Methods and compositions for activation of gamma delta T cells

Abstract

translated from Japanese

The present invention relates generally to methods and compositions for gene therapy and immunotherapy that activate gamma delta T cells and can be used in particular in the treatment of various cancers and infectious diseases. In one aspect, a method for activating GD T cells is provided. The method includes infecting a target cell with a viral delivery system encoding at least one genetic element in the presence of GD T cells. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of enzymes involved in the mevalonate pathway.

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Claims (25)

Hide Dependent

translated from Japanese

A viral vector comprising at least one encoded genetic element comprising a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. The viral vector according to claim 1, wherein the enzyme is farnesyl diphosphate synthase (FDPS). 2. The viral vector of claim 1, wherein the at least one encoded genetic element comprises microRNA or shRNA. The shRNA is
4. A viral vector according to claim 3 comprising a sequence having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. The shRNA is
The viral vector according to claim 4, comprising: The microRNA is
4. A viral vector according to claim 3 comprising a sequence having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. The microRNA is
The viral vector according to claim 6, comprising: The viral vector according to any one of claims 1 to 7, which is a lentiviral vector. The viral vector according to claim 1, which is an adeno-associated viral vector. 2. The viral vector of claim 1, further comprising a second encoded genetic element comprising at least one cytokine or chemokine. 11. The at least one cytokine is selected from the group consisting of IL-18, TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17 and IL-12. The described viral vector. The viral vector according to claim 10, wherein the at least one chemokine is a CC chemokine, a CXC chemokine, a CX3C chemokine or an XC chemokine. A lentiviral vector system for expressing lentiviral particles,
a. A lentiviral vector according to claim 8;
b. At least one envelope plasmid for expressing an envelope protein optimized to infect cells; and c. comprising at least one helper plasmid for expressing gag, pol and rev genes, wherein said lentiviral vector, said at least one envelope plasmid and said at least one helper plasmid are transfected into a packaging cell A lentiviral vector system, wherein viral particles are produced by the packaging cells, the lentiviral particles can infect target cells and inhibit enzymes involved in the mevalonate pathway in the target cells. The lentiviral vector system according to claim 13, comprising a first helper plasmid for expressing the gag and pol genes and a second plasmid for expressing the rev gene. A lentiviral particle capable of infecting cells, comprising an envelope protein optimized to infect target cells and the lentiviral vector according to claim 8. 16. The lentiviral particle of claim 15, wherein the envelope protein is optimized to infect target cells, and the target cells are cancer cells. 16. The lentiviral particle of claim 15, wherein the envelope protein is optimized to infect target cells, and the target cells are cells that infect infectious diseases. A method of activating gamma delta T cells comprising infecting a target cell with a viral delivery system encoding at least one genetic element in the presence of said GD T cells, said at least one encoded The genetic element comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway, and when the enzyme is inhibited in the target cell, the target cell activates the GD T cell How to turn. A method of treating cancer in a subject 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: When the enzyme contains a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway, and the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell A method of activating cells and thereby treating said cancer. 20. The method according to claim 18 or 19, wherein the enzyme is farnesyl diphosphate synthase (FDPS). 20. A method according to claim 18 or 19, wherein the at least one encoded genetic element comprises microRNA or shRNA. The shRNA is
23. The method of claim 21, comprising a sequence having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. The microRNA is
23. The method of claim 21, comprising a sequence having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. 20. The method of claim 18 or 19, further comprising administering a therapeutically effective amount of an aminobisphosphonate drug to the subject. 25. The method of claim 24, wherein the aminobisphosphonate drug is zoledronic acid.

Description

translated from Japanese

This application is filed on January 15, 2016 and claims priority to US Provisional Patent Application No. 62 / 279,474 entitled “Methods and Compositions for Activation of Gamma Delta T Cells”. And incorporated herein by reference.

The present disclosure relates generally to the fields of gene therapy and immunotherapy, particularly related to increased activation of gamma delta (“GD”) T cells.

Human T cells are differentiated based on T cell receptor structure. A major population comprising CD4 + and CD8 + subsets express receptors composed of alpha and beta chains. A smaller subset expresses T cell receptors composed of gamma and delta chains. Gamma delta (“GD”) T cells constitute 3-10% circulating lymphocytes, and the Vδ2 + subset constitutes 75% of GD T cells in the blood. Vδ2 + cells recognize non-peptide epitopes and do not require antigen presentation by the major

histocompatibility complex (“MHC”) or human leukocyte antigen (“HLA”). The vast majority of Vδ2 + T cells also express the Vγ9 chain and are stimulated by exposure to 5-carbon pyrophosphate compounds, intermediates in the mevalic acid and non-mevalic acid sterol / isoprenoid synthesis pathways. Response to isopentenyl pyrophosphate (5-carbon) is universal in healthy humans.

Vδ1 +, another subset of GD T cells, constitutes a rather small percentage of T cells circulating in the blood, but Vδ + 1 cells are commonly found in epithelial mucosa and skin.

In general, GD T cells have several functions, including the death of tumor cells and pathogen-infected cells. Stimulation through their unique T cell receptor (“TCR”), which is composed of two glycoprotein chains γ and δ, improves the ability of cytotoxicity, cytokine secretion and other effector functions. The TCR of GD T cells has a unique specificity and the cells themselves are present at a high clonal frequency, thus allowing a rapid innate immune-like response against tumors and pathogens.

Of aminobisphosphonate drug (“ABP”) and farnesyl diphosphate synthase (“FDPS”) downstream of isopentenyl pyrophosphate (“IPP”) in the mevalonate pathway (see FIG. 1 for an example) Other inhibitors have been used to treat various diseases including cancer, particularly those involving bone metastasis. ABP includes trade names such as Zometa (registered trademark) (Novatis) and Fosamax (registered trademark) (Merck).

ABP has also been used to stimulate GD T cells. This may be because FDPS is inhibited in bone marrow cells and IPP begins to accumulate, reducing the downstream product geranylgeranyl pyrophosphate (“GGPP”) of FDPS that suppresses inflammasome pathway activation. Reduction in GGPP removes inhibitors of the caspase-dependent inflammasome pathway and allows secretion of mature cytokines including interleukin-beta and interleukin-18, the latter being particularly important for gamma delta T cell activation It is.

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

However, ABP is not only associated with inflammation and osteonecrosis, but also has poor bioavailability due to their chemistry. Similarly, IPP has a very short half-life and is difficult to synthesize. Both types of compounds require systemic administration in the individual. Therefore, both ABP in general, and specifically IPP, are often unsatisfactory for therapeutic purposes.

In one aspect, a method for activating GD T cells is provided. The method includes infecting a target cell with a viral delivery system encoding at least one genetic element in the presence of GD T cells. In embodiments, the at least one genetic element comprises a small RNA capable of inhibiting the production of enzymes involved in the mevalonate pathway. In an embodiment, the enzyme is FDPS. In embodiments, if 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 infected with an infectious agent. In a preferred embodiment, activation of GD T cells results in GD T cells that kill cancer cells or cells infected with infectious agents. In embodiments, the at least one encoded genetic element comprises microRNA or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate drug. In an embodiment, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method for 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 genetic element comprises a small RNA capable of inhibiting the production of enzymes involved in the mevalonate pathway. In a further embodiment, 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, thereby treating the cancer. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises microRNA or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate drug. In an embodiment, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method for 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 genetic element comprises a small RNA capable of inhibiting the production of enzymes involved in the mevalonate pathway. In a further embodiment, if the enzyme is inhibited in cells infected with an infectious agent in the presence of GD T cells, the infected cells activate GD T cells, thereby causing infected cells and infectious diseases. Take action. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises microRNA or shRNA. In a further embodiment, the target cell is also contacted with an aminobisphosphonate drug. In an embodiment, the aminobisphosphonate drug is zoledronic acid.

In another aspect, the at least one encoded genetic element is
And a shRNA having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. In a preferred embodiment, the shRNA is including.

In another aspect, the at least one encoded genetic element is
And a microRNA having a percent identity of at least 80%, or at least 85%, or at least 90%, or at least 95%. In a preferred embodiment, the microRNA is including.

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

In another aspect, the at least one encoded genetic element comprises a shRNA that has a percent identity with v of at least 80%, or at least 85%, or at least 90%, or at least 95%. In preferred embodiments, the shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In another aspect, the at least one encoded genetic element is SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; MicroRNAs having a percent identity of at least 90%, or at least 95%. In a preferred embodiment, the microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9;

In embodiments, the viral vector is comprised of any vector that can efficiently transduce small RNAs into target cells. In embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated viral vector.

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

In another aspect, a lentiviral vector system for expressing lentiviral particles is provided. The system includes a lentiviral vector, at least one envelope plasmid for expressing an envelope protein optimized to infect cells; and at least one helper plasmid for expressing the gag, pol and rev genes. . Lentiviral particles are produced by the packaging cell when the lentiviral vector, at least one envelope plasmid and at least one helper plasmid are transfected into the packaging cell. In embodiments, the lentiviral particles can infect target cells and inhibit enzymes involved in the mevalonate pathway within the target cells. In an embodiment, the enzyme involved in the mevalonate pathway is FDPS. In an embodiment, 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 to infect target cells. In an embodiment, the target cell is a cancer cell. In other embodiments, the target cell is a cell that is infected with an infectious agent.

FIG. 1 outlines the major steps in the mevalonate pathway for steroid and isoprenoid biosynthesis.

FIG. 2 shows an exemplary 3-vector lentiviral vector system in circular form.

FIG. 3 shows an exemplary 4-vector lentiviral vector system in circular form.

FIG. 4 shows (A) a linear map of a lentiviral vector expressing an FDPS shRNA targeting sequence; and (B) a linear map of a lentiviral vector expressing a synthetic microRNA having an FDPS targeting sequence.

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

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

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

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

FIG. 9 shows data demonstrating activation of Vδ2 + T cells by HepG2 cancer cells with lentiviruses expressing FDPS shRNA # 1 (SEQ ID NO: 1) or FDPS shRNA # 4 (SEQ ID NO: 4) described herein. Indicates.

FIG. 10 shows data demonstrating activation of Vδ2 + T cells by THP-1 leukemia cells having a lentivirus expressing miR30 FDPS # 1 (SEQ ID NO: 5) as described herein.

FIG. 11 shows data demonstrating the percent specific lysis versus E: T ratio for the various experimental conditions described herein.

FIG. 12 shows data demonstrating shRNA-based RNA interference targeting the human FDPS gene delivered by lentivirus.

FIG. 13 shows data demonstrating miR-based RNA interference targeting the human FDPS gene delivered by lentivirus.

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

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

SUMMARY OF THE DISCLOSURE The present disclosure relates to farnesyl diphosphate synthase ("FDPS") required to convert isopentenyl pyrophosphate (IPP) to farnesyl diphosphate (FDP), for example as shown in FIG.) Gene therapy construct and its delivery to cells. In embodiments, one or more viral vectors are provided with microRNA or small homologous RNA (shRNA) that targets FDPS, thereby reducing the expression level of this enzyme. Viral vectors include lentiviral vectors and AAV vectors. The result of modulating FDPS expression is to increase the accumulation of IPP, a stimulator of GD T cell proliferation and differentiation. Thus, the constructs provided herein are used to activate GD T cells and are used to treat cancer and infectious diseases.
Definition and interpretation

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure 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. In general, the nomenclature used in conjunction with the cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are such as They are well known and commonly used in the field. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and described in various general and more specific references cited and discussed throughout this specification, unless otherwise specified. The For example, Sambrook J. et al. And Russell D. et al. Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.A. Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Son. (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 reaction or purification technique is performed in accordance with the manufacturer's specifications commonly accomplished in the art or as described herein. The nomenclature used in conjunction with analytical chemistry, synthetic organic chemistry and medicinal chemistry and pharmaceutical chemistry, and their laboratory procedures and techniques described herein are those commonly used in the art. .

As used in the specification and appended claims, the singular forms "a", "an", and "the" unless the context clearly indicates otherwise. They are used interchangeably and are intended to include the plural forms and fall within the meaning of each. Also, as used herein, "and / or" means any and all possible combinations of one or more of the listed items, as well as alternative interpretations ("or"). Refers to the lack of combinations, including these.

All numerical designations, including ranges, pH, temperature, time, concentration and molecular weight are approximations that vary (+) or (-) in 0.1 increments. It should be understood that the term "about" precedes all numerical designations, although not necessarily explicitly stated. 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 is also to be understood that although not necessarily explicitly stated, the reagents described herein are exemplary only and their equivalents are known in the art.

As used herein, the term "about" is understood by one of ordinary skill in the art and varies to some extent depending on the context in which it is used. Where there is a use of a term that is not obvious to one skilled in the art, "about" means up to plus or minus 10% of the particular term, given the context in which it is used.

The term "administration" of an active agent or "administering" an active agent is a therapeutically useful form and a form that can be introduced into an individual's body in a therapeutically effective amount in a subject in need of treatment. It should be understood to mean providing.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not the others. "Consisting essentially of", when used to define compositions and methods, means excluding other elements of any essential importance to the composition or method. "Consisting of" shall

mean excluding non-traceable elements of the claimed composition and other ingredients for substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure. Thus, the methods and compositions may include additional steps and components (comprising), or include and may not include steps and compositions (consisting essentially of) It is intended that only method steps or compositions are intended (consisting of).

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

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

The term “gamma delta T cell” may be referred to herein as a γδ T cell and may further be referred to as a GD T cell. The term “gamma delta T cell activation” refers to any measurable biological phenomenon associated with gamma delta T cells that are representative of such T cells being activated. Non-limiting examples of such biological phenomena include increased cytokine production, changes in qualitative or quantitative composition of cell surface proteins, increases in T cell proliferation, and / or T cell effector functions, such as target cells Or increase in assisting another effector cell to kill the target cell.

The terms “individual”, “subject” and “patient” are used interchangeably herein and refer to any individual mammalian subject, eg, a cow, dog, cat, horse or human.

The term “miRNA” refers to microRNA and may also be referred to herein as “miR”.

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

The term “percent identity” means in the context of two or more nucleic acid or polypeptide sequences the sequence comparison algorithms described below (eg, BLASTP and BLASTN or other available to those skilled in the art). Two having a specified percentage of nucleotide or amino acid residues that are the same when compared and aligned for maximum correspondence when measured using one of the algorithms) or by visual inspection Or more sequences or subsequences. Depending on the application, “percent identity” may exist over a region of the sequences being compared, eg, across functional domains, or alternatively may exist over the entire length of the two sequences being 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 entered 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) compared to the reference sequence, based on the designated program parameters.

The optimal alignment of sequences for comparison is described, for example, in Smith and Waterman, Adv. Appl. Math. 2: 482 (1981), according to the Needleman and Wunsch, J. et al. Mol. Biol. 48: 443 (1970) by the homology alignment algorithm of Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA 85: 2444 (1988) by the search method for similarities, the computer implementations of these algorithms (Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis., GAP, BEST. And TFASTA) or by visual inspection (Ausubel et al., See generally below).

An example of a suitable algorithm for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. Mol. Biol. 215: BLAST algorithm described on pages 403-410 (1990). Software for performing BLAST analyzes is publicly available through the National Center for Biotechnology Information website.

The percent identity between two nucleotide sequences is determined by NWSgapdna. Using the CMP matrix and gap weights of 40, 50, 60, 70 or 80 and length weights of 1, 2, 3, 4, 5 or 6, the GAP program in the GCG software package (http: // available on www.gcg.com). The percent identity between two nucleotide or amino acid sequences is the E. coli incorporated into the ALIGN program (version 2.0) using the PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4. Meyers and W.M. It can also be determined using the algorithm of Miller (CABIOS, 4: 11-17 (1989)). Furthermore, the percent identity between the two amino acid sequences is either the Blossum 62 matrix or the PAM250 matrix, and the gap weight of 16, 14, 12, 10, 8, 6 or 4, and 1, 2, 3, 4, Needleman and Wunsch (J. Mol. Biol. (48) incorporated into the GAP program in the GCG software package (available at http://www.gcg.com) using 5 or 6 length weights. Volume): 444-453 (1970)).

The nucleic acid and protein sequences of the present disclosure can be further used as “query sequences” to perform searches against public databases, for example, to identify related sequences. Such a search is described in Altschul et al. (1990) J. Mol. Mol. Biol. 215: Can be implemented using the NBLAST and XBLAST programs (version 2.0) on pages 403-10. A BLAST nucleotide search can be performed using the NBLAST program, score = 100, word length = 12, in order to obtain nucleotide sequences homologous to the nucleic acid molecules provided in this disclosure. A BLAST protein search can be performed using the XBLAST program, score = 50, word

length = 3 to obtain amino acid sequences that are homologous to the protein molecules of the present disclosure. To obtain a gapped alignment for comparison purposes, Gapped BLAST was developed by Altschul et al. (1997) Nucleic Acids Res. 25 (17): 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of representative programs (eg, XBLAST and NBLAST) can be used. http: // www. ncbi. nlm. nih. See gov.

As used herein, “pharmaceutically acceptable” means reasonably free of excessive toxicity, irritation, allergic response or other problems or complications, commensurate with a reasonable benefit / risk ratio. Refers to compounds, materials, compositions and / or dosage forms suitable for use in contact with human and animal tissues, organs and / or body fluids within reasonable medical judgment.

As used herein, “pharmaceutically acceptable carrier” refers to any and all physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and It refers to absorption delaying agents and the like. The composition may include pharmaceutically acceptable salts, such as acid addition or base addition salts (see, eg, Berge et al. (1977) J Pharm Sci 66: 1-19).
As used herein, the term “SEQ ID NO” is synonymous with the term “SEQ ID NO”.

As used herein, “small RNA” refers to non-coding RNA that possesses a silencing or interference function, generally less than about 200 nucleotides in length or less. 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. It is. Such RNA includes micro RNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA) and small hairpin RNA (shRNA). The “small RNA” of the present disclosure should be capable of inhibiting or knocking down the gene expression of the target gene, generally through a pathway that results in the destruction of the target gene mRNA.

The term “therapeutically effective amount” is sufficient to treat or prevent the progression or prevention of complications found in a patient suffering from a given discomfort, injury, disease or condition, Refers to the amount of active agent of the present disclosure in a suitable composition and in a suitable dosage form. The therapeutically effective amount will vary depending on the condition of the patient or its severity and the condition, such as age, weight, etc. of the subject being treated. A therapeutically effective amount can vary depending on any of a number of factors, including, for example, the route of administration, the condition of the subject, and other factors understood by those of skill in the art.

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

“Treatment” is intended to target and combat the disease state, ie ameliorate or prevent the disease state. Thus, the specific treatment depends on the disease state being targeted, as well as the current and future states of drug therapy and therapeutic approaches. Treatment can be toxic.

The term “treatment” or “treating” generally refers to an intervention that attempts to alter the natural course of the subject being treated and may be performed for prevention or during the course of clinical pathology. Desired effects include preventing the occurrence or recurrence of the disease, reducing symptoms, suppressing, attenuating or inhibiting any direct or indirect pathological consequences of the disease, disease status This includes, but is not limited to, ameliorating or alleviating and causing a remission or improved prognosis.
Description of aspects of the disclosure

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

In an embodiment, the at least one encoded genetic element is
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least ShRNAs having a percent identity of 92%, at least 93%, at least 94%, at least 95% or higher. In a preferred embodiment, the shRNA is
including.

In another aspect, the at least one encoded genetic element is
And at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at

least Includes microRNAs having percent identity of 92%, at least 93%, at least 94%, at least 95% or higher. In a preferred embodiment, the microRNA is including.

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 for 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 comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell activates the GD T cell, thereby treating the cancer. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises microRNA or shRNA.

In another aspect, a method for 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 comprises a small RNA capable of inhibiting the production of an enzyme involved in the mevalonate pathway. In a further embodiment, if the enzyme is inhibited in cells infected with an infectious agent in the presence of GD T cells, the infected cells activate GD T cells, thereby causing infected cells and infectious diseases. Take action. In an embodiment, the enzyme is FDPS. In embodiments, the at least one encoded genetic element comprises microRNA or shRNA.

In embodiments, at least one encoded genetic element is at least 80%, at least 81%, at least 82%, at least 83%, at least 84% of SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4 , At least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or higher percentages Includes shRNAs with identity. In preferred embodiments, the shRNA comprises 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 is at least 80%, at least 81%, at least with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; 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% A microRNA having a percent identity of at least 95% or higher. In a preferred embodiment, the microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9;

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

In another aspect, the at least one encoded genetic element is at least 80%, at least 81%, at least 82%, at least 83%, at least 84 with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; %, At least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or higher Includes shRNAs with percent identity. In preferred embodiments, the shRNA comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In another aspect, the at least one encoded genetic element is at least 80%, at least 81%, at least 82 with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; %, 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%, MicroRNAs having a percent identity of at least 95% or higher. In a preferred embodiment, the microRNA comprises SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9;

In embodiments, viral vectors include any vector that can efficiently transduce small RNAs. 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 comprises a second encoded genetic element. In embodiments, the second genetic element comprises at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from the group consisting of IL-18, TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least one chemokine is a CC chemokine, CXC chemokine, cCX3 chemokine or 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 lentiviral particles is provided. The system includes a lentiviral vector, at least one envelope plasmid for expressing an envelope protein optimized to infect cells; and at least one helper plasmid for expressing the gag, pol and rev genes. . Lentiviral particles are produced by the packaging cell when the lentiviral vector, at least one envelope plasmid and at least one helper plasmid are transfected into the packaging cell. In embodiments, the

lentiviral particles can infect target cells and inhibit enzymes involved in the mevalonate pathway within the target cells. In an embodiment, the enzyme involved in the mevalonate pathway is FDPS. In an embodiment, 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 to infect target cells. In an embodiment, the target cell is a cancer cell. In other embodiments, the target cell is a cell that infects an infectious disease.

cancer

The compositions and methods provided herein are used to treat cancer. The cell, tissue or target may be a cancer cell, cancerous tissue, may possess cancerous tissue, or is diagnosed with or at risk of developing a disease or condition It can be. In certain embodiments, the cells can be epithelial, endothelial, mesothelial, glial, stromal or mucosal cells. Cancer cell populations include brain, neurons, blood, endometrium, meninges, esophagus, lung, cardiovascular, liver, lymphatic system, breast, bone, connective tissue, fat, retina, thyroid, gland, adrenal gland, pancreas , Stomach, intestinal tract, kidney, bladder, colon, prostate, uterus, ovary, cervix, testis, spleen, skin, smooth muscle, cardiac muscle or striated muscle cells. In yet a further aspect, the cancer includes astrocytoma, acute myeloid leukemia, anaplastic large cell lymphoma, acute lymphocytic leukemia, angiosarcoma, B cell lymphoma, Burkitt lymphoma, breast cancer, bladder cancer, head and neck Carcinoma, cervical cancer, chronic lymphoblastic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, squamous cell carcinoma of the esophagus, Ewing sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, Gastric cancer, hepatoblastoma, hepatocellular carcinoma, Kaposi's sarcoma, Hodgkin's lymphoma, laryngeal squamous cell carcinoma, larynx cancer, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, mantle cell lymphoma, marrow Blastoma, mesothelioma, myxofibrosarcoma, myeloid leukemia, mucosa-associated lymphoid tissue B-cell lymphoma, multiple myeloma, high-risk myelodysplastic syndrome, nasopharyngeal carcinoma, neuroblastoma, Transfibroma, high-grade non-Hodgkin lymphoma, non-Hodgkin lymphoma, lung cancer, non-small cell lung cancer, ovarian cancer, esophageal cancer, osteosarcoma, pancreatic cancer, pheochromocytoma, prostate cancer, renal cell carcinoma, retinoblastoma, lateral Examples include, but are not limited to, rhabdomyosarcoma, salivary gland tumor, Schwannoma, small cell lung cancer, squamous cell carcinoma of the head and neck, testicular tumor, thyroid cancer, urothelial cancer and Wilms tumor.

The compositions and methods provided herein include NSCLC (Non-Small Cell Lung Cancer), childhood malignancy, cervical and other tumors caused or promoted by human papillomavirus (HPV), melanoma, Barrett's esophagus (It is also used to treat premalignant syndromes), adrenal and skin cancers and autoimmune, neoplastic skin diseases.

Infectious disease

The compositions and methods disclosed herein can be used to treat infectious diseases. The term “infectious disease” includes any disease caused by an infectious agent. “Infectious agents” include any exogenous pathogen, including, without limitation, bacteria, fungi, viruses, mycoplasmas and parasites. Infectious agents that can be treated with the compositions provided in this disclosure include organisms such as bacteria that are Gram-negative or Gram-positive cocci or bacilli, DNA viruses such as papillomavirus, parvovirus, adenovirus, herpesvirus And vaccinia viruses, and RNA viruses such as arenaviruses, coronaviruses, rhinoviruses, respiratory multinucleated viruses, influenza viruses, picornaviruses, paramyxoviruses, reoviruses, retroviruses and rhabdoviruses Any art-recognized infectious organism that causes pathogenesis in an animal, including but not limited to DNA and RNA viruses. Examples of fungi that can be treated with the compositions and methods of the present disclosure include, for example, diseases such as ringworm, histoplasmosis, blastosomiasis, aspergillosis, cryptococcosis, sporotrichum, coccidioidomycosis, paracoccidioidomycosis and candidiasis Including fungi that cause, fungi that grow as molds or that are yeast-like. The compositions and methods provided herein include parasitic infections including but not limited to somatic tapeworms, schistosomiasis, roundworms, amoeba, and Plasmodium, Trypanosoma, Leishmania, and Toxoplasma species. Can be used to treat.

Method for GD T cell activation

Provided herein are compositions and methods for activating GD T cells in an individual, and methods for treating tumors and infectious diseases. For example, in embodiments, activated GD T cells contain natural mechanisms for tumor immune surveillance, so the compositions and methods provided herein treat all known cancers. (See, for example, Pauza et al. 2014 Frontiers in Immunol. 5: 687). Similarly, in embodiments, the compositions and methods provided herein include flaviviruses, influenza viruses, human retroviruses, mycobacteria, malaria parasites and various other viral, fungal and bacterial infections. It can be used to treat infectious diseases not limited to these (see, eg, Pauza and Cairo, 2015 Cell Immunol. 296 (1)).

In general, vector systems are used to transfect or transduce target cell populations using the disclosed constructs to reduce FDPS expression and, in other embodiments, to increase chemokine or cytokine expression. Administered to an individual. Administration and transfection / transduction can occur in vivo or ex vivo, and the transfected cells are later administered back to the subject in a later scenario.

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

The disclosed methods can also increase the ability 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 immune and inflammatory responses. In addition, GD T cells are known to induce dendritic cell maturation, recruit B cells and macrophages, and are involved in the secretion of various cytolytic activities, such as interferon-γ and TNF-α.

In embodiments, the disclosed compositions and methods provided herein constitute one form of gene therapy for activating GD T cells at the site of a tumor or infectious disease pathology. In one aspect, the compositions and methods provided herein provide specific lytic activity necessary for activating GD T cells and killing cancer cells or treating infectious diseases. By promoting the production of cytokines, they support their growth, differentiation and functional capacity.

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

In embodiments, the therapeutic vector may comprise a single construct or at least 2, at least 3, at least 4 or at least 5 different constructs. If more than one construct is present in the vector, these constructs may be the same or different. For example, the constructs can vary with respect to their promoter, the presence or absence of integration elements, and / or their sequences. In some embodiments, the therapeutic vector comprises at least one construct encoding a small RNA capable of knocking down expression of FDPS. In embodiments, the therapeutic vector is a specific cytokine, including but not limited to TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, IL-18, or IL-12. Code (s) and / or chemokine (s) are also encoded. In some embodiments, the single construct is a small RNA capable of knocking down FDPS expression, and TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL. It may encode both specific cytokines or chemokines including but not limited to -17, IL-18 or IL-12.

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

In embodiments, the disclosed constructs and vectors are low in ability to reduce or knock down expression of FDPS and / or geranyl pyrophosphate synthase (“GPPS”) and / or farnesyl transferase (“FT”) genes. Includes molecular homology region RNA (“shRNA”), microRNA (“miRNA”) or siRNA. By down-regulating these genes that control steroid and isoprenoid synthesis, isopentenyl pyrophosphate ("IPP") levels are increased. IPP elevation and accumulation is a known mechanism for increasing GD T cell activation. Furthermore, downregulation of these pyrophosphate synthase genes eliminates key negative regulators of inflammasome function, which in turn results in increased expression of cytokines important for GD T cell activation and effector cell function .

In embodiments, the disclosed constructs are regulated by specific promoters capable of producing interleukin-2 and / or interleukin-15 to sustain GD T cell proliferation. Furthermore, the disclosed constructs are specific 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 functions. It can be regulated by a promoter. Desired effector cell functions include direct cytotoxic cell killing of tumors and / or infected cells, secretion of beneficial cytokines and / or chemokines, NK receptors required to recognize cancerous or infected cells As well as the ability of increased expression of the Fc receptor required to bind the targeted antibody to co-localize GD T cells with cancerous or infected cell targets.

In embodiments, the disclosed methods produce an indirect effect of activating GD T cells and increasing the ability of NK cells to attack and destroy cancerous cells, tumors or infected cells. Activation of NK cells is stimulated to proliferate and differentiate, and to express the 4-1BBL costimulatory ligand required to engage with the 4-1BB costimulatory receptor on NK cells. Requires cells. This form of crosstalk is known as an important mechanism for activating NK cells and is achieved herein 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 tissue. Alone, neither GD T cells nor NK cells are able to destroy dendritic cells, but when the crosstalk interaction described above occurs, NK cells become cytotoxic to inflammatory dendritic cells Will be changed as follows. This immune regulatory mechanism relies on the strong activation and proliferation of GD T cells.

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

In an embodiment, the therapeutic vector is co-administered with an aminobisphosphonate (ABP) drug to achieve synergistic activation of gamma delta T cells. Synergy can allow for alternating, modified or reduced doses of ABP and can reduce 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 Vδ2 + GD T cells and expression of IL-18, which is also important in activation of GD T cells. Inhibition of farnesyltransferase results in decreased prenylation of the protein. The disclosed constructs can be transfected or transduced into specific target cells such as tumor cells or infected cells, where they inhibit the translation of FDPS and encode cytotoxic cytokines or chemokines And expressing RNA sequences (ie, siRNA, shRNA or microRNA).

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

Expression of cytokines and chemokines, such as those listed above, results in localized cytotoxic destruction of tumor cells or cells that infect pathogenic organisms. Thus, expression of such constructs by tumor cells or infected cells results in unwanted cells that assist in their own destruction.

Similarly, when the disclosed constructs are expressed in tumor cells or infected cells, reducing FDPS and FT expression can result in activation of GD T cells and GD T cell activation at the site of tumor or cell infection. Cause mobilization. Increasing RANTES expression further attracts GD T cells to the intended tissue location. Since GD T cells can kill a wide range of tumors of epithelial origin and many leukemias and lymphomas and can further produce high levels of the anti-tumor cytokine IFNγ, GD T cells to the site of the tumor The recruitment of can be a particularly effective means of inducing anti-tumor immunity.

Reduced expression of FDPS can be achieved via shRNA, microRNA, siRNA, or other means known in the art. For example, shRNAs according to SEQ ID NO: 1, 2, 3 or 4 or variants thereof can be used in the disclosed constructs and methods, but this example is not limiting. The coding region of RNA to reduce the expression of FDPS and FT, and the coding region of cytokines and chemokines can be in the same construct or on different constructs.

The classical approach for the production of gene regulatory molecules, including recombinant polypeptides or small RNAs, is the use of stable expression constructs. These constructs are based on non-integrating viral vectors that also have chromosomal integration of the transduced expression plasmid (or at least a portion thereof), short plasmid transfection, or limited half-life into the genome of the host cell. The site of gene integration is generally random, and the number and rate of gene integration at any particular site is often unpredictable; similarly, non-integrated plasmids or viral vectors also produce nuclear DNA However, these species usually lack the sequences necessary for DNA replication and sustained maintenance. Thus, constructs that rely on chromosomal integration result in permanent maintenance of the recombinant gene that can exceed the treatment interval.

An alternative to a stable expression construct for gene expression is a transient expression construct. Expression of the latter gene expression construct is based on non-integrated plasmids, and thus expression is typically lost when cells undergo division or when plasmid vectors are destroyed by endogenous nucleases. .

The disclosed construct is preferably a transiently expressed episomal construct. Episomal constructs are disrupted or diluted over time so that they do not undergo permanent changes to the genome of interest and are not incorporated into the chromosome of the 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-deficient constructs can have background frequency of integration, and any DNA molecule can be found to be less homologous to recombine with host sequences; Exceptionally low and generally not clinically significant.

Accordingly, in some embodiments, the disclosed vectors are 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. Supports delivery of active genes and / or small RNAs over a period of weeks. In some embodiments, the disclosed vectors are about 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months or Supports delivery of active genes and / or small RNAs over longer periods of time. Any combination of these periods can also be used in the methods of the invention, such as 1 month and 1 week, or 3 months and 2 weeks.

However, in some embodiments, the construct comprises an integration element that is dependent on the retroviral integrase gene so that the construct is integrated into the chromosome of the subject. Retrotransposition and transposition are further examples of the mechanism by which mobile genetic elements are integrated or inserted into the chromosome. Plasmids can be 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.

The construct may include specific promoters for expression of cytokines involved in the maintenance of GD T cells (ie IL-2, IL-7, IL-17 and IL-15). For example, promoters that can be incorporated into the disclosed constructs include TATA-box promoter, CpG-box promoter, CCAAT-box promoter, TTGACA-box promoter, BRE-box promoter, INR-box promoter, AT-based promoter, These include 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. It is not limited. See Gagnec and Ionescu-Tirgoviste, Eukaryotic Genomes May Exhibit Up to 10 generic classes of gene promoters, BMC GENOMICS 13: 512 (2012).

Treatment vector

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

Viral vectors can be preferentially targeted to cell types useful for the disclosed methods (ie, tumor cells or bone marrow cells). Viral vectors can be used to transduce genes into target cells, thanks to specific viral envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for gene transfer into many different cell types including whole embryos, fertilized eggs, isolated tissue samples, in situ tissue targets and cultured cell lines. . The ability to introduce foreign genes into cells and express them is used for gene expression studies and cell lineage elucidation, as well as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy Is useful to provide the potential for therapeutic interventions such as: A pox vector comprising a Papovaviridae (eg bovine papillomavirus, ie BPV) or herpesviridae (eg Epstein-Barr virus, ie EBV) or Hepadnaviridae (eg, hepatitis B virus, ie HBV) or vaccinia Viral components derived from viruses such as can be used in the disclosed vectors.

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

Infectious lentiviruses have two regulatory genes, including three major genes gag, pol and env, and tat and rev that code for pathogenic proteins. Depending on the particular serotype and virus, there may be additional accessory genes that encode proteins involved in the regulation, synthesis and / or processing of viral nucleic acids, and other replication functions.

In addition, lentiviruses contain a long terminal repeat (LTR) region that can be approximately 600 nt long. The LTR can be segmented into U3, R and U5 regions. The LTR can mediate integration of retroviral DNA into the host chromosome through the action of integrase. Alternatively, the LTR can be used to circularize viral nucleic acids when the integrase is not functioning.

Viral proteins involved in the early stages of lentiviral replication include reverse transcriptase and integrase. Reverse transcriptase is a virus-encoded RNA-dependent DNA polymerase. This enzyme uses the viral RNA genome as a template for the synthesis of complementary DNA copies. Reverse transcriptase also has RNase H activity for RNA-template destruction. Integrase binds both viral cDNA and host DNA produced by reverse transcriptase. Integrases process the LTR before inserting the viral genome into host DNA. tat acts as a transactivator during transcription to enhance initiation and elongation. The rev responsive element acts after transcription to regulate mRNA splicing and transport to the protoplasm.

Viral vectors generally include glycoproteins, and various glycoproteins can provide specific affinity. For example, VSVG peptides can increase transfection into bone marrow cells. Alternatively, viral vectors can also have targeting moieties such as antibodies conjugated to their shell peptides. Targeted antibodies can be specific for antigens that are overexpressed on tumors such as, for example, HER-2, PSA, CEA, M2-PK and CA19-9.

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

Lentiviral vector system

Lentiviral virions (particles) are expressed by a vector system that encodes the viral proteins necessary to produce virions (viral particles). There is at least one vector comprising a nucleic acid sequence encoding a lentiviral pol protein required for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pol protein is expressed by multiple vectors. There are also vectors comprising a nucleic acid sequence encoding a lentiviral gag protein necessary to form a viral capsid operably linked to a promoter. In certain embodiments, the gag nucleic acid sequence is on a separate vector from at least a portion of the pol nucleic acid sequence. In another embodiment, the gag nucleic acid is on a separate vector from all pol nucleic acid sequences that encode the pol protein.

Numerous modifications can be made to the vector used to create the particles to further minimize the possibility of generating wild type revertants. These include, but are not limited to, deletion of the U3 region of the LTR, tat deletion and matrix (MA) deletion.

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

The vector (s) that form the particles preferably do not include a nucleic acid sequence derived from a lentiviral genome that expresses an envelope protein. Preferably, another vector containing 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 a lentivirus but from a different virus. The resulting particles are called pseudotyped particles. By appropriate selection of the envelope, virtually any cell can be “infected”. For example, influenza virus, VSV-G, alphavirus (Semliki Forest virus, Sindbis virus), arena virus (lymphocytic choriomeningitis virus), flavivirus (tick-borne encephalitis virus, dengue virus, hepatitis C virus, GB Env encoding envelope proteins that target the endocytic compartment, such as those of viruses), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus) Genes can be used. Other envelopes that may preferably be used include those derived from Moloney leukemia viruses such as MLV-E, MLV-A and GALV. These latter envelopes are particularly preferred when the host cell is a primary cell. Other envelope proteins can be selected depending on the desired host cell. For example, targeting specific receptors such as dopamine receptors can be used for brain delivery. Another target may be vascular endothelium. These cells can be targeted using the filovirus envelope. For example, Ebola GP becomes a GP and GP $_2$ glycoprotein by post-transcriptional modification. In another embodiment, different lentiviral capsids with pseudotyped envelopes can be used (eg, FIV or SHIV [US Pat. No. 5,654,195]). SHIV pseudotyped vectors can be readily used in animal models such as monkeys.

As detailed herein, lentiviral vector systems typically include at least one helper plasmid that includes at least one of the gag, pol, or rev genes. Each of the gag, pol and rev genes can be provided on individual plasmids, or one or more genes can be provided together on the same plasmid. In one embodiment, the gag, pol and rev genes are provided on the same plasmid (eg, FIG. 2). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (eg, FIG. 3). Thus, both 3-vector and 4-vector systems can be used to produce lentiviruses, as described in the Examples section and elsewhere herein. The therapeutic vector, envelope plasmid and at least one helper plasmid are transfected into the packaging cell line. A non-limiting example of a packaging cell line is the 293T / 17 HEK cell line. When the therapeutic vector, envelope plasmid and at least one helper plasmid are transfected into the packaging cell line, lentiviral particles are finally produced.

In another aspect, a lentiviral vector system for expressing lentiviral particles is disclosed. This system comprises a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized to infect cells; and at least one for expressing gag, pol and rev genes A lentiviral particle is produced by the packaging cell line when the lentiviral vector comprises a helper plasmid and the lentiviral vector, envelope plasmid and at least one helper plasmid are transfected into the packaging cell line, It is possible to inhibit the production of the chemokine receptor CCR5 or target the HIV RNA sequence.

In another aspect, as detailed in FIG. 2, a lentiviral vector, also referred to herein as a therapeutic vector, may include the following elements: a hybrid 5 ' long terminal repeat (RSV / 5 ' LTR) (SEQ ID NO: 11-12), psi sequence (RNA packaging site) (SEQ ID NO: 13), RRE (Rev response element) (SEQ ID NO: 14), cPPT (polyprint lact) (SEQ ID NO: 15), H1 promoter (SEQ ID NO: 15) 16), FDPS shRNA (SEQ ID NO: 1, 2, 3, 4), Marmot post-transcriptional regulatory element (WPRE) (SEQ ID NO: 17) and 3 ' delta LTR (SEQ ID NO: 18). In another aspect, sequence variations due to substitutions, deletions, additions or mutations can be used to modify the sequence references herein.

In another aspect, as detailed herein, the helper plasmid is 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 can be modified to include a first helper plasmid for expressing the gag and pol genes and a second separate plasmid for expressing the rev gene. In another aspect, sequence variations due to substitutions, deletions, additions or mutations can be used to modify the sequence references herein.

In another aspect, as detailed herein, the envelope plasmid is designed to contain the following elements from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 25) and vesicular Stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 26). In another aspect, sequence variations due to substitutions, deletions, additions or mutations can be used to modify the sequence references herein.

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

Notably, lentiviral packaging systems can be obtained commercially (eg, Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, MD) and designed as described herein. You can also Furthermore, it is within the skill of the artisan to substitute or modify the properties of the lentiviral packaging system to improve any number of related factors, including the efficiency of lentiviral particle production.

Dosage and dosage form

The disclosed vectors allow short-, medium- or long-term expression of the gene or sequence of interest as well as episomal maintenance of the disclosed vectors. Thus, the dosage regimen may vary based on the condition being treated and the method of administration.

In one embodiment, the transduction vector can be administered to a subject in need at varying doses. Specifically, subjects can be administered at an infection dose of about $\geqq 10^{6}$ (on average one dose is required to transduce one target cell). More specifically, the subject can be administered at an infectious dose of about $\geqq 10^{7}$, about $\geqq 10^{8}$, about $\geqq 10^{9}$ or about $\geqq 10^{10}$, or administered at any number of doses intermediate these values Can be done. The upper limit of transduction vector dosing is determined for each disease indicator and depends on the toxicity / safety profile for each individual product or product lot.

Furthermore, the vectors of the present disclosure can be administered periodically, for example once or twice daily, or any other suitable period. For example, the vector is once a week, once every two weeks, once every three weeks, once a month, every two months, every three months, every six months, every nine months, every year Once every 18 months, every 2 years, every 30 months or every 3 years, it can be administered to a subject in need.

In one embodiment, the disclosed vector is administered as a pharmaceutical composition. In some embodiments, a pharmaceutical composition comprising the disclosed vectors can be used in a wide variety of dosage forms including, but not limited to, nasal, pulmonary, oral, topical or parenteral dosage forms for clinical application. It can be formulated in form. Each dosage form may contain various solubilizers, disintegrants, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. . A pharmaceutical composition comprising the vector can also be formulated for injection, insufflation, infusion or intradermal exposure. For example, injectable formulations can include the disclosed vectors in aqueous or non-aqueous solutions at the appropriate pH and osmotic pressure.

The disclosed vectors can be administered to a subject via direct injection at the tumor site or at the site of infection. In some embodiments, the vector can be administered systemically. In some embodiments, the vector can be administered via guided cannulation to the tissue directly surrounding the site of the tumor or infection.

The disclosed vector compositions can be administered using any pharmaceutically acceptable method, eg, intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenous, intradermal) , Intramuscular, subcutaneous, intraperitoneal), lung, intravaginal, topical administration, topical administration, topical administration after ablation, via aerosol, in a semi-solid medium such as agarose or gelatin, or It can be administered to the mucosa via a buccal or nasal spray formulation.

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

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

In further embodiments, a pharmaceutical composition comprising the vector can be formulated as a sublingual or buccal dosage form. Such dosage forms include sublingual tablets or solution compositions administered under the tongue, and buccal tablets placed between the cheek and gingiva.

In some embodiments, a pharmaceutical composition comprising a vector can be formulated as a nasal dosage form. Such dosage forms of the invention include solutions, suspensions and gel compositions for nasal delivery.

In some embodiments, a pharmaceutical composition comprising a vector can be formulated in a liquid dosage form for oral administration, such as a suspension, emulsion or syrup. In some embodiments, the liquid dosage form contains various excipients such as water retention agents, sweeteners, fragrances or preservatives in addition to commonly used simple diluents such as water and liquid paraffin. May be included. In certain embodiments, a composition comprising a vector can be formulated to be suitable for administration to pediatric patients.

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

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

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

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

Example 1

Development of Lentiviral Vector System A lentiviral vector system as summarized in FIG. 4 was developed (circular form). Lentiviral particles were produced in 293T / 17 HEK cells (purchased from American Type Culture Collection, Manassas, Va.) After transfection with therapeutic vectors, envelope plasmids and helper plasmids. Transfection of 293T / 17 HEK cells that produced functional viral particles used the reagent poly (ethyleneimine) (PEI) to increase the efficiency of plasmid DNA uptake. Plasmid and DNA were first added separately in serum-free culture medium at a ratio of 3: 1 (mass ratio of PEI to DNA). After 2-3 days, cell culture 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 units of transduction units / ml (TU / ml). TU determination can be done by measuring HIV p24 levels (p24 protein is incorporated into lentiviral particles) in the culture fluid and measuring the number of viral DNA copies per cell by quantitative PCR, or cells And using light (when the vector encodes a luciferase or fluorescent protein marker).

As described above, a 3-vector system (ie, a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic diagram of the 3-vector system is shown in FIG. Briefly, referring to FIG. 2, the top vector is a helper plasmid that in this case contains Rev. The vector that appears in the center of FIG. 2 is an envelope plasmid. The bottom vector is a therapeutic vector as described herein.

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

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

Synthesis of a 2-vector lentiviral packaging system containing a helper (+ Rev) plasmid and an envelope plasmid.
Materials and methods:

Construction of helper plasmid: The helper plasmid was constructed by initial PCR amplification of DNA fragments from pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol and integrase genes. Primers were designed to amplify fragments with EcoRI and NotI restriction sites that could be used to insert at the same site in the pCDNA3 plasmid (Invitrogen). The forward primer is
Met.
The sequences of the Gag, Pol and integrase fragments were as follows:

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

Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG enhancer / promoter + 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 Operan. The DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows:

Construction of VSV-G envelope plasmid:

Vesicular stomatitis Indianavirus glycoprotein (VSV-G) sequences with flanking EcoRI restriction sites were synthesized by MWG Operan. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and the correct orientation was determined by sequencing using CMV specific primers. The DNA sequence was as follows:

A 4-vector system (ie, a 3-vector lentiviral packaging system) was also designed and produced using the methods and materials described herein. A schematic diagram of the 4-vector system is shown in FIG. Briefly, referring to FIG. 3, the top vector is a helper plasmid that in this case does not contain Rev. The second vector from the top is another Rev plasmid. The second vector from the bottom is an envelope plasmid. The bottom vector is the previously described therapeutic vector.

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

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

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

Synthesis of 3-vector lentiviral packaging system including helper plasmid, Rev plasmid and envelope plasmid Materials and Methods:
Construction of a helper plasmid without Rev:

A helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin polyA sequences. This sequence with adjacent XbaI and XmaI restriction sites was synthesized by MWG Operan. The RRE / rabbit polyAbeta globin sequence was then inserted into a helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

Rev plasmid construction:

The RSV promoter and HIV Rev sequence with adjacent MfeI and XbaI restriction sites were synthesized by MWG Operan as a single DNA fragment. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites, replacing the CMV promoter with the RSV promoter. The DNA sequence was as follows:

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

Promoter: Elongation factor-1 (EF-1) (SEQ ID NO: 41), phosphoglycerate kinase (PGK) (SEQ ID NO: 42) and ubiquitin C (UbC) (SEQ ID NO: 43) are CMV (SEQ ID NO: 25) or CAG The promoter (SEQ ID NO: 19) can be replaced. These sequences can also be further varied by additions, substitutions, deletions or mutations.

Poly A sequence: SV40 poly A (SEQ ID NO: 44) and bGH poly A (SEQ ID NO: 45) can replace rabbit beta globin poly A (SEQ ID NO: 29). These sequences can also be further varied by additions, substitutions, deletions or mutations.

HIV Gag, Pol and integrase sequences: HIV sequences in helper plasmids can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 20) from the Bal strain; HIV Pol (SEQ ID NO: 21); and HIV Int (SEQ ID NO: 22) are gags contained in the helper / helper + Rev plasmids outlined herein, Can be exchanged for pol and int sequences. These sequences can also be further varied by additions, substitutions, deletions or mutations.

Envelope: VSV-G glycoprotein is derived from feline endogenous virus (RD114) (SEQ ID NO: 46), gibbon leukemia virus (GALV) (SEQ ID NO: 47), rabies (FUG) (SEQ ID NO: 48), lymphocytic choroid meninges Flame virus (LCMV) (SEQ ID NO: 49), influenza A poultry plague virus (FPV) (SEQ ID NO: 50), Ross River alpha virus (RRV) (SEQ ID NO: 51), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 52) Alternatively, it can be replaced with a membrane glycoprotein from Ebola virus (EboV) (SEQ ID NO: 53). The sequences of these envelopes are identified in the sequence portion herein. Furthermore, these sequences can also be further varied by additions, substitutions, deletions or mutations.

In summary, the 3-vector system versus the 4-vector system can be compared and contrasted in part as follows. The 3-vector lentiviral vector system includes: Helper plasmids: HIV Gag, Pol, integrase and Rev / Tat; 2. 2. Envelope plasmid: VSV-G / FUG envelope; Therapeutic vectors: RSV 5 ' LTR, psy packaging signal, Gag fragment, RRE, Env fragment, cPPT, WPRE and 3'δ LTR. 4- Vector lentiviral vector system includes: Helper plasmids: HIV Gag, Pol and integrase; Rev plasmid: Rev; 3. Envelope plasmid: VSV-G / FUG envelope; Therapeutic vectors: RSV 5 ' LTR, psy packaging signal, Gag fragment, RRE, Env fragment, cPPT, WPRE and 3 ' delta LTR. Sequences corresponding to the above elements are identified in the sequence listing portion of the specification.
(Example 2)
Development of lentiviral vector expressing FDPS

The purpose of this example was to develop an FDPS lentiviral vector.

Inhibitory RNA design: The sequence of Homo sapiens farnesyl diphosphate synthase (FDPS) (NM — 001004.3) mRNA was used to search for potential siRNA or shRNA candidates that knock down FDPS levels in human cells. Potential RNA interference sequences can be obtained from the GPP Web Portal (http://portals.broadinstate.org/gpp/public/) hosted by Broad Institute or the BLOCK-iT RNAiess. Selected from the selected candidates, such as by siRNA or shRNA design programs such as from / rnaiexpress /). Each selected shRNA sequence is inserted into a lentiviral vector immediately 3 'to an RNA polymerase III promoter, eg, H1 (SEQ ID NO: 16), U6 (SEQ ID NO: 54) or 7SK (SEQ ID NO: 55). , Regulated shRNA expression. These lentiviral shRNA constructs were used to transduce cells and measure changes in specific mRNA levels. The most potent shRNAs to reduce mRNA levels were individually embedded within the microRNA backbone to allow expression by either the EF-1 alpha or CMV RNA polymerase II promoter. The microRNA backbone is mirbase. org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using lentiviral vectors.

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

The shRNA sequence was then assembled into a synthetic microRNA (miR) under the control of the EF-1 alpha promoter. Briefly, miR hairpin sequences, eg, miR30, miR21 or miR185, detailed below, are mirbase. from org. A synthetic miR sequence was constructed using a 19-22 mer shRNA target sequence. The miR sequences were aligned as antisense-target sequence-hairpin loop sequence (specific for each microRNA) -sense target sequence.

The following miR sequences were developed:

(Example 3)

FDPS knockdown over 3 days in THP1 monocytic leukemia by shRNA # 4

This example demonstrates that knockdown of FDPS in THP1 monocytic leukemia cells by FDPS shRNA # 4 expressing lentivirus (LV) stimulates TNF-α expression in gamma delta T cells as shown in FIG. Show.

THP1 cells (1×10^5 cells) were transduced with LV-control or LV-FDPS shRNA # 4 for 3 days. Two days after transduction, cells were or were not treated with 1 μM zoledronic acid. After 24 hours, transduced THP-1 cells were co-cultured with 5×10^5 PBMC cells and IL-2 for 4 hours in round bottom 96-well plates. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of 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)

Knockdown of FDPS over 14 days in THP1 leukemia cells by shRNA # 4

This example shows that knockdown of FDPS over 14 days in THP1 leukemia cells by FDPS shRNA # 4 expressing lentivirus (LV) stimulates TNF-α expression in GD T cells as shown in FIG. ing.

THP1 cells (1×10^5 cells) were transduced for 14 days with LV-control or LV-FDPS shRNA # 4. Two days after transduction, cells were or were not treated with 1 uM zoledronic acid. After 24 hours, transduced THP-1 cells were co-cultured with 5×10^5 PBMC cells and IL-2 for 4 hours in round bottom 96-well plates. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the 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)

Knockdown of FDPS over 3 days in PC3 prostate cancer cells by shRNA # 1

This example shows that knockdown of FDPS in PC3 prostate cancer cells by FDPS shRNA # 1-expressing lentivirus (LV) stimulates TNF-α expression in GD T cells as shown in FIG. Show.

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 or were not treated with 1 uM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96-well plate for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of 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)

Knockdown of FDPS over 3 days in PC3 prostate cancer cells by shRNA # 4

This example shows that knockdown of FDPS in PC3 prostate cancer cells by FDPS shRNA # 4 expressing lentivirus (LV) stimulates TNF-α expression in GD T cells as shown in FIG. Show.

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 or were not treated with 1 uM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with 5×10^5 PBMC cells and IL-2 in a round bottom 96-well plate for 4 hours. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of 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)

Knockdown of FDPS over 3 days in HepG2 liver cancer cells by shRNA # 1 and # 4

This example shows that FDPS knockdown in HepG2 liver cancer cells by FDPS shRNA # 1 (SEQ ID NO: 1) expressing lentivirus (LV) and shRNA # 4 (SEQ ID NO: 4) expressing lentivirus (LV) over 3 days, As shown in FIG. 9, it shows stimulating TNF-α expression in GD T cells.

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 or were not treated with 1 uM zoledronic acid. After 24 hours, transduced HepG2 cells were co-cultured with 5×10^5 PBMC cells and IL-2 for 4 hours in round bottom 96-well plates. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, the 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) were 0 respectively. Stimulated 7% and 0.9%. With zoledronic acid treatment, the 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. I was stimulated.

(Example 8)

3 days FDPS knockdown in THP1 leukemia by microRNA-30

This example demonstrates that knockdown of FDPS over 3 days in THP1 leukemia cells by FDPS targeted synthetic microRNA-30 expressing lentivirus (LV) results in TNF-α expression in gamma delta T cells as shown in FIG. Indicates irritation.

THP1 cells (1×10^5 cells) were transduced with LV-control or LV-miR30 FDPS # 1 (SEQ ID NO: 5) for 3 days. Two days after transduction, cells were or were not treated with 1 uM zoledronic acid. After 24 hours, transduced THP-1 cells were co-cultured with 5×10^5 PBMC cells and IL-2 for 4 hours in round bottom 96-well plates. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2 and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram. In the absence of zoledronic acid, LV-control stimulated 0.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS stimulated 8.1%. With zoledronic acid treatment, LV-control stimulated 5.3% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS # 1 (SEQ ID NO: 5) stimulated 67.3%.

Example 9

E: T ratio obtained from a mixture of THP-1 cells, cultured human GD T cells and / or Zometa (Zol)

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

The monocyte-like cell line THP-1 was treated with a control lentiviral vector (LV), LV (LV-FDPS), zoledronic acid (Zol) or a combination that suppresses farnesyl diphosphate synthase gene expression. The legends shown in FIG. 11 were as follows: lentiviral control vector (LV-control), lentiviral vector expressing microRNA down-regulating FDPS (LV-FPPS), Zometa (Zol), Zometa + Lenti Virus control (Zol + LV-control), or a lentiviral vector (Zol + LV-FPPS) expressing microRNAs that down-regulate Zometa + FPPS.

Anonymous donor-derived human GD T cells were cultured and added to the treated THP-1 cells at a ratio of 4: 1, 2: 1 or 1: 1 (GD T: THP-1) for 4 hours. Cell killing was measured by 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 greatly increased compared to either treatment alone. When LV-FDPS treatment alone was compared to Zol treatment alone, LV-FDPS resulted in greater killing, but> 3 fold below tumor cell killing after combination treatment. The combined LV-FDPS + Zol treatment caused nearly 70% tumor cell killing at a 4: 1 ratio; this was more than three times higher than the second best treatment (LV-FDPS alone).

(Example 10)

ShRNA-based RNA interference targeting the human farnesyl diphosphate synthase (FDPS) gene delivered by lentivirus

As shown in FIG. 12, HepG2 human hepatocellular carcinoma cells were infected with a H1 promoter and a lentiviral vector containing either non-targeted or four different FDPS shRNA sequences. After 48 hours, RNA was extracted from the cells and converted to cDNA. FDPS cDNA expression was determined by quantitative PCR using SYBR Green and FDPS primers. FDPS expression was normalized to actin levels for each sample.

H1 promoter and non-targeting sequences

FDPS targeted lentiviral vectors containing any one of these were produced in 293 T cells.

HepG2 human hepatocellular carcinoma cells were then infected with a lentiviral vector to determine the effectiveness of FDPS knockdown. After 48 hours, RNA was extracted from the cells using the RNeasy RNA isolation kit (Qiagen) and converted to cDNA using the SuperScript VILO cDNA synthesis kit (Thermo Scientific). Expression of FDPS cDNA was performed using SYBR Green PCR mix (Thermo Scientific) and FDPS primer (forward primer: 5'-AGGAATTGATGGCGAGAAGGG-3 ' (SEQ ID NO: 61) and reverse primer: 5'-CCCAAAAGGGTCCAAGGTATACA-3 ' (SEQ ID NO: 62). As determined by quantitative PCR on an Applied Biosystems StepOne qPCR instrument. FDPS expression was brought to the actin level for each sample using actin primers (forward primer: 5'-AGCGCGGCTACAGCTTCA-3 ' (SEQ ID NO: 63) and reverse primer: 5'-GGCGGACGTAGCACAGCTTCT-3 ' (SEQ ID NO: 64)). Standardized. Set the relative FDPS RNA expression of the shCon sample to 100%. There was a 85% (FDPS sequence # 1), 89% (FDPS sequence # 2), 46% (FDPS sequence # 3) and 98% (FDPS sequence # 4) reduction in FDPS expression.

(Example 11)

Lentivirally delivered miR-based RNA interference targeting the human farnesyl diphosphate synthase (FDPS) gene

As shown in FIG. 13, HepG2 human hepatocellular carcinoma cells were treated with either the H1 promoter (SEQ ID NO: 16), the FDPS shRNA # 4 (SEQ ID NO: 4) sequence or the EF-1α promoter (SEQ ID NO: 41) and miR30-based Lentiviral vectors containing FDPS sequences were infected. After 48 hours, cells were lysed and immunoblots were performed using anti-FDPS (Thermo Scientific) and anti-actin (Sigma) antibodies as protein loading controls.

More specifically, in HepG2 human hepatocellular carcinoma cells, the H1 promoter (SEQ ID NO: 16) and FDPS shRNA sequence

Or EF-1 alpha promoter (SEQ ID NO: 41) and miR30 based FDPS sequence

Lentiviral vectors containing any of the above were infected.

After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified using Bio-Rad protein assay reagent. 50 micrograms of protein samples were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific) and transferred to PVDF membranes (EMD Millipore). Immunoblots were performed using anti-FDPS (Thermo Scientific) and anti-actin (Sigma) antibodies as protein loading controls. The antibody was conjugated with HRP-conjugated secondary antibody and detected using a Licor c-DiGit Blot scanner using Immobilon Western ECL reagent (EMD Millipore). Immunoblot band concentration measurements were quantified using NIH imaging 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 in FDPS protein expression.

(Example 12)

Knockdown of FDPS over 3 days in HepG2 liver cancer cells by adeno-associated virus (AAV) expressing FDPS shRNA # 4

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

HepG2 cells were transduced with control or AAV-FDPS shRNA # 4 (SEQ ID NO: 8) for 3 days. Two days after transduction, cells were or were not treated with 1 uM zoledronic acid. After 24 hours, transduced HepG2 cells were co-cultured with 5×10^5 PBMC cells and IL-2 for 4 hours in round bottom 96-well plates. PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand the Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti-TCR-Vδ2

and anti-TNF-α antibodies, the cells were analyzed via flow cytometry. Live cells were gated and Vδ2 + and TNF-α + cells were selected on a dot blot. Activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytogram (FIG. 14, panel B).

AAV vector construction. The FDPS shRNA sequence # 4 (SEQ ID NO: 4) was inserted into the pAAV plasmid (Cell Biolabs). The FDPS oligonucleotide sequence containing BamHI and EcoRI restriction sites was synthesized by Eurofins MWG Operan. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 ° C. to room temperature. pAAV was digested with the restriction enzymes BamHI and EcoRI for 1 hour at 37 ° C. The digested pAAV plasmid was purified by agarose gel electrophoresis and extracted from the gel using a Thermo Scientific DNA gel extraction kit. DNA concentration was determined, vector to oligo (3: 1 ratio) mixed, annealed and ligated. The ligation reaction was performed for 30 minutes at room temperature using T4 DNA ligase. 2.5 microliters of ligation mix was added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat shock at 42 ° C. Bacterial cells were spread on agar plates containing ampicillin and drug resistant colonies (indicating the presence of ampicillin resistant plasmid) were collected and expanded in LB broth. To check for oligo sequence insertion, plasmid DNA was extracted from the harvested bacterial cultures using a Thermo Scientific DNA mini prep kit. The insertion of the shRNA sequence in the pAAV plasmid was verified by DNA sequencing using primers specific for the promoter used to regulate shRNA expression. Exemplary having H1 promoter (SEQ ID NO: 16), shFDPS sequence (eg, SEQ ID NO: 4), left terminal inverted sequence (left ITR; SEQ ID NO: 65) and right terminal inverted sequence (right ITR; SEQ ID NO: 66) AAV vectors can be found in FIG. 14, panel A.

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

(Example 13)

Reduced RAP1 prenylation in cells transduced with LV-shFDPS and treated with zoledronic acid

This example shows that lentivirally 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. Inhibition of the enzymatic activity of FDPS by zoledronic acid or reduced protein expression by shRNA-mediated knockdown results in reduced farnesyl diphosphate levels. Farnesylation of cellular proteins requires farnesyl diphosphate. RAP1A is a protein that is modified by farnesylation that can be used as a biomarker for the level of cellular farnesyl diphosphate. FDPS activity was measured after transduction with LV-shFDPS alone or in combination with zoledronic acid using an antibody that specifically recognizes reduced RAP1A farnesylation. HepG2 human hepatocellular carcinoma cells were infected with a lentiviral vector containing FDPS shRNA sequence # 4. For zoledronic acid treated cells, zoledronic acid (Sigma) was added over the last 24 hours. After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified using Bio-Rad protein assay reagent. 50 micrograms of protein samples were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific) and transferred to PVDF membranes (EMD Millipore). Immunoblots were performed using anti-FDPS (Thermo Scientific), anti-RAP1A (Santa Cruz) and anti-actin (Sigma) antibodies as protein loading controls. The antibody was conjugated with HRP-conjugated secondary antibody and detected using a Licor c-DiGit Blot scanner using Immobilon Western ECL reagent (EMD Millipore). An increase in RAP1A band intensity correlates with decreased farnesylation. RAP1A defarnesylation occurred only in cells transduced with LV-shFDPS and treated with zoledronic acid.

(Example 14)

Treatment of subjects with cancer LV-FDPS is a genetic drug delivered by a lentiviral vector via local administration to the site of late unresectable hepatocellular carcinoma.

A phase I clinical trial of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound-guided cannulation of the patient's liver without concomitant radiotherapy or chemotherapy Test safety and feasibility. This study is reasonably expected to result in successful treatment of HCC. This study was an open-label 4 × 3 dose escalation to identify the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with stage III / IV unresectable HCC (4 One dose range, up to 3 subjects per dose).

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

Subjects who have a target lesion with a longest diameter ≧ 1 cm (measured by helical CT) and a maximum diameter of ≦ 4.9 cm and meet the inclusion and exclusion criteria detailed below are in the next available medication category sign up. A maximum of 3 subjects are recruited for each dosage group. The dose is several transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with a volume not exceeding 25 mL. Minimum dose is 1×10^{9} transducing units, increasing is 10 times until the next dose of $1 \times {}^{10}10$ transducing units, the next dose is $1 \times {}^{10}11$ transducing units, for HCC Based on reported experience with recombinant adenovirus therapy, it is the maximum dose of 1×10^{12} transducing units (Sangro et al., A phase I clinical triglycine kinase-based gene therapy in heparc cell 20 Year, Cancer Gene Ther., 17: 837-43). Subjects are enrolled, treated, and evaluated over 3 months. All safety assessments are completed for each group before enrolling and treating subjects at the next higher dose level. Registration and dose escalation continues until the maximum tolerated dose is achieved or the study is complete.

Cannulation is through the left subclavian artery until the catheter tip is at the appropriate hepatic artery junction. Cannulation is guided by ultrasonography, as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapeutic with cis citrate, mitomycin C, leucovor and 5-Fluorourc. Chin.Med.Assoc.67: 602-10).
Primary evaluation items

Safety: Systemic and local area adverse events are graded according to CTCAS and encoded according to MedRA. Adverse event data for all subjects in a single dose range will be evaluated prior to dose escalation. The final safety assessment captures data from all dose ranges.
Secondary evaluation items

• Lesion distribution and LV-FDPS retention after local regional administration, and subsequent biopsy or autopsy to obtain tissue.
Response rate (ORR) at the target and measurable non-local lesions (if any) by physical analysis, medical imaging or biopsy for 3 months after treatment.
Levels of LV-FDPS in the bloodstream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
Disease-free survival over historical controls (no LV-FDPS) patients in ad hoc analysis.
Inclusion criteria

• Includes older than 18 years old, both men and women.
Confirmed by histology or cytology or based on currently accepted clinical standards for parenchymal cell hepatocellular carcinoma not suitable for resection, transplantation or other potentially curative therapy at the time of screening Diagnosed.
• The attending physician determines that the lesion is suitable for localized regional targeted delivery.
The target lesion must show measurable disease with a one-dimensional longest diameter of ≧ 1.0 cm by computed tomography; the maximum longest diameter is ≦ 5.0 cm.
Karnofsky performance score, 60-80% of ECOG value.
-Life expectancy ≥ 12 weeks.
Hematopoietic function: WBC ≧ 2,500 / mm^3 ; ANC ≧ 1000 / mm^3 ; hemoglobin ≧ 8 g / dL; platelet count ≧ 50,000 / mm^3 ; coagulation INR ≦ 1.3.
AST and ALT <ULN 5 times; ALPS <ULN 5 times. Bilirubin ≦ 1.5 times ULV; creatine ≦ 1.5 times ULN and eGFR ≧ 50.
Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.
• Renal, cardiovascular and respiratory function as appropriate in the opinion of the attending physician.
Immunological function: circulating V gamma 9V delta2 + T cells ≧ 30 / mm^3 ; no immunodeficiency disease.
HIV negative by serology and viral RNA test.
・ Written informed consent.
Exclusion criteria

A target lesion adjacent to, surrounding or infiltrating the blood vessel.
Primary HCC suitable for excision, transplantation or other potentially curative therapy.
-Liver surgery or chemoembolization within the last 4 months.

-Liver irradiation or whole body radiation therapy within the last 4 months.
• Chemotherapy within 4 weeks or any use of nitrosourea, mitomycin C or cisplatin.
• Acceptance of aminobisphosphonate therapy within the current or past 4 weeks.
Study drug within 4 weeks or <5 drug half-life.
-Wound healing disorders due to diabetes.
• Significant psychosis, alcohol dependence or illegal drug use.
• Do not follow study protocols and reporting requirements.
-Aminobisphosphonate treatment within the last 4 months.
-Clinically significant cardiovascular, cerebrovascular (stroke), immunological (excluding hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current Encephalopathy; varicose bleeding that requires hospitalization or blood transfusion within the past 4 months.
History of HIV or acquired immune deficiency syndrome.
• Current or previous treatment with antiretroviral drug therapy.
• Refusal to incorporate pregnancy, breastfeeding, or use of barrier-type or chemical contraceptives throughout the study and follow-up period.

LV-FDPS is a gene drug delivered by a lentiviral vector via local administration to the site of late unresectable hepatocellular carcinoma—adjunctive administration of aminobisphosphonate

Phase I clinical trials have shown the safety of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound-guided cannulation of the patient's liver with combined aminobisphosphonate chemotherapy and Test feasibility. This study is reasonably expected to result in successful treatment of HCC. This study was an open-label 4 × 3 dose escalation to identify the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with stage III / IV unresectable HCC (4 One dose range, up to 3 subjects per dose).

LV-FDPS is a gene therapy designed to reduce the expression of the enzyme farnesyl diphosphate synthase in tumor cells. Experimental studies show that tumor cells modified by LV-FDPS induce anti-tumor activity of human gamma delta T cells, including the ability to kill tumors by cytotoxicity. Previous experimental studies have also shown the potential for positive interaction of LV-FDPS with certain aminobisphosphonate drugs that can be prescribed in primary or metastatic disease. For this study, subjects were subject to a persistent standard with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference. Along with therapeutic dosing, receive dose escalating doses of LV-FDPS.

A subject with a target lesion with a longest diameter ≧ 1 cm (measured by helical CT) and a maximum diameter of ≦ 4.9 cm and meeting the inclusion and exclusion criteria detailed below is enrolled and aminobisphosphonate treatment begins. Reassess the size 30 days after the target lesion to ensure that the subject still meets the LV-FDPS initiation criteria. Subjects who do not have an objective clinical response to aminobisphosphonates are enrolled in the next available LV-FDPS dosage category. A maximum of 3 subjects will be recruited into each dosage group and all will continue aminobisphosphonate for the duration of the study 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 a volume not exceeding 25 mL. Minimum dose is 1×10^{9} transducing units, increasing is 10 times until the next dose of $1 \times {}^{10}10$ transducing units, the next dose is $1 \times {}^{10}11$ transducing units, for HCC Based on reported experience with recombinant adenovirus therapy, it is the maximum dose of 1×10^{12} transducing units (Sangro et al., A phase I clinical triglycine kinase-based gene therapy in heparc cell 20 Year, Cancer Gene Ther., 17: 837-43). Subjects are enrolled, treated, and evaluated over 3 months. All safety assessments are completed for each group before enrolling and treating subjects at the next higher dose level. Registration and dose escalation continues until the maximum tolerated dose is achieved or the study is complete.

Cannulation is through the left subclavian artery until the catheter tip is at the appropriate hepatic artery junction. Cannulation is guided by ultrasonography, as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapeutic with cis citrate, mitomycin C, leucovor and 5-Fluorourc. Chin.Med.Assoc.67: 602-10).
Primary evaluation items

Safety: Systemic and local area adverse events are graded according to CTCAS and encoded according to MedRA. Adverse event data for all subjects in a single dose range will be evaluated prior to dose escalation. The final safety assessment captures data from all dose ranges.
Secondary evaluation items

• Lesion distribution and LV-FDPS retention after local regional administration, and subsequent biopsy or autopsy to obtain tissue.
Response rate (ORR) at the target and measurable non-local lesions (if any) by physical analysis, medical imaging or biopsy for 3 months after treatment.
Levels of LV-FDPS in the bloodstream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
Disease-free survival over historical controls (no LV-FDPS) patients in ad hoc analysis.
Inclusion criteria

• Includes older than 18 years old, both men and women.
Confirmed by histology or cytology or based on currently accepted clinical standards for parenchymal cell hepatocellular carcinoma not suitable for resection, transplantation or other potentially curative therapy at the time of screening Diagnosed.
• The attending physician determines that the lesion is suitable for localized regional targeted delivery.
The target lesion must show measurable disease with a one-dimensional longest diameter of ≧ 1.0 cm by computed tomography; the maximum longest diameter is ≦ 5.0 cm.
Karnofsky performance score, 60-80% of ECOG value.
-Life expectancy ≥ 12 weeks.
Hematopoietic function: WBC ≧ 2,500 / mm ^{3} ; ANC ≧ 1000 / mm ^{3} ; hemoglobin ≧ 8 g / dL; platelet count ≧ 50,000 / mm ^{3} ; coagulation INR ≦ 1.3.
AST and ALT <ULN 5 times; ALPS <ULN 5 times. Bilirubin ≦ 1.5 times ULV; creatine ≦ 1.5 times ULN and eGFR ≧ 50.
Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.
• Renal, cardiovascular and respiratory function as appropriate in the opinion of the attending physician.
Immunological function: circulating V gamma 9V delta2 + T cells ≧ 30 / mm ^{3} ; no immunodeficiency disease.
HIV negative by serology and viral RNA test.
・ Written informed consent.
Exclusion criteria

Intolerance to aminobisphosphonate adjuvant, or do not attempt to continue aminobisphosphonate adjuvant.
Objective clinical response after aminobisphosphonate treatment.
A target lesion adjacent to, surrounding or infiltrating the blood vessel.
Primary HCC suitable for excision, transplantation or other potentially curative therapy.
-Liver surgery or chemoembolization within the last 4 months.
-Liver irradiation or whole body radiation therapy within the last 4 months.
Chemotherapy with the exception of aminobisphosphonate or any use of nitrosourea, mitomycin C or cisplatin within 4 weeks.
Study drug within 4 weeks or <5 drug half-life.
-Wound healing disorders due to diabetes.
• Significant psychosis, alcohol dependence or illegal drug use.
• Do not follow study protocols and reporting requirements.
-Clinically significant cardiovascular, cerebrovascular (stroke), immunological (excluding hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current Encephalopathy; varicose bleeding that requires hospitalization or blood transfusion within the past 4 months.
History of HIV or acquired immune deficiency syndrome.
• Current or previous treatment with antiretroviral drug therapy.
• Refusal to incorporate pregnancy, breastfeeding, or use of barrier-type or chemical contraceptives throughout the study and follow-up period.
(Example 15)
Treatment of subjects with chronic viral disease (s) of the liver LV-FDPS is via local administration to the liver for the treatment of hepatitis B virus, hepatitis C virus, HIV or other viral infections of the liver. A gene drug delivered by a lentiviral vector

Phase I clinical trials test the safety and feasibility of delivering LV-FDPS to virus-infected livers using ultrasound guided cannulation. This study is reasonably expected to result in successful treatment of liver infection. This study was an open-label 4 × 3 dose escalation to identify the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with chronic viral disease of the liver resistant to chemotherapy (4 dose ranges, up to 3 subjects per dose).

LV-FDPS is a gene therapy designed to reduce the expression of the enzyme farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that tumor cells modified by LV-FDPS induce human gamma delta T cells, including cytotoxic ability against virus-infected cells.

Subjects with confirmed viral infections of the liver, including hepatitis B virus, hepatitis C virus, HIV or other viruses, are enrolled in the next available LV-FDPS dosage 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 a volume not exceeding 25 mL. Minimum dose is 1×10^{9} transducing units, increasing is 10 times until the next dose of $1 \times {}^{10}10$ transducing units, the next dose is $1 \times {}^{10}11$ transducing units, for HCC Based on reported experience with recombinant adenovirus therapy, it is the maximum dose of 1×10^{12} transducing units (Sangro et al., A phase I clinical triglycine kinase-based gene therapy in heparc cell 20 Year, Cancer Gene Ther., 17: 837-43). Subjects are enrolled, treated, and evaluated over 3 months. All safety assessments are completed for each group before enrolling and treating subjects at the next higher dose level. Registration and dose escalation continues until the maximum tolerated dose is achieved or the study is complete.

Cannulation is through the left subclavian artery until the catheter tip is at the appropriate hepatic artery junction. Cannulation is guided by ultrasonography, as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapeutic with cis citrate, mitomycin C, leucovor and 5-Fluorourc. Chin.Med.Assoc.67: 602-10).
Primary evaluation items

Safety: Systemic and local area adverse events are graded according to CTCAS and encoded according to MedRA. Adverse event data for all subjects in a single dose range will be evaluated prior to dose escalation. The final safety assessment captures data from all dose ranges.
Secondary evaluation items

• Lesion distribution and LV-FDPS retention after local regional administration, and subsequent biopsy or autopsy to obtain tissue.
Response rate (ORR) measured as an internal or systemic persistent viral response (SVR) for 3 months after treatment.
Levels of LV-FDPS in the bloodstream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
Disease-free survival over historical controls (no LV-FDPS) patients in ad hoc analysis.
Inclusion criteria

• Includes older than 18 years old, both men and women.
Confirmed by histology or cytology, or at the time of screening, based on currently accepted clinical standards for chronic viral infections of the liver that are not amenable to excision, transplantation or other potentially curative therapy Diagnosis.
• The attending physician determines that the lesion is suitable for localized regional targeted delivery.
Karnofsky performance score, 60-80% of ECOG value.
-Life expectancy ≥ 12 weeks.
Hematopoietic function: WBC ≧ 2,500 / mm^3 ; ANC ≧ 1000 / mm^3 ; hemoglobin ≧ 8 g / dL; platelet count ≧ 50,000 / mm^3 ; coagulation INR ≦ 1.3.
AST and ALT <ULN 5 times; ALPS <ULN 5 times. Bilirubin ≦ 1.5 times ULV; creatine ≦ 1.5 times ULN and eGFR ≧ 50.
Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.
• Renal, cardiovascular and respiratory function as appropriate in the opinion of the attending physician.
Immunological function: circulating V gamma 9V delta2 + T cells ≧ 30 / mm^3 ; no immunodeficiency disease.
HIV negative by serology and viral RNA test.
・ Written informed consent.
Exclusion criteria

• Chronic viral disease suitable for excision, transplantation or other potentially curative therapy.
-Liver surgery or chemoembolization within the last 4 months.
-Liver irradiation or whole body radiation therapy within the last 4 months.
Study drug within 4 weeks or <5 drug half-life.
• Current (within the past 4 weeks) or ongoing acceptance of aminobisphosphonate therapy.
-Wound healing disorders due to diabetes.

• Significant psychosis, alcohol dependence or illegal drug use.
• Do not follow study protocols and reporting requirements.
• Clinically significant cardiovascular, cerebrovascular (stroke), immunological (excluding viral infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; within the past 4 months Varicose bleeding requiring hospitalization or blood transfusion.
• Refusal to incorporate pregnancy, breastfeeding, or use of barrier-type or chemical contraceptives throughout the study and follow-up period.

LV-FDPS is a genetic drug delivered by a lentiviral vector via topical administration to the liver for the treatment of hepatitis B virus, hepatitis C virus, HIV or other viral infections of the liver-combination aids Aminobisphosphonate treatment

Phase I clinical trials test the safety and feasibility of delivering LV-FDPS to virus-infected livers using ultrasound guided cannulation. This study is reasonably expected to result in successful treatment of liver infection. This study was an open-label 4 × 3 dose escalation to identify the maximum tolerated dose of LV-FDPS in patients 18 years of age or older with chronic viral disease of the liver resistant to chemotherapy (4 dose ranges, up to 3 subjects per dose).

LV-FDPS is a gene therapy designed to reduce the expression of the enzyme farnesyl diphosphate synthase in tumor cells. Experimental studies have shown that tumor cells modified by LV-FDPS induce human gamma delta T cells, including cytotoxic ability against virus-infected cells. Previous experimental studies have also shown the potential for positive interactions between LV-FDPS and certain aminobisphosphonate drugs that can be prescribed during infectious diseases. For this study, subjects were subject to a persistent standard with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference. Along with therapeutic dosing, receive dose escalating doses of LV-FDPS.

Subjects with confirmed viral infections of the liver, including hepatitis B virus, hepatitis C virus, HIV or other viruses, are rescreened to meet registration criteria for LV-FDPS treatment of infectious diseases Begin aminobisphosphonate treatment over the previous 45 days. Eligible subjects are enrolled in the next available LV-FDPS medication 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 a volume not exceeding 25 mL. Minimum dose is 1×10^{9} transducing units, increasing is 10 times until the next dose of $1 \times {}^{10}{}^{10}$ transducing units, the next dose is $1 \times {}^{10}{}^{11}$ transducing units, for HCC Based on reported experience with recombinant adenovirus therapy, it is the maximum dose of 1×10^{12} transducing units (Sangro et al., A phase I clinical triglycine kinase-based gene therapy in heparc cell 20 Year, Cancer Gene Ther., 17: 837-43). Subjects are enrolled, treated, and evaluated over 3 months. All safety assessments are completed for each group before enrolling and treating subjects at the next higher dose level. Registration and dose escalation continues until the maximum tolerated dose is achieved or the study is complete.

Cannulation is through the left subclavian artery until the catheter tip is at the appropriate hepatic artery junction. Cannulation is guided by ultrasonography, as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapeutic with cis citrate, mitomycin C, leucovor and 5-Fluorourc. Chin.Med.Assoc.67: 602-10).
Primary evaluation items

Safety: Systemic and local area adverse events are graded according to CTCAS and encoded according to MedRA. Adverse event data for all subjects in a single dose range will be evaluated prior to dose escalation. The final safety assessment captures data from all dose ranges.
Secondary evaluation items

• Lesion distribution and LV-FDPS retention after local regional administration, and subsequent biopsy or autopsy to obtain tissue.
Response rate (ORR) measured as an internal or systemic persistent viral response (SVR) for 3 months after treatment.
Levels of LV-FDPS in the bloodstream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
Changes in liver function markers including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
Disease-free survival over historical controls (no LV-FDPS) patients in ad hoc analysis.
Inclusion criteria

• Includes older than 18 years old, both men and women.
Confirmed by histology or cytology, or at the time of screening, based on currently accepted clinical standards for chronic viral infections of the liver that are not amenable to excision, transplantation or other potentially curative therapy Diagnosis.
• The attending physician determines that the lesion is suitable for localized regional targeted delivery.

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

Hematopoietic function: WBC ≧ 2,500 / mm ^{3} ; ANC ≧ 1000 / mm ^{3} ; hemoglobin ≧ 8 g / dL; platelet count ≧ 50,000 / mm ^{3} ; coagulation INR ≦ 1.3.
AST and ALT <ULN 5 times; ALPS <ULN 5 times. Bilirubin ≦ 1.5 times ULV; creatine ≦ 1.5 times ULN and eGFR ≧ 50.
Thyroid function: total T3 or free T3, total T4 or free T4 and THC ≦ CTCAE grade 2 abnormalities.
• Renal, cardiovascular and respiratory function as appropriate in the opinion of the attending physician.

Immunological function: circulating V gamma 9V delta2 + T cells ≧ 30 / mm ^{3} ; no immunodeficiency disease.
HIV negative by serology and viral RNA test.
・ Written informed consent.
Exclusion criteria

• Chronic viral disease suitable for excision, transplantation or other potentially curative therapy.
-Liver surgery or chemoembolization within the last 4 months.
-Liver irradiation or whole body radiation therapy within the last 4 months.
Study drug within 4 weeks or <5 drug half-life.
-Wound healing disorders due to diabetes.
• Significant psychosis, alcohol dependence or illegal drug use.
• Do not follow study protocols and reporting requirements.
• Clinically significant cardiovascular, cerebrovascular (stroke), immunological (excluding viral infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; within the past 4 months Varicose bleeding requiring hospitalization or blood transfusion.
• Refusal to incorporate pregnancy, breastfeeding, or use of barrier-type or chemical contraceptives throughout the study and follow-up period.
The following sequences are referred to herein:

While certain of the preferred embodiments of the present invention have been described and specifically listed above, it is not intended that the invention be limited to such embodiments. Various modifications may be made to the invention without departing from the scope and spirit of the invention.

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US20050019927A1	2003-07-13	2005-01-27	Markus Hildinger	DECREASING GENE EXPRESSION IN A MAMMALIAN SUBJECT IN VIVO VIA AAV-MEDIATED RNAi EXPRESSION CASSETTE TRANSFER
US20050138677A1	2003-09-16	2005-06-23	Pfister Herbert J.	Transgenic animal model for the treatment of skin tumors
WO2005028634A2	2003-09-18	2005-03-31	Emory University	Improved mva vaccines
WO2005033282A2	2003-10-01	2005-04-14	Pharmacia & Upjohn Company Llc	Polyamide compositions and therapeutic methods for treatment of human papilloma virus
US20080039413A1	2003-10-21	2008-02-14	Morris David W	Novel compositions and methods in cancer
EP1753777B1	2004-02-25	2014-05-07	Dana-Farber Cancer Institute, Inc.	METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF HIV INFECTION USING TRIM5a
EP1737956A2	2004-03-01	2007-01-03	Massachusetts Institute of Technology	Rnai-based therapeutics for allergic rhinitis and asthma

TWI439284B	2004-04-09	2014-06-01	Abbvie Biotechnology Ltd	Multiple-variable dose regimen for treating tnfα-related disorders
US20080227736A1	2004-06-03	2008-09-18	Regents Of The University Of California,	Targeting Pseudotyped Retroviral Vectors
WO2006012221A2	2004-06-25	2006-02-02	The Regents Of The University Of California	Target cell-specific short interfering rna and methods of use thereof
WO2006023491A2	2004-08-16	2006-03-02	The Cbr Institute For Biomedical Research, Inc.	Method of delivering rna interference and uses thereof
WO2006039721A2 *	2004-10-08	2006-04-13	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods for bone resorption diseases, cancer, bone pain, immune disorders, and infectious diseases
EP1647595A1	2004-10-15	2006-04-19	Academisch Medisch Centrum bij de Universiteit van Amsterdam	Nucleic acids against viruses, in particular HIV
WO2006048215A1	2004-11-02	2006-05-11	Istituto Di Ricerche Di Biologia Molecolare P Angeletti Spa	Adenoviral amplicon and producer cells for the production of replication-defective adenoviral vectors, methods of preparation and use thereof
US7790446B2	2005-02-11	2010-09-07	Kosagen Cell Factory Oü	Vectors, cell lines and their use in obtaining extended episomal maintenance replication of hybrid plasmids and expression of gene products
DK2002003T3	2005-05-27	2016-03-21	Ospedale San Raffaele Srl	Gene vector comprising miRNA
US20070032443A1 *	2005-08-02	2007-02-08	Jaeseob Kim	Therapy for Alzheimer's disease
WO2007015122A1 *	2005-08-02	2007-02-08	Genexel, Inc.	Therapy for alzheimer’s disease
WO2007056388A2	2005-11-07	2007-05-18	The General Hospital Corporation	Compositions and methods for modulating poly (adp-ribose) polymerase activity
WO2007133674A2	2006-05-12	2007-11-22	Lentigen Corporation	Lentiviral vector compositions, methods and applications
US8535897B2 *	2006-06-19	2013-09-17	The Trustees Of Columbia University In The City Of New York	Assays for non-apoptotic cell death and uses thereof
US20080003225A1	2006-06-29	2008-01-03	Henri Vie	Method for enhancing the antibody-dependent cellular cytotoxicity (ADCC) and uses of T cells expressing CD16 receptors
WO2008008719A2	2006-07-10	2008-01-17	Alnylam Pharmaceuticals, Inc.	Compositions and methods for inhibiting expression of the myc gene
EP1878440A1	2006-07-13	2008-01-16	INSERM (Institut National de la Santé et de la Recherche Médicale)	Methods and compositions for increasing the efficiency of therapeutic antibodies using gamma delta cell activator compounds
CN101516365A *	2006-07-26	2009-08-26	诺瓦提斯公司	Inhibitors of undecaprenyl pyrophosphate synthase

US20080199961A1 *	2006-08-25	2008-08-21	Avi Biopharma, Inc.	ANTISENSE COMPOSITION AND METHOD FOR INHIBITION OF miRNA BIOGENESIS
WO2008100292A2 *	2006-10-16	2008-08-21	Genelux Corporation	Modified vaccinia virus strains for use in diagnostic and therapeutic methods
ES2639568T3	2007-01-23	2017-10-27	Janssen Pharmaceutica Nv	Method to design a drug regimen for HIV-infected patients
CA2682694A1 *	2007-04-12	2008-10-23	The Board Of Trustees Of The University Of Illinois	Bisphosphonate compounds and methods with enhanced potency for multiple targets including fpps, ggpps, and dpps
US20080293142A1	2007-04-19	2008-11-27	The Board Of Regents For Oklahoma State University	Multiple shRNA Expression Vectors and Methods of Construction
EP2008656A1	2007-06-28	2008-12-31	Bergen Teknologioverforing AS	Compositions for the treatment of hyperphenylalaninemia
US8673477B2	2008-06-16	2014-03-18	Polyplus Battery Company	High energy density aqueous lithium/air-battery cells
WO2009026328A2	2007-08-21	2009-02-26	Immune Disease Institute, Inc.	Methods of delivery of agents to leukocytes and endothelial cells
CA3018281C	2007-09-28	2022-02-22	Anthrogenesis Corporation	Tumor suppression using human placental perfusate and human placenta-derived intermediate natural killer cells
EP2090659A1	2008-02-14	2009-08-19	Fraunhofer-Gesellschaft zur Förderung der angewandten Forschung e.V.	Infectious particle, process for its preparation and use thereof
GB0810209D0	2008-06-04	2008-07-09	Cambridge Entpr Ltd	Pluripotency associated epigenetic factor
US8629334B2	2008-07-16	2014-01-14	University Of Florida Research Foundation, Inc.	Viral-based transient-expression vector system for trees
WO2010022195A2	2008-08-20	2010-02-25	Virxsys Corporation	Non-integrating lenti/adeno-associated virus hybrid vector system
EP2342321B1	2008-09-17	2018-04-11	Isogenis, Inc.	Construction of fully-deleted adenovirus-based gene delivery vectors and uses thereof
WO2010045659A1	2008-10-17	2010-04-22	American Gene Technologies International Inc.	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules
US8734795B2	2008-10-31	2014-05-27	Biogen Idec Ma Inc.	Light targeting molecules and uses thereof
WO2010051521A1	2008-10-31	2010-05-06	Lentigen Corporation	Cell therapy product for the treatment of hiv infection
WO2011071476A2	2008-11-14	2011-06-16	Life Technologies Corporation	Compositions and methods for engineering cells
EP2191834A1	2008-11-26	2010-06-02	Centre National De La Recherche Scientifique (Cnrs)	Compositions and methods for treating retrovirus infections

US20120114618A1	2009-03-26	2012-05-10	The Regents Of The University Of California	Mesenchymal Stem Cells Producing Inhibitory RNA for Disease Modification
WO2010117974A2	2009-04-09	2010-10-14	Stemcyte Inc.	Hiv-resistant stem cells and uses thereof
EP2419113B1	2009-04-13	2017-05-10	Apceth GmbH & Co. KG	Engineered mesenchymal stem cells and method of using same to treat tumors
EP2425001A4	2009-04-30	2012-11-14	Univ California	Combination anti-hiv vectors, targeting vectors, and methods of use
EP3329772B1	2009-07-15	2019-10-16	Calimmune, Inc.	Dual vector for inhibition of human immunodeficiency virus
US20120027725A1 *	2009-11-30	2012-02-02	Galvin Jeffrey A	Safe lentiviral vectors for targeted delivery of multiple therapeutic molecules to treat liver cancer
CN102782136A	2010-02-18	2012-11-14	爱默蕾大学	Vectors expressing HIV antigens and GM-CSF and related methods for generating an immune response
WO2011119942A1	2010-03-25	2011-09-29	Vistagen Therapeutics, Inc.	Induction of ips cells using transient episomal vectors
WO2011133687A2 *	2010-04-20	2011-10-27	President And Fellows Of Harvard College	Methods and compositions for inhibition of beta2-adrenergic receptor degradation
LT2561078T	2010-04-23	2019-01-10	Cold Spring Harbor Laboratory	NOVEL STRUCTURALLY DESIGNED shRNAs
WO2012020757A1	2010-08-10	2012-02-16	タカラバイオ株式会社	Production method for cell populations
US20130281493A1	2010-10-07	2013-10-24	The Trustees Of The University Of Columbia In The City Of New York	Method for Treating Cancer Harboring a p53 Mutation
WO2012061075A2	2010-10-25	2012-05-10	The Regents Of The University Of California	Hiv resistant and functional hematopoietic stem/progenitor cells and macrophages from induced pluripotent stem cells
CN108744262A	2010-11-23	2018-11-06	普莱萨格生命科学公司	Treatment and composition for for physical delivery
WO2012115980A1	2011-02-22	2012-08-30	California Institute Of Technology	Delivery of proteins using adeno-associated virus (aav) vectors
US9226976B2 *	2011-04-21	2016-01-05	University Of Massachusetts	RAAV-based compositions and methods for treating alpha-1 anti-trypsin deficiencies
US9358250B2	2011-10-15	2016-06-07	Genentech, Inc.	Methods of using SCD1 antagonists
EP2782596A4	2011-11-22	2015-07-29	Philadelphia Children Hospital	Virus vectors for highly efficient transgene delivery
US9745631B2 *	2011-12-20	2017-08-29	Dana-Farber Cancer Institute, Inc.	Methods for diagnosing and treating oncogenic kras-associated cancer
BR112014019431A8	2012-02-07	2017-07-11	Global Bio Therapeutics Usa Inc	COMPARTMENTALIZED METHOD OF DELIVERY OF NUCLEIC ACID AND COMPOSITIONS AND USES

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WO2013174404A1	2012-05-23	2013-11-28	Ganymed Pharmaceuticals Ag	Combination therapy involving antibodies against claudin 18.2 for treatment of cancer
AU2013273483A1	2012-06-06	2014-12-11	Bionor Immuno As	Vaccine
WO2014016817A2	2012-07-17	2014-01-30	Universite De Geneve	Nucleic acids for down-regulation of gene expression
CA2922005A1	2012-09-27	2014-04-03	Population Diagnostics, Inc.	Methods and compositions for screening and treating developmental disorders
JP6391582B2	2012-11-13	2018-09-19	コディアック バイオサイエンシズ インコーポレイテッド	Methods for delivering therapeutic agents
CA2892448A1	2012-12-05	2014-06-12	Sangamo Biosciences, Inc.	Methods and compositions for regulation of metabolic disorders
US9642921B2	2012-12-20	2017-05-09	Tocagen Inc.	Cancer combination therapy and recombinant vectors
WO2014117050A2 *	2013-01-26	2014-07-31	Mirimus, Inc.	Modified mirna as a scaffold for shrna
CN103184224A	2013-04-03	2013-07-03	衡阳师范学院	Triple minRNA for resisting virus infection of aids and construction method thereof
WO2014187881A1	2013-05-21	2014-11-27	Max-Planck Gesellschaft zur Förderung der Wissenschaften e.V.	Isoforms of gata6 and nkx2-1 as markers for diagnosis and therapy of cancer and as targets for anti-cancer therapy
KR20160011645A	2013-06-03	2016-02-01	떼라벡띠스	LENTIVIRAL VECTORS CONTAINING AN MHC CLASS I, MHC CLASS II OR β2 MICROGLOBULIN UPSTREAM PROMOTER SEQUENCE
AU2014296059B2	2013-08-02	2020-12-10	The Regents Of The University Of California	Engineering antiviral T cell immunity through stem cells and chimeric antigen receptors
WO2015042308A2	2013-09-18	2015-03-26	City Of Hope	Rna-based hiv inhibitors
AU2014340083B2	2013-10-22	2019-08-15	Translate Bio, Inc.	mRNA therapy for phenylketonuria
CN106459995B	2013-11-07	2020-02-21	爱迪塔斯医药有限公司	CRISPR-associated methods and compositions using dominant grnas
EP2878674A1	2013-11-28	2015-06-03	Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)	Stable episomes based on non-integrative lentiviral vectors
WO2015148926A1	2014-03-28	2015-10-01	Regents Of The University Of Minnesota	Polypeptides, cells, and methods involving engineered cd16
CA2946312A1	2014-04-23	2015-10-29	Juno Therapeutics, Inc.	Methods for isolating, culturing, and genetically engineering immune cell populations for adoptive therapy

DK3851537T3	2014-04-25	2024-03-18	Genethon	TREATMENT OF HYPERBILIRUBINAMIA
CA2955254A1	2014-08-29	2016-03-03	Immunomedics, Inc.	Identification of cancer genes by in-vivo fusion of human cancer cells and animal cells
SI3197472T1	2014-09-22	2022-01-31	Tanea Medical Ab	Recombinant phe-free proteins for use in the treatment of phenylketonuria
AU2015329696A1	2014-10-10	2017-04-27	The United States Of America, As Represented By The Secretary, Department Of Health And Human Services	Methods to eliminate cancer stem cells by targeting CD47
CN107405357B	2014-10-14	2021-12-31	德克萨斯科技大学系统	Multiple shRNAs and application thereof
WO2016069716A1	2014-10-30	2016-05-06	The Scripps Research Institute	Compositions and methods comprising tyrosyl-trna synthetases and resveratrol compounds
GB201509202D0	2015-05-28	2015-07-15	Ge Healthcare Bio Sciences Ab	Semi-static cell culture
JP6924487B2	2015-06-10	2021-08-25	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-embedded virus delivery system and how to use it
WO2017007994A1	2015-07-08	2017-01-12	American Gene Technologies International Inc.	Hiv pre-immunization and immunotherapy
JP6780870B2	2015-08-13	2020-11-04	北昊干細胞与再生医学研究院有限公司Ｂｅｉｈａｏ ＳｔｅｍＣｅｌｌ ＡｎｄＲｅｇｅｎｅｒａｔｉｖｅＭｅｄｉｃｉｎｅＲｅｓｅａｒｃｈＩｎｓｔｉｔｕｔｅＣｏ．，Ｌｔｄ．	Induced expanded pluripotent stem cells, how to make and use
CN105112370B *	2015-08-25	2019-02-05	杭州优善生物科技有限公司	A kind of method and its application of stimulated in vitro peripheral blood gamma delta T cells high efficiently multiplying
JP7059179B2	2015-10-20	2022-04-25	アンスティチュ ナショナル ドゥ ラ サンテ エ ドゥ ラ ルシェルシュ メディカル	Methods and products for genetic engineering
US11389546B2	2015-12-09	2022-07-19	Modernatx, Inc.	Heterologous UTR sequences for enhanced mRNA expression
US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
EP4310500A3	2016-01-15	2024-04-03	American Gene Technologies International Inc.	Methods and compositons for the activation of gamma-delta t-cells
EP3413926A4	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
WO2017156311A2	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
BR112018069090A2	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 (hmsc)
WO2017173453A1	2016-04-01	2017-10-05	The Brigham And Women's Hospital, Inc.	Stimuli-responsive nanoparticles for biomedical applications
JP7173548B2	2016-06-08	2022-11-16	アメリカン ジーン テクノロジーズ インターナショナル インコーポレイテッド	Non-Integrating Viral Delivery Systems and Related Methods
AU2017292582C1	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
KR20190100318A	2016-12-30	2019-08-28	더 트러스티스 오브 더 유니버시티 오브 펜실바니아	Gene therapy to treat phenylketonuria
EP3565564A4	2017-01-09	2020-09-23	American Gene Technologies International Inc.	Hiv immunotherapy with no pre-immunization step
CN110621322A	2017-02-08	2019-12-27	达纳-法伯癌症研究所有限公司	Modulatable endogenous protein degradation with heterobifunctional compounds
BR112019017839A2 *	2017-02-28	2020-04-14	Univ Pennsylvania	clade f vector of adeno-associated virus (aav) and its use
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
US20200181645A1	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
CN111433368A	2017-10-02	2020-07-17	美国基因技术国际有限公司	Vector with promoter and enhancer combination for treating phenylketonuria
DE112019000348B4	2018-01-05	2021-10-28	Nantbio, Inc.	REPROGRAMMED T-CELLS SIMILAR NK-CELLS
WO2020011247A1	2018-07-13	2020-01-16	Nanjing Legend Biotech Co., Ltd.	Co-receptor systems for treating infectious diseases
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
KR20220068954A	2019-05-31	2022-05-26	아메리칸 진 테크놀로지스 인터내셔널 인코포레이티드	Optimized phenylalanine hydroxylase expression
IL296096A	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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US10137144B2	2016-01-15	2018-11-27	American Gene Technologies International Inc.	Methods and compositions for the activation of gamma-delta T-cells
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EP3413926A4	2016-02-08	2019-10-09	American Gene Technologies International, Inc.	Hiv vaccination and immunotherapy
WO2017156311A2	2016-03-09	2017-09-14	American Gene Technologies International Inc.	Combination vectors and methods for treating cancer
AU2017292582C1	2016-07-08	2021-11-11	American Gene Technologies International Inc.	HIV pre-immunization and immunotherapy
EP3487507A4	2016-07-21	2020-04-08	American Gene Technologies International, Inc.	Viral vectors for treating parkinson's disease
US11820999B2	2017-04-03	2023-11-21	American Gene Technologies International Inc.	Compositions and methods for treating phenylketonuria
US20200181645A1 *	2017-06-16	2020-06-11	American Gene Technologies International Inc.	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells
KR20200051011A *	2017-09-08	2020-05-12	제너레이션 바이오 컴퍼니	Modified closed-terminated DNA (CEDNA)
US11352646B2	2018-11-05	2022-06-07	American Gene Technologies International Inc.	Vector system for expressing regulatory RNA
CN109363955B *	2018-11-14	2021-09-21	广州玮弘祺生物科技有限公司	Solid styling spray for hair and preparation method thereof
CN109456993A *	2018-11-28	2019-03-12	上海安民生物技术有限公司	The albumin expression vectors of the promoter containing CAG

WO2020237219A1 *	2019-05-23	2020-11-26	Rocket Pharmaceuticals, Ltd.	Gene therapy vectors for infantile malignant osteopetrosis

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Publication	Publication Date	Title
US11519006B2	2022-12-06	Methods and compositions for the activation of gamma-delta T-cells
US11534450B2	2022-12-27	Methods and compositions for the activation of gamma-delta T-cells
US11242527B1	2022-02-08	Combination vectors and methods for treating cancer
US20200181645A1	2020-06-11	Methods and compositions for the activation of tumor cytotoxicity via human gamma-delta t-cells

Priority And Related Applications

Child Applications (1)

Application	Priority date	Filing date	Relation	Title
JP2021045605A	2016-01-15	2021-03-19	Division	Methods and compositions for activation of gamma-delta t-cells

Applications Claiming Priority (3)

Application	Filing date	Title
US201662279474P	2016-01-15	
US62/279,474	2016-01-15	
PCT/US2017/013399	2017-01-13	Methods and compositons for the activation of gamma-delta t-cells

Legal Events

Date	Code	Title	Description
2020-01-10	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20200110
2020-01-10	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621

			Effective date: 20200110
2020-05-19	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20200519
2020-05-19	A871	Explanation of circumstances concerning accelerated examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A871 **Effective date**: 20200519
2020-06-05	A975	Report on accelerated examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A971005 **Effective date**: 20200605
2020-06-26	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20200626
2020-09-18	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20200918
2020-11-19	A02	Decision of refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A02 **Effective date**: 20201119
2021-03-19	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20210319
2021-03-19	C60	Trial request (containing other claim documents, opposition documents)	**Free format text**: JAPANESE INTERMEDIATE CODE: C60 **Effective date**: 20210319
2021-03-29	C11	Written invitation by the commissioner to file amendments	**Free format text**: JAPANESE INTERMEDIATE CODE: C11 **Effective date**: 20210329
2021-06-14	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A821 **Effective date**: 20210428
2021-06-16	A911	Transfer to examiner for re-examination before appeal (zenchi)	**Free format text**: JAPANESE INTERMEDIATE CODE: A911 **Effective date**: 20210615

2021-06-16	C21	Notice of transfer of a case for reconsideration by examiners before appeal proceedings	**Free format text**: JAPANESE INTERMEDIATE CODE: C21 **Effective date**: 20210616
2021-08-10	A912	Re-examination (zenchi) completed and case transferred to appeal board	**Free format text**: JAPANESE INTERMEDIATE CODE: A912 **Effective date**: 20210806
2021-08-11	C211	Notice of termination of reconsideration by examiners before appeal proceedings	**Free format text**: JAPANESE INTERMEDIATE CODE: C211 **Effective date**: 20210811
2022-05-11	C22	Notice of designation (change) of administrative judge	**Free format text**: JAPANESE INTERMEDIATE CODE: C22 **Effective date**: 20220511
2022-07-07	C302	Record of communication	**Free format text**: JAPANESE INTERMEDIATE CODE: C302 **Effective date**: 20220707
2022-07-11	C13	Notice of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: C13 **Effective date**: 20220711
2022-07-11	C28A	Non-patent document cited	**Free format text**: JAPANESE INTERMEDIATE CODE: C2838 **Effective date**: 20220711
2022-10-07	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20221007
2022-11-10	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20221110
2022-12-09	A601	Written request for extension of time	**Free format text**: JAPANESE INTERMEDIATE CODE: A601 **Effective date**: 20221209
2023-01-11	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20230111
2023-01-13	C302	Record of communication	**Free format text**: JAPANESE INTERMEDIATE CODE: C302 **Effective date**: 20230113

2023-01-27	C302	Record of communication	**Free format text**: JAPANESE INTERMEDIATE CODE: C302 **Effective date**: 20230127
2023-01-30	C13	Notice of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: C13 **Effective date**: 20230130
2023-03-03	A521	Request for written amendment filed	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20230303
2023-03-07	C23	Notice of termination of proceedings	**Free format text**: JAPANESE INTERMEDIATE CODE: C23 **Effective date**: 20230307
2023-03-30	C03	Trial/appeal decision taken	**Free format text**: JAPANESE INTERMEDIATE CODE: C03 **Effective date**: 20230330
2023-03-30	C30A	Notification sent	**Free format text**: JAPANESE INTERMEDIATE CODE: C3012 **Effective date**: 20230330
2023-04-04	A61	First payment of annual fees (during grant procedure)	**Free format text**: JAPANESE INTERMEDIATE CODE: A61 **Effective date**: 20230331
2023-04-11	R150	Certificate of patent or registration of utility model	**Ref document number**: 7260898 **Country of ref document**: JP **Free format text**: JAPANESE INTERMEDIATE CODE: R150

Concepts

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Name	Image	Sections	Count	Query match
method		title,claims,abstract,description	67	0.000
gamma-delta t lymphocyte		title,claims,abstract,description	15	0.000
mixture		title,abstract,description	45	0.000
activation		title,description	19	0.000

cell	claims,abstract,description	239	0.000
T-lymphocyte	claims,abstract,description	106	0.000
genetic effect	claims,abstract,description	51	0.000
Neoplasm	claims,abstract,description	50	0.000
Enzymes	claims,abstract,description	39	0.000
Enzymes	claims,abstract,description	39	0.000
virological effect	claims,abstract,description	28	0.000
pathway	claims,abstract,description	26	0.000
Bacterial small RNA	claims,abstract,description	22	0.000
(R)-mevalonate	claims,abstract,description	21	0.000
DL-mevalonic acid	claims,abstract,description	21	0.000
manufacturing process	claims,abstract,description	20	0.000
Communicable disease	claims,abstract,description	17	0.000
inhibitory effect	claims,abstract,description	16	0.000
activating effect	claims,abstract,description	10	0.000
vector	claims,description	156	0.000
Geranyltranstransferase	claims,description	118	0.000
Farnesyl pyrophosphate synthase	claims,description	115	0.000
plasmid	claims,description	97	0.000
zoledronic acid	claims,description	50	0.000
zoledronic acid	claims,description	42	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

microRNA	claims,description	37	0.000
viral vector	claims,description	37	0.000
MicroRNAs	claims,description	35	0.000
cancer	claims,description	31	0.000
particle	claims,description	31	0.000
drug	claims,description	30	0.000
drug	claims,description	30	0.000
packaging method and process	claims,description	28	0.000
Chemokines	claims,description	22	0.000
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Cytokines	claims,description	22	0.000
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Interleukin-2	claims,description	22	0.000
Interleukin-2	claims,description	22	0.000
Envelope protein	claims,description	15	0.000
Protein X	claims,description	15	0.000
small Interfering RNA	claims,description	14	0.000
Small hairpin RNA	claims,description	12	0.000
gag Genes	claims,description	12	0.000
pol Genes	claims,description	12	0.000
rev Genes	claims,description	12	0.000
Interleukin-18	claims,description	9	0.000
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Interferon-gamma	claims,description	8	0.000
Interferon-gamma	claims,description	8	0.000

Interleukin-15	claims,description	8	0.000
Interleukin-15	claims,description	8	0.000
interferon gamma	claims,description	8	0.000
Interleukin-1	claims,description	6	0.000
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Interleukin-12	claims,description	6	0.000
Interleukin-12	claims,description	6	0.000
Interleukin-17	claims,description	6	0.000
Interleukin-17	claims,description	6	0.000
rev gene	claims,description	5	0.000
CC Chemokines	claims,description	3	0.000
CC Chemokines	claims,description	3	0.000
CXC Chemokine	claims,description	3	0.000
CXC chemokine	claims,description	3	0.000
Syncytin-1	claims	4	0.000
treatment	abstract,description	38	0.000
gene therapy	abstract,description	17	0.000
immunotherapy	abstract,description	3	0.000

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