

# Methods and compositions for the activation of gamma-delta t-cells

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## Claims (25)

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1. CLAIMS WHAT IS CLAIMED IS: 1. A viral vector comprising at least one encoded genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway.

2. The viral vector of claim 1, wherein the enzyme is farnesyl diphosphate synthase (FDPS).

3. The viral vector of claim 1, wherein the at least one encoded genetic element comprises a microRNA or a shRNA.

4. The viral vector of claim 3, wherein the shRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with: a. GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); b. GCAGGATTTTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); c.

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or d. GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4).

5. The viral vector of claim 4, wherein the shRNA comprises: a. GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); b. GCAGGATTTTCGTTCAAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); c. GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or d. GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4).

6. The viral vector of claim 3, wherein the microRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with: a. AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCT CTTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); b. AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); c. TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); d. CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGAGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); e. CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); or f. GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCCG (SEQ ID NO: 10).

7. The viral vector of claim 6, wherein the microRNA comprises: a. AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); b. AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); c. TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); d. CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCT CTTTCTGCTTTTGGCCACTGACTGAGCAGAAGGAGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); e. CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); or f. GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCCG (SEQ ID NO: 10).

8. The viral vector of any one of claims 1-7, wherein the viral vector is a lentiviral vector.

9. The viral vector of claim 1, wherein the viral vector is an adeno-associated virus vector.

10. The viral vector of claim 1, further comprising a second encoded genetic element, wherein the second genetic element comprises at least one cytokine or chemokine.

11. The viral vector of claim 10, wherein the at least one cytokine is selected from the group consisting of: IL-18, TNF- $\alpha$ , interferon- $\gamma$ , IL-1, IL-2, IL-15, IL-17, and IL-12.

12. The viral vector of claim 10, wherein the at least one chemokine is a CC chemokine, a CXC chemokine, a CX3C chemokine, or a XC chemokine.

13. A lentiviral vector system for expressing a lentiviral particle, the system comprising: a. a lentiviral vector according to claim 8; b. at least one envelope plasmid for expressing an envelope protein optimized for infecting a cell; and c. at least one helper plasmid for expressing gag, pol, and rev genes, wherein 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, wherein the lentiviral particle is capable of infecting a targeting cell, and inhibiting an enzyme involved in the mevalonate pathway within the target cell.

14. The lentiviral vector system of claim 13, wherein the system comprises a first helper plasmid for expressing the gag and pol genes, and a second plasmid for expressing the rev gene.

15. A lentiviral particle capable of infecting a cell, the lentiviral particle comprising an envelope protein optimized for infecting a target cell, and a lentiviral vector according to claim 8.

16. The lentiviral particle of claim 15, wherein the envelope protein is optimized for infecting a target cell, and wherein the target cell is a cancer cell.

17. The lentiviral particle of claim 15, wherein the envelope protein is optimized for infecting a target cell, and wherein the target cell is a cell that is infected with an infectious disease.

18. A method of activating a gamma delta T cell, the method comprising infecting, in the presence of the GD T cell, a target cell with a viral delivery system encoding at least one genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway, and wherein when the enzyme is inhibited in the target cell, the target cell activates the GD T cell.

19. A method of treating cancer in a subject, the method comprising administering to the subject a therapeutically-effective amount of a viral delivery system encoding at least one genetic element, wherein the at least one encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway, and wherein 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.

20. The method of claim 18 or 19, wherein the enzyme is farnesyl diphosphate synthase (FDPS).

21. The method of claim 18 or 19, wherein the at least one encoded genetic element comprises a microRNA or a shRNA.

22. The method of claim 21, wherein the shRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with: a.

GTCTTGGAGTACAATGCCATTCTCGAGAATGGCATTGTA CT CAGGACTTTTT (SEQ ID NO: 1); b.

GCAGGATTTTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); c.

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or d.

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4).

23. The method of claim 21, wherein the microRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with: a.

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); b.

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCCTACTGCCTCGGACTTCAAGGGGCT

(SEQ ID NO: 6); c. TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCCTACTGCCTCGGA (SEQ ID NO: 7); d.

CCTGGAGGCTTGTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA

(SEQ ID NO: 8); e.

CATCTCCATGGCTGTACCACCTGTTCGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); or f.

GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCCTCCCAATGACCGCGTCTTCGTGCG (SEQ ID NO: 10).

24. The method of claim 18 or 19, further comprising administering to the subject 1 a therapeutically effective amount of an aminobisphosphonate drug.

25. The method of claim 24, wherein the aminobisphosphonate drug is zoledronic acid.

## Description

METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLSCROSS-REFERENCE TO RELATED APPLICATION This application claims priority to: U.S. Provisional Patent Application No. 62/279,474, filed on January 15, 2016, and entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells", which is incorporated herein by reference.

FIELD OF THE INVENTION The present disclosure relates generally to the fields of gene therapy and immunotherapy, specifically in relation to increased activation 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% circulating lymphocytes, and 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, Vδ1+, make up a much smaller percentage of the T cells circulating in the blood, but Vδ1+ cells are commonly found in the epithelial mucosa and the skin. 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. Aminobisphosphonate drugs ("ABPs") and other inhibitors of farnesyl diphosphate synthase ("FDPS"), which are downstream from isopentenyl pyrophosphate ("IPP") in the mevalonate pathway (see, for e.g., Figure 1), have been used to treat various diseases, including cancers, specifically those involving bone metastasis. ABPs include trade names such as Zometa® (Novartis) and Fosamax® (Merck). ABPs have also been used to stimulate 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 ABPs will proliferate rapidly, express a number of cytokines and chemokines, and can function to cytotoxicity destroy tumor cells or cells infected with pathogenic microorganisms. However, ABPs 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 ABPs in general, and IPP specifically, leave a great deal to be desired for therapeutic purposes.

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 an aminobisphosphonate drug. In embodiments, the aminobisphosphonate 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 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 an aminobisphosphonate drug. In embodiments, the aminobisphosphonate 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 an aminobisphosphonate drug. In embodiments, the aminobisphosphonate 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 GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTCGTTCCAGCACTTCTCGAGAAGTGCTGAACGAA ATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAA TTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGA GAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In a preferred embodiment, the shRNA includes GTCCTGGAGTACAATGCCATTCTCGAG AATGGCATTGACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTCGTTC GCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCA TGTACATGGCAGGAATTCTCGAGAATTCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCTCCTT 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 AAGGTATATTGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); CATCTCCATGGCTGTACCACCTTGTCCGGACTTTCTCAGCCTCCTTCTGCCTGTTG AATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCTTCCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). In a preferred embodiment, the microRNA includes AAGGTATATTGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTGTTGACAGTGAGCGGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAA GGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); CATCTCCATGGCTGTACCACCTTGTCCGGACTTTCTCAGCCTCCTT CTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATC TTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCTTCCCCTCCCAATGACCGCGTCTTCGTCG (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- $\alpha$ , interferon- $\gamma$ , 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.

**BRIEF DESCRIPTION OF THE DRAWINGS** Figure 1 depicts an overview of the major steps in the mevalonate pathway for biosynthesis of steroids and isoprenoids.

Figure 2 depicts an exemplary 3-vector lentiviral vector system in a circularized form. Figure 3 depicts an exemplary 4-vector lentiviral vector system in a circularized form. Figure 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. Figure 5 depicts data demonstrating activation of V $\delta$ 2+ T cells THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein. Figure 6 depicts data demonstrating activation of V $\delta$ 2+ T cells by THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein. Figure 7 depicts data demonstrating activation of V $\delta$ 2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #1 (SEQ ID NO: 1), as described herein. Figure 8 depicts data demonstrating activation of V $\delta$ 2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein. Figure 9 depicts data demonstrating activation of V $\delta$ 2+ 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. Figure 10 depicts data demonstrating activation of V $\delta$ 2+ T

cells by THP-1 leukemia cells with a lentivirus expressing miR30 FDPS #1 (SEQ ID NO: 5), as described herein. Figure 11 depicts data demonstrating the percent of specific lysis versus an E:T ratio for a variety of experimental conditions, as described herein. Figure 12 depicts data demonstrating lentiviral-delivered shRNA-based RNA interference targeting the human FDPS gene. Figure 13 depicts data demonstrating lentiviral-delivered miR-based RNA interference targeting the human FDPS gene. Figure 14 depicts data demonstrating activation of V $\delta$ 2+ T cells by HepG2 carcinoma cells with an adeno-associated virus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein. Figure 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 Figure 1. In embodiments, one or more viral vectors are provided with microRNAs or short homology 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. The terms "administration of" or "administering" an active agent should be understood to mean providing an active agent to the subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount. As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method. "Consisting of" shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure. Accordingly, 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, "expression," "expressed," or "encodes" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification. The term "farnesyl diphosphate synthase" may also be referred to herein as FDPS, and may also be referred to herein as farnesyl pyrophosphate synthase or FPPS. The term "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 as killing or a target cell or assisting another effector cell to kill a target cell. The terms "individual," "subject," and "patient" are used interchangeably herein, and refer to any individual mammal subject, e.g., bovine, canine, feline, equine, or human. 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. The term "percent 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* 85:24(1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 5Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*). One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website. 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-(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 XBLAST 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, wordlength = 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., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. As used herein, "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, a "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 "SEQ ID NO" is synonymous with the term "Sequence ID No." As used herein, "small RNA" refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). "Small RNA" of the disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, generally through pathways that result in the destruction of the target gene mRNA. 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. "A treatment" is intended to target the disease state and combat it, i.e., ameliorate or prevent the disease state. The particular treatment thus will depend on the disease state to be targeted and the

current or future state of medicinal therapies and therapeutic approaches. A treatment may be associated toxicities. The term "treatment" or "treating" generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

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 GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTCGTTTCAGCACTTCTCGAGAAGTGC TGAACGAA ATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAA TTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGA GAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In a preferred embodiment, the shRNA includes GTCCTGGAGTACAATGCCATTCTCGAG AATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTCGTTCA GCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCA TGTACATGGCAGGAATTCTCGAGAATTCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTT 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 AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA AGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGTGAGCGACACT TTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTGTTGACAGTG AGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCTTGCTGAAG GCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); CATCTCCATGGCTGTACCACCTTGTCTGGGACTTTCTCAGCCTCCTTCTGCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCT CAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). In a preferred embodiment, the microRNA includes AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCT CCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 5); AAGGTATATTGCTGTTGACAGT GAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 6); TGCTG TTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (SEQ ID NO: 7); CCTGGAGGCT TGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTTGGCCACTGACTG AGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGCACTCA (SEQ ID NO: 8); CATCTCCATGGCTGTACCACCTTGTCTGGGACTTTCTCAGCCTCCTT CTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA (SEQ ID NO: 9); or GGGCCTGGCTCGAGCAGGGGGCGAGGG ATACTTTCTCAGCCTCCTTCTGCTGGTCCCCTCCCCGAGAAGGAGGCTGAGAAA GTCCTTCCCCTCCCAATGACCGCGTCTTCGTCG (SEQ ID NO: 10). In another aspect, the target cell is also contacted with an aminobisphosphonate drug. In a preferred embodiment, the aminobisphosphonate 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 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.

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- $\alpha$ , interferon- $\gamma$ , IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least one chemokine is a CC chemokine, CXC chemokine, c 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. 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. 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, oesophageal carcinoma, osteosarcoma, pancreatic carcinoma, pheochromocytoma, prostate carcinoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, Schwannoma, small cell lung cancer, squamous cell carcinoma of the head and neck, testicular tumor, thyroid carcinoma, urothelial carcinoma, and Wilm's 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, picomaviruses, 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 yeastlike, including, for example, fungi that cause diseases such as ringworm, histoplasmosis, blastomycosis, aspergillosis, cryptococcosis, sporotrichosis, coccidioidomycosis, paracoccidio-idomycosis, 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- $\gamma$  and TNF- $\alpha$ . 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 an 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- $\alpha$ , interferon- $\gamma$ , IL-1, IL-2, IL-15, IL-17, IL- 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- $\alpha$ , interferon- $\gamma$ , 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 homology region 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 inflammasome function that in turn results in increased expression of cytokines that are important for GD T cell activation and effector cell function. 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 an 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 concurrently with aminobisphosphonate (ABP) drugs to achieve synergistic activation of gamma delta T cells. The synergism can allow alternate, modified or reduced doses of ABP and may decrease adverse reactions to ABP including acute inflammatory responses and chronic diseases.

Constructs for GD T Cell Activation Inhibition of FDPS results in IPP accumulation, resulting in activation of V $\delta$ 2+ 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, IFN $\gamma$ , 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 homologies 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, 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 Gagniuc 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, 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, VSVG 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 vectors 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. Patent 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., Figure 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., Figure 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 Figure 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 (WPRE) (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  $\geq 10$  infectious doses (where 1 dose is needed on average to transduce 1 target cell). More specifically, a subject may be administered about  $\geq 10$ , about  $\geq 10$ , about  $\geq 10$ , or about  $\geq 10$  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, emulsions, non-aqueous solutions or suppositories. In some embodiments, the solutions or suspensions can include propyleneglycol, polyethyleneglycol, 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 Figure 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 Figure 2. Briefly, and with reference to Figure 2, the top-most vector is a helper plasmid, which, in this case, includes Rev. The vector appearing in the middle of Figure 2 is the envelope plasmid. The bottom-most vector is the therapeutic vector, as described herein. Referring more specifically to Figure 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).  
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 ATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 32) and reverse primer was (5'-CCATACAATGAATGGACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 33). The sequence for the Gag, Pol, Integrase fragment was as follows:

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAG  
GTACAGTATTAGTAGGACCTACACCTGTCAACATAAATGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAG  
CCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCA  
TTGACAGAAGAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAG  
AAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTACAGAACTTAATAAGAGAAGTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTG  
CAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAACAA  
TGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAACAAAATCC  
AGACATAGTCATCTATCAATACATGGATGATTTGTAT  
GTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTACCACACCAGACAAAAACATCAGAAAGAACCTCCATTC  
CTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGG  
CAAGTCAGATTTATGCAGGGATTAAGTAAGGCAA  
TTATGTAACTTCTTAGGGGAACCAAGCACTAACAGAAGTAGTACCCTAACAGAAGAAGCAGAGCTAGAAGTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGT  
GTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAG  
AATGAAGGGTGCCACACTAATGATGTGAAACAATT  
AACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGAGTATTGGCA  
AGCCACCTGGATTCTGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAAGAACCATAATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGC  
CAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT  
AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGAC  
AGACTCACAAATATGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT  
ACCAGCACACAAGGAATTGGAGGAAATGAACAAGTA  
GATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGGCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTAACC  
TACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCAGGAATATGGCAGCTAGATTGTACAC  
ATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTTCATGTA  
GCCAGTGGATATATAGAAGCAGAAGTAATTCAGCAGAGACAGGGCAAGAAAACAGCATACTTCCTCTTAAATAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGC  
AGCAATTCACCAGTACTACAGTTAAGGCCCGCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT  
AGAATCTATGAATAAAGAATTAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGG  
GATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGA  
CAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT  
CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAGGTGATGATTG  
TGTGGCAAGTAGACAGGATGAGGATTAA (SEQ ID NO: 34) 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:

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGC  
CCGAAGGAATAGAAGAAGAAGGTGGAGAGAGACAGAGACAGAT  
CCATTCGATTAGTGAACGGATCCTTGGCCTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTACAGTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAT  
TCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAA  
GCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGG  
CCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA  
TCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAA  
ATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTTGTG  
TCTCTACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATA  
AAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTGTTATTTTTTTCTTTAACATC  
CCTAAAATTTTCTTACATGTTTTACTAGCCAGATTTTT

CTCTCCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTCTATGATCCCTCGACCTGCGTAAGCTTGGCGTAATCATGGTCATGACTGTTTCTGCTGTGAAATTGTTAT  
CCGCTCACAAATCCACACAACATACGAGCCGGAAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTG  
CCCGCTTCCAGTCGGGAAACCTGTGCTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCAGTTCGGCCC  
ATTCTCCGCCCATGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCA  
AAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATA

AAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTACTGCATTCTAGTTGTGGTTGTCCAACTCATCAATGTATCTTATCAGCGGCCGCCCGGG (SEQ ID NO: 35)

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:

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAAATTACGGTAAATGGCCCCGCTGGCTGACCGCCA  
ACGACCCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATTTACGGTAACTGCCCACTTGGCAGTACA  
TCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTAC  
GTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCC  
CACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTGTATTTATTTATTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGGCCAG  
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGG  
CGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCT  
GCGTTGCCCTTGCCTCGTCCCCGCTCCGCGCCGCTCGCGCCGCCCGCCCGGCTCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCT  
GTAATTAGCGCTTGGTTAATGACGGCTCGTTTCTTTCTGTGGCTGCGTGAAGCCTTAAAGGGCTCCGGGAGGGCCCTTGTGCGGGGGGAGCGGCTCGGGGGTGCCTGCGT  
GTGTGTGTCGCTGGGGAGCGCCGCTGCGGCCCGCTGCCG  
GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGCGGTGCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAA  
AGGCTGCGTGCAGGGTGTGTGCGTGGGGGGTGAAGAGGGGTGTGGGCGCGCGGTCCGGCTGTAACCCCCCTGCACCCCCCTCCCCAGTTGCTGAGC  
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGTGGCGGCAGGTGGGGTGCAGGGCGGGGCGGGGCCCTCGGGCC  
GGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCGGAGCGCCGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTTATGGTAATCGTGCAGAGGGGCGAGGGACTTC  
CTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC

GCCGCACCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTGCAGCGCGCCCGTCCCTTCTCCATCTCCAGCCT  
CGGGGCTGCCGACGGGGACGGCTGCCTTCGGGGGGACGGGGCAGGGCGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC (SEQ ID NO: 36)

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:

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTTCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAACTGGAAAAATGTTCTTCTAATTACC  
ATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCAAGAGTACAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGC  
TTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGG  
ACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAG  
TTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTACAGTTCATCAACGGAAA  
ATGCAGCAATTACATATGCCCCACTGTCCATAACTCTAC  
AACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAGGGCACAGG  
GTTTCAAGTAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGCCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAAGTCCCATCAGGTGTCTGGTTGAGATGGCTGATAAGGA  
TCTCTTTGCTGCAGCCAGATTCCCTGAATGCCAGAAGG  
GTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGATTATTCCCTCTGCCAAGAACTGGAGCAAAATCAGAGCGGGTCTT  
CCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAACCCAGGAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCCAG  
ATACATCAGAGTGCATATTGCTGCTCAATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACTACCACAGAAAGGAACTGTGGGATGACTGGGCACCATATGAAGACGTGGAAAT  
TGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCAACAT  
CCTCACATTCAAGACGCTGCTTCGCAACTTCTGATGAT

GAGAGTTTTATTTTTGGTGATACTGGGCTATCCAAAATCCAATCGAGCTTGTAGAAGTTGGTTTCAAGTGTGGAAAAGCTCTATTGCCTTTTTTTCTTTATCATAGGGTTAATCAT  
TGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATTAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC (SEQ ID NO: 37) 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 Figure 3. Briefly, and with reference to Figure 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 Figure 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 (SEQ ID NO: 38); a HIV Rev (SEQ ID NO: 39); 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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TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGC
AGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA
CAGCTCCTAGATCTTTTTCCCTCTGCCAAAATTATGGGGA
CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAAT
CATTTAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCT
GTCCATTCTTATTCCATAGAAAAGCCTTGACTTGAGGT
TAGATTTTTTTTATATTTTGTGTTTATTTTTTCTTTAACATCCCTAAAATTTTCTTACATGTTTTACTAGCCAGATTTTTCTCTCTCCTGACTACTCCCAGTCATAGCTGTCC
CTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATCCACACAACATACGAGCCGGAA
GCATAAAGTGTAAGCCTGGGGTGCTAATGAGTGAGCTAA
CTCACATTAATTGCGTTGCGCTCACTGCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCC
CGCCCCTAACTCCGCCAGTCCGCCATTCTCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAG
GCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCACCC
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GGG (SEQ ID NO: 35) 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: CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTT AGGGCAAAAAGCGGGGCTTCGGTTGTACGCGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTAGTCTTGCA ACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGCCTTATTAGGA AGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCGCATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGACCATTACCACATTG GTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGACCGATCCAGCCTCCCCTCGAAG CTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAG

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ACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCCCAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTG
GAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTACGCTACCACCGCTTGAGAGACTTACTCTTGA
TTGTAACGAGGATTGTGGAACCTTCTGGGACGCAGGGGGT GGAAGCCCTCAAATATTGGTGGAAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA (SEQ ID NO:
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40) 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: 41), phosphoglycerate kinase (PGK) (SEQ ID NO: 42), and ubiquitin C (Ubc) (SEQ ID NO: 43) 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: 44) and bGH poly A (SEQ ID NO: 45) 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: 46), gibbon ape leukemia virus (GALV) (SEQ ID NO: 47), Rabies (FUG) (SEQ ID NO: 48), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 49), influenza A fowl plague virus (FPV) (SEQ ID NO: 50), Ross River alphavirus (RRV) (SEQ ID NO: 51), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 52), or Ebola virus (EboV) (SEQ ID NO: 53). 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'δ 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 corresponding 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: 54), or 7SK (SEQ ID NO: 55) 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-1 alpha 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: GTCCTGGAGTACAATGCCATT (FDPS target sequence #1; SEQ ID NO: 56); GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGACTCCAGGACTTTTT (FDPS shRNA sequence #1; SEQ ID NO: 1); GCAGGATTCGTTCCAGCACTT (FDPS target sequence #2; SEQ ID NO: 57); GCAGGATTCGTTCCAGCACTTCTCGAGAAGTCTGAACGAAATCCTGCTTTTT (FDPS shRNA sequence #2; SEQ ID NO: 2); GCCATGTACATGGCAGGAATT (FDPS target sequence #3; SEQ ID NO: 58); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (FDPS shRNA sequence #3; SEQ ID NO: 3); GCAGAAGGAGGCTGAGAAAGT (FDPS target sequence #4; SEQ ID NO: 59); and GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (FDPS shRNA sequence #4; SEQ ID NO: 4). 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 19-22mer 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: AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (miR30 FDPS sequence #1; SEQ ID NO: 5) AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT (miR30 FDPS sequence #2; SEQ ID NO: 6) TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA (miR30 FDPS sequence #3; SEQ ID NO: 7) CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACTAGC ACTCA (miR155 FDPS sequence #1; SEQ ID NO: 8) CATCTCCATGGCTGTACCACCTTGTCTGGGACTTTCTCAGCCTCCTTCTGCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTTATCTGACCA (miR21 FDPS sequence #1; SEQ ID NO: 9) GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCTGGTCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCCTCCCAATGACCGCTTCTGCTCG (miR185 FDPS sequence #1; SEQ ID NO: 10) **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 Figure 5. THP1 cells (1x10<sup>6</sup> 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 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 3.1% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 5%. With zoledronic acid treatment, LV-control stimulated 7.2% of TNF-α expressing Vγ9Vδ2 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 Figure 6. THP1 cells (1x10<sup>6</sup> 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 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.9% of TNF-α expressing Vγ9Vδ2 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 Vγ9Vδ2 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 Figure 7. PC3 cells were transduced with LV-control or LV-FDPS shRNA #1 (SEQ ID NO: 1) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #1 stimulated 0.5%. With zoledronic acid treatment, LV-control stimulated 1.7% of TNF-α expressing Vγ9Vδ2 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 Figure 8. PC3 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.5% of TNF-α expressing Vγ9Vδ2 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 Vγ9Vδ2 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 Figure 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 μM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.4% of TNF-α expressing Vγ9Vδ2 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 Vγ9Vδ2 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 Figure 10. THP1 cells (1x10<sup>6</sup> cells) were transduced with LV-control or LV-miR30 FDPS # (SEQ ID NO: 5) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with 5x10<sup>6</sup> 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 Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in

the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF- $\alpha$  expressing Vy9V $\delta$ 2 T cells and LV-miR30 FDPS stimulated 8.1%. With zoledronic acid treatment, LV-control stimulated 5.3% of TNF- $\alpha$  expressing Vy9V $\delta$ 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 Figure 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 Figure 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 Figure 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 non-targeting sequence (5'- GCCGCTTTGTAGGATAGAGCTCGAGCTCTATCCTACAAAGCGGCTTTTT-3') (SEQ ID NO: 60) or one of four different FDPS shRNA sequences GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGACTCCAGGACTTTTT (FDPS shRNA sequence #1; SEQ ID NO: 1); GCAGGATTCGTTCCAGCACTTCTCGAGAAGTGTGAACGAAATCCTGCTTTTT (FDPS shRNA sequence #2; SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCTGCCATGTACATGGCTTTTT (FDPS shRNA sequence #3; SEQ ID NO: 3); and GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (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: 61) and Reverse primer: 5'- CCCAAAGAGGTCAAGGTAATCA-3' (SEQ ID NO: 62)). FDPS expression was normalized to actin levels for each sample using the actin primers (Forward primer: 5'-AGCGCGCTACAGCTTCA-3' (SEQ ID NO: 63) and Reverse primer: 5'- GGCGACGTAGCACAGCTTCT-3') (SEQ ID NO: 64). 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 Figure 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 $\alpha$  promoter (SEQ ID NO: 41) 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 GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (FDPS shRNA sequence #4; SEQ ID NO: 4) or the EF-1 $\alpha$  promoter (SEQ ID NO: 41) and miR30-based FDPS sequences AAGGTATATTGCTGTTGACAGTGAGCGGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGTGCTGCTACTGCCTCGGACTTCA AGGGGCT (miR30 FDPS sequence #1; SEQ ID NO: 5) and AAGGTATATTGCTGTTGACAGTGAGCGGACACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGTGCTGCTACTGCCTCGGACTTCAAGGGGCT (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- $\alpha$  expression in GD T cells (Figure 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 $\mu$ M zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with 5X10 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 $\gamma$ 9V $\delta$ 2 T cells. After staining for V $\gamma$ 9V $\delta$ 2 and TNF- $\alpha$  using fluorophore-conjugated anti TCR-V $\delta$ 2 and anti-TNF- $\alpha$  antibody, cells were analyzed via flow cytometry. Live cells were gated, and V $\delta$ 2+ and TNF- $\alpha$ + cells were selected on a dot blot. The activated cytotoxic V $\gamma$ 9V $\delta$ 2 T cells appeared in the upper right quadrant of flow cytograms (Figure 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 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: 65), and Right Inverted Terminal Repeat (Right ITR; SEQ ID NO: 66) can be found in Figure 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 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). 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 intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is 1x10<sup>8</sup> transducing units and escalation is 10-fold to a next dose of 1x10<sup>9</sup> transducing units, the next dose is 1x10<sup>10</sup> transducing units, and a maximum dose of 1x10<sup>11</sup> 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 > 2,500/mm<sup>3</sup>; ANC > 1000/mm<sup>3</sup>; Hemoglobin > g/dL; Platelet count > 50,000/mm<sup>3</sup>; Coagulation INR < 1.3. • AST and ALT < 5 times ULN; ALPS < 5 times ULN. Bilirubin <1.5 times ULN; Creatinine < 1.5 times ULN and eGFR > 50. • Thyroid function: Total T3 or free T3, total T4 or free T4 and TSH < CTCAE Grade abnormality. • Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician. • Immunological function: Circulating Vgamma9Vdelta2+ T cells > 30/mm<sup>3</sup>; 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 aminobisphosphonate 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.

• Aminobisphosphonate 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 aminobisphosphonate 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 aminobisphosphonate 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 aminobisphosphonate 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 aminobisphosphonate 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 aminobisphosphonate 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 aminobisphosphonate 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 1x10 transducing units and escalation is 10-fold to a next dose of 1x10 transducing units, the next dose is 1x10 transducing units, and a maximum dose of 1x10 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 > 2,500/mm; ANC > 1000/mm; Hemoglobin > 8 g/dL; Platelet count > 50,000/mm; Coagulation INR < 1.3. • AST and ALT < 5 times ULN; ALPS < 5 times 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 TSH < CTCAE Grade abnormality. • Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician. • Immunological function: Circulating Vgamma9Vdelta2+ T cells > 30/mm; no immunodeficiency disease. • Negative for HIV by serology and viral RNA test. • Written informed consent.

Exclusion criteria • Intolerant to or unwilling to continue aminobisphosphonate adjunct therapy. • Objective clinical response after aminobisphosphonate 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 aminobisphosphonate, 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 1x10 transducing units and escalation is 10-fold to a next dose of 1x10 transducing units, the next dose is 1x10 transducing units, and a maximum dose of 1x10 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) 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 > 2,500/mm<sup>3</sup>; ANC > 1000/mm<sup>3</sup>; Hemoglobin > g/dL; Platelet count > 50,000/mm<sup>3</sup>; Coagulation INR < 1.3. • AST and ALT < 5 times ULN; ALPS < 5 times 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 TSH < CTCAE Grade abnormality. • Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician. • Immunological function: Circulating Vgamma9Vdelta2+ T cells > 30/mm<sup>3</sup>; 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 aminobisphosphonate 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. • 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.

## Patent Citations (152) ▲

Publication number	Priority date	Publication date	Assignee	Title
Family To Family Citations				
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<a href="#">WO1993024632A1</a>	1992-05-22	1993-12-09	Dana Farber Cancer Institute	Hybrid siv/hiv-1 viral vectors and monkey model for aids
<a href="#">AU6014094A</a>	1992-12-02	1994-06-22	Baylor College Of Medicine	Episomal vectors for gene therapy
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<a href="#">WO1999009139A1</a>	1997-08-15	1999-02-25	Rubicon Laboratory, Inc.	Retrovirus and viral vectors



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<a href="#">DK1115290T3</a>	1998-10-01	2009-06-22	Univ Southern California	Retroviral gene delivery system and methods for its use
<a href="#">US6156514A</a>	1998-12-03	2000-12-05	Sunol Molecular Corporation	Methods for making recombinant cells
<a href="#">US6410013B1</a>	1999-01-25	2002-06-25	Musc Foundation For Research Development	Viral vectors for use in monitoring HIV drug resistance
<a href="#">WO2000072886A1</a>	1999-05-26	2000-12-07	Dana-Farber Cancer Institute, Inc.	Episomally replicating lentiviral vectors
<a href="#">AU2001257611A1</a>	2000-04-28	2001-11-12	Avigen, Inc.	Polynucleotides for use in recombinant adeno-associated virus virion production
<a href="#">AU2001261515A1</a>	2000-05-12	2001-11-26	The Regents Of The University Of California	Treatment of human papillomavirus (hpv)-infected cells
<a href="#">WO2001091802A1</a> *	2000-05-30	2001-12-06	Baylor College Of Medicine	Chimeric viral vectors for gene therapy
<a href="#">NO314588B1</a>	2000-09-04	2003-04-14	Bionor Immuno As	HIV peptides, antigens, vaccine composition, immunoassay test kits and a method for detecting antibodies induced by HIV
<a href="#">US7122181B2</a> *	2000-12-19	2006-10-17	Research Development Foundation	Lentiviral vector-mediated gene transfer and uses thereof
<a href="#">US20030119770A1</a>	2001-08-02	2003-06-26	Zhennan Lai	Intercellular delivery of a herpes simplex virus VP22 fusion protein from cells infected with lentiviral vectors
<a href="#">WO2003015708A2</a>	2001-08-18	2003-02-27	Myriad Genetics, Inc	Composition and method for treating hiv infection
<a href="#">US7737124B2</a>	2001-09-13	2010-06-15	California Institute Of Technology	Method for expression of small antiviral RNA molecules with reduced cytotoxicity within a cell
<a href="#">WO2003040311A2</a>	2001-10-25	2003-05-15	The Government Of The United States Of America As Represented By The Secretary Of Health And Human Services	Efficient inhibition of hiv-1 viral entry through a novel fusion protein including of cd4
<a href="#">US20070203333A1</a>	2001-11-30	2007-08-30	Mcswiggen James	RNA interference mediated inhibition of vascular endothelial growth factor and vascular endothelial growth factor receptor gene expression using short interfering nucleic acid (siNA)
<a href="#">CA2479530A1</a>	2002-03-20	2003-10-02	Massachusetts Institute Of Technology	Hiv therapeutic
<a href="#">US20040142416A1</a>	2002-04-30	2004-07-22	Laipis Philip J.	Treatment for phenylketonuria
<a href="#">WO2004037847A2</a>	2002-05-07	2004-05-06	Chiron Corporation	Hiv envelope-cd4 complexes and hybrids

<a href="#">US7199107B2</a>	2002-05-23	2007-04-03	Isis Pharmaceuticals, Inc.	Antisense modulation of kinesin-like 1 expression
<a href="#">US20040161412A1</a>	2002-08-22	2004-08-19	The Cleveland Clinic Foundation	Cell-based VEGF delivery
<a href="#">DK1545204T3</a>	2002-09-06	2016-11-14	The Government Of The Us Secretary Dept Of Health And Human Services	Immunotherapy with in vitro selected antigen-specific lymphocytes following non-myeloablative lymphodepletive chemotherapy
<a href="#">JP2006505288A</a>	2002-11-04	2006-02-16	ユニバーシティー オブ マサチューセッツ	Allele-specific RNA interference
<a href="#">AU2003283174A1</a>	2002-12-11	2004-06-30	Cytos Biotechnology Ag	Method for protein production
<a href="#">WO2004104591A2</a> *	2003-05-23	2004-12-02	Institut National De La Sante Et De La Recherche Medicale	Improvements to gamma delta t cell-mediated therapy
<a href="#">EP1644508A1</a>	2003-07-11	2006-04-12	Cytos Biotechnology AG	Gene expression system
<a href="#">US20050019927A1</a>	2003-07-13	2005-01-27	Markus Hildinger	DECREASING GENE EXPRESSION IN A MAMMALIAN SUBJECT IN VIVO VIA AAV-MEDIATED RNAi EXPRESSION CASSETTE TRANSFER
<a href="#">US20050138677A1</a>	2003-09-16	2005-06-23	Pfister Herbert J.	Transgenic animal model for the treatment of skin tumors
<a href="#">WO2005028634A2</a>	2003-09-18	2005-03-31	Emory University	Improved mva vaccines
<a href="#">WO2005033282A2</a>	2003-10-01	2005-04-14	Pharmacia & Upjohn Company Llc	Polyamide compositions and therapeutic methods for treatment of human papilloma virus
<a href="#">US20080039413A1</a>	2003-10-21	2008-02-14	Morris David W	Novel compositions and methods in cancer
<a href="#">EP1753777B1</a>	2004-02-25	2014-05-07	Dana-Farber Cancer Institute, Inc.	METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HIV INFECTION USING TRIM5a
<a href="#">EP1737956A2</a>	2004-03-01	2007-01-03	Massachusetts Institute of Technology	Rnai-based therapeutics for allergic rhinitis and asthma
<a href="#">TWI439284B</a>	2004-04-09	2014-06-01	Abbvie Biotechnology Ltd	Multiple-variable dose regimen for treating tnfa-related disorders
<a href="#">US20080227736A1</a>	2004-06-03	2008-09-18	Regents Of The University Of California,	Targeting Pseudotyped Retroviral Vectors
<a href="#">WO2006012221A2</a>	2004-06-25	2006-02-02	The Regents Of The University Of California	Target cell-specific short interfering rna and methods of use thereof
<a href="#">WO2006023491A2</a>	2004-08-16	2006-03-02	The Cbr Institute For Biomedical Research, Inc.	Method of delivering rna interference and uses thereof
<a href="#">WO2006039721A2</a> *	2004-10-08	2006-04-13	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods for bone resorption diseases, cancer, bone pain, immune disorders, and infectious diseases

<a href="#">EP1647595A1</a>	2004-10-15	2006-04-19	Academisch Medisch Centrum bij de Universiteit van Amsterdam	Nucleic acids against viruses, in particular HIV
<a href="#">WO2006048215A1</a>	2004-11-02	2006-05-11	Istituto Di Ricerche Di Biologia Molecolare P Angeletti Spa	Adenoviral amplicon and producer cells for the production of replication-defective adenoviral vectors, methods of preparation and use thereof
<a href="#">US7790446B2</a>	2005-02-11	2010-09-07	Kosagen Cell Factory Oü	Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products
<a href="#">EP2573185A3</a> *	2005-02-16	2013-06-05	Lentigen Corporation	Lentiviral vectors and their use
<a href="#">DK2002003T3</a>	2005-05-27	2016-03-21	Ospedale San Raffaele Srl	Gene vector comprising miRNA
<a href="#">US20070032443A1</a> *	2005-08-02	2007-02-08	Jaeseob Kim	Therapy for Alzheimer's disease
<a href="#">WO2007015122A1</a> *	2005-08-02	2007-02-08	Genexel, Inc.	Therapy for alzheimer's disease
<a href="#">WO2007056388A2</a>	2005-11-07	2007-05-18	The General Hospital Corporation	Compositions and methods for modulating poly (adp-ribose) polymerase activity
<a href="#">WO2007133674A2</a>	2006-05-12	2007-11-22	Lentigen Corporation	Lentiviral vector compositions, methods and applications
<a href="#">US8535897B2</a> *	2006-06-19	2013-09-17	The Trustees Of Columbia University In The City Of New York	Assays for non-apoptotic cell death and uses thereof
<a href="#">US20080003225A1</a>	2006-06-29	2008-01-03	Henri Vie	Method for enhancing the antibody-dependent cellular cytotoxicity (ADCC) and uses of T cells expressing CD16 receptors
<a href="#">WO2008008719A2</a>	2006-07-10	2008-01-17	Alnylam Pharmaceuticals, Inc.	Compositions and methods for inhibiting expression of the myc gene
<a href="#">EP1878440A1</a>	2006-07-13	2008-01-16	INSERM (Institut National de la Santé et de la Recherche Médicale)	Methods and compositions for increasing the efficiency of therapeutic antibodies using gamma delta cell activator compounds
<a href="#">CN101516365A</a> *	2006-07-26	2009-08-26	诺瓦提斯公司	Inhibitors of undecaprenyl pyrophosphate synthase
<a href="#">US20080199961A1</a> *	2006-08-25	2008-08-21	Avi Biopharma, Inc.	ANTISENSE COMPOSITION AND METHOD FOR INHIBITION OF miRNA BIOGENESIS
<a href="#">WO2008100292A2</a> *	2006-10-16	2008-08-21	Genelux Corporation	Modified vaccinia virus strains for use in diagnostic and therapeutic methods
<a href="#">ES2639568T3</a>	2007-01-23	2017-10-27	Janssen Pharmaceutica Nv	Method to design a drug regimen for HIV-infected patients
<a href="#">CA2682694A1</a> *	2007-04-12	2008-10-23	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods with enhanced potency for multiple targets including fpps, ggpps, and dpps

<a href="#">US20080293142A1</a>	2007-04-19	2008-11-27	The Board Of Regents For Oklahoma State University	Multiple shRNA Expression Vectors and Methods of Construction
<a href="#">EP2008656A1</a>	2007-06-28	2008-12-31	Bergen Teknologioverforing AS	Compositions for the treatment of hyperphenylalaninemia
<a href="#">US8673477B2</a>	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
<a href="#">WO2009026328A2</a>	2007-08-21	2009-02-26	Immune Disease Institute, Inc.	Methods of delivery of agents to leukocytes and endothelial cells
<a href="#">CA3018281C</a>	2007-09-28	2022-02-22	Anthrogenesis Corporation	Tumor suppression using human placental perfusate and human placenta-derived intermediate natural killer cells
<a href="#">EP2090659A1</a>	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.	Infectious particle, process for its preparation and use thereof
<a href="#">GB0810209D0</a>	2008-06-04	2008-07-09	Cambridge Entpr Ltd	Pluripotency associated epigenetic factor
<a href="#">US8629334B2</a>	2008-07-16	2014-01-14	University Of Florida Research Foundation, Inc.	Viral-based transient-expression vector system for trees
<a href="#">WO2010022195A2</a>	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system
<a href="#">EP2342321B1</a>	2008-09-17	2018-04-11	Isogenis, Inc.	Construction of fully-deleted adenovirus-based gene delivery vectors and uses thereof
<a href="#">WO2010045659A1</a>	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
<a href="#">US8734795B2</a>	2008-10-31	2014-05-27	Biogen Idec Ma Inc.	Light targeting molecules and uses thereof
<a href="#">WO2010051521A1</a>	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection
<a href="#">WO2011071476A2</a>	2008-11-14	2011-06-16	Life Technologies Corporation	Compositions and methods for engineering cells
<a href="#">EP2191834A1</a>	2008-11-26	2010-06-02	Centre National De La Recherche Scientifique (Cnrs)	Compositions and methods for treating retrovirus infections
<a href="#">US20120114618A1</a>	2009-03-26	2012-05-10	The Regents Of The University Of California	Mesenchymal Stem Cells Producing Inhibitory RNA for Disease Modification
<a href="#">WO2010117974A2</a>	2009-04-09	2010-10-14	Stemcyte Inc.	Hiv-resistant stem cells and uses thereof
<a href="#">EP2419113B1</a>	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
<a href="#">EP2425001A4</a>	2009-04-30	2012-11-14	Univ California	Combination anti-hiv vectors, targeting vectors, and methods of use

<a href="#">EP3329772B1</a>	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
<a href="#">US20120027725A1</a> *	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules to treat liver cancer
<a href="#">CN101805750B</a> *	2009-12-29	2011-11-30	浙江大学	Construction and application of farnesyl pyrophosphoric acid synthetase RNA (Ribonucleic Acid) interference recombinant lentivirus vector
<a href="#">CN102782136A</a>	2010-02-18	2012-11-14	爱默雷大学	Vectors expressing HIV antigens and GM-CSF and related methods for generating an immune response
<a href="#">WO2011119942A1</a>	2010-03-25	2011-09-29	Vistagen Therapeutics, Inc.	Induction of ips cells using transient episomal vectors
<a href="#">WO2011133687A2</a> *	2010-04-20	2011-10-27	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-adrenergic receptor degradation
<a href="#">LT2561078T</a>	2010-04-23	2019-01-10	Cold Spring Harbor Laboratory	NOVEL STRUCTURALLY DESIGNED shRNAs
<a href="#">US20110293571A1</a> *	2010-05-28	2011-12-01	Oxford Biomedica (Uk) Ltd.	Method for vector delivery
<a href="#">WO2012020757A1</a>	2010-08-10	2012-02-16	タカラバイオ株式会社	Production method for cell populations
<a href="#">US20130281493A1</a>	2010-10-07	2013-10-24	The Trustees Of The University Of Columbia In The City Of New York	Method for Treating Cancer Harboring a p53 Mutation
<a href="#">WO2012061075A2</a>	2010-10-25	2012-05-10	The Regents Of The University Of California	Hiv resistant and functional hematopoietic stem/progenitor cells and macrophages from induced pluripotent stem cells
<a href="#">CN108744262A</a>	2010-11-23	2018-11-06	普莱萨格生命科学公司	Treatment and composition for for physical delivery
<a href="#">WO2012115980A1</a>	2011-02-22	2012-08-30	California Institute Of Technology	Delivery of proteins using adeno-associated virus (aav) vectors
<a href="#">US9226976B2</a> *	2011-04-21	2016-01-05	University Of Massachusetts	RAAV-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
<a href="#">US9358250B2</a>	2011-10-15	2016-06-07	Genentech, Inc.	Methods of using SCD1 antagonists
<a href="#">EP2782596A4</a>	2011-11-22	2015-07-29	Philadelphia Children Hospital	Virus vectors for highly efficient transgene delivery
<a href="#">US9745631B2</a> *	2011-12-20	2017-08-29	Dana-Farber Cancer Institute, Inc.	Methods for diagnosing and treating oncogenic kras-associated cancer
<a href="#">BR112014019431A8</a>	2012-02-07	2017-07-11	Global Bio Therapeutics Usa Inc	COMPARTMENTALIZED METHOD OF DELIVERY OF NUCLEIC ACID AND COMPOSITIONS AND USES THEREOF
<a href="#">WO2013174404A1</a>	2012-05-23	2013-11-28	Ganymed Pharmaceuticals Ag	Combination therapy involving antibodies against claudin 18.2 for treatment of cancer

<a href="#">AU2013273483A1</a>	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
<a href="#">WO2014016817A2</a>	2012-07-17	2014-01-30	Universite De Geneve	Nucleic acids for down-regulation of gene expression
<a href="#">CA2922005A1</a>	2012-09-27	2014-04-03	Population Diagnostics, Inc.	Methods and compositions for screening and treating developmental disorders
<a href="#">JP6391582B2</a>	2012-11-13	2018-09-19	コーディアック バイオサイエンシズ インコーポレイテッド	Methods for delivering therapeutic agents
<a href="#">CA2892448A1</a>	2012-12-05	2014-06-12	Sangamo Biosciences, Inc.	Methods and compositions for regulation of metabolic disorders
<a href="#">US9642921B2</a>	2012-12-20	2017-05-09	Tocagen Inc.	Cancer combination therapy and recombinant vectors
<a href="#">WO2014117050A2 *</a>	2013-01-26	2014-07-31	Mirimus, Inc.	Modified mirna as a scaffold for shrna
<a href="#">CN103184224A</a>	2013-04-03	2013-07-03	衡阳师范学院	Triple minRNA for resisting virus infection of aids and construction method thereof
<a href="#">WO2014187881A1</a>	2013-05-21	2014-11-27	Max-Planck Gesellschaft zur Förderung der Wissenschaften e.V.	Isoforms of gata6 and nkx2-1 as markers for diagnosis and therapy of cancer and as targets for anti-cancer therapy
<a href="#">KR20160011645A</a>	2013-06-03	2016-02-01	떼라백띠스	LENTIVIRAL VECTORS CONTAINING AN MHC CLASS I, MHC CLASS II OR $\beta$ 2 MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE
<a href="#">AU2014296059B2</a>	2013-08-02	2020-12-10	The Regents Of The University Of California	Engineering antiviral T cell immunity through stem cells and chimeric antigen receptors
<a href="#">WO2015042308A2</a>	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors
<a href="#">AU2014340083B2</a>	2013-10-22	2019-08-15	Translate Bio, Inc.	mRNA therapy for phenylketonuria
<a href="#">CN106459995B</a>	2013-11-07	2020-02-21	爱迪塔斯医药有限公司	CRISPR-associated methods and compositions using dominant grnas
<a href="#">EP2878674A1</a>	2013-11-28	2015-06-03	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)	Stable episomes based on non-integrative lentiviral vectors
<a href="#">WO2015148926A1</a>	2014-03-28	2015-10-01	Regents Of The University Of Minnesota	Polypeptides, cells, and methods involving engineered cd16
<a href="#">CA2946312A1</a>	2014-04-23	2015-10-29	Juno Therapeutics, Inc.	Methods for isolating, culturing, and genetically engineering immune cell populations for adoptive therapy
<a href="#">PL3689899T3 *</a>	2014-04-25	2022-01-31	2Seventy Bio, Inc.	Mnd promoter chimeric antigen receptors
<a href="#">DK3851537T3</a>	2014-04-25	2024-03-18	Genethon	TREATMENT OF HYPERBILIRUBINAMIA

<a href="#">CA2955254A1</a>	2014-08-29	2016-03-03	Immunomedics, Inc.	Identification of cancer genes by in-vivo fusion of human cancer cells and animal cells
<a href="#">SI3197472T1</a>	2014-09-22	2022-01-31	Tanea Medical Ab	Recombinant phe-free proteins for use in the treatment of phenylketonuria
<a href="#">AU2015329696A1</a>	2014-10-10	2017-04-27	The United States Of America, As Represented By The Secretary, Department Of Health And Human Services	Methods to eliminate cancer stem cells by targeting CD47
<a href="#">CN107405357B</a>	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
<a href="#">WO2016069716A1</a>	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds
<a href="#">GB201509202D0</a>	2015-05-28	2015-07-15	Ge Healthcare Bio Sciences Ab	Semi-static cell culture
<a href="#">JP6924487B2</a>	2015-06-10	2021-08-25	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-embedded virus delivery system and how to use it
<a href="#">WO2017007994A1</a>	2015-07-08	2017-01-12	American Gene Technologies International Inc.	Hiv pre-immunization and immunotherapy
<a href="#">JP6780870B2</a>	2015-08-13	2020-11-04	北吳干細胞与再生医学研究院有限公司 B e i h a o S t e m C e l l A n d R e g e n e r a t i v e M e d i c i n e R e s e a r c h I n s t i t u t e C o . , L t d .	Induced expanded pluripotent stem cells, how to make and use
<a href="#">CN105112370B *</a>	2015-08-25	2019-02-05	杭州优善生物科技有限公司	A kind of method and its application of stimulated in vitro peripheral blood gamma delta T cells high efficiently multiplying
<a href="#">JP7059179B2</a>	2015-10-20	2022-04-25	アンスティチュ ナショナル ドゥ ラ サンテ エ ドゥ ラ ルシエルシュ メディカル	Methods and products for genetic engineering
<a href="#">US11389546B2</a>	2015-12-09	2022-07-19	Modernatx, Inc.	Heterologous UTR sequences for enhanced mRNA expression
<a href="#">US10137144B2</a>	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
<a href="#">EP4310500A3</a>	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta t-cells
<a href="#">EP3413926A4</a>	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
<a href="#">WO2017156311A2</a>	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
<a href="#">BR112018069090A2</a>	2016-03-23	2019-01-29	Univ New York State Res Found	cancer treatment based on distribution of oligos through communicating junctions from human mesenchymal stem cells (hmssc)

<a href="#">WO2017173453A1</a>	2016-04-01	2017-10-05	The Brigham And Women's Hospital, Inc.	Stimuli-responsive nanoparticles for biomedical applications
<a href="#">JP7173548B2</a>	2016-06-08	2022-11-16	アメリカン ジーン テクノ ロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
<a href="#">AU2017292582C1</a>	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
<a href="#">EP3487507A4</a>	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
<a href="#">KR20190100318A</a>	2016-12-30	2019-08-28	더 트러스티스 오브 더 유니버시티 오브 펜실바니아	Gene therapy to treat phenylketonuria
<a href="#">EP3565564A4</a>	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
<a href="#">CN110621322A</a>	2017-02-08	2019-12-27	达纳-法伯癌症研究所有限公司	Modulatable endogenous protein degradation with heterobifunctional compounds
<a href="#">BR112019017839A2</a> *	2017-02-28	2020-04-14	Univ Pennsylvania	clade f vector of adeno-associated virus (aav) and its use
<a href="#">US11820999B2</a>	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
<a href="#">US20200181645A1</a>	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
<a href="#">CN111433368A</a>	2017-10-02	2020-07-17	美国基因技术国际有限公司	Vector with promoter and enhancer combination for treating phenylketonuria
<a href="#">DE112019000348B4</a>	2018-01-05	2021-10-28	Nantbio, Inc.	REPROGRAMMED T-CELLS SIMILAR NK-CELLS
<a href="#">WO2020011247A1</a>	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
<a href="#">US11352646B2</a>	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
<a href="#">KR20220068954A</a>	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression
<a href="#">IL296096A</a>	2020-03-03	2022-11-01	American Gene Tech Int Inc	On demand expression of exogenous factors in lymphocytes to treat hiv

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<a href="#">US10137144B2</a>	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
<a href="#">EP4310500A3</a>	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta t-cells
<a href="#">EP3413926A4</a>	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
<a href="#">WO2017156311A2</a>	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
<a href="#">AU2017292582C1</a>	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
<a href="#">EP3487507A4</a>	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
<a href="#">US11820999B2</a>	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
<a href="#">US20200181645A1</a> *	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
<a href="#">KR20200051011A</a> *	2017-09-08	2020-05-12	제너레이션 바이오 컴퍼니	Modified closed-terminated DNA (CEDNA)
<a href="#">US11352646B2</a>	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
<a href="#">CN109363955B</a> *	2018-11-14	2021-09-21	广州玮弘祺生物科技有限公司	Solid styling spray for hair and preparation method thereof
<a href="#">CN109456993A</a> *	2018-11-28	2019-03-12	上海安民生物技术有限公司	The albumin expression vectors of the promoter containing CAG
<a href="#">WO2020237219A1</a> *	2019-05-23	2020-11-26	Rocket Pharmaceuticals, Ltd.	Gene therapy vectors for infantile malignant osteopetrosis

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

## Similar Documents



Publication	Publication Date	Title
<a href="#">US11519006B2</a>	2022-12-06	Methods and compositions for the activation of gamma-delta T-cells
<a href="#">US11534450B2</a>	2022-12-27	Methods and compositions for the activation of gamma-delta T-cells
<a href="#">US11242527B1</a>	2022-02-08	Combination vectors and methods for treating cancer

## Priority And Related Applications

### Applications Claiming Priority (2) ▲

Application	Filing date	Title
US201662279474P	2016-01-15	
<a href="#">PCT/US2017/013399</a>	2017-01-13	Methods and compositons for the activation of gamma-delta t-cells

## Concepts ▲

machine-extracted

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Name	Image	Sections	Count	Query match
■ method		title,claims,description	68	0.000
■ gamma-delta t lymphocyte		title,claims,description	13	0.000
■ mixture		title,description	45	0.000
■ activation		title,description	19	0.000
■ cell		claims,description	233	0.000
■ vector		claims,description	155	0.000
■ Geranyltranstransferase		claims,description	137	0.000
■ Farnesyl pyrophosphate synthase		claims,description	135	0.000
■ T-lymphocyte		claims,description	104	0.000
■ plasmid		claims,description	94	0.000
■ genetic effect		claims,description	55	0.000
■ zoledronic acid		claims,description	47	0.000
■ Neoplasm		claims,description	45	0.000
■ zoledronic acid		claims,description	40	0.000

■ (3s)-4-[[[(2s)-1-[[[(2s)-1-[[(1s)-1-carboxy-2-hydroxyethyl]amino]-4-methyl-1-oxopentan-2-yl]amino]-5-(diaminomethylideneamino)-1-oxopentan-2-yl]amino]-3-[[2-[[[(2s)-2,6-diaminohexanoyl]amino]acetyl]amino]-4-oxobutanoic acid	claims,description	39	0.000
■ Tumor Necrosis Factor	claims,description	39	0.000
■ Tumor Necrosis Factor-alpha	claims,description	39	0.000
■ Enzymes	claims,description	38	0.000
■ Enzymes	claims,description	38	0.000
■ MicroRNAs	claims,description	38	0.000
■ microRNA	claims,description	38	0.000
■ viral vector	claims,description	37	0.000
■ particle	claims,description	33	0.000
■ packaging method and process	claims,description	28	0.000
■ cancer	claims,description	26	0.000
■ pathway	claims,description	25	0.000
■ drug	claims,description	23	0.000
■ Chemokines	claims,description	22	0.000
■ Chemokines	claims,description	22	0.000
■ Cytokines	claims,description	22	0.000
■ Cytokines	claims,description	22	0.000
■ DL-mevalonic acid	claims,description	22	0.000
■ virological effect	claims,description	22	0.000
■ (R)-mevalonate	claims,description	21	0.000
■ Bacterial small RNA	claims,description	21	0.000
■ Interleukin-2	claims,description	20	0.000
■ Interleukin-2	claims,description	20	0.000
■ drug	claims,description	20	0.000

<ul style="list-style-type: none"> <li>inhibitory effect</li> </ul>	claims,description	19	0.000
<ul style="list-style-type: none"> <li>manufacturing process</li> </ul>	claims,description	19	0.000
<ul style="list-style-type: none"> <li>infectious disease</li> </ul>	claims,description	18	0.000
<ul style="list-style-type: none"> <li>targeting</li> </ul>	claims,description	17	0.000
<ul style="list-style-type: none"> <li>Envelope protein</li> </ul>	claims,description	15	0.000
<ul style="list-style-type: none"> <li>Protein X</li> </ul>	claims,description	15	0.000
<ul style="list-style-type: none"> <li>4-amino-1-[(2r)-6-amino-2-[[[(2r)-2-[[[(2r)-2-amino-3-phenylpropanoyl]amino]-3-phenylpropanoyl]amino]-4-methylpentanoyl]amino]hexanoyl]piperidine-4-carboxylic acid</li> </ul>	claims,description	14	0.000
<ul style="list-style-type: none"> <li>Communicable disease</li> </ul>	claims,description	14	0.000
<ul style="list-style-type: none"> <li>small Interfering RNA</li> </ul>	claims,description	14	0.000
<ul style="list-style-type: none"> <li>gag Genes</li> </ul>	claims,description	12	0.000
<ul style="list-style-type: none"> <li>pol Genes</li> </ul>	claims,description	12	0.000
<ul style="list-style-type: none"> <li>rev Genes</li> </ul>	claims,description	12	0.000
<ul style="list-style-type: none"> <li>Dependoparvovirus</li> </ul>	claims,description	8	0.000
<ul style="list-style-type: none"> <li>Interferon-gamma</li> </ul>	claims,description	8	0.000
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<ul style="list-style-type: none"> <li>Small hairpin RNA</li> </ul>	claims,description	8	0.000
<ul style="list-style-type: none"> <li>interferon gamma</li> </ul>	claims,description	8	0.000
<ul style="list-style-type: none"> <li>Interleukin-17</li> </ul>	claims,description	7	0.000
<ul style="list-style-type: none"> <li>Interleukin-17</li> </ul>	claims,description	7	0.000
<ul style="list-style-type: none"> <li>activating effect</li> </ul>	claims,description	7	0.000
<ul style="list-style-type: none"> <li>Interleukin-1</li> </ul>	claims,description	6	0.000

■ Interleukin-1	claims,description	6	0.000
■ Interleukin-12	claims,description	6	0.000
■ Interleukin-12	claims,description	6	0.000
■ rev gene	claims,description	6	0.000
■ CC Chemokines	claims,description	4	0.000
■ CC Chemokines	claims,description	4	0.000
■ CXC Chemokine	claims,description	3	0.000
■ CXC chemokine	claims,description	3	0.000
■ Syncytin-1	claims	4	0.000
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