctggg acgatctgcg gagcctgtgc ctcttcagct accaccgctt	240
gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca gggggtgagg	300
agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa agaatagagg	360
agctttgttc cttgggttct tgggagcagc aggaagcact atgggagcag cgtcaatgac	420
gctgacggta caggccagac aattattgtc tggatatgtg cagcagcaga acaatttgct	480
gagggtctatt gaggcgcaac agcatctgtt gcaactcaca gtctggggca tcaagcagct	540
ccaggcaaga atcctggctg tggaaagata cctaaaggat caacagctcc tagatctttt	600
tccctctgcc aaaaattatg gggacatcat gaagcccctt gagcatctga cttctggcta	660
ataaagggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgc tctcactcgg	720
aaggacatat gggaggggcaa atcatttaaa acatcagaat gagtatttgg tttagagttt	780
ggcaacatat gccatatgct ggctgccatg aacaaagggtg gctataaaga ggatcatcagt	840
atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg acttgagggt	900
agattttttt tatattttgt tttgtgttat tttttctttt aacatcccta aaattttcct	960
tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc	1020
ctcttctctt atgaagatcc ctgcacctgc agcccaagct tggcgtaatc atggatcatag	1080
ctgtttctctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc	1140
ataaagtgtg aagcctgggg tgcctaataa gtgagctaac tcacattaat tgcgttgccg	1200
tcactgcccg ctttccagtc gggaaacctg tctgtccagc ggatccgcat ctcaattagt	1260
cagcaacctt agtcccggcc ctaactccgc ccatcccgcc cctaactccg cccagttccg	1320
cccattctcc gccccatggc tgactaattt tttttattta tgcagaggcc gaggcgcct	1380
cggcctctga gctattccag aagtagtgag gaggtttttt tggaggccta ggcttttgca	1440
aaaagctaac ttgtttattg cagcttataa tgggtacaaa taaagcaata gcatcacaaa	1500
tttcacaaat aaagcatttt tttcactgca ttctagttgt gggtttgtcca aactcatcaa	1560
tgtatcttat cagcggccgc cccggg	1586

<210> SEQ ID NO 36

<211> LENGTH: 1614

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA fragment containing the CAG
enhancer/promoter/intron sequence

<400> SEQUENCE: 36

acgcgttagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga	60
gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgcca acgacccccg	120
cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg	180
acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca	240
tatgccaaagt acgcccccta ttgacgtcaa tgacggtaaa tggccgcctt ggcattatgc	300

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ccagttacatg accttatggg acttttcctac ttggcagtag atctacgtat tagtcatcgc	360
tattaccatg ggtcgagggtg agccccacgt tctgcttcac tctcccatc tccccccct	420
ccccacccc aattttgtat ttatttattt tttaattatt ttgtgcagcg atggggcg	480
gggggggggg ggcgcgcgcgc aggcggggcg gggcggggcg agggcgggcg cgggcgagg	540
cggagagggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtctcc ttttatggcg	600
agggcgcggc ggcggcggcgc ctataaaaag cgaagcgcgc ggcggcgagg agtcgctgcg	660
ttgccttcgc cccgtgcccc gctccgcgcgc gcctcgcgcgc gcccgccccg gctctgactg	720
accgcgttac tcccacaggt gagcggggcg gacggccctt ctctccggg ctgtaattag	780
cgtctggttt aatgaaggct cgtttctttt ctgtggctgc gtgaaagcct taaagggtc	840
cgggaggggc ctttgtgcgg gggggagcgg ctcggggggt gcgtgcgtgt gtgtgtgcgt	900
ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgtgcgg gcgcggcgcg	960
gggctttgtg cgtcccgctg gtgcgcgagg gagcgcggc cggggcgagg gcccccgagg	1020
gcgggggggg tgcgagggga acaaaaggctg cgtgcgggggt gtgtgcgtgg gggggtgagc	1080
agggggtgtg ggcgcggcgcg tcgggctgta accccccct gcacccccct ccccgagttg	1140
ctgagcacgg cccggttcg ggtgcggggc tccgtgcggg gcgtggcgcg gggctcgccg	1200
tgcggggcg ggggtgcccg caggtggggg tgcggggcg ggcggggcg cctcgggcg	1260
gggagggctc gggggagggg cgcggcgccc ccggagcgcc ggcggctgtc gaggcgcggc	1320
gagcgcgagc cattgccttt tatggtaatc gtgcgagagg gcgcaggagc ttcctttgtc	1380
ccaaatctgg cggagccgaa atctgggagg cgcgcgcga cccctctag cgggcgcggg	1440
cgaagcgggtg cggcgcgggc aggaaggaaa tgggcgggga gggccttcgt gcgtcgcgcg	1500
gccgcgctcc ccttctccat ctccagctc ggggctgcgg cagggggagc gctgccttcg	1560
ggggggacgg ggcaggcgcg ggttcggctt ctggcgtgtg accggcgga attc	1614

<210> SEQ ID NO 37

<211> LENGTH: 1531

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: DNA fragment containing VSV-G

<400> SEQUENCE: 37

gaattcatga agtgcccttt gtacttagcc tttttattca ttggggtgaa ttgcaagttc	60
accatagttt ttccacacaa ccaaaaagga aactggaaaa atgttccttc taattacat	120
tattgcccggt caagctcaga tttaaattgg cataatgact taataggcac agccttacia	180
gtcaaaatgc ccaagagtca caaggctatt caagcagacg gttggatgtg tcatgcttc	240
aaatgggtca ctacttgtga tttccgctgg tatggaccga agtatataac acattccatc	300
cgatccttca ctccatctgt agaacaatgc aaggaaagca ttgaacaaac gaaacaagga	360
acttggtgta atccaggctt cctcctcaa agttgtggat atgcaactgt gacggatgcc	420
gaagcagtga ttgtccagggt gactcctcac catgtgctgg ttgatgaata cacaggagaa	480
tgggttgatt cacagttcat caacggaaaa tgcagcaatt acatatgcc cactgtccat	540
aactctacaa cctggcattc tgactataag gtcaaaaggc tatgtgatc taacctcatt	600
tccatggaca tcaccttctt ctccaggagc ggagagctat catccctggg aaaggagggc	660
acagggttca gaagtaacta ctttgcctat gaaactggag gcaaggcctg caaaatgcaa	720
tactgcaagc attggggagt cagactccca tcagggtgtc ggttcgagat ggctgataag	780

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gatctctttg ctgcagccag attcctgaa tgcccagaag ggtcaagtat ctctgctcca	840
tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt ggattattcc	900
ctctgccaaag aaacctggag caaaatcaga gcgggtcttc caatctctcc agtggatctc	960
agctatcttg ctctaaaaa cccaggaacc ggtcttgctt tcaccataat caatgggtacc	1020
ctaaaatact ttgagaccag atacatcaga gtcgatattg ctgctccaat cctctcaaga	1080
atggtcggaa tgatcagtg aactaccaca gaaagggaaac tgtgggatga ctgggcacca	1140
tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg atataagttt	1200
cctttataca tgattggaca tggtagttg gactccgac ttcattcttag ctcaaaggct	1260
caggtgtctg aacatctca cattcaagac gctgcttcgc aacttctga tgatgagagt	1320
ttattttttg gtgatactgg gctatccaaa aatccaatcg agctttaga aggttggttc	1380
agtagttgga aaagctctat tgcctctttt ttctttatca tagggttaat cattggacta	1440
ttcttggttc tccgagtgg tatccatctt tgcattaaat taaagcacac caagaaaaga	1500
cagatttata cagacataga gatgagaatt c	1531

<210> SEQ ID NO 38
 <211> LENGTH: 884
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: RSV promoter and HIV Rev

<400> SEQUENCE: 38

caattgcgat gtacgggccg gatatacgcg tatctgaggg gactagggtg tgtttaggcg	60
aaaagcgggg cttcggttgt acgcggttag gagtcccctc aggatatagt agtttcgctt	120
ttgcataggg agggggaaat gtagtcttat gcaatacact tgtagtcttg caacatggta	180
acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg ccgattgggtg	240
gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct gacatggatt	300
ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct agctcgatac	360
aataaaacgcc atttgacctc tcaccacatt ggtgtgcacc tccaagctcg agctcgttta	420
gtgaaccgtc agatcgccctg gagacgccat ccacgtgtt ttgacctcca tagaagacac	480
cgggaccgat ccagcctccc ctccaagcta gcgattagcg atctcctatg gcaggaagaa	540
gcggagacag cgacgaagaa ctctcaagg cagtcagact catcaagttt ctctatcaaa	600
gcaaccaccc tccaatccc gaggggaccc gacaggcccg aaggaataga agaagaaggt	660
ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg	720
gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt	780
gtaacgagga ttgtggaact tctgggacgc aggggggtggg aagccctcaa atattggtgg	840
aatctcctac aatattggag tcaggagcta aagaatagtc taga	884

<210> SEQ ID NO 39
 <211> LENGTH: 1104
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

<400> SEQUENCE: 39

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc	60
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gcctttttcc cgaggggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc	120
tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc	180
ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg	240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct	300
tgcgcttaag gagcccttc gccctgtgct tgagttgagg cctggcctgg gcgctggggc	360
cgccgcgtgc gaatctgggt gcaccttcgc gccctgtctg ctgctttcga taagtctcta	420
gccatttaaa atttttgatg acctgtctcg acgctttttt tctggcaaga tagtcttgta	480
aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg	540
gcccgtgcgt cccagcgca atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga	600
atcggacggg ggtagtctca agctggccgg cctgtctctg tgccctggcct cgcgcgcgg	660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagtgc gtgagcggaa	720
agatggccgc tcccggccc tgtgcaggg agctcaaaat ggaggacgcg gcgctcggga	780
gagcgggcgg gtgagtcacc cacacaaagg aaaaggcct ttcgctcctc agccgtcgt	840
tcatgtgact ccacggagta ccgggcgcgg tccaggcacc tcgattagtt ctcgagcttt	900
tggagtacgt cgtctttagg ttggggggag gggttttatg cgatggagtt tccccacact	960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt	1020
gccctttttg agtttgatc ttggttcatt ctcaagcctc agacagtggg tcaaagtttt	1080
ttttctccat ttcaggtgtc gtga	1104

<210> SEQ ID NO 40
 <211> LENGTH: 511
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Promoter; PGK

<400> SEQUENCE: 40

gggggttggg ttgcgccttt tccaaggcag cccctgggtt ggcgaggac gcggtgctc	60
tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgacat tcttcacgtc	120
cgttcgcagc gtcacccgga tcttcgccgc tacccttggt ggccccccgg cgacgcttcc	180
tgtccgccc ctaagtggg aaggttcctt gcggttcgcy gcgtgccgga cgtgacaaac	240
ggaagccgca cgtctcacta gtaccctcgc agacggacag cgccaggag caatggcagc	300
gcgcgcagcg cgatgggctg tgcccaatag cggctgctca gcagggcgcy ccgagagcag	360
cggccgggaa ggggcgggtg gggaggcggg gtgtggggcg gtatgtggg cctgttcct	420
gcccgcgcgg tgttccgcat tctgaagcc tccggagcgc acgtcgagc tcggctccct	480
cgttgaccga atcaccgacc tctctcccca g	511

<210> SEQ ID NO 41
 <211> LENGTH: 1162
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Promoter; UbC

<400> SEQUENCE: 41

gcgcggggtt ttggcgctc ccgcgggcgc cccctcctc acggcgagcg ctgccacgtc	60
agacgaaggc gcgaggagcg ttcctgatcc ttccgcccg acgtcagga cagcggcccg	120
ctgctcataa gactcggcct tagaacccca gtatcagcag aaggacattt taggacggga	180

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cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg aggaaaagta 240
gtcccttctc ggcgattctg cggagggatc tccgtggggc ggtgaacgcc gatgattata 300
taaggacgcg ccgggtgtgg cacagctagt tccgtcgagc ccgggatttg ggtcgcggtt 360
cttgttttgt gatcgctgtg atcgtcactt ggtgagttgc gggctgctgg gctggccggg 420
gctttcgtgg ccgccgggcc gctcgggtgg acggaagcgt gtggagagac cgccaagggc 480
tgtagtctgg gtccgcgagc aagggtgccc tgaactgggg gttgggggga gcgcacaaaa 540
tggcggtgtg tcccgagtct tgaatggaag acgcttgtaa ggcgggctgt gaggtcgttg 600
aaacaagggtg gggggcatgg tgggcggcaa gaaccaagg tcttgaggcc ttcgctaata 660
cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc ctgacgtgaa 720
gtttgtcact gactggagaa ctccgggttg tcgtctggtt gcggggcgcg cagttatgcg 780
gtgccgttgg gcagtgcacc cgtacctttg ggagcgcgcg cctcgctctg tcgtgacgtc 840
acccgttctg ttgcttata atgcaggggtg gggccacctg ccggtagggtg tgcggtaggc 900
ttttctccgt cgcaggacgc aggggttcggg 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

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<210> SEQ ID NO 42
<211> LENGTH: 120
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A; SV40

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<400> SEQUENCE: 42

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gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa 60
agcatttttt tcaactgcatt ctagtgtggg tttgtccaaa ctcacaaatg tatcttatca 120

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<210> SEQ ID NO 43
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A; bGH

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<400> SEQUENCE: 43

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gactgtgcct tctagttgcc agccatctgt tgtttgcccc tccccgtgc cttecttgac 60
cctggaaggt gccactccca ctgtcctttc ctaataaaat gaggaaattg catcgcatg 120
tctgagtagg tgtcattcta ttctgggggg tgggggtggg caggacagca agggggagga 180
tggggaagac aatagcaggc atgctgggga tgcggtgggc tctatgg 227

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<210> SEQ ID NO 44
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope; RD114

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<400> SEQUENCE: 44

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atgaaactcc caacaggaat ggtcatttta tgtagcctaa taatagtctg ggcagggttt 60

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gacgaccccc gcaaggctat cgcattagta caaaaacaac atggtaaacc atgcgaatgc	120
agcggaggggc aggtatccga ggccccaccg aactccatcc aacaggtaac ttgccaggc	180
aagacggcct acttaatgac caacaaaaa tggaaatgca gagtcaactcc aaaaaatctc	240
accctagcgg ggggagaact ccagaactgc cctgtgaaca cttccagga ctcgatgcac	300
agttcttggt atactgaata ccggcaatgc agggcgaata ataagacata ctacacggcc	360
accttgctta aaatacggtc tgggagcctc aacgaggta agatattaca aaaccccaat	420
cagctcctac agtccccttg taggggctct ataaatcagc ccgtttgctg gagtgccaca	480
gccccatcc atactccga tggtaggagga cccctcgata ctaagagagt gtggacagtc	540
caaaaaaggc tagaacaat tcataaggct atgcacctg aacttaata ccacccctta	600
gccctgccc aagtcagaga tgaccttagc ctgatgcac ggacttttga taccctgaat	660
accactttta gggtactcca gatgtccaat tttagccttg cccaagattg ttggctctgt	720
ttaaaactag gtaccttac cctcttgcg ataccactc cctctttaac ctactcccta	780
gcagactccc tagcgaatgc ctctgtcag attatactc cctcttggg tcaaccgatg	840
cagttctcca actcgtcctg tttatcttc cctttcatta acgatacgg acaaatagac	900
ttagtgtagc tcaccttac taactgcacc tctgtagcca atgtcagtag tctttatgt	960
gccctaaacg ggtcagctt cctctgtgga aataacatgg catacaccta ttaccccaa	1020
aactggacag gactttgcgt ccaagcctc ctccctcccg acattgacat catcccgagg	1080
gatgagccag tccccattcc tgccattgat cattatatac atagacctaa acgagctgta	1140
cagttcatcc ctttactagc tggactggga atcacgcag cattcaccac cggagctaca	1200
ggcctagggt tctccgtcac ccagtataca aaattatccc atcagttaat atctgatgtc	1260
caagtcttat ccggtacct acaagattta caagaccagg tagactcgtt agctgaagta	1320
gttctccaaa ataggagggg actggacct ctaacggcag aacaaggagg aatttgttta	1380
gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag aaacaaaata	1440
agaaccttac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa cctctctgg	1500
accgggctgc agggctttct tccgtacct ctacctctc tgggacccct actcaccctc	1560
ctactcatc taaccattgg gccatgcgtt ttcaatcgat tggtoaat tgtaaaagac	1620
aggatctcag tggtcaggc tctggttttg actcagcaat atcaccagct aaaaccata	1680
gagtagcagc catga	1695

<210> SEQ ID NO 45

<211> LENGTH: 2013

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; GALV

<400> SEQUENCE: 45

atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtctgg gagctggaaa	60
agactgatca tcctcttaag ctgcgtatc ggagacggca aaacgagtct gcagaataag	120
aacccccacc agcctgtgac cctcacctgg caggtaactgt cccaaactgg ggacgttgct	180
tgggacaaaa aggcagtcga gcccttttgg acttggtggc cctctcttac acctgatgta	240
tgtgccctgg cggcggtct tgagtctgg gatatccgg gatccgatgt atcgtcctct	300
aaaagagtta gacctctga ttcagactat actgccgctt ataagcaaat cacctgggga	360
gccatagggt gcagctaccc tcgggctagg accaggatgg caaatcccc cttctacgtg	420

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tgccccgag ctggccgaac ccattcagaa gctaggaggt gtgggggggt agaatcccta	480
tactgtaaag aatggagttg tgagaccacg ggtaccgttt attggcaacc caagtcccta	540
tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa atttcaaaag	600
tgtgaacaaa ccgctgggtg taacccccctc aagatagact tcacagaaaa aggaaaaactc	660
tccagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata tggacacca	720
ggcatacagt tgactatccg cttagagggtc actaacatgc cggttgtggc agtgggcccc	780
gacctgtcc ttgcggaaca gggacctcct agcaagcccc tcaactctccc tctctcccca	840
cggaaagcgc cggccacccc tctacccccg cgggctagtg agcaaacccc tgcggtgcat	900
ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact ctttggcctt	960
gtgcaggggg ccttcctaac cttgaatgct accaaccagc gggccactaa gtcttctgtg	1020
ctctgttttg gcatgagccc cccttattat gaagggatag cctcttcagg agaggctcgt	1080
tatacctcca accatacccg atgccactgg ggggccccag gaaagcttac cctcactgag	1140
gtctccggac tcgggtcatg catagggaag gtgctcttta cccatcaaca tctttgcaac	1200
cagaccttac ccatcaattc ctctaaaaac catcagtatc tgctccctc aaaccatagc	1260
tgggtgggct gcagcactgg cctcaccccc tgctctccca cctcagtttt taatcagtct	1320
aaagacttct gtgtccaggt ccagctgac ccccgcatct attaccatc tgaagaaacc	1380
ttgttacaag cctatgacaa atcaccccc aggtttaaaa gagagcctgc ctcacttacc	1440
ctagctgtct tcctgggggt agggattgag gcaggtatag gtactggctc aacggcccta	1500
attaaggggc ccatagacct ccagcaaggc ctaaccagcc tccaaatgc cattgacgct	1560
gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac ttccctatct	1620
gaggtagtac tccaaaatag gagaggcctt gacttactat tccttaaaga aggaggcctc	1680
tgcgcgcccc taaaagaaga gtgctgtttt tatgtagacc actcaggtgc agtacgagac	1740
tccatgaaaa aacttaaaga aagactagat aaaagacagt tagagcgcca gaaaaacca	1800
aactgggatg aagggtgggt caataactcc ccttggttta ctaccctact atcaaccatc	1860
gctgggcccc tattgtctct ccttttggtta ctactcttg ggcctgcat catcaataaa	1920
ttaatccaat tcataatga taggataagt gcagtcacaaa ttttagtctc tagacagaaa	1980
tatcagaccc tagataacga ggaaaacctt taa	2013

<210> SEQ ID NO 46

<211> LENGTH: 1530

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; FUG

<400> SEQUENCE: 46

atggttccgc aggttctttt gtttgtactc cttctgggtt tttcgttggtg tttcgggaag	60
ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga catacaccat	120
ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct gtccgagttc	180
tcctacatgg aactcaaaagt gggatacatc tcagccatca aagtgaacgg gttcacttgc	240
acaggtgttg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca	300
ttcaagagaa agcatttccg cccaccccc gacgcagtga gagccgcgta taactggaag	360
atggccgggtg accccagata tgaagagtcc ctacacaatc cataccccga ctaccactgg	420

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cttcgaactg taagaaccac caaagagtcc ctcattatca tatccccaag tgtgacagat	480
ttggacccat atgacaaatc ccttcactca agggctctcc ctggcggaag gtgctcagga	540
ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg gatgcccgag	600
aatccgagac caaggacacc ttgtgacatt ttaccaata gcagagggaa gagagcatcc	660
aacgggaaca agacttgcgg ctttgtggat gaaagaggcc tgtataagtc tctaaaagga	720
gcctgcaggg tcaagttatg tggagttctt ggacttagac ttatggatgg aacatgggtc	780
gcgatgcaaa catcagatga gaccaaattg tgccctccag atcagttggg gaatttgac	840
gactttcgct cagacgagat cgagcatctc gttgtggagg agttagttaa gaaaagagag	900
gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt cagacgtctc	960
agtcacctga gaaaacttgt cccagggttt ggaaaagcat ataccatatt caacaaaacc	1020
ttgatggagg ctgatgtcca ctacaagtca gtccggacct ggaatgagat catccctca	1080
aaaggggtgt tgaaagtgg aggaagggtc catcctcatg tgaacggggt gtttttcaat	1140
ggataatat tagggcctga cgaccatgtc ctaatccag agatgcaatc atccctctc	1200
cagcaacata tggagttgtt ggaatcttca gttatcccc tgatgcaccc cctggcagac	1260
ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt tcacctcccc	1320
gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg aaagtatgta	1380
ttgatgactg caggggcat gattggcctg gtgttgatat tttccctaat gacatgggtc	1440
agagttggtg tccatctttg cattaaatta aagcacacca agaaaagaca gatttatata	1500
gacatagaga tgaaccgact tggaaagtaa	1530

<210> SEQ ID NO 47

<211> LENGTH: 1497

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; LCMV

<400> SEQUENCE: 47

atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatcgatga ggtgatcaac	60
attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa ttttgccacc	120
tgtgggatat tcgcattgat cagtttctca cttctggctg gcaggctcctg tggcattgac	180
ggtcttaagg gacccgacat ttacaaagga gtttaccat ttaagtcagt ggagtttgat	240
atgtcacatc tgaacctgac catgcccaac gcatgttcag ccaacaactc ccaccattac	300
atcagtatgg ggacttcttg actagaattg accttcacca atgattccat catcagtcac	360
aacttttgca atctgacctc tgccttcaac aaaaagacct ttgaccacac actcatgagt	420
atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc agtatcctgc	480
gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg acaaagtgtc	540
cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac tgccttcggg	600
gggaaataca tgaggagtgg ctggggctgg acaggctcag atggcaagac cacctgggtg	660
agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa ccactgcaca	720
tatgcaggtc cttttgggat gtccaggatt ctcccttccc aagagaagac taagtctctc	780
actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg ggtggagaat	840
ccagtggtt attgcctgac caaatggatg attcttctg cagagcttaa gtgtttcggg	900
aacacagcag ttgcgaaatg caatgtaat catgatgccg aattctgtga catgctgcga	960

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ctaattgact	acaacaaggc	tgctttgagt	aagttcaaag	aggacgtaga	atctgccttg	1020
cacttattca	aaacaacagt	gaattctttg	atttcagatc	aactactgat	gaggaaccac	1080
ttgagagatc	tgatgggggt	gccatattgc	aattactcaa	agttttggta	cctagaacat	1140
gcaaagaccg	gcgaactag	tgccccaaag	tgctggcttg	tcaccaatgg	ttcttactta	1200
aatgagaccc	acttcagtga	tcaaategaa	caggaagccg	ataacatgat	tacagagatg	1260
ttgaggaagg	attacataaa	gaggcagggg	agtaccccc	tagcattgat	ggaccttctg	1320
atgttttcca	catctgcata	tctagtcagc	atcttctctg	accttgtaaa	aataccaaca	1380
cacaggcaca	taaaagggtg	ctcatgtcca	aagccacacc	gattaaccaa	caaaggaatt	1440
tgtagttgtg	gtgcatttaa	ggtgcctggg	gtaaaaaccg	tctggaaaag	acgctga	1497

<210> SEQ ID NO 48

<211> LENGTH: 1692

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; FPV

<400> SEQUENCE: 48

atgaacactc	aaatcctggg	tttcgccctt	gtggcagtc	tccccacaaa	tgagacaaa	60
atttgtcttg	gacatcatgc	tgtatcaaat	ggcaccaaag	taaacacact	cactgagaga	120
ggagtagaag	ttgtcaatgc	aacggaaaca	gtggagcggg	caaacatccc	caaaatttgc	180
tcaaaaggga	aaagaaccac	tgatcttggc	caatgcggac	tgtaggggac	cattaccgga	240
ccacctcaat	gcgaccaatt	tctagaattt	tcagctgac	taataatcga	gagacgagaa	300
ggaaatgatg	tttgttacc	ggggaagttt	gttaatgaag	aggcattg	acaaatctc	360
agaggatcag	gtgggattga	caaagaaaca	atgggattca	catatagtgg	aataaggacc	420
aacggaacaa	ctagtgc	tagaatga	gggtcttcat	tctatgcaga	aatggagtgg	480
ctcctgtcaa	atacagacaa	tgctgctttc	ccacaaatga	caaatcata	caaaaacaca	540
aggagagaat	cagctctgat	agtctgggga	atccaccatt	caggatcaac	caccgaacag	600
accaaactat	atgggagtgg	aaataaactg	ataacagtcg	ggagtccaa	atatcatcaa	660
tcttttgtgc	cgagtccagg	aacacgaccg	cagataaatg	gccagtccgg	acggattgat	720
tttcattggg	tgatcttggg	tcccaatgat	acagttactt	ttagtttcaa	tggggctttc	780
atagctccaa	atcgtgccag	cttcttgagg	ggaaagtcca	tggggatcca	gagcgatgtg	840
caggttgatg	ccaattgcga	aggggaatgc	taccacagtg	gagggaactat	aacaagcaga	900
tgctcttttc	aaaacatcaa	tagcagagca	gttggcaaat	gcccagata	tgtaaacag	960
gaaagtttat	tattggcaac	tgggatgaag	aacgttcccg	aaccttccaa	aaaaaggaaa	1020
aaaagaggcc	tgtttgccgc	tatagcaggg	tttattgaaa	atggttgagg	aggtctggtc	1080
gacgggtggg	acggtttcag	gcatacagaat	gcacaaggag	aaggaactgc	agcagactac	1140
aaaagcacc	aatcggaat	tgatcagata	accggaaggt	taaatagact	cattgagaaa	1200
accaaccagc	aatttgagct	aatagataat	gaattcactg	aggtggaaaa	gcagattggc	1260
aatttaatta	actggaccaa	agactccatc	acagaagtat	ggctttacaa	tgctgaactt	1320
cttggtggca	tggaaaacca	gcacactatt	gatttggctg	attcagagat	gaacaagctg	1380
tatgagcgag	tgaggaaaca	attaagggaa	aatgctgaag	aggatggcac	tggttgcttt	1440
gaaatttttc	ataaatgtga	cgatgattgt	atggctagta	taaggacaaa	tacttatgat	1500

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cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgaccc agtcaaatg	1560
agtagtggtt acaaatggtt gatacttttg tttagcttcg gggcatcatg ctttttgctt	1620
cttgccattg caatgggctt tgttttcata tgtgtgaaga acggaaacat gcggtgcact	1680
attgtatat aa	1692

<210> SEQ ID NO 49
 <211> LENGTH: 1266
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope; RRV

<400> SEQUENCE: 49

agtgtaacag agcacttta tgtgtataag gctactagac catacctagc acattgcgcc	60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag	120
gcgtctgatg gcattgctta gatccaagtc tccgccccaa taggtctgga caaggcaggc	180
acccacgccc acacgaagct ccgatatatg gctggctcatg atgttcagga atctaagaga	240
gattccttga ggggtgtacac gtccgcagcg tgctccatag atgggacgat gggacacttc	300
atcgctgcac actgtccacc aggcgactac ctcaaggttt cggttcagga cgcagattcg	360
cacgtgaagg catgtaagg ccaatacaag cacaatccat tgccggtggg tagagagaag	420
ttcggtggtt gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg	480
gtcccccagg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg	540
ctatcacaga cggcggggaa cgtcaaaata acagcaggcg gcaggactat caggatcaac	600
tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagacat caacacatgc	660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca	720
tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg	780
actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggttaagaag	840
gaggtgaccc tgagattaca ccagatcat ccgacgctct tctcctatag gagtttagga	900
gccgaaccgc acccgtaaga ggaatgggtt gacaagttct ctgagcgcat catcccagt	960
acggaagaag ggattgagta ccagtggtg cacaacccgc cggctctgct gtggcgcaa	1020
ctgacgaccg agggcaaac ccattggtg ccacatgaaa tcattcagta ctattatgga	1080
ctataccccc ccgcccactat tgccgcagta tccggggcga gtctgatggc cctcctaact	1140
ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgccctaac accgtacgcc	1200
ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc accgagggcg	1260
aatgca	1266

<210> SEQ ID NO 50
 <211> LENGTH: 1938
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope; MLV 10A1

<400> SEQUENCE: 50

atggaaggtc cagcgttctc aaaacccctt aaagataaga ttaacccgtg gaagtcctta	60
atggtcatgg gggctctattt aagagtaggg atggcagaga gccccatca ggtctttaat	120
gtaacctgga gagtcaccaa cctgatgact gggcgtaccg ccaatgccac ctccctttta	180
ggaactgtac aagatgcctt cccaagatta tattttgatc tatgtgatct ggtcggagaa	240

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gagtgggacc	cttcagacca	ggaaccatat	gtcgggtatg	gctgcaaata	ccccggagg	300
agaaagcgga	ccccgacttt	tgacttttac	gtgtgccctg	ggcataccgt	aaaatcgagg	360
tgtggggggc	caagagaggg	ctactgtggt	gaatgggggt	gtgaaaccac	cggacaggct	420
tactggaagc	ccacatcatc	atgggaccta	atctccctta	agcgcggtaa	caccccttgg	480
gacacgggat	gctccaaaat	ggcttgtggc	ccctgctacg	acctctccaa	agtatccaat	540
tccttocaag	gggtactctg	agggggcaga	tgcaaccctc	tagtctaga	attcactgat	600
gcaggaaaaa	aggctaattg	ggacggggcc	aaatcggtgg	gactgagact	gtaccggaca	660
ggaacagatc	ctattaccat	gttctccctg	acccgccagg	tcctcaatat	agggccccgc	720
atccccattg	ggcctaatac	cgtgatcact	ggtcaactac	ccccctcccg	accgtgcag	780
atcaggctcc	ccaggctcc	tcagctcct	cctacaggcg	cagcctctat	agtccctgag	840
actgccccac	cttctcaaca	acctgggacg	ggagacaggc	tgctaaacct	ggtagaagga	900
gcctatcagg	cgcttaacct	caccaatccc	gacaagacct	aagaatgttg	gctgtgctta	960
gtgtcgggac	ctccttatta	cgaaggagta	gcggctgttg	gcacttatac	caatcattct	1020
accgccccgg	ccagctgtac	ggccacttcc	caacataagc	ttaccctatc	tgaagtgaca	1080
ggacaggggc	tatgcatggg	agcactacct	aaaactcacc	aggccttatg	taacaccacc	1140
caaagtgcgg	gctcaggatc	ctactacctt	gcagcaccgg	ctggaacaat	gtgggcttgt	1200
agcactggat	tgactccctg	cttgtccacc	acgatgctca	atctaaccac	agactattgt	1260
gtattagtgt	agctctggcc	cagaataatt	taccactccc	ccgattatat	gtatggtcag	1320
cttgaacagc	gtaccaaata	taagaggggag	ccagtatcgt	tgacctggc	ccttctgcta	1380
ggaggattaa	ccatgggagg	gattgcagct	ggaataggga	cggggaccac	tgccctaata	1440
aaaaccacgc	agtttgagca	gcttcacggc	gctatccaga	cagacctcaa	cgaagtcgaa	1500
aaatcaatta	ccaacctaga	aaagtcactg	acctcgttgt	ctgaagtagt	cctacagaac	1560
cgaagaggcc	tagatttgct	cttcctaaaa	gagggaggtc	tctgocgagc	cctaaaagaa	1620
gaatgttggt	tttatgcaga	ccacacggga	ctagtgaagc	acagcatggc	caaactaagg	1680
gaaaggctta	atcagagaca	aaaactatct	gagtcaggcc	aagggttggt	cgaagggcag	1740
tttaatatgat	ccccctgggt	taccacctta	atctccacca	tcattgggac	tctaataagta	1800
ctcttactga	tcttactctt	tgagacctgc	attctcaatc	gattggtcca	atttgttaaa	1860
gacaggatct	cagtgggtcca	ggctctgggt	ttgactcaac	aatatcacca	gctaaaaacct	1920
atagagtacg	agccatga					1938

<210> SEQ ID NO 51

<211> LENGTH: 2030

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Envelope; Ebola

<400> SEQUENCE: 51

atgggtgtta	caggaatatt	gcagttacct	cgtgatcgat	tcaagaggac	atcattcttt	60
ctttgggtta	ttatcctttt	ccaaagaaca	ttttccatcc	cacttgaggt	catccacaat	120
agcacattac	aggttagtga	tgtcgacaaa	ctggtttgcc	gtgacaaact	gtcatccaca	180
aatcaattga	gatcagttgg	actgaatctc	gaagggaatg	gagtggcaac	tgacgtgcca	240
tctgcaacta	aaagatgggg	cttcagggtcc	ggtgtccccc	caaagggtgt	caattatgaa	300

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gctggtgaat gggctgaaaa ctgctacaat cttgaaatca aaaaacctga cgggagtgag	360
tgtctaccag cagcgccaga cgggattcgg ggcttcccc ggtgccgga tgtgcacaaa	420
gtatcaggaa cgggaccgtg tgccggagac ttgctctcc acaaagaggg tgctttcttc	480
ctgtatgacc gacttgcttc cacagttatc taccgaggaa cgactttcgc tgaaggtgtc	540
gttgcatctc tgatactgcc ccaagctaag aaggacttct tcagctcaca ccccttgaga	600
gagccggtca atgcaacgga ggaccgtct agtggtact attctaccac aattagatat	660
caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga caatttgacc	720
tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa tgagacaata	780
tatacaagtg ggaaggag caataccacg gaaaactaa tttggaagg caacccgaa	840
attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaacctc actagaaaaa	900
ttcgcagtg agagtgtct ttcacagctg tatcaaacag agccaaaaac atcagtggtc	960
agagtcgggc gcgaacttct tccgaccag ggaccaacac aacaactgaa gaccacaaaa	1020
tcatggcttc agaaaattcc tctgcaatgg ttcaagtgc cagtcaggga agggaagctg	1080
cagtgctgca tctgacaacc ctggccaaa tctccacgag tcctcaacct cccacaacca	1140
aaccaggtcc ggacaacagc accacaata caccgtgtg taaacttgac atctctgagg	1200
caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc tccgacactc	1260
cccccgccac gaccgcagcc ggaccctaa aagcagagaa caccaacacg agcaagggtg	1320
cgcacctct ggacccgccc accacaacaa gtccccaaaa ccacagcgag accgctggca	1380
acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg aagctaggct	1440
taattaccaa tactattgct ggagtcgag gactgatcac aggcgggagg agagctcgaa	1500
gagaagcaat tgtcaatgct caaccctaat gcaacctaa tttacattac tggactactc	1560
aggatgaagg tgctgcaatc ggactggcct ggataccata ttctgggcca gcagccgagg	1620
gaatttcat agaggggctg atgcacaatc aagatgggtt aatctgtggg ttgagacagc	1680
tggccaacga gacgactcaa gctcttcaac tgctctgag agccacaacc gagctacgca	1740
ccttttcaat cctcaaccgt aaggcaattg atttcttct gcagcgatgg ggcggcacat	1800
gccacatttt gggaccggac tgctgtatcg aaccacatga ttggaccaag aacataacag	1860
acaaaattga tcagattatt catgattttg ttgataaaac ccttcgggac cagggggaca	1920
atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga gttacaggcg	1980
ttataattgc agttatcgct ttattctgta tatgcaaat tgtcttttag	2030

<210> SEQ ID NO 52

<211> LENGTH: 237

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Polymerase III shRNA promoters; U6 promoter

<400> SEQUENCE: 52

tttcccatga ttccttcata ttgcatata cgatacaagg ctgttagaga gataattgga	60
attaatttga ctgtaaacac aaagatatta gtacaaaata cgtgacgtag aaagtaataa	120
tttcttgggt agtttgcagt tttaaaatta tggttttaaa tggactatca tatgcttacc	180
gtaacttgaa agtatttoga tttcttggct ttatatatct tgtggaaagg acgaaac	237

<210> SEQ ID NO 53

<211> LENGTH: 243

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polymerase III shRNA promoters; 7SK promoter

<400> SEQUENCE: 53

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

<210> SEQ ID NO 54
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #1

<400> SEQUENCE: 54

gtcctggagt acaatgccat t                                                                           21

<210> SEQ ID NO 55
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #2

<400> SEQUENCE: 55

gcaggatttc gttcagcaat t                                                                           21

<210> SEQ ID NO 56
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #3

<400> SEQUENCE: 56

gccatgtaca tggcaggaat t                                                                           21

<210> SEQ ID NO 57
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #4

<400> SEQUENCE: 57

gcagaaggag gctgagaaag t                                                                           21

<210> SEQ ID NO 58
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Non-targeting sequence

<400> SEQUENCE: 58

gcgcgtttgt aggatagagc tcgagctcta tcctacaaag cggtttttt      49

<210> SEQ ID NO 59

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 59

aggaattgat ggcgagaagg                20

<210> SEQ ID NO 60
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 60

cccaaagagg tcaaggtaat ca            22

<210> SEQ ID NO 61
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 61

agcgcggcta cagcttca                18

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 62

ggcgacgtag cacagcttct            20

<210> SEQ ID NO 63
<211> LENGTH: 130
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Left Inverted Terminal Repeat (Left ITR)

<400> SEQUENCE: 63

cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgctcg ggcgaccttt    60
ggtcgccccg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact    120
aggggttcct                                     130

<210> SEQ ID NO 64
<211> LENGTH: 151
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Right Inverted Terminal Repeat (Right ITR)

<400> SEQUENCE: 64

gagcgggcgc aggaaccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg    60
ctcactgagg ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggccgctca    120
gtgagcgagc gagcgcgcag ctgcctgcag g                                     151

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<210> SEQ ID NO 65
<211> LENGTH: 1227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: RRE/rabbit poly A beta globin

<400> SEQUENCE: 65

tctagaagga gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc    60
gtcaatgacg ctgacggtag aggccagaca attattgtct ggtatagtgc agcagcagaa    120
caatttgcctg agggctattg aggcgcaaca gcattctgtt caactcacag tctggggcat    180
caagcagctc caggcaagaa tcttggtgtt ggaaagatac ctaaaggatc aacagctcct    240
agatcttttt ccctctgcc aaaaattatgg ggacatcatg aagccccctg agcatctgac    300
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttgaatt ttttgtgtct    360
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggg    420
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaagggtg ctataaagag    480
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga    540
cttgagggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa    600
aattttcctt acatgtttta ctagccagat ttttctcct ctctgacta ctcccagtca    660
tagctgtccc tcttctctta tgaagatccc tcgacctgca gcccaagctt ggcgtaatca    720
tggtcatagc tgtttctgtg gtgaaattgt tatccgctca caattccaca caacatacga    780
gccggaagca taaagtgtaa agcctggggt gcctaagtga tgagctaact cacattaatt    840
gcggtgcgct cactgcccgc ttccagctg ggaaacctgt cgtgccagcg gatccgcac    900
tcaattagtc agcaaccata gtcccgcgcc taactccgcc catcccgcgc ctaactccgc    960
ccagttccgc ccattctccg ccccatggct gactaatttt ttttatttat gcagaggccg    1020
aggcgcctc ggctctgag ctattccaga agtagtgagg aggtttttt ggaggcctag    1080
gcttttgcaa aaagctaaact tgttatttgc agcttataat ggttaciaat aaagcaatag    1140
catcacaat ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa    1200
actcatcaat gtatcttctc acccgggg                                1227

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What is claimed is:

1. A method of activating a GD T cell comprising: contacting a target cell with a bisphosphonate drug; and infecting, in the presence of the GD T cell, the target cell with a viral delivery system that encodes at least one genetic element comprising a small RNA capable of inhibiting production of FDP S, wherein the GD T cell is activated.
2. The method of claim 1, further comprising killing of the target cell by the activated GD T cell.
3. The method of claim 1, wherein the target cell is a cancer cell.
4. The method of claim 3, wherein the cancer cell is derived from a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof.
5. The method of claim 1, wherein activating the GD T cell comprises increasing tumor necrosis factor TNF- α expression by the GD T cell.
6. The method of claim 1, wherein the small RNA is a microRNA or a shRNA.
7. The method of claim 6, wherein the shRNA comprises a sequence having at least 80% identity with SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.
8. The method of claim 7, wherein the shRNA comprises SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.
9. The method of claim 6, wherein the microRNA comprises a sequence having at least 80% identity with SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.
10. The method of claim 9, wherein the microRNA comprises SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.
11. The method of claim 1, wherein the bisphosphonate drug comprises zoledronic acid.
12. The method of claim 1, wherein the contacting step and the infecting step occur simultaneously.
13. The method of claim 1, wherein the contacting step and the infecting step occur sequentially.
14. A method of activating a lymphocyte cell comprising: contacting a target cell with a bisphosphonate drug; and infecting, in the presence of the lymphocyte cell, the target cell with a viral delivery system that encodes

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at least one genetic element comprising a small RNA capable of inhibiting production of an enzyme of the mevalonate pathway,

wherein the lymphocyte cell is activated.

15. The method of claim 14, further comprising killing of the target cell by the activated lymphocyte. 5

16. The method of claim 14, wherein the enzyme of the mevalonate pathway is FDPS.

17. The method of claim 14, wherein the lymphocyte is a GD T cell. 10

18. The method of claim 17, wherein activating the lymphocyte comprise increasing TNF- α expression by the GD T cell.

19. The method of claim 14, wherein the target cell is a cancer cell. 15

20. The method of claim 19, wherein the cancer cell is derived from a carcinoma, a leukemia, a lymphoma, a sarcoma, a myeloma, a mesothelioma, a mixed type, or mixtures thereof.

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