

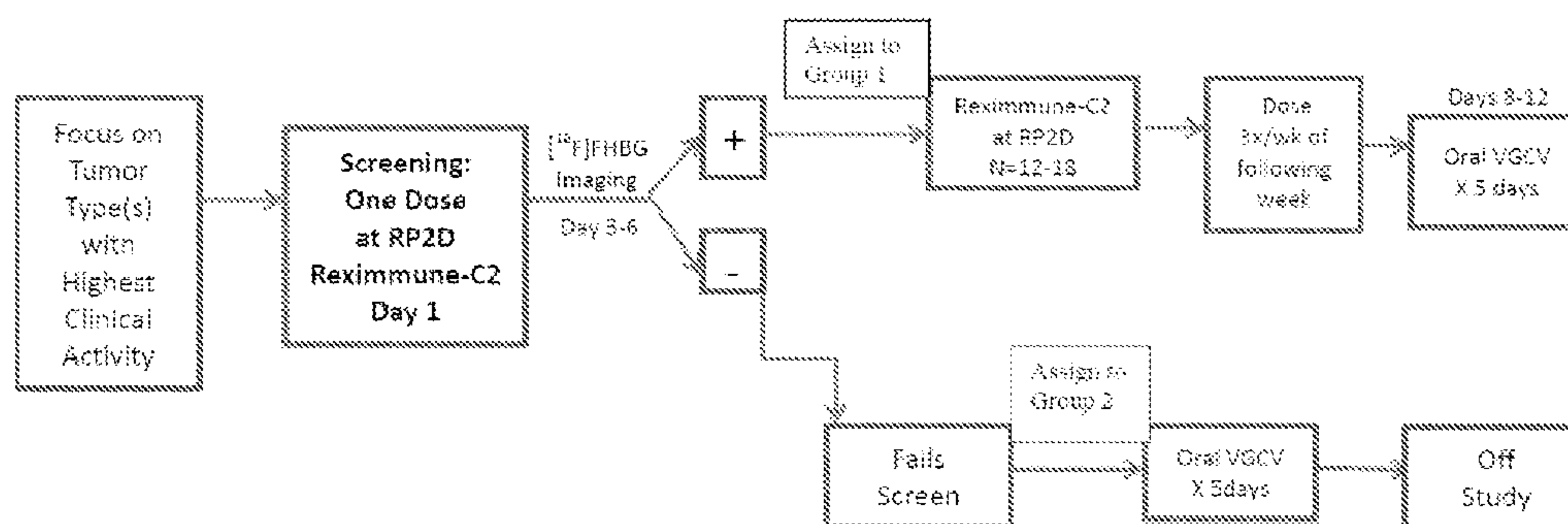


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(54) **Titre : DOSAGE DIAGNOSTIQUE DES THYMIDINE KINASES POUR APPLICATIONS DE THERAPIE GENIQUE**
(54) **Title: THYMIDINE KINASE DIAGNOSTIC ASSAY FOR GENE THERAPY APPLICATIONS**

FIG. 4



(57) **Abrégé/Abstract:**

Nucleic acid sequences encoding improved Herpes Simplex Virus Thymidine Kinases are provided, including their use in diagnostic and therapeutic applications. The thymidine kinases may be mutated using conservative mutations, non-conservative mutations, or both. Also provided are gene therapeutic systems, including viral and retroviral particles.



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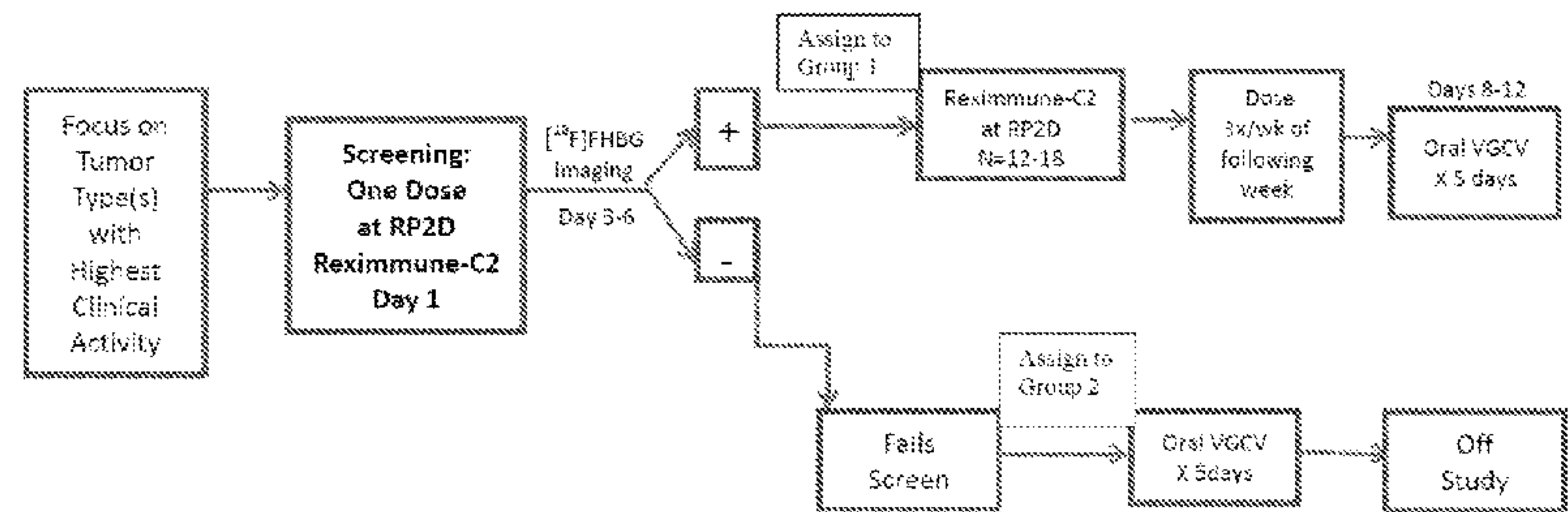
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(54) **Title:** THYMIDINE KINASE DIAGNOSTIC ASSAY FOR GENE THERAPY APPLICATIONS

FIG. 4



(57) **Abstract:** Nucleic acid sequences encoding improved Herpes Simplex Virus Thymidine Kinases are provided, including their use in diagnostic and therapeutic applications. The thymidine kinases may be mutated using conservative mutations, non-conservative mutations, or both. Also provided are gene therapeutic systems, including viral and retroviral particles.

WO 2014/153205 A1

THYMIDINE KINASE DIAGNOSTIC ASSAY FOR GENE THERAPY APPLICATIONS**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Application No. 61/784,901, filed on March 14, 2013, which is incorporated herein by reference in its entirety.

[0002] This application is related to the following co-pending patent applications: application Serial No. [not yet assigned], Attorney Docket No. 30863-722.201, filed the same day herewith, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Proliferative diseases, such as cancer, pose a serious challenge to society. Cancerous growths, including malignant cancerous growths, possess unique characteristics such as uncontrollable cell proliferation resulting in, for example, unregulated growth of malignant tissue, an ability to invade local and even remote tissues, lack of differentiation, lack of detectable symptoms and most significantly, the lack of effective therapy and prevention.

[0004] Cancer can develop in any tissue of any organ at any age. The etiology of cancer is not clearly defined but mechanisms such as genetic susceptibility, chromosome breakage disorders, viruses, environmental factors and immunologic disorders have all been linked to a malignant cell growth and transformation. Cancer encompasses a large category of medical conditions, affecting millions of individuals worldwide. Cancer cells can arise in almost any organ and/or tissue of the body. Worldwide, more than 10 million people are diagnosed with cancer every year and it is estimated that this number will grow to 15 million new cases every year by 2020. Cancer causes six million deaths every year or 12% of the deaths worldwide.

SUMMARY OF THE INVENTION

[0005] Provided herein are methods and compositions for identifying subjects or patients that are capable of benefitting from gene therapy treatment. More specifically, provided herein are methods and compositions for identifying subjects or patients that express in sufficient quantities a therapeutic protein included in a gene therapy agent. Preferably the therapeutic protein is an enzyme, more specifically viral thymidine kinase or mutant viral thymidine kinase.

[0006] Accordingly, provided herein are methods for identifying a patient capable of benefitting from gene therapy treatment comprising administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide to the patient; administering to the patient a substrate of HSV-TK attached to a radioactive tracer; measuring the relative amount and location of the radioactive signal present in the patient; and determining the location of lesions in the patient, wherein patients with: radioactive signals above a certain threshold, and location of the

radioactive signal correlating with lesions measured in step (d) of the patient, are identified as capable of benefitting from gene therapy treatment.

[0007] In some embodiments, the substrate of HSV-TK is chosen from the group consisting of FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains. In yet other embodiments, the substrate of HSV-TK is FHBG (9-(4-fluoro-3-(hydroxymethyl)butyl]guanine).

[0008] In still other embodiments, the radioactive tracer is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$. In other embodiments, the radioactive tracer is ^{18}F .

[0009] In one embodiment, the HSV-TK substrate is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine). In still other embodiments, the radioactive tracer signal is measured using positron emission tomography (PET) scanning.

[0010] In still other embodiments, the threshold level is at least above 2.0 SUV (standardized uptake value) or at least 20% above background on a PET scan, or between about 1.0 SUV and about 3.0 SUV, or between about 20% to about 40% above background on a PET scan.

[0011] In some embodiments, the methods disclosed herein further comprises treating the patient with the HSV-TK retroviral particle.

[0012] In still other embodiments, the viral nuclear localization sequence (NLS) of the encoded HSV-TK polynucleotide is mutated. In yet other embodiments, the thymidine kinase polynucleotide is mutated to include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein. In one embodiment, the thymidine kinase polynucleotide is mutated to increase substrate binding of the expressed thymidine kinase protein. In another embodiment, the mutation is A168H.

[0013] In still other embodiments, the methods disclosed herein further comprises mutating the thymidine kinase polynucleotide to remove the viral nuclear localization sequence (NLS) and include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein. In some embodiments, the HSV-thymidine kinase polynucleotide is SEQ ID NO: 18.

[0014] In one embodiment, the methods disclosed herein further comprises a targeting protein expressed on the viral envelope. In some embodiments, the targeting protein binds to collagen, laminin, fibronectin, elastin, glycosaminoglycans, proteoglycans or RGD. In still other embodiments, the targeting protein binds to collagen. In yet other embodiments, the targeting protein is SEQ ID NO: 25.

[0015] Also provided herein are methods and compositions for identifying a patient or subject in need of treatment for lesions and capable of benefitting from gene therapy treatment: a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells from the patient with the polynucleotide encoding HSV-thymidine kinase; b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; c) measuring the relative amount of radioactive signal present in target tissue; d) identifying patients wherein the level of radioactively-labelled HSV-TK substrate is above a threshold; e) determining the location of lesions in the patient; and f) treating said patient or subject with the gene therapy retroviral particle comprising an HSV-TK polynucleotide when the measured radioactive signal in the patient is above a certain threshold, and the location of the measured radioactive signal correlates with lesions measured in step (e) of the patient.

[0016] Provided herein are methods and compositions for measuring the enzymatic activity of a transduced HSV-thymidine, the method comprising: a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells from the patient with the polynucleotide encoding HSV-thymidine kinase; b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; and c) measuring the relative amount of radioactive signal present in target tissue.

[0017] In addition, provided herein are methods and compositions for determining the level of a tracer signal in a subject or patient after administration of a gene therapy particle, and selecting the subject or patient for treatment with the gene therapy particle when the level of the tracer signal is above a set threshold. In some embodiments, the tracer is a radioactive, luminescent or a fluorescent signal. In some embodiments, the radioactive tracer element is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$.

[0018] In yet other embodiments, provided herein are methods and compositions for determining the level of a radiotracer signal in a subject or patient after administration of a thymidine kinase gene therapy construct, and selecting the subject or patient for treatment with the gene therapy construct when the level of the tracer signal is above a set threshold. In some embodiments, the tracer is a radioactive tracer. In other embodiments, the radioactive tracer element is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$. In yet other embodiments, the radioactive tracer element is coupled to a nucleoside or synthetic nucleoside

target to form a radioactive target. In some embodiments, the nucleoside target is FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, val-ganciclovir, acyclovir, val-aciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains. Preferably the radioactive target is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-[hydroxymethyl]butyl)guanine).

[0019] Also provided herein are methods comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; and (b) selecting the subject for treatment with a composition wherein the level of FHBG is above a threshold level. In some embodiments, the threshold level is at least about 2.0 SUV (standardized uptake values) or at least 20% above background signal on a PET scan.

[0020] Additionally provided herein is a method comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; (b) excluding the subject from treatment with a composition wherein the level of FHBG in the subject is greater than about 2.0 SUV or greater than about 20% above background signal on a PET scan; and (c) administering to said subject an anti-cancer agent.

[0021] In some embodiments, the invention provides a method for identifying a subject that is susceptible to a cancer treatment, the method comprising: a) identifying expression of [^{18}F]FHBG in the subject; b) treating the subject.

[0022] Provided herein is a method of measuring HSV-TK-FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, val-ganciclovir, acyclovir, val-aciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-like, ganciclovir-like and penciclovir-like side chains-mediated bystander effect, the method comprising: a) transducing cells with a polynucleotide encoding HSV-TK and a first fluorescent

protein; b) transducing the cells with a polynucleotide encoding a second or bioluminescent protein that is optically discernible from the first fluorescent or bioluminescent protein; c) treating the cells with an agent that becomes cytotoxic upon being phosphorylated by HSV-TK; and d) measuring the relative amount of expression of the first fluorescent protein and the second fluorescent protein. In one embodiment, step d) comprises a Perkin Elmer Plate reader, a fluorimeter; a fluorescent activated cell sorter (FACS); a cellometer; or a spectrophotometer. In another embodiment, step d) comprises measuring fluorescent output of the second fluorescent or bioluminescent protein *in vivo* in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0024] **Figure 1** illustrates the structure of 9-[4- ^{18}F Fluoro-3-(hydroxymethyl)butyl]guanine (^{18}F FHBG) and its mechanism of inhibition.

[0025] **Figure 2** is whole body coronal images of ^{18}F FHBG biodistribution in a healthy human subject at four distinct time periods after 4.53 mCi i.v. injection of the ^{18}F FHBG.

[0026] **Figure 3** is a schematic for a Phase IA clinical trial.

[0027] **Figure 4** is a schematic for a Phase IB clinical trial.

[0028] **Figure 5** is a patient's response to treatment with HSV-TK in AAV retroviral vector particle.

[0029] **Figure 6** measuring the response of a patient to administration of ^{18}F FHBG in PET Scan (top panel), CT Scan (middle panel) and fusion of signals (bottom panel).

[0030] **Figure 7** is a fluorescent image of the biodistribution of the HSV-TK retroviral vector particles in animals.

[0031] **Figure 8** is a comparison of coronal three hour images of 5 mm slices in rats administered Reximmune C1 and C2. The tumor on the left expressed Reximmune-C2 and on the right is Reximmune-C1.

[0032] **Figure 9** is a graphical representation of Figure 8, showing the averages of the mean within tumors for one and three hour images. Error bars are standard error of the averages. B12 is the Reximmune-C2 expressing tumor and A9 the Reximmune-C1 expressing tumor. C6 is the native cell line tumor.

DETAILED DESCRIPTION OF THE INVENTION

[0033] Provided herein are methods and compositions for identifying a patient susceptible to treatment with a gene therapy delivery system. Also provided herein are methods and compositions for identifying subjects or patients that are capable of benefitting from gene therapy treatment. Moreover, provided herein are methods and compositions for identifying subjects or patients that express in sufficient quantities a therapeutic protein included in a gene therapy construct. The identification of subjects or patients that are capable of expressing sufficient quantities of a therapeutic protein allows a practitioner to screen and identify patients that can benefit from a particular gene therapy treatment. By doing so, patients and subjects are identified at an early stage that are capable of delivering anti-cancer agents via gene therapy particles to treat, for example, primary and metastatic lesions.

[0034] In some embodiments, anti-cancer agents expressed from gene therapy constructs included in viral particles can be administered to patients by intravenous infusion. In yet other embodiments, anti-cancer agents expressed from gene therapy constructs can be administered to patients via inter-arterial infusion. In yet other embodiments, the viral particles containing anti-cancer agents can be administered intra-tumoral. In still other embodiments, anti-cancer agents expressed from gene therapy constructs can be selectively transduced *in vitro* into target cells.

[0035] In yet other embodiments, anti-cancer agents expressed from gene therapy constructs can be targeted to primary and metastatic lesions, thereby delivering a tumor-killing gene to primary and metastatic lesions while sparing normal cells and tissues. In some embodiments, the targeting of gene therapy constructs is specific. In yet other embodiments, the targeting of gene therapy constructs is to a cell-surface or extracellular protein. In some embodiments, the cell-surface or extracellular protein is collagen. In yet other embodiments, the targeting of gene therapy constructs is to a specific protein expressed by tumor cells. Such anti-cancer agents provide a powerful tool that can specifically target cancer cells, thereby mitigating the unwanted side-effects of other known cancer therapies.

[0036] In some embodiments, the gene therapy construct is a retrovirus. Retroviruses typically have three common open reading frames, gag, pol, and env, which encode the matrix, gag and nucleocapsid structural proteins, encode enzymes including reverse transcriptase, integrase and protease, and encode envelope proteins and transmembrane fusogenic proteins, respectively. Typically, retroviral vector particles are produced by packaging cell lines that provide the necessary gag, pol, and env gene products in trans. (Miller, et al., Human Gene Therapy, Vol. 1, pgs. 5-14 (1990)). This approach results in the production of retroviral vector particles which transduce mammalian cells, but are incapable of further replication after they have integrated into the genome of the cell.

[0037] In some embodiments, the retrovirus comprises at least one therapeutic protein or payload delivered by the gene therapy construct. In some embodiments, the therapeutic protein or payload is an enzyme. In yet other embodiments, the therapeutic protein or payload is thymidine kinase. In still other embodiments, the thymidine kinase is HSV (herpes simplex virus) thymidine kinase. In yet other embodiments, the thymidine kinase is HSV (herpes simplex virus) thymidine kinase-1.

[0038] In some embodiments, the HSV-TK gene therapy construct is optimized with respect to maximal gene expression and tumor kill activity both *in vitro* and *in vivo* including cancer gene therapy. In some embodiments, the HSV-TK gene is codon-optimized. In still other embodiments, the HSV-TK gene therapy construct is targeted to a specific tumor cell or tissue. In yet other embodiments, the HSV-TK gene therapy construct is targeted to a cell-surface protein specifically expressed in tumor cells. In still other embodiments, the HSV-TK gene therapy construct is targeted to a cell-surface protein expressed in tumor tissue or cells. In other embodiments, the HSV-TK gene therapy construct is targeted to collagen.

[0039] When expressed *in vivo* in cells, HSV-TK enzymatically cleaves a co-administered nucleoside agent, such as ganciclovir, penciclovir, val-ganciclovir, acyclovir and val-aciclovir, and subsequently transforms the co-administered agent into a cytotoxic agent. Mammalian thymidine kinases are insensitive to these co-administered agents. Sensitivity to the cytotoxic agent is therefore only conferred upon tumor cells after expression of the HSV-TK gene. Ganciclovir is converted by the resulting HSV-TK to the monophosphorylated product, which is then converted to di- and triphosphates by host kinases, leading to cytotoxicity and tumor cell death. Viral thymidine kinase therapy has been previously shown to have promise in the treatment of several cancers, including gliomas, hepatoma and melanoma.

[0040] HSV-TK also selectively phosphorylates the nucleoside analogue of, for example, 9-[4-¹⁸F-fluoro-3-(hydroxymethyl)butyl]guanine ([¹⁸F]FHBG) (FIG. 1), which cleaves the radioactive tracer ¹⁸F from the FHBG molecule. HSV-TK expression can therefore be closely monitored with positron emission tomography (PET) scans.

[0041] Accordingly provided herein are methods and compositions for determining the level of a tracer signal in a subject or patient after administration of a gene therapy vector, and selecting the subject or patient for treatment with the gene therapy vector when the level of the tracer signal is above a set threshold. In some embodiments, the tracer is a radioactive, luminescent or a fluorescent signal. In some embodiments, the radioactive tracer element is ¹⁸F, ⁶⁴Cu, ^{99m}Te, ¹¹C, ¹⁴C, ¹²⁴I, ¹²³I, ¹³¹I, ¹⁵O, ¹³N and/or ⁸²RbCl.

[0042] In yet other embodiments, provided herein are methods and compositions for determining the level of a radiotracer signal in a subject or patient after administration of a

thymidine kinase gene therapy vector, and selecting the subject or patient for treatment with the gene therapy vector when the level of the tracer signal is above a set threshold. In some embodiments, the tracer is a radioactive tracer. In other embodiments, the radioactive tracer element is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$. In yet other embodiments, the radioactive tracer element is coupled to a nucleoside or synthetic nucleoside target to form a radioactive target. In some embodiments, the nucleoside target is FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains. Preferably the radioactive target is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-[hydroxymethyl]butyl)guanine).

[0043] Also provided herein are methods comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; and (b) selecting the subject for treatment with a gene therapy composition wherein the level of [^{18}F]FHBG is at least about 2.0 SUV or at least 20% above background on a PET scan.

[0044] Additionally provided herein is a method comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; (b) including the subject with treatment with a composition wherein the level of FHBG in the subject is greater than about 2.0 SUV on PET scan; and (c) administering to said subject an anti-cancer agent.

[0045] In some embodiments, the invention provides a method for identifying a subject that is susceptible to a cancer treatment, the method comprising: a) identifying expression of [^{18}F]FHBG in the subject; b) treating the subject.

[0046] Provided herein is a method of measuring HSV-TK-mediated FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-

hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains-mediated bystander effect, the method comprising: a) transducing cells with a polynucleotide encoding HSV-TK and a first fluorescent protein; b) transducing the cells with a polynucleotide encoding a second fluorescent or bioluminescent protein that is optically discernible from the first fluorescent or bioluminescent protein; c) treating the cells with an agent that becomes cytotoxic upon being phosphorylated by HSV-TK; and d) measuring the relative amount of expression of the first fluorescent protein and the second fluorescent protein. In one embodiment, step d) comprises a Perkin Elmer Plate reader, a fluorimeter; a fluorescent activated cell sorter (FACS); a cellometer; or a spectrophotometer. In another embodiment, step d) comprises measuring fluorescent output of the second fluorescent or bioluminescent protein *in vivo* in the subject.

[0047] Also provided herein are methods for determining the level of [^{18}F]FHBG signal in a subject and selecting the subject for treatment with a gene therapy composition wherein the level of [^{18}F]FHBG is at least about 2.0 SUV or at least 20% above background on a PET scan.

DEFINITIONS

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. All patents, patent applications, published applications and publications, GenBank sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information can be found by searching the internet. Reference thereto evidences the availability and public dissemination of such information.

[0049] As used herein, “nucleic acid” refers to a polynucleotide containing at least two covalently linked nucleotide or nucleotide analog subunits. A nucleic acid is generally a deoxyribonucleic acid (DNA), a ribonucleic acid (RNA), or an analog of DNA or RNA. Nucleotide analogs are commercially available and methods of preparing polynucleotides containing such nucleotide analogs are known (Lin *et al.* (1994) *Nucl. Acids Res.* 22:5220-5234; Jellinek *et al.* (1995) *Biochemistry* 34:11363-11372; Pagratis *et al.* (1997) *Nature Biotechnol.* 15:68-73). The nucleic acid is generally single-stranded, double-stranded, or a mixture thereof. For purposes herein, unless specified otherwise, the nucleic acid is double-stranded, or it is apparent from the context.

[0050] As used herein, “DNA” is meant to include all types and sizes of DNA molecules including cDNA, plasmids and DNA including modified nucleotides and nucleotide analogs.

[0051] As used herein, “nucleotides” include nucleoside mono-, di-, and triphosphates.

Nucleotides also include modified nucleotides, such as, but are not limited to, phosphorothioate nucleotides and deazapurine nucleotides and other nucleotide analogs.

[0052] The term “polynucleotide” as used herein means a polymeric form of nucleotide of any length, and includes ribonucleotides and deoxyribonucleotides. Such term also includes single- and double-stranded DNA, as well as single- and double-stranded RNA. The term also includes modified polynucleotides such as methylated or capped polynucleotides.

[0053] As used herein, the term “subject” refers to animals, plants, insects, and birds into which the large DNA molecules are introduced. Included are higher organisms, such as mammals and birds, including humans, primates, rodents, cattle, pigs, rabbits, goats, sheep, mice, rats, guinea pigs, cats, dogs, horses, chicken and others.

[0054] As used herein, “administering to a subject” is a procedure by which one or more delivery agents and/or large nucleic acid molecules, together or separately, are introduced into or applied onto a subject such that target cells which are present in the subject are eventually contacted with the agent and/or the large nucleic acid molecules.

[0055] As used herein, “delivery vector” or “delivery vehicle” or “therapeutic vector” or “therapeutic system” refers to both viral and non-viral particles that harbor and transport exogenous nucleic acid molecules to a target cell or tissue. Viral vehicles include, but are not limited to, retroviruses, adenoviruses, lentiviral viruses, herpes viruses and adeno-associated viruses. Non-viral vehicles include, but are not limited to, microparticles, nanoparticles, virosomes and liposomes. “Targeted,” as used herein, refers to the use of ligands that are associated with the delivery vehicle and target the vehicle to a cell or tissue. Ligands include, but are not limited to, antibodies, receptors and collagen-binding domains.

[0056] As used herein, “delivery,” which is used interchangeably with “transduction,” refers to the process by which exogenous nucleic acid molecules are transferred into a cell such that they are located inside the cell. Delivery of nucleic acids is a distinct process from expression of nucleic acids.

[0057] As used herein, a “multiple cloning site (MCS)” is a nucleic acid region in a plasmid that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector. “Restriction enzyme digestion” refers to catalytic cleavage of a nucleic acid molecule with an enzyme that functions only at specific locations in a nucleic acid molecule. Many of these restriction enzymes are commercially available. Use of such enzymes is widely understood by those of skill in the art. Frequently, a

vector is linearized or fragmented using a restriction enzyme that cuts within the MCS to enable exogenous sequences to be ligated to the vector.

[0058] As used herein, “origin of replication” (often termed “ori”), is a specific nucleic acid sequence at which replication is initiated. Alternatively an autonomously replicating sequence (ARS) can be employed if the host cell is yeast.

[0059] As used herein, “selectable or screenable markers” confer an identifiable change to a cell permitting easy identification of cells containing an expression vector. Generally, a selectable marker is one that confers a property that allows for selection. A positive selectable marker is one in which the presence of the marker allows for its selection, while a negative selectable marker is one in which its presence prevents its selection. An example of a positive selectable marker is a drug resistance marker.

[0060] Usually the inclusion of a drug selection marker aids in the cloning and identification of transformants, for example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selectable markers. In addition to markers conferring a phenotype that allows for the discrimination of transformants based on the implementation of conditions, other types of markers including screenable markers such as GFP, whose basis is colorimetric analysis, are also contemplated. In some embodiments, screenable enzymes such as herpes simplex virus thymidine kinase (tk) or chloramphenicol acetyltransferase (CAT) are utilized. One of skill in the art would also know how to employ immunologic markers, possibly in conjunction with FACS analysis. The marker used is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selectable and screenable markers are well known to one of skill in the art.

[0061] As used herein, “expression” refers to the process by which nucleic acid is translated into peptides or is transcribed into RNA, which, for example, can be translated into peptides, polypeptides or proteins. If the nucleic acid is derived from genomic DNA, expression includes, if an appropriate eukaryotic host cell or organism is selected, splicing of the mRNA. For heterologous nucleic acid to be expressed in a host cell, it must initially be delivered into the cell and then, once in the cell, ultimately reside in the nucleus.

[0062] As used herein, a “therapeutic course” refers to the periodic or timed administration of the vectors disclosed herein within a defined period of time. Such a period of time is at least one day, at least two days, at least three days, at least five days, at least one week, at least two weeks, at least three weeks, at least one month, at least two months, or at least six months. Administration could also take place in a chronic manner, *i.e.*, for an undefined period of time.

The periodic or timed administration includes once a day, twice a day, three times a day or other set timed administration.

[0063] As used herein, the terms “co-administration,” “administered in combination with” and their grammatical equivalents or the like are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are administered by the same or different route of administration or at the same or different times. In some embodiments, a therapeutic agent as disclosed in the present application will be co-administered with other agents. These terms encompass administration of two or more agents to an animal so that both agents and/or their metabolites are present in the animal at the same time. They include simultaneous administration in separate compositions, administration at different times in separate compositions, and/or administration in a composition in which both agents are present. Thus, in some embodiments, a therapeutic agent and the other agent(s) are administered in a single composition. In some embodiments, a therapeutic agent and the other agent(s) are admixed in the composition. In further embodiments, a therapeutic agent and the other agent(s) are administered at separate times in separate doses.

[0064] The term “host cell” denotes, for example, microorganisms, yeast cells, insect cells, and mammalian cells, that can be, or have been, used as recipients for multiple constructs for producing a delivery vector. The term includes the progeny of the original cell which has been transfected. Thus, a “host cell” as used herein generally refers to a cell which has been transfected with an exogenous DNA sequence. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0065] As used herein, “genetic therapy” involves the transfer of heterologous DNA to the certain cells, target cells, of a mammal, particularly a human, with a disorder or conditions for which therapy or diagnosis is sought. The DNA is introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. In some embodiments, the heterologous DNA, directly or indirectly, mediates expression of DNA that encodes the therapeutic product. In some embodiments, the heterologous DNA encodes a product, such as a peptide or RNA that mediates, directly or indirectly, expression of a therapeutic product. In some embodiments, genetic therapy is used to deliver a nucleic acid encoding a gene product to replace a defective gene or supplement a gene product produced by the mammal or the cell in which it is introduced. In some embodiments, the introduced nucleic acid encodes a therapeutic compound, such as a growth factor or inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor therefore, that is not

generally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. In some embodiments, the heterologous DNA encoding the therapeutic product is modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

[0066] As used herein, “heterologous nucleic acid sequence” is generally DNA that encodes RNA and proteins that are not normally produced *in vivo* by the cell in which it is expressed or that mediates or encodes mediators that alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes. Any DNA that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which it is expressed is herein encompassed by heterologous DNA. Examples of heterologous DNA include, but are not limited to, DNA that encodes traceable marker proteins, such as a protein that confers drug resistance, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies. In some embodiments, antibodies that are encoded by heterologous DNA is secreted or expressed on the surface of the cell in which the heterologous DNA has been introduced.

[0067] As used herein, the term “thymidine kinase mutant” refers to not only the specific protein described herein (as well as the nucleic acid sequences which encode these proteins), but derivatives thereof which may include various structural forms of the primary protein which retain biological activity.

[0068] As used herein, “unmutated thymidine kinase” refers to a native or wild-type thymidine kinase polypeptide sequence.

[0069] As used herein, “suicide gene” refers to a nucleic acid encoding a product, wherein the product causes cell death by itself or in the presence of other compounds.

[0070] As used herein, the term “mutated” or “replaced by another nucleotide” means a nucleotide at a certain position is replaced at that position by a nucleotide other than that which occurs in the unmutated or previously mutated sequence. That is, in some instances, specific modifications may be made in different nucleotides. In some embodiments, the replacements are made such that the relevant splice donor and/or acceptor sites are no longer present in a gene.

[0071] As used herein, a “polar amino acid” refers to amino acid residues Asp(N), Cys (C), Gln (Q), Gly (G), Ser (S), Thr (T) or Tyr (Y).

[0072] As used herein, a “non-polar amino acid” refers to amino acid residues Ala (A), Ile (I), Leu (L), Met (M), Phe (F), Pro (P), Trp (W), or Val (V).

[0073] As used herein, a “basic amino acid” refers to amino acid residues Arg (R), His (H), or Lys (K).

[0074] As used herein, an “acidic amino acid” refers to amino acid residues Asp (D) or Glu (E).

GENE THERAPY

[0075] Gene therapy involves the transfer of heterologous DNA to certain cells of a mammal, particularly a human, with a disorder or conditions for which therapy or diagnosis is sought. The DNA is introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced.

[0076] In some embodiments, the heterologous DNA, directly or indirectly, mediates expression of DNA that encodes the therapeutic product. In some embodiments, the heterologous DNA encodes a product, such as a peptide or RNA that mediates, directly or indirectly, expression of a therapeutic product. In some embodiments, the introduced nucleic acid encodes a therapeutic compound, such as a growth factor or inhibitor thereof, or a tumor necrosis factor or inhibitor thereof, such as a receptor therefore, that is not generally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time.

[0077] Non-viral and viral methods have been used to deliver heterologous therapeutic DNA into the cell, including viral vector particles derived from retrovirus, adenovirus, adeno-associated viral particles, herpes virus particles, vaccinia virus, lentivirus, pox virus, Semliki virus and pseudotyped viruses.

[0078] Accordingly, provided herein are viral constructs for gene transfer to cells either *in vivo*, *ex vivo* or *in vitro* for gene therapy. Such viral vector particles include, but are not limited to retroviral vector particles, adenoviral vector particles, adeno-associated virus particles, herpes virus particles, pseudotyped viruses, lentiviral vector particles, pox virus vector particles, vaccinia virus vector particles and non-viral vectors. Preferably, the viral vector particle is a retroviral vector particle.

RETROVIRAL CONSTRUCTS

[0079] In some embodiments, the vector particle employed for gene therapy use is a retroviral vector particle. In still other embodiments, the retroviral vector particle is derived from Moloney Murine Leukemia Virus and is of the LN series of vectors, such as those hereinabove mentioned, and described further in Bender, et al., J. Virol., Vol. 61, pgs. 1639-1649 (1987) and Miller, et al., Biotechniques, Vol. 7, pgs 980-990 (1989). Such vectors, have a portion of the packaging signal derived from a mouse sarcoma virus, and a mutated gag initiation codon. The term "mutated" as used herein means that the gag initiation codon has been deleted or altered such that the gag protein or fragments or truncations thereof, are not expressed.

[0080] In some embodiments, the retroviral vector particle includes a modified envelope, including at least one polynucleotide encoding at least one heterologous polypeptide to be expressed in a desired cell. The heterologous polypeptide may, in one embodiment, be a therapeutic agent. The therapeutic agent is thymidine kinase, more preferably HSV-TK.

[0081] In still other embodiments, therapeutic agents include, but are not limited to, growth factors such as, for example, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), erythropoietin, G-CSF, GM-CSF, TGF α , TGF- β , and fibroblast growth factor, cytokines, including, but not limited to, interleukins and tumor necrosis factors. Other therapeutic agents include, but are not limited to, anticoagulants, anti-platelet agents, anti-inflammatory agents, tumor suppressor proteins, clotting factors, including Factor VII, Factor VIII and Factor IX, protein S, protein C, antithrombin III and von Willebrand Factor.

[0082] In some embodiments, the polynucleotide encoding the therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; the histone promoter; the polIII promoter, the β -actin promoter; inducible promoters, such as the MMTV promoter, the metallothionein promoter; heat shock promoters; adenovirus promoters; the albumin promoter; the ApoAI promoter; B19 parvovirus promoters; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex Virus thymidine kinase promoter; retroviral LTRs; human growth hormone promoters, and the MxIFN inducible promoter. The promoter also may be the native promoter which controls the polynucleotide encoding the therapeutic agent.

[0083] The polynucleotides encoding the modified envelope polypeptide and the therapeutic agent may be placed into an appropriate vector by genetic engineering techniques known to those skilled in the art. When the modified vector is a retroviral vector particle, the polynucleotides encoding the modified envelope polypeptide and the therapeutic agent are placed into an appropriate retroviral plasmid vector.

[0084] The retroviral plasmid vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., *Biotechniques*, Vol. 7, No. 9, 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

[0085] In one embodiment, the retroviral plasmid vector, which includes a polynucleotide encoding the modified envelope and a polynucleotide encoding a therapeutic agent, is employed to transduce a packaging cell line to form a producer cell line, which will generate infectious retroviral vector particles. In one embodiment, the packaging cell line is a "pre-packaging" cell line which includes polynucleotides encoding the gag and pol retroviral proteins, but not the envelope, or env, protein. Such cell lines, upon transduction with the retroviral plasmid vector, generates infectious retroviral particles including the modified, or chimeric, envelope and a polynucleotide encoding the therapeutic agent. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, and use of liposomes, such as hereinabove described, and CaPO₄ precipitation. Such producer cells generate infectious retroviral vector particles which include the modified envelope, the wild-type retroviral envelope, a polynucleotide encoding the modified, or chimeric, envelope, and a polynucleotide encoding a therapeutic agent.

[0086] In another embodiment, there is provided a packaging cell which includes a nucleic acid sequence encoding a modified chimeric envelope in accordance with the invention, and which may further include nucleic acid sequences encoding the gag and pol proteins. A producer cell for generating viral particles which includes a modified envelope in accordance with the invention is produced by introducing into such packaging cell either a retroviral vector particle or a retroviral plasmid vector, in each case including a polynucleotide encoding a therapeutic agent. The producer cell line thus generates infectious retroviral particles including the modified chimeric envelope and the polynucleotide encoding the therapeutic agent.

TARGETED RETROVIRAL VECTOR DELIVERY

[0087] In some embodiments, provided herein are vector particles having a modified viral surface protein, such as, for example, a modified viral envelope polypeptide, for targeting the vector particle to an extracellular matrix component. The viral surface protein is modified to include a targeting polypeptide including a binding region which binds to, an extracellular matrix component.

[0088] In some embodiments, the targeting polypeptide is inserted between two consecutively numbered amino acid residues of the native (i.e., unmodified) receptor binding region of the retroviral envelope. In yet other embodiments, amino acid residues of the receptor binding region may be removed and replaced with the targeting polypeptide.

[0089] As an alternative to modifying the receptor binding region, or in addition to the modified receptor binding region, the retroviral particles may have modifications in other regions of the envelope protein such that other regions of the envelope may include the targeting polypeptide, such as, for example, the secretory signal or "leader" sequence, the hinge region, or the body

portion. Such modifications may include deletions or substitutions of amino acid residues in the retroviral envelope wherein amino acid residues from regions other than the receptor binding region of the envelope are removed and replaced with the targeting polypeptide, or the targeting polypeptide is placed between consecutively numbered amino acid residues of regions other than the receptor binding region of the viral envelope.

[0090] In another alternative embodiment, the retroviral envelope, prior to modification thereof to include the targeting polypeptide which binds to the extracellular matrix component, may be an envelope which includes regions of different tropisms. For example, the retroviral envelope may be a Moloney Murine Leukemia Virus envelope which includes a gp70 protein having an ecotropic portion and an amphotropic and/or xenotropic portion.

[0091] In general, the targeting polypeptide includes a binding region which binds to an extracellular matrix component, including, but not limited to, collagen (including collagen Type I and collagen Type IV), laminin, fibronectin, elastin, glycosaminoglycans, proteoglycans, and sequences which bind to fibronectin, such as arginine-glycine-aspartic acid, or RGD, sequences. Binding regions which may be included in the targeting polypeptide include, but are not limited to, polypeptide domains which are functional domains within von Willebrand Factor or derivatives thereof, wherein such polypeptide domains bind to collagen. In one embodiment, the binding region is a polypeptide having the following structural formula: Trp-Arg-Glu-Pro-Ser-Phe-Met-Ala-Leu-Ser. (SEQ ID NO: 25).

[0092] In addition to the binding region, the targeting polypeptide may further include linker sequences of one or more amino acid residues, placed at the N-terminal and/or C-terminal of the binding region, whereby such linkers increase rotational flexibility and/or minimize steric hindrance of the modified envelope polypeptide.

HSV-TK

[0093] Thymidine kinase is a salvage pathway enzyme which phosphorylates natural nucleoside substrates as well as nucleoside analogues. Generally, thymidine kinase is used therapeutically by administration of a nucleoside analogue such as ganciclovir or acyclovir to a cell expressing thymidine kinase, wherein the thymidine kinase phosphorylates the nucleoside analogue, creating a toxic product capable of killing the cell.

[0094] Polynucleotide sequences encoding exogenous thymidine kinase as used herein may be prepared from a wide variety of thymidine kinases. In some embodiments, the thymidine kinase mutant is derived from *Herpesviridae* thymidine kinase including, for example, both primate herpes viruses, and non-primate herpes viruses such as avian herpes viruses. Representative examples of suitable herpes viruses include, for example, Herpes Simplex Virus (HSV) Type 1, Herpes Simplex Virus Type 2, Varicella zoster Virus, marmoset herpes virus, feline herpes virus

type 1, pseudorabies virus, equine herpes virus type 1, bovine herpes virus type 1, turkey herpes virus, Marek's disease virus, herpes virus saimir and Epstein-Barr virus.

[0095] IMPROVEMENTS TO TK GENE

[0096] Disclosed herein, in some embodiments, is a polynucleotide sequence encoding HSV-TK. In some embodiments, the polynucleotide sequence encodes a wild-type HSV-TK amino acid sequence. In some embodiments, the polynucleotide sequence encodes a mutated HSV-TK amino acid sequence.

[0097] Exemplary procedures that may be used in preparation of an optimized polynucleotide sequence provided herein include, but are not limited to: codon optimization; correction of splice sites, removal of poly-pyrimidine tracts and excess GC content; addition of single Kozak sequence, removal of unwanted Kozak sequences; inclusion of restriction sites for subcloning into retroviral or other vectors; removal of nuclear localization sequences or addition of nuclear export sequences; addition of mutation sequences; addition of double stop codon sequences; addition of tags, linkers and fusion sequences; preparation of sequence file for submission to gene synthesis company; subcloning of synthesized gene into retroviral vectors; inclusion of fluorescent protein genes into retroviral vectors; inclusion of selectable marker genes into retroviral vectors; preparation of Maxiprep plasmid DNA; transfection of retroviral producer or other cells; lab, pilot or GMP scale production of retrovirus; transduction of target cells with retrovirus; GCV or analogous pro-drug mediated cell kill assay; Hypoxanthine/Aminopterin/Thymidine (HAT) selection assay; selectable marker drug selection procedure to produce retroviral transduced cell lines; fluorescent microscopy and photography to detect and document retroviral transduced target cells; quantitative fluorescent detection of retroviral transduced target cells; Western protein expression assay; other procedures and assays as needed for HSV-TK analysis; or a combination thereof. Protocols for such methods are described herein, are commercially available or are described in the public literature and databases.

[0098] In some embodiments, described herein is a method of obtaining an improved HSV-TK sequence. In some embodiments, the method comprises: a) correction and/or removal of splice sites; and/or b) adjustment to a single Kozak sequence. Optionally, in some embodiments, the method further comprises inclusion of restriction sites for sub-cloning of the HSV-TK sequence. Optionally, or in addition, in some embodiments, the method further comprises removal of nuclear localization sequences.

[0099] Provided herein is a polynucleotide sequence encoding a mutated form of viral thymidine kinase from human simplex virus (HSV-TK), wherein the encoded HSV-TK is mutated at amino acid residue 25, 26, 32, 33, 167, 168, , or a combination thereof, wherein the

polynucleotide sequence is mutated compared to a polynucleotide sequence of SEQ ID NO: 1 or 3. In such sequences, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 mutations may be made.

[00100] Modifications may be conservative or non-conservative mutations. A mutation may be made such that the encoded amino acid is modified to a polar, non-polar, basic or acidic amino acid.

[00101] Provided herein is a polynucleotide sequence encoding a mutated form of thymidine kinase from human simplex virus (HSV-TK), wherein the encoded HSV-TK includes a nuclear export sequence. Provided herein is a polynucleotide sequence encoding a mutated form of thymidine kinase from human simplex virus (HSV-TK), where the encoded HSV-TK is improved in function compared to wild-type HSV-TK and comprises A168H dmNES (CL system = CMV enhancer properly fused to LTR promoter regions), where NES refers to a nuclear export sequence. In one embodiment, a mutant HSV-TKA168HdmNES is a mutant HSV-TK gene for inclusion in Reximmune-C2. In one embodiment, the NES is derived from MAP Kinase Kinase (MAPKK). In yet another embodiment, the polynucleotide sequence for NES is CTGCAGAAAAAGCTGGAAGAGCTGGAAGCTGGATGGC (SEQ ID NO: 23). In other embodiments, the NES polypeptide sequence is LQKKLEELELDG (SEQ ID NO: 24).

[00102] In some embodiments, disclosed herein are mutations to a polynucleotide sequence encoding Human Simplex Virus Thymidine Kinase (HSV-TK) wherein mutations are not made to the polypeptide sequence of wildtype HSV-TK.

[00103] Nucleotide positions are referred to by reference to a position in SEQ ID NO: 1 (wildtype (wt) HSV1-TK nucleotide sequence) or SEQ ID NO: 3 (HSV-TK in Reximmune-C HSV-TK; SR39 mutant and R25G-R26S Mutation of the HSV-TK nuclear localization signal (NLS)).

[00104] In one embodiment, a Sac I-Kpn I restriction sites bounding the clonable double stranded oligonucleotides of the mutant HSV-TK SR39 mutant region is provided. See, for example, SEQ ID NOS: 6 and 7, where the Sac I and Kpn I sites are shown on the left and right, respectively. Bold, underlining illustrates the sites where mutations may be made. SEQ ID NOS: 8 and 9 illustrate an exemplary sequence after cutting with Sac I and Kpn I. Exemplary forward and reverse primers that may be used to make the mutations are shown as SEQ ID NOS: 10 and 11.

[00105] Exemplary optimized HSV-TK polynucleotide sequences are provided, for example, as SEQ ID NOS: 12-22.

[00106] However, when such references are made, the invention is not intended to be limited to the exact sequence as set out in SEQ ID NO: 1 or 3, but includes variants and derivatives thereof. Thus, identification of nucleotide locations in other thymidine kinase sequences are

contemplated (*i.e.*, identification of nucleotides at positions which the skilled person would consider to correspond to positions recited in SEQ ID NO: 1 or 3).

[00107] In some embodiments, nucleotides are replaced by taking note of the genetic code such that a codon is changed to a different codon which codes for the same amino acid residue. In some embodiments, nucleotides are replaced within coding regions of a HSV-TK encoding nucleic acid sequence, yet the nucleic acid sequence maintains wild type HSV-TK protein expression.

[00108] In such embodiments, 5/21 codons contain “C or G” in third position (24%); 0/21 codons contain “C” in third position (0 %); 5/21 codons contain “G” in third position (24%); and 16/21 codons contain “A or T” in third position (76%).

[00109] In yet other embodiments, 16/21 codons contain “C or G” in third position (76%); 11/21 codons contain “C” in third position (52 %); 5/21 codons contain “G” in third position (24%); and 5/21 codons contain “A or T” in third position (24%).

[00110] In some embodiments, the following rare codons are not used or are avoided in the coding region of a polynucleotide encoding HSV-TK, or a variant thereof: GCG for alanine; CGA or CGT for arginine; TTA or CTA for leucine; CCG for proline; TCG for serine; ACG for threonine; and GTA for valine.

[00111] In some embodiments, altering codons as described herein results in about 2%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or greater percentage increase in activity.

[00112] In some embodiments, disclosed herein is a nucleic acid sequence encoding a thymidine kinase wherein at least one nucleotide corresponding to a splice donor site is replaced by another nucleotide. In further embodiments, the nucleotides of the splice acceptor sites are not altered. In some embodiments, at least one nucleotide corresponding to a splice acceptor site is replaced by another nucleotide.

[00113] In some embodiments, disclosed herein is a nucleic acid sequence encoding a thymidine kinase wherein at least one of the nucleotides corresponding to splice donor site nucleotides at positions 329 and 330 of a polynucleotide sequence (*e.g.*, SEQ ID NO: 1 or 3) is replaced by another nucleotide. In some embodiments, both of the nucleotides at positions 327 and 555 are replaced by other nucleotides. For example, position 327 may be mutated to an amino acid residue selected from: G to A. Alternately, or in addition, position 555 may be mutated to an amino acid residue selected from: G to A. In one embodiment, the modified HSV-TK has a polynucleotide sequence of SEQ ID NO: 18, in which HSV-TK was improved in the following ways:

HSV-TK NESdmNLS A168H, CO & SC

NES = nuclear export sequence from MAP Kinase Kinase (MAPKK)

dmNLS = double mutated HSV-TK Nuclear Localization Sequence

CO = codon optimized

SC = splice donor/acceptor site corrected at 327 and 555,

Underlined sequence

SEQ ID NO: 18

gtcaGCGGCCGCAACCGGTACGCGTCCACCATGGCCCTGCAGAAAAAGCTGGAAGAGCTGGAAC
 GGATGGCAGCTACCCCGGCCACCAGCACGCCAGCGCCTTCGACCAGGCCGCCCGCAGCCGCGGC
 CACAGCAACGGCAGCACCGCaCTGCGgCCaGGATCTCAGCAGGAGGCCACCGAGGTGCGCCCCG
 AGCAGAAGATGCCCCACCCTGCTGCGCGTGTACATCGACGGaCCaCACGGCATGGGCAAGACCAC
 CACCACCCAGCTGCTGGTGGCCCTGGGCAGCCGCGACGACATCGTGTACGTGCCCCGAGCCCATG
 ACCTACTGGCGCGTGCTGGGCGCCAGCGAGACCATCGCCAACATCTACACCACCCAGCACCGCC
 TGGACCAaGGCGAGATCAGCGCCGGCGACGCCGCCGTGGTGATGACCAGCGCCCAGATtACaAT
 GGGCATGCCCTACGCCGTGACCGACGCCGTGCTGGCaCCaCACATCGGCGGGCGAGGCCGGCAGC
 AGCCACGCaCCaCCaCCaGCaCTGACCCTGATCTTCGACCGgCACCCaATCGCaCACCTGCTGT
 GCTACCCgGCaGCaCGCTACCTGATGGGctccATGACaCCaCAaGCCGTGCTGGCCTTCGTGGC
 CCTGATCCCaCCaACaCTGCCCCGGCACCAACATCGTGCTGGGCGCCCTGCCCGAGGACCGCCAC
 ATCGACCGCCTGGCCAAGCGCCAGCGCCCCGGCGAGCGCCTGGACCTGGCCATGCTGGCCGCCA
 TCCGCCGCGTGTTACGGCCTGCTGGCCAACACCGTGCGCTACCTGCAGTGCGGCGGCAGCTGGCG
 CGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCCaCCaCAGGGCGCCGAGCCaCAGAGCAAC
 GCCGGaCCaCGaCCaCACATCGGCGACACCCTGTTCACCCTGTTCGGgGCaCCaGAGCTGCTGG
 CaCCaAACGGCGACCTGTACAACGTGTTTCGCCCTGGGCCCCTGGACGTGCTGGCCAAGCGCCTGCG
 CtccATGCACGTGTTTCATCCTGGACTACGACCAGtcaCCgGCCGGCTGCCGCGACGCCCTGCTG
 CAGCTGACCAGCGGCATGGTGCAGACCCACGTGACaACaCCCGGCAGCATCCCaACaATCTGCG
 ACCTGGCCCCGCACCTTCGCCCCGCGAGATGGGCGAGGCCAACTAATAGGGATCCCTCGAGAAGCT
 Tgtca

[00114] In some embodiments, disclosed herein is a nucleic acid sequence encoding a thymidine kinase wherein at least one of the nucleotides corresponding to splice acceptor site nucleotides at positions 554 and 555, or at least one of the nucleotides corresponding to splice acceptor site nucleotides at positions 662 and 663, or at least one of the nucleotides corresponding to splice acceptor sites at positions 541 and 542 of the wild type sequence is replaced by another nucleotide. For example, position 541 may be mutated to an amino acid residue selected from: G to A. Position 542 may be mutated to an amino acid residue selected from: G to A. Position 554 may be mutated to an amino acid residue selected from: G to A. Position 555 may be mutated to an amino acid residue selected from: G to A. Position 662 may be mutated to an amino acid residue selected from: G to A. Position 663 may be mutated to an amino acid residue selected from: G to A.

[00115] A Kozak sequence flanks the AUG start codon within mRNA and influences the recognition of the start codon by eukaryotic ribosomes. In some embodiments, a polynucleotide sequence encoding HSV-TK comprises no more than one Kozak sequence. In some

embodiments, the Kozak sequence is upstream of the coding portion of the DNA sequence. In some embodiments, the Kozak sequence of a polynucleotide encoding HSV-TK is modified to produce a Kozak sequence with a higher efficiency of translation initiation in a mammalian cell. In some embodiments, modification of the Kozak sequence does not produce an amino acid substitution in the encoded HSV-TK polypeptide product. In some embodiments, modification of the Kozak sequence results in at least one amino acid substitution in the encoded HSV-TK polypeptide product. In one embodiment, the modified HSV-TK has a polynucleotide sequence of SEQ ID NO: 18 or 22.

[00116] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 60, 75, 80, 85, 90, 95, 100 or more codon substitutions. In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 60, 75, 80, 85, 90, 95, 100 or more codon substitutions, wherein the codon substitutions comprise the substitution of a codon having a higher frequency of usage in a mammalian cell than the wild type codon at that position. However, in some embodiments, less favored codons may be chosen for individual amino acids depending upon the particular situation.

[00117] In some embodiments, the polynucleotide sequence encoding HSV-TK comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 60, 75, 80, 85, 90, 95, 100 or more codon substitutions has less than about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 1 or 3 wherein the sequence identity is determined over the full length of the coding sequence using a global alignment method. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00118] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 60, 75, 80, 85, 90, 95, 100 or more codon substitutions, wherein the codon substitutions comprise the substitution of a codon having the highest frequency of usage in a mammalian cell for the wild type codon at that position. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00119] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more codon substitutions, wherein the substituted codons have a frequency of usage greater than or equal to about 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35 or higher. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00120] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises less than about 45, 40, 35, 30, 25, 20 or fewer codons, wherein the codons have a frequency of usage less than about 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24 or 0.25. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00121] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% or more of codons having a frequency of usage greater than or equal to about 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, or higher. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00122] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises at least 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or more of codons having the highest frequency of usage in a mammalian cell. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00123] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises less than about 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10% or less of codons having a frequency of usage less than about 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17,

0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24 or 0.25. In some embodiments, the polynucleotide sequence comprises less than about 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10% or less of codons having a frequency of usage less than about 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24 or 0.25 in a mammalian cell. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00124] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises codon substitutions, wherein at least 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the codons have been changed as compared to the wild type sequence. In some embodiments, the polynucleotide sequence encoding HSV-TK comprises codon substitutions, wherein at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the codons have been changed to a codon having a higher frequency of usage in a mammalian cell as compared to the wild type sequence. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00125] In some embodiments, the polynucleotide sequence encoding HSV-TK comprises codon substitutions, wherein at least 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of the codons have been changed to a codon having the highest frequency of usage in a mammalian cell as compared to the wild type sequence. In some embodiments, the corresponding encoded polypeptide sequence has at least 75 %, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a HSV-TK amino acid sequence, *e.g.*, SEQ ID NO: 2 or 4.

[00126] The viral thymidine kinase gene from the selected herpesvirus may be readily isolated and mutated as described below, in order to construct nucleic acid molecules encoding a thymidine kinase enzyme comprising one or more mutations which increases biological activity of the thymidine kinase, as compared to unmutated wild-type thymidine kinase. The biological

activity of a thymidine kinase may be readily determined utilizing any of the assays known in the art, including for example, determination of the rate of nucleoside analogue uptake or determination of the rate of nucleoside or nucleoside analogue phosphorylation. In addition, thymidine kinase mutants may be readily selected which are characterized by other biological properties, such as thermostability and protein stability.

[00127] In some embodiments, the polynucleotide sequence encoding HSV-TK is modified to remove or modify a predicted signal sequence. In some embodiments, the polynucleotide is modified to remove or modify a nuclear localization sequence (NLS). In some embodiments, the polynucleotide is modified to remove the nuclear localization sequence. In some embodiments, the polynucleotide is modified to modify the NLS so that it no longer functions to localize HSV-TK exclusively to the nucleus.

[00128] In some embodiments, a HSV-TK polypeptide sequence is mutated at amino acid residues 167, 168, or both. In one example, the sequence is mutated at amino acid residue 167. In another example, the sequence is mutated at amino acid residue 168. In another example, the sequence is mutated at amino acid residues 167 and 168. Amino acid residue 167 may be mutated to serine or phenylalanine. Amino acid residue 168 may be mutated to histidine, lysine, cysteine, serine or phenylalanine. In some embodiments, a HSV-TK polypeptide sequence is mutated at amino acid residues 25 and/or 26. Amino acid residues 25 and/or 26 may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. In some embodiments, the HSV-TK polypeptide sequence is mutated at amino acid residues 32 and/or 33. Amino acid residues 32 and/or 33 may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. In some embodiments, the HSV-TK polypeptide is mutated at amino acid residues 25, 26, 32, and/or 33. Amino acid residues 25, 26, 32, and/or 33, may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. Amino acid residue modifications may be made in comparison to a polypeptide sequence of SEQ ID NOS: 2 or 4.

[00129] In accordance with the present invention, mutant thymidine kinase enzymes which are encoded by the above-described nucleic acid molecules are provided, as well as vectors which are capable of expressing such molecules. In some embodiments, expression vectors are provided comprising a promoter operably linked to a nucleic acid molecule of the present invention. In some embodiments, the vector is a viral vector capable of directing the expression of a nucleic acid molecule. Representative examples of such viral vectors include herpes simplex viral vectors, adenoviral vectors, adenovirus-associated viral vectors, pox vectors, parvoviral vectors, baculovirus vectors and retroviral vectors. In some embodiments, viral vectors are provided which are capable of directing the expression of a nucleic acid molecule

which encodes a thymidine kinase enzyme comprising one or more mutations, at least one of the mutations encoding an amino acid substitution which increases a biological activity of thymidine kinase, as compared to unmutated (*i.e.*, wild-type) thymidine kinase.

[00130] In some embodiments, a nucleic acid molecule provided herein encodes a thymidine kinase enzyme capable of phosphorylating a nucleoside analogue at a level at least 10% greater than the level of phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme. In some embodiments, the thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue at a level at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 100%, at least 150%, at least 200%, at least 300%, or at least 500% greater than the level of phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme. Representative examples of suitable nucleoside analogues include gancyclovir, acyclovir, famciclovir, buciclovir, penciclovir, valciclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2, 5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC. In some embodiments, the improved TK mutant lacks thymidine kinase activity.

[00131] In some embodiments, the K_m value thymidine kinase activity of a disclosed HSV-TK mutant is at least 2.5 μm . In some embodiments, the K_m value of a disclosed HSV-TK mutant is at least 5 μm , at least 10 μm , at least 15 μm , at least 20 μm , at least 25 μm , at least 30 μm , at least 40 μm , at least 50 μm , at least 60 μm , at least 70 μm , at least 80 μm , at least 90 μm , at least 100 μm , at least 150 μm , at least 200 μm , at least 250 μm , at least 300 μm , at least 400 μm , at least 500 μm , at least 600 μm , at least 700 μm , at least 800 μm , at least 900 μm , or at least 1000 μm . In some embodiments, the percent K_m of a disclosed HSV-TK mutant compared to wild-type HSV-TK is at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 100%, at least 150%, at least 200%, at least 300%, or at least 500%.

[00132] Within one embodiment of the present invention, truncated derivatives of HSV-TK mutants are provided. For example, site-directed mutagenesis may be readily performed in order to delete the N-terminal 45 amino acids of a thymidine kinase mutant, thereby constructing a truncated form of the mutant which retains its biological activity.

[00133] Mutations in nucleotide sequences constructed for expression of derivatives of thymidine kinase mutants should preserve the reading frame phase of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which would adversely affect translation of the receptor mRNA. Such derivatives may be readily constructed using a wide variety of techniques, including those discussed above.

[00134] In some embodiments, a HSV-TK polypeptide sequence is mutated at amino acid residues 167, 168, or both. In one example, the sequence is mutated at amino acid residue 167. In another example, the sequence is mutated at amino acid residue 168. In another example, the sequence is mutated at amino acid residues 167 and 168. Amino acid residue 167 may be mutated to serine or phenylalanine. Amino acid residue 168 may be mutated to histidine, lysine, cysteine, serine or phenylalanine. In some embodiments, a HSV-TK polypeptide sequence is mutated at amino acid residues 25 and/or 26. In amino acid residues 25 and/or 26 may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. In some embodiments, the HSV-TK polypeptide sequence is mutated at amino acid residues 32 and/or 33. Amino acid residues 32 and/or 33 may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. In some embodiments, the HSV-TK polypeptide is mutated at amino acid residues 25, 26, 32, and/or 33. Amino acid residues 25, 26, 32, and/or 33, may be mutated to an amino acid chosen from the group consisting of: glycine, serine, and glutamate. Amino acid residue modifications may be made in comparison to a polypeptide sequence of SEQ ID NOS: 2 or 4.

[00135] In accordance with the present invention, mutant thymidine kinase enzymes which are encoded by the above-described nucleic acid molecules are provided, as well as vectors which are capable of expressing such molecules. In some embodiments, expression vectors are provided comprising a promoter operably linked to a nucleic acid molecule of the present invention. In some embodiments, the vector is a viral vector capable of directing the expression of a nucleic acid molecule. Representative examples of such viral vectors include herpes simplex viral vectors, adenoviral vectors, adenovirus-associated viral vectors, pox vectors, parvoviral vectors, baculovirus vectors and retroviral vectors. In some embodiments, viral vectors are provided which are capable of directing the expression of a nucleic acid molecule which encodes a thymidine kinase enzyme comprising one or more mutations, at least one of the mutations encoding an amino acid substitution which increases a biological activity of thymidine kinase, as compared to unmutated (*i.e.*, wild-type) thymidine kinase.

[00136] In some embodiments, a nucleic acid molecule provided herein encodes a thymidine kinase enzyme capable of phosphorylating a nucleoside analogue at a level at least 10% greater than the level of phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme. In some embodiments, the thymidine kinase enzyme is capable of phosphorylating a nucleoside analogue at a level at least 15%, at least 20%, at least 25%, at least 50%, at least 75%, at least 100%, at least 150%, at least 200%, at least 300%, or at least 500% greater than the level of phosphorylation of the nucleoside analogue by a wild-type thymidine kinase enzyme. Representative examples of suitable nucleoside analogues include gancyclovir,

acyclovir, famciclovir, buciclovir, penciclovir, valciclovir, trifluorothymidine, 1-[2-deoxy, 2-fluoro, beta-D-arabino furanosyl]-5-iodouracil, ara-A, araT 1-beta-D-arabinofuranoxyl thymine, 5-ethyl-2'-deoxyuridine, 5-iodo-5'-amino-2, 5'-dideoxyuridine, idoxuridine, AZT, AIU, dideoxycytidine and AraC. In some embodiments, the improved TK mutant lacks thymidine kinase activity.

[00137] Within one embodiment of the present invention, truncated derivatives of thymidine kinase mutants are provided. For example, site-directed mutagenesis may be readily performed in order to delete the N-terminal 45 amino acids of a thymidine kinase mutant, thereby constructing a truncated form of the mutant which retains its biological activity.

[00138] Mutations in nucleotide sequences constructed for expression of derivatives of thymidine kinase mutants should preserve the reading frame phase of the coding sequences. Furthermore, the mutations will preferably not create complementary regions that could hybridize to produce secondary mRNA structures, such as loops or hairpins, which would adversely affect translation of the receptor mRNA. Such derivatives may be readily constructed using a wide variety of techniques, including those discussed above.

[00139] Using the methods described herein, the inventors determined that the majority of the candidates for optimized HSV-TK genes appeared to be compatible with a retroviral expression system and produce biologically useful retroviral titers.

[00140] Furthermore, the optimized HSV-TK genes which incorporated most of these optimizations (SEQ ID NO: 18) exhibited pro-drug GCV enzyme activity and selectivity for their ability to kill cancer cells following retroviral transduction delivery. The mutant HSV-TK gene A168H, which was codon optimized and splice corrected appeared to have the highest GCV mediated cancer kill activity (SEQ ID NOs: 12, 16, 18, or 22). The same version of this HSV-TK gene A168H and mutated at amino acids 159-161 from LIF to IFL exhibited GCV mediated cancer cell kill activity.

[00141] The mutant HSV-TK gene A167F (SEQ ID NOs: 13, 17, or 19), which was codon optimized and splice corrected had very high GCV mediated cancer kill activity following retroviral transduction delivery, but more surprisingly had NO thymidine kinase activity as determined by expressing this gene following retroviral transduction delivery in 3T3 TK(-) cells selected with HAT medium. To our knowledge, this is the most GCV selective HSV-TK synthetic gene product for GCV activation which has no Thymidine activity (HAT assay) ever evaluated biologically.

[00142] The double mutant HSV-TK gene A167F + A168H (SEQ ID NO: 14) unexpectedly ablates both GCV and Thymidine enzyme activity by exhibiting very little GCV mediated cancer kill activity and very little thymidine activity (HAT assay),

[00143] The present inventors identified that it is possible to produce functional HSV-TK fusions of genes such as bacterial cytosine deaminase, yeast cytosine deaminase, neomycin phosphotransferase and include linker sequences and retain HSV-TK GCV mediated cancer cell killing activity.

[00144] In one embodiment, a codon optimized HSV-TK gene with GCV-mediated cancer killing activity may be made which retains one or more nuclear localization sequences which is not fused to one or more other therapeutic genes.

[00145] Additional modifications to and/or evaluations of an optimized HSV-TK gene described herein may include one or more of the following: removal of known nuclear localization sequences within HSV-TK; increased pro-drug GCV enzyme activity and selectivity for their ability to kill cancer cells, evaluate the use of more tags, fusion proteins and linkers of HSV-TK to other genes and proteins, co-expression of HSV-TK optimized genes with other optimized suicide and cancer killer genes in cancer cells, include optimized HSV-TK genes in a Reximmune-C type retroviral vector system; production and testing of a Reximmune-C type GMP product, or any combination thereof.

[00146] In one embodiment, a polynucleotide sequence described herein comprises a nuclear export signal. For example, a polynucleotide sequence may comprise TK168dmNES.

[00147] In another embodiment, a retroviral vector for use in the methods described herein comprises one or more splice site modifications.

[00148] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A167Fsm (SEQ ID NO: 13).

[00149] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A168Hsm (SEQ ID NO: 12).

[00150] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A167Fdm (SEQ ID NO: 17).

[00151] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A168dm (SEQ ID NO: 16).

[00152] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A167Fdm and an NES (SEQ ID NO: 19).

[00153] In another embodiment, a retroviral vector for use in the methods described herein comprises HSV-TK A168Hdm and an NES (SEQ ID NO: 18). In such an embodiment, the sequence comprises HSV-TK A168H.

[00154] In another embodiment, a retroviral vector for use in the methods described herein comprises a HSV-TK, wherein such vector comprises an upgraded substrate binding domain and a mNLS/NES set.

[00155] In another embodiment, a retroviral vector for use in the methods described herein comprises a HSV-TK, wherein the vector comprises a selectable marker, a glowing gene and/or one or more kill genes.

[00156] In another embodiment, a retroviral vector for use in the methods described herein comprises at least two modifications.

[00157] Using the methods described herein, the inventors determined that the majority of the optimized HSV-TK genes appeared to be compatible with a retroviral expression system and produce biologically useful retroviral titers.

[00158] The mutant HSV-TK gene A167F (SEQ ID NOs: 13, 17, or 19), which was codon optimized and splice corrected had very high GCV mediated cancer kill activity following retroviral transduction delivery, but more surprisingly had no thymidine kinase activity as determined by expressing this gene following retroviral transduction delivery in 3T3 TK(-) cells selected with HAT medium. This is highly GCV selective HSV-TK synthetic gene.

[00159] The double mutant HSV-TK gene A167F + A168H (SEQ ID NO: 14) exhibited very little GCV mediated cancer kill activity and very little thymidine activity; thus, a proper double mutant may have surprising null properties.

[00160] The present inventors identified that it is possible to produce functional HSV-TK fusions of genes such as bacterial cytosine deaminase, yeast cytosine deaminase, neomycin phosphotransferase and include linker sequences and retain HSV-TK GCV mediated cancer cell killing activity.

[00161] In one embodiment, a fully codon optimized HSV-TK gene with GCV-mediated cancer killing activity may be made which retains one or more nuclear localization sequences which is not fused to one or more other therapeutic genes.

[00162] Additional modifications to and/or evaluations of an optimized HSV-TK gene described herein may include one or more of the following: removal of known nuclear localization sequences within HSV-TK; increased pro-drug GCV enzyme activity and selectivity for their ability to kill cancer cells, evaluate the use of more tags, fusion proteins and linkers of HSV-TK to other genes and proteins, co-expression of HSV-TK optimized genes with other optimized suicide and cancer killer genes in cancer cells, include optimized HSV-TK genes in a Reximmune-C retroviral vector system; production and testing of a Reximmune-C GMP product, or any combination thereof.

[00163] The therapeutic vectors may be administered alone or in conjunction with other therapeutic treatments or active agents. Examples of other active agents that may be used include, but are not limited to, chemotherapeutic agents, anti-inflammatory agents, protease inhibitors, such as HIV protease inhibitors, nucleoside analogs, such as AZT. In some

embodiments, the methods of treatment further comprise administering to the subject a chemotherapeutic agent, a biologic agent, or radiotherapy prior to, contemporaneously with, or subsequent to the administration of the therapeutic viral particles. One of skill in the art will appreciate that the retroviral particles described herein may be administered either by the same route as the one or more agents (*e.g.*, the retroviral vector and the agent are both administered intravenously) or by different routes (*e.g.*, the retroviral vector is administered intravenously and the one or more agents are administered orally).

[00164] The dosage of the therapeutic viral particles lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. A therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal infection or a half-maximal inhibition) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by RT-qPCR or ddPCR methods.

[00165] An effective amount or therapeutically effective of the retroviral particles disclosed herein to be administered to a subject in need of treatment may be determined in a variety of ways. By way of example, the amount may be based on viral titer or efficacy in an animal model. Alternatively the dosing regimes used in clinical trials may be used as general guidelines.

[00166] In some embodiments, the daily dose may be administered in a single dose or in portions at various hours of the day. In some embodiments, a higher dosage may be required and may be reduced over time when the optimal initial response is obtained. In some embodiments, treatment may be continuous for days, weeks, or years, or may be at intervals with intervening rest periods. In some embodiments, the dosage is modified in accordance with other treatments the individual may be receiving. However, the method of treatment is in no way limited to a particular concentration or range of the retroviral particle and may be varied for each individual being treated and for each derivative used.

[00167] Individualization of dosage may be required to achieve the maximum effect for a given individual. In some embodiments, the dosage administered to an individual being treated varies depending on the individual's age, severity or stage of the disease and response to the course of treatment. In some embodiments, clinical parameters for determining dosage include, but are not limited to, tumor size, alteration in the level of tumor markers used in clinical testing for particular malignancies. In some embodiments, the treating physician determines the therapeutically effective amount to be used for a given individual. In some embodiments, the

therapies disclosed herein are administered as often as necessary and for the period of time judged necessary by the treating physician.

[00168] The therapeutic vectors, including but not limited to the therapeutic retroviral particles that are specifically to the cell or system of interest, may be systemically or regionally (locally) delivered to a subject in need of treatment. For example, the therapeutic vectors may be systemically administered intravenously. Alternatively, the therapeutic vectors may also be administered intra-arterially. The therapeutic vectors may also be administered topically, intravenously, intra-arterially, intra-tumorally, intracolonicallly, intratracheally, intraperitoneally, intranasally, intravascularly, intrathecally, intracranially, intramarrowly, intrapleurally, intradermally, subcutaneously, intramuscularly, intraocularly, intraosseously and/or intrasynovially or stereotactically. A combination of delivery modes may also be used, for example, a patient may receive the therapeutic vectors both systemically and regionally (locally) to improve tumor responses with treatment of the therapeutic vectors.

[00169] In some embodiments, multiple therapeutic courses (*e.g.*, first and second therapeutic course) are administered to a subject in need of treatment. In some embodiments, the first and/or second therapeutic course is administered intravenously. In other embodiments, the first and/or second therapeutic course is administered via intra-arterial infusion, including but not limited to infusion through the hepatic artery, cerebral artery, coronary artery, pulmonary artery, iliac artery, celiac trunk, gastric artery, splenic artery, renal artery, gonadal artery, subclavian artery, vertebral artery, axillary artery, brachial artery, radial artery, ulnar artery, carotid artery, femoral artery, inferior mesenteric artery and/or superior mesenteric artery. Intra-arterial infusion may be accomplished using endovascular procedures, percutaneous procedures or open surgical approaches. In some embodiments, the first and second therapeutic course may be administered sequentially. In yet other embodiments, the first and second therapeutic course may be administered simultaneously. In still other embodiments, the optional third therapeutic course may be administered sequentially or simultaneously with the first and second therapeutic courses.

[00170] In some embodiments, the therapeutic vectors disclosed herein may be administered in conjunction with a sequential or concurrently administered therapeutic course(s) in high doses on a cumulative basis. For example, in some embodiments, a patient in need thereof may be systemically administered, *e.g.*, intravenously administered, with a first therapeutic course of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a cumulative basis. The first therapeutic course may be

systemically administered. Alternatively, the first therapeutic course may be administered in a localized manner, *e.g.*, intra-arterially, for example a patient in need thereof may be administered via intra-arterial infusion with at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a cumulative basis.

[00171] In yet other embodiments, a subject in need thereof may receive a combination, either sequentially or concurrently, of systemic and intra-arterial infusions administration of high doses of delivery vector. For example, a patient in need thereof may be first systemically administered with at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} , at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a cumulative basis, followed by an additional therapeutic course of intra-arterial infusion, *e.g.*, hepatic arterial infusion, administered delivery vector of at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP on a cumulative basis. In still another embodiment, a patient in need thereof may receive a combination of intra-arterial infusion and systemic administration of delivery vector in high doses. For example, a patient in need thereof may be first be administered via intra-arterial infusion with at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a cumulative basis, followed by an additional therapeutic course of systemically administered delivery vector of at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP on a cumulative basis. The therapeutic courses may also be administered simultaneously, *i.e.*, a therapeutic course of high doses of delivery vector, for example, at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a

cumulative basis, together with a therapeutic course of intra-arterial infusion, *e.g.*, hepatic arterial infusion, administered delivery vector of at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP on a cumulative basis.

[00172] In still other embodiments, a subject in need thereof may additionally receive, either sequentially or concurrently with the first and second therapeutic courses, additional therapeutic courses (*e.g.*, third therapeutic course, fourth therapeutic course, fifth therapeutic course) of cumulative dose of delivery vector, for example, at least of at least 1×10^9 TVP, at least 1×10^{10} TVP, at least 1×10^{11} TVP, at least 1×10^{12} TVP, at least 1×10^{13} TVP, at least 1×10^{14} TVP, at least 1×10^{15} TVP, at least 1×10^{16} TVP, at least 1×10^{17} TVP, at least 1×10^{18} TVP, at least 1×10^{19} TVP, at least 1×10^{20} TVP, at least 1×10^{21} TVP or at least 1×10^{22} TVP delivery vector on a cumulative basis.

[00173] In some embodiments, the subject in need of treatment is administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{11} TVP, followed by the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{11} TVP. In other embodiments, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a cumulative dose of at least 1×10^{12} TVP, followed by the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{12} TVP. In one embodiment, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{13} TVP, followed by the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{13} TVP. In yet other embodiments, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{14} TVP, concurrently with the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{14} TVP. In still other embodiments, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{15} TVP, together with the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{15} TVP. In yet other embodiments, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{16} TVP, concurrently with the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{16} TVP. In still other embodiments, the patient in need of treatment may be administered systemically (*e.g.*, intravenously) a dose of at least 1×10^{13} TVP, together with the administration via intra-arterial infusion (*e.g.*, hepatic-arterial infusion) of a dose of at least 1×10^{17} TVP.

[00174] A subject in need of treatment may also be administered, either systemically or localized (for example intra-arterial infusion, such as hepatic arterial infusion) a therapeutic course of delivery vector for a defined period of time. In some embodiments, the period of time may be at least one day, at least two days, at least three days, at least four days, at least five days, at least six days, at least seven days, at least one week, at least two weeks, at least three weeks, at least four weeks, at least five weeks, at least six weeks, at least seven weeks, at least eight weeks, at least 2 months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, at least one year, at least two years, at least three years, at least four years, or at least five years. Administration could also take place in a chronic manner, *i.e.*, for an undefined or indefinite period of time.

[00175] Administration of the therapeutic vector may also occur in a periodic manner, *e.g.*, at least once a day, at least twice a day, at least three times a day, at least four times a day, at least five times a day. Periodic administration of the delivery vector may be dependent upon the time of delivery vector as well as the mode of administration. For example, parenteral administration may take place only once a day over an extended period of time, whereas oral administration of the delivery vector may take place more than once a day wherein administration of the delivery vector takes place over a shorter period of time.

[00176] In one embodiment, the subject is allowed to rest 1 to 2 days between the first therapeutic course and second therapeutic course. In some embodiments, the subject is allowed to rest 2 to 4 days between the first therapeutic course and second therapeutic course. In other embodiments, the subject is allowed to rest at least 2 days between the first and second therapeutic course. In yet other embodiments, the subject is allowed to rest at least 4 days between the first and second therapeutic course. In still other embodiments, the subject is allowed to rest at least 6 days between the first and second therapeutic course. In some embodiments, the subject is allowed to rest at least 1 week between the first and second therapeutic course. In yet other embodiments, the subject is allowed to rest at least 2 weeks between the first and second therapeutic course. In one embodiment, the subject is allowed to rest at least one month between the first and second therapeutic course. In some embodiments, the subject is allowed to rest at least 1-7 days between the second therapeutic course and the optional third therapeutic course. In yet other embodiments, the subject is allowed to rest at least 1-2 weeks between the second therapeutic course and the optional third therapeutic course.

DIAGNOSING A PATIENT THAT IS SUSCEPTIBLE TO THYMIDINE KINASE GENE THERAPY TREATMENT

[00177] Imaging tests, including the use of radioactive tracers, contrast imaging technology and other imaging technology can be used to identify patients that are susceptible to gene therapy treatment, including thymidine kinase gene therapy treatment, and thus more likely to benefit from such therapeutic measures.

[00178] In a preferred embodiment, positron emission tomography (PET) scans are used to identify patients capable of transducing retroviral vector particles containing thymidine kinase constructs for expression *in vivo*. A PET scan produces 3-dimensional images of functional processes in the body by detecting pairs of gamma rays emitted indirectly by radioactive tracers placed on a biological active molecule. PET scans detect energy emitted by positively charged particles (positrons).

[00179] Patients administered a retroviral vector particle containing thymidine kinase polynucleotide are co-administered a radiotracer agent capable of being cleaved by expressed thymidine kinase. An example is [^{18}F]FHBG (9-[4-[^{18}F]fluoro-3-(hydroxymethyl)butyl]guanine), which is a high affinity substrate for HSV-TK enzyme, with relative low affinity for mammalian TK enzymes. See Yaghoubi and Gambhir, *Nat. Protocol* 1:3069-75 (2006); Green et al., *J. Nucl. Med.* 45:1560-70 (2004). [^{18}F]FHBG is phosphorylated by HSV1-TK or HSV1-sr39TK, which is then trapped within cells expressing thymidine kinase enzyme. Cleavage of this substrate *in vivo* in patients administered retroviral vectors containing a thymidine kinase polynucleotide, including the mutated and/or optimized thymidine kinase constructs described herein, thus indicates efficient transduction of the retroviral vector particles by the subjects and patients, and thus an initial of patient or subject susceptibility to thymidine kinase-mediated gene therapy.

[00180] Alternatively, other methods for measuring viral TK activity include chemical exchange saturation transfer magnetic resonance imaging with 5-methyl-5,6-dihydrothymidine and related compounds.

[00181] Accordingly, in some embodiments disclosed herein, provided are methods and compositions for detecting thymidine kinase expression in patients administered a retroviral viral particle containing a polynucleotide encoding a thymidine kinase protein. In some embodiments, the thymidine kinase is derived from *Herpesviridae* thymidine kinase. In some embodiments, the thymidine kinase is HSV-TK. In other embodiments, the thymidine kinase is HSV-TK1. In still other embodiments, the thymidine kinase is an optimized version of HSV-TK1.

[00182] In some embodiments, the HSV-TK gene is codon optimized for efficient expression and/or transduction. In other embodiments, the amino terminus of the thymidine kinase is altered to remove or eliminate the nuclear localization sequence (NLS) of the viral thymidine kinase sequence. In other embodiments, the thymidine kinase nucleotide sequence includes a nuclear export sequence (NES) attached to the amino terminus. In some embodiments, the nuclear export sequence is LQKKLEELELDG (SEQ ID NO: 24).

[00183] In yet other embodiments, the thymidine kinase coding sequence is mutated to increase substrate binding of the expressed thymidine kinase protein. In still other embodiments, the thymidine kinase coding sequence includes an A168H mutation.

[00184] Other examples of substrates targeted by thymidine kinase, including HSV-TK protein, include: FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains. Examples of radiotracers that can be used to determine if a therapeutic protein, such as thymidine kinase, is expressed in an individual treated with the thymidine kinase gene therapy vectors described herein may also include ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$.

[00185] Clinical trials for 9-[4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine (FHBG) for PET can be found on the following website: www.clinicaltrials.gov. Methods of measuring FHBG with PET in clinical use can be found in clinical trials NCT00871702, NCT00185848 and NCT01082926.

[00186] Briefly patients will receive a dose of therapeutic drug product on Day 1. On Day 3 to 6, preferably day 4, or at a time period after receiving the dose of therapeutic drug product encoding a modified HSV-TK as disclosed herein, they will be infused with [^{18}F]FHBG intravenously and imaged by PET scan 1-5 hours later, preferably 0.5, 1.0, 1.5, 2, 2.5, 3.0, 3.5 or 4.0 hours later or other appropriate time after administration for scanning, for accumulation in the tumor sites where HSV-1 TK is shown to be expressed. Patients that show uptake of the FHBG will be enrolled in the trial; those that do not will be excluded as disclosed herein. The amount of FHBG will be determined and based on previous studies. Additional protocols for FHBG/PET may be found, for example, in references 15-39 below.

[00187] Accordingly provided herein is are methods and compositions for measuring a tagged substrate of thymidine kinase, including HSV-TK, including FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains, the method comprising: a) transducing cells with a polynucleotide encoding HSV-TK; b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; and c) measuring the relative amount of radioactive signal present in target tissue. In one embodiment, step c) comprises measuring the output of the radioactive tracer *in vivo* in the subject using PET (positron emission tomography) scanning.

[00188] Also provided herein is are methods and compositions for identifying a patient or subject capable of benefitting from gene therapy treatment, comprising measuring a tagged substrate of thymidine kinase, including HSV-TK, including FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains. In some embodiments, the method comprises: a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells with the polynucleotide encoding HSV-thymidine kinase; b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; c) measuring the relative amount of radioactive signal present in target tissue; and d) identifying patients where the level of radioactively-labelled HSV-TK substrate is above a set threshold. In one embodiment, step c) comprises measuring the output of the radioactive tracer *in vivo* in the subject using PET (positron emission tomography) scanning. In some embodiments, patients capable of benefitting from a gene therapy protocol include patients or subjects exhibiting a level above a set threshold

on a PET scan. In some embodiments, the level of radioactive HSV-TK substrate is at least about 2.0 SUV or at least 20% above background on a PET scan. In some embodiments, the level of radioactive HSV-TK substrate is at least about 1.9 SUV or at least 20% above background on a PET scan. In yet other embodiments, the level of radioactive HSV-TK substrate is at least about 1.0 SUV, about 1.5 SUV, about 2.0 SUV or about 2.5 SUV or more, or at least 10% above background, at least 20% above background, at least 30% above background, at least 40% above background or at least 50% above background or more on a PET scan.

[00189] In some embodiments, provided herein are methods and compositions for identifying a patient or subject in need of treatment for benign or metastatic lesions and capable of benefitting from gene therapy treatment. In some embodiments, the method for identifying patients capable of benefitting from gene therapy for the treatment of benign or metastatic lesions include measuring a tagged substrate of thymidine kinase, including HSV-TK, including FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains after administration of the gene therapy treatment. In some embodiments, the method comprises: a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells with the polynucleotide encoding HSV-thymidine kinase; b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; c) measuring the relative amount of radioactive signal present in target tissue; d) identifying patients where the level of radioactively-labelled HSV-TK substrate is above a set threshold; and e) treatment said patient or subject with the gene therapy retroviral particle. In one embodiment, step c) comprises measuring the output of the radioactive tracer *in vivo* in the subject using PET (positron emission tomography) scanning. In some embodiments, patients capable of benefitting from a gene therapy protocol include patients or subjects exhibiting a level above a set threshold on a PET scan. In some embodiments, the level of [^{18} F]FHBG signal is at least about 2.0 SUV or at least 20% above background on a PET scan. In some embodiments, the level of radioactive HSV-TK substrate is at least about 1.9 SUV or at least 20% above background on a PET scan. In yet other embodiments, the level of radioactive HSV-TK substrate is at least about 1.0 SUV, about 1.5 SUV, about 2.0 SUV or about 2.5 SUV or

more, or at least 10% above background, at least 20% above background, at least 30% above background, at least 40% above background or at least 50% above background or more on a PET scan.

[00190] Also provided herein are methods comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; and (b) selecting the subject for treatment with a composition wherein the level of FHBG is at least about 2.0 SUV or at least 20% above background on a PET scan. In some embodiments, the level of radioactive HSV-TK substrate is at least about 1.9 SUV or at least 20% above background on a PET scan. In yet other embodiments, the level of radioactive HSV-TK substrate is at least about 1.0 SUV, about 1.5 SUV, about 2.0 SUV or about 2.5 SUV or more, or at least 10% above background, at least 20% above background, at least 30% above background, at least 40% above background or at least 50% above background or more on a PET scan.

[00191] Additionally provided herein is a method comprising: (a) determining the level of [^{18}F]FHBG signal in a subject; (b) excluding the subject from treatment with a composition wherein the level of FHBG in the subject is greater than about 2.0 SUV or at least above 20% above background on a PET scan; and (c) administering to said subject an anti-cancer agent. In some embodiments, the level of radioactive HSV-TK substrate is at least about 1.9 SUV or at least 20% above background on a PET scan. In yet other embodiments, the level of radioactive HSV-TK substrate is at least about 1.0 SUV, about 1.5 SUV, about 2.0 SUV or about 2.5 SUV or more, or at least 10% above background, at least 20% above background, at least 30% above background, at least 40% above background or at least 50% above background or more on a PET scan.

[00192] In some embodiments, the invention provides a method for identifying a subject that is susceptible to a cancer treatment, the method comprising: a) identifying expression of [^{18}F]FHBG in the subject; b) treating the subject.

[00193] Also provided herein are compositions and methods of measuring HSV-TK-mediated FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains phosphorylation using a fluorescent imaging

system. In some embodiments, the method comprises: a) transducing cells with a polynucleotide encoding HSV-TK and a first fluorescent protein; b) transducing the cells with a polynucleotide encoding a second fluorescent or bioluminescent protein that is optically discernible from the first fluorescent or bioluminescent protein; c) treating the cells with an agent that becomes cytotoxic upon being phosphorylated by HSV-TK; and d) measuring the relative amount of expression of the first fluorescent protein and the second fluorescent protein. In one embodiment, step d) comprises a Perkin Elmer Plate reader, a fluorimeter; a fluorescent activated cell sorter (FACS); a cellometer; or a spectrophotometer. In another embodiment, step d) comprises measuring fluorescent output of the second fluorescent or bioluminescent protein *in vivo* in the subject using a fluorescent or bioluminescent imaging system.

THYMIDINE KINASE DIAGNOSTIC USES

[00194] In some embodiments, disclosed herein is a method of selecting a patient for therapy, or for excluding a patient from therapy. In one embodiment, the thymidine kinase gene therapy. In other embodiments, the thymidine kinase is herpes simplex virus thymidine kinase (HSV-TK). In yet other embodiments, the thymidine kinase is HSV-TK1.

[00195] As described herein, [^{18}F]FHBG and other HSV-TK labeled substrates may be used as a marker for selection or exclusion of subjects for gene therapy. For example, cells expressing HSV-TK after administration of a retroviral vector particle comprising a polynucleotide encoding HSV-TK will selectively phosphorylate the nucleoside analogue 9-[4-fluoro-3-(hydroxymethyl) butyl]guanine ([^{18}F]FHBG). *See, e.g.,* Yaghoubi and Gambhir, *Nat. Protocols* 1:3069-75 (2006). [^{18}F]FHBG imaging above a certain threshold can then be used to identify HSV-TK positive cells and to select or exclude a patient for gene therapy.

[00196] Accordingly, in one embodiment, a subject is administered a gene therapy composition, wherein the gene therapy composition encodes an HSV-TK polypeptide. The subject is administered a labeled nucleoside analog HSV-TK substrate after a predetermined period of time, and monitored until background of the labeled substrate is reached in the subject. The label activity is measured, and compared against a scan detecting lesions in the subject. If the imaging activity: 1) is above a set threshold; and 2) correlates with the lesion location in the subject, then the subject is a candidate for HSV-TK gene therapy.

[00197] In some embodiments, the nucleoside analog is FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir,

valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir- and penciclovir-like side chains. In some embodiments, the label is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$. Preferably, the labeled nucleoside analog HSV-TK substrate is [^{18}F]FHBG ((9-[4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine).

[00198] In some embodiments, the clearance pattern in a subject determines the length of time delay for measuring background and determining, for example, [^{18}F]FHBG imaging activity. In humans, for example, [^{18}F]FHBG background rapidly decreases from most tissues outside the lower abdomen, as seen in FIG. 2. As seen, between 7.6 and 42.6 minutes after administration of [^{18}F]FHBG, levels are high in the liver, kidneys and bladder. This is in contrast to the heart and lungs, for example, which show virtually no background [^{18}F]FHBG signal after administration. After 45.3 minutes to 80.3 minutes, [^{18}F]FHBG levels have decreased significantly in the liver and kidneys, with persistent high signal in the bladder. From 83.3 minutes to 155.6 minutes, levels in the liver and kidneys and decreased even further, with maintenance of high signal levels in the bladder. Accordingly, depending upon the organ and subject individual, some time may be required for background levels to decrease in order to measure HSV-TK gene expression. No time may be needed for measurement in organ systems outside of the lower abdomen region, as seen in the heart and lungs. In these organ systems, a sufficient threshold for gene therapy suitability may be, for example, at least above 1.0 SUV, at least above 1.5 SUV, at least above 2.0 SUV, at least above 2.5 SUV, at least above 3.0 SUV, at least above 3.5 SUV or at least above 4.0 SUV.

[00199] In contrast, some delay may be needed in order to image signals above background levels in, for example, the liver and kidneys. *See Yaghoubi et al. Nat. Protocols* at vol. 1, p. 3073. Because of the background signals in these organ systems, a sufficient threshold for determination of suitability for gene therapy treatment may be, for example, at least 10% above background, at least 15% above background, at least 20% above background, at least 25% above background, at least 30% above background, at least 35% above background, at least 40% above background, at least 45% above background, at least 50% above background, at least 55% above background, at least 60% above background, at least 65% above background, at least 70% above background, at least 75% above background, at least 80% above background, at least 85% above background, at least 90% above background, at least 95% above background or at least 100% or more above background when measured after a predetermined amount of time. For example, as seen in FIG. 2, background signals in the liver are considerably less after at least 1 to 1-1/2 hours after administration of [^{18}F]FHBG. Accordingly, [^{18}F]FHBG signal measurements in the

liver should not be taken until after [^{18}F]FHBG signal levels have decreased to background, approximately 1 to 1-1/2 hours, depending upon clearance rate in each individual subject.

[00200] In some organ systems, HSV-TK gene expression may not be measurable, for example, in the bladder, where high background signal levels are maintained over time.

[00201] In other embodiments, a ratio of administered [^{18}F]FHBG to measured [^{18}F]FHBG signal is measured to determine if a subject should be included or excluded from a gene therapy protocol. For example, if a subject that is injected with, for example, 500 MBq of [^{18}F]FHBG and exceeds the threshold of, for example, 50 MBq of [^{18}F]FHBG signal, the subject is capable of producing a therapeutically effective amount of phosphorylated ganciclovir, or a derivative thereof, from a construct described herein to be therapeutic, indicating that the subject may respond in a gene therapy situation. In such an embodiment, the subject is a candidate for treatment with a gene therapy construct described herein.

[00202] In other embodiments, the subject is injected with 100-750 MBq or 100-600 MBq or 100-500 MBq or 200-500 MBq or 200-400 MBq, or 2.0 to 15.5 MBq/kg or 2.0 to 12.0 MBq/kg or 2.0 to 10.0 MBq/kg or 2.0 to 7.5 MBq/kg of [^{18}F]FHBG and exceeds the threshold of, for example, 10-100 MBq or 10-90 MBq or 10-80 MBq or 10-70 MBq or 10-60 MBq or 20-50 MBq or 20-40 MBq of [^{18}F]FHBG signal. In some embodiments, the subject is injection with 200-500 MBq of [^{18}F]FHBG and exceeds the threshold of, for example, 20-50 MBq of [^{18}F]FHBG signal. In some embodiments, the ratio of [^{18}F]FHBG signal injected to [^{18}F]FHBG signal measured is 2:1, 5:1, 10:1, 20:1 30:1, 40:1 or 50:1. In some embodiments, the ratio of [^{18}F]FHBG signal injected to [^{18}F]FHBG signal measured is from about 2:1 to about 50:1, from about 2:1 to about 40:1, from about 5:1 to about 30:1, from about 5:1 to about 20:1, from about 5:1 to about 10:1 or about 10:1.

[00203] In another embodiment, if a subject produces sufficient phosphorylated FHBG to generate a signal of greater than 2.0 SUV or at least 20% above background on PET scan, the subject is likely to produce a therapeutically effective amount of TK from a construct described herein and the subject is likely respond in a gene therapy situation. In some embodiments, the subject may be selected for combination therapy with another anti-cancer agent or treatment described herein.

[00204] In other embodiments, the subject produces sufficient phosphorylated FHBG to generate a signal of greater than about 1.5 SUV, greater than about 2.0 SUV, greater than about 2.5 SUV, greater than about 3.0 SUV, greater than about 4.0 SUV or greater than about 5.0 SUV. In yet other embodiments, the subject generates a signal of at least 10% above background, at least 20% above background, at least 30% above background, at least 40% above background or at least 50% or more above background.

CANCERS

[00205] Non-limiting examples of cancers can include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocyoma of bone/osteosarcoma, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocyoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, skin carcinoma merkel cell, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor.

[00206] In other embodiments, the cancers to be treated are chosen from the group consisting of primary hepatocellular carcinoma, metastatic breast carcinoma to liver, metastatic pancreatic

cancer to liver, metastatic gastric cancer to liver, metastatic esophageal cancer to liver, metastatic lung cancer to liver, metastatic melanoma to liver, metastatic ovarian carcinoma to liver and metastatic kidney cancer to liver.

FORMULATIONS

[00207] Pharmaceutical compositions comprising a therapeutic vector can be formulated in any conventional manner by mixing a selected amount of the therapeutic vector with one or more physiologically acceptable carriers or excipients. For example, the therapeutic vector may be suspended in a carrier such as PBS (phosphate buffered saline). The active compounds can be administered by any appropriate route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid, semi-liquid or solid form and are formulated in a manner suitable for each route of administration.

[00208] In some embodiments, the therapeutic vector and physiologically acceptable salts and solvates are formulated for administration by inhalation or insufflation (either through the mouth or the nose) or for oral, buccal, parenteral or rectal administration. In some embodiments, for administration by inhalation, the therapeutic vector is delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In some embodiments, a pressurized aerosol dosage unit or a valve to deliver a metered amount. In some embodiments, capsules and cartridges (*e.g.*, of gelatin) for use in an inhaler or insufflator are formulated containing a powder mix of a therapeutic compound and a suitable powder base such as lactose or starch.

[00209] In some embodiments, the pharmaceutical compositions are formulated for oral administration as tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). In some embodiments, the tablets are coated by methods well known in the art. In some embodiments, liquid preparations for oral administration are in the form of, for example, solutions, syrups or suspensions, or they are formulated as a dry product for constitution with water or other suitable vehicle before use. In some embodiments, such liquid preparations are prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-

hydroxybenzoates or sorbic acid). In some embodiments, the preparations also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. In some embodiments, pharmaceutical compositions are formulated oral administration to give controlled release of the active compound. In some embodiments, the pharmaceutical compositions are formulated for buccal in the form of tablets or lozenges formulated in conventional manner.

[00210] In some embodiments, the therapeutic vector is formulated for parenteral administration by injection, *e.g.*, by bolus injection, or continuous infusion. In some embodiments, formulations for injection are in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. In some embodiments, the compositions are formulated as suspensions, solutions or emulsions in oily or aqueous vehicles. In some embodiments, the formulations comprise formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, in some embodiments, the active ingredient is in powder lyophilized form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00211] In some embodiments, the therapeutic vector is formulated as a depot preparation. In some embodiments, such long acting formulations are administered by implantation (for example, subcutaneously, intramuscularly or directly into or in close proximity to a tumor) or by intramuscular injection. Thus, for example, in some embodiments, the therapeutic compounds are formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00212] In some embodiments, the active agents are formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. In some embodiments, such solutions, particularly those intended for ophthalmic use, are formulated as 0.01%-10% isotonic solutions, pH about 5-9, with appropriate salts. In some embodiments, the compounds are formulated as aerosols for topical application, such as by inhalation.

[00213] The concentration of active compound in the drug composition will depend on absorption, inactivation and excretion rates of the active compound, the dosage schedule, and amount administered as well as other factors known to those of skill in the art.

[00214] In some embodiments, the compositions are presented in a pack or dispenser device which comprise one or more unit dosage forms containing the active ingredient. In some embodiments, the pack may comprises metal or plastic foil, such as a blister pack. In some embodiments, the pack or dispenser device is accompanied by instructions for administration.

[00215] In some embodiments, the active agents are packaged as articles of manufacture containing packaging material, an agent provided herein, and a label that indicates the disorder for which the agent is provided.

ANIMAL MODELS

[00216] In some embodiments, the retroviral vector particles, hereinabove described are administered to an animal *in vivo* as part of an animal model for the study of the effectiveness of a gene therapy treatment. In some embodiments, the retroviral vector particles are administered in varying doses to different animals of the same species. The animals then are evaluated for *in vivo* expression of the desired therapeutic or diagnostic agent. In some embodiments, from the data obtained from such evaluations, a person of ordinary skill in the art determines the amount of retroviral vector particles to be administered to a human patient.

KITS

[00217] Also provided are kits or drug delivery systems comprising the compositions for use in the methods described herein. All the essential materials and reagents required for administration of the retroviral particles disclosed herein may be assembled in a kit (*e.g.*, packaging cell construct or cell line, cytokine expression vector). The components of the kit may be provided in a variety of formulations as described above. The one or more therapeutic retroviral particles may be formulated with one or more agents (*e.g.*, a chemotherapeutic agent) into a single pharmaceutically acceptable composition or separate pharmaceutically acceptable compositions.

[00218] The components of these kits or drug delivery systems may also be provided in dried or lyophilized forms. When reagents or components are provided as a dried form, reconstitution generally is by the addition of a suitable solvent, which may also be provided in another container means.

[00219] Container means of the kits may generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the at least one substance can be placed.

[00220] The kits disclosed herein may also comprise instructions regarding the dosage and or administration information for the retroviral particle. Instructions can include instructions for practicing any of the methods described herein including treatment methods. Instructions can additionally include indications of a satisfactory clinical endpoint or any adverse symptoms that may occur, or additional information required by regulatory agencies such as the Food and Drug Administration for use on a human subject.

[00221] The instructions may be on “printed matter,” *e.g.*, on paper or cardboard within or affixed to the kit, or on a label affixed to the kit or packaging material, or attached to a vial or tube containing a component of the kit. Instructions may additionally be included on a computer

readable medium, such as a disk (floppy diskette or hard disk), optical CD such as CD- or DVD-ROM/RAM, magnetic tape, electrical storage media such as RAM and ROM, IC chip and hybrids of these such as magnetic/optical storage media.

[00222] In some embodiments, the kits or drug delivery systems include a means for containing the vials in close confinement for commercial sale such as, *e.g.*, injection or blow-molded plastic containers into which the desired vials are retained. Irrespective of the number or type of containers, the kits may also comprise, or be packaged with, an instrument for assisting with the injection/administration or placement of the ultimate complex composition within the body of a subject. Such an instrument may be an applicator, inhalant, syringe, pipette, forceps, measured spoon, eye dropper or any such medically approved delivery vehicle.

[00223] Packages and kits can further include a label specifying, for example, a product description, mode of administration and/or indication of treatment. Packages provided herein can include any of the compositions as described herein. The package can further include a label for treating one or more diseases and/or conditions.

[00224] The term “packaging material” refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampules, *etc.*). The label or packaging insert can include appropriate written instructions. Kits, therefore, can additionally include labels or instructions for using the kit components in any method described herein. A kit can include a compound in a pack, or dispenser together with instructions for administering the compound in a method described herein.

EXAMPLES

[00225] In order that those in the art may be better able to practice the compositions and methods described herein, the following examples are provided for illustration purposes.

Example 1: Clinical Trial.

[00226] A dose escalation trial was conducted to evaluate the safety, pharmacokinetics, and pharmacodynamics of Reximmune-C2 (Thymidine Kinase and GM-CSF Genes) in refractory subjects with primary hepatocellular carcinoma or tumors metastatic to the liver.

Background and Rationale

[00227] Reximmune-C2 is comprised of a genetic delivery platform containing an internal payload that encodes for therapeutic proteins of interest. The genetic delivery platform has been dosed in over 280 subjects worldwide; approximately 270 subjects were treated with the vector containing dnG1 as a payload (Rexin-G) and 16 subjects with thymidine kinase (vTK) and the immune stimulator Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) as a payload (Reximmune-C). The genetic delivery platform is a highly engineered non-recombinant Mouse

Moloney Viral vector (MoMLV). Previously, a Phase 1 dose escalation trial was performed investigating the combination of Rexin-G and Reximmune-C in subjects with refractory primary or metastatic solid tumors (Genevieve Trial). This proposed Phase I clinical trial (entitled Genevieve 2 Trial) is an extension of a trial undertaken investigating Reximmune-C2 alone – without the Rexin-G – utilizing an improved form of thymidine kinase in a thymidine kinase plus GM-CSF combination.

[00228] In the original Genevieve trial, sixteen subject were recruited over 3 dose levels with the mean exposure in the highest dose group being 8.0×10^{10} cfus (# of pts = 7) and the longest duration 6 cycles (range of cycles 3-6). For Part A of the study, treatment consisted of a previously determined safe and effective (optimal) dose of Rexin-G, and escalating doses of Reximmune-C. Specifically, Rexin-G, 2×10^{11} cfu, on Days 1, 3, 5, 8, 10 and 12, Reximmune-C, 1.0, 2.0 or 3.0×10^{10} cfu on Day 3 (Dose Levels I, II, III respectively), and valacyclovir at 1 gm p.o. three times a day on Days 6-19, as one cycle. For the Part B part of the study, subjects who had no toxicity or in whom toxicity had resolved to Grade 1 or less could receive additional cycles of therapy up to a total of 6 treatment cycles.

[00229] There were no dose-limiting toxicities at any dose level. Unrelated adverse events were reported for the 16 subjects in the study, but the number of events was low (in most cases 1 or 2 occurrences per preferred term), and most were Grade 1 or 2. Related non-serious adverse events occurred in 2 subjects and both were Grade 2. Four subjects experienced serious adverse events, all of which were deemed not related to the study drug.

[00230] The rationale for continuation of this Phase 1 trial is that: (1) thymidine kinase itself could prove to be an effective anticancer agent particularly in subjects whose tumors demonstrate a bystander effect; (2) administration of the genetic delivery platform to date to an international group of subjects has demonstrated a very high degree of safety; and (3) biodistribution in animals suggests a high biodistribution to the liver. Moreover, the addition of GM-CSF could contribute to an immunological effect and enhanced tumor cell kill through tumor associated antigens through recruitment of the appropriate immune cells.

[00231] The biodistribution of the viral particles is highest to the liver, followed by spleen, then lung – this is the rationale for focusing initially on hepatocellular tumors where the dose intensity should be the highest. There is also a high clinical unmet need for effective anticancer agents for these cancers.

[00232] It is understood that the embodiments disclosed herein are not limited to the particular methods and components and other processes described as these may vary. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. It must be

noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to a "protein" is a reference to one or more proteins, and includes equivalents thereof known to those skilled in the art and so forth.

[00233] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Specific methods, devices, and materials are described, although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

[00234] All publications cited herein are hereby incorporated by reference including all journal articles, books, manuals, published patent applications, and issued patents. In addition, the meaning of certain terms and phrases employed in the specification, examples, and appended claims are provided. The definitions are not meant to be limiting in nature and serve to provide a clearer understanding of certain aspects of the present invention.

Example 2: TK diagnostic assay for gene therapy applications.

[00235] Animal and human studies have previously shown the utility of measuring vTK expression by PET imaging using [^{18}F]-FHBG. These imaging tools will be utilized as a personalized surrogate test in accessing appropriate dosing and exposure and used in the IB portion to determine which subjects have the best opportunity to benefit from the drug candidates.

[00236] This clinical trial is divided into two phases: Phase IA in which Reximmune-C2 was administered as a single intravenous dose on three out of five days and the presence of the HSV-TK-m2 expression potentially monitored by [^{18}F]FHBG PET scanning after 3-8 days (Schematic for Phase IA is illustrated in FIG. 3). Valganciclovir (the oral form of ganciclovir) dosing is initiated on day 8 for 5 days irrespective of the PET scan results. An approximately one week drug holiday follows. Each cycle will be of three weeks duration.

[00237] There will be three patients in the first and subsequent cohorts until a patient experiences Dose Limiting Toxicity (DLT) or two instances of NCI-CTC Grade 2 toxicities attributed to the study drug (except nausea/vomiting, fatigue, anorexia, alopecia, or anemia). If there are no DLTs, patients will move to the next dose level. If there is a DLT, the cohort will be expanded to 6 patients and the dose level will not be exceeded if 2 or more patients exhibit DLTs.

[00238] Once the Maximum Administered Dose (MAD) is reached, a modified Fibonacci schedule will be followed starting with the cohort dose which had no DLTs and continuing until

dose-limiting toxicities are observed in two patients at a dose level. Once the Recommended Phase 2 Dose (RP2D) is defined, 6-12 patients will be recruited.

[00239] Phase IB is designed to explore the activity of Reximmune-C2 in patients of a defined tumor type and stage based on the Phase IA data and who are [^{18}F]FHBG scan positive day three to six after one dose (RP2D) of Reximmune-C2. If the scan is positive, the patient is accepted into the Phase IB treatment phase of the protocol and the RP2D is given as three doses within 5 days, followed by 5 days of valganciclovir beginning on day 8 of that phase, followed by a one week drug holiday. Each cycle is of three week duration. Patients who have a negative [^{18}F]FHBG PET scan after one single dose of Reximmune-C2 will be dosed with 5 days of valganciclovir and will not continue in the study.

[00240] The patient DLT will be defined as the occurrence of any of the following events which is attributed to Reximmune-C2 and occurring during the first cycle (3 weeks) of drug administration:

[00241] Grade 4 neutropenia (i.e., absolute neutrophil count (ANC) $< 500 \text{ cells/mm}^3$) for 7 or more consecutive days or febrile neutropenia (i.e., fever $\geq 38.5^\circ \text{C}$ with an ANC $< 1000 \text{ cells/mm}^3$); Grade 4 thrombocytopenia ($< 25,000 \text{ cells/mm}^3$ or bleeding episode requiring platelet transfusion); Grade 3 or greater nausea and/or vomiting despite the use of adequate/maximal medical intervention and/or prophylaxis; Any Grade 3 or greater non-hematological toxicity (except Grade 3 injection site reaction, alopecia, fatigue); Retreatment delay of more than 3 weeks due to delayed recovery from a toxicity related to treatment with Reximmune-C2; and Grade 3 or greater hypersensitivity reaction despite the appropriate use of premedications (by Common Toxicity Criteria defined as “symptomatic bronchospasm, requiring parenteral medications(s), with or without urticaria; allergy-related edema-angioedema”).

[00242] Reximmune-C2 is infused intravenously over 15-60 minutes (depending on the dose) via an infusion pump. Reximmune-C2 is provided in 30 ml vials stored at $-80^\circ \text{C} \pm 10^\circ \text{C}$.

[00243] In this Phase I trial, the safety, pharmacokinetics, and pharmacodynamics of escalating doses of Reximmune-C2 will be investigated. The maximum tolerated dose will be identified and a recommended Phase 2 dose will be defined for Reximmune C2. Any antitumor activity and clinical responses to Reximmune-C2 treatment will be described.

[00244] The starting dose in this trial is based on: human clinical safety experience with the related vector platform drug products Rexin-G and Reximmune-C and the results of the 21 day rat GLP toxicology study for Reximmune-C2.

Objectives

[00245] The primary objective of the study is to determine the maximum tolerated dose (MTD), dose limiting toxicity (DLT), safety, and a recommended Phase 2 dose (RP2D) of Reximmune-C2 administered over a three week cycle consisting of a series of three doses given intravenously within five days in week 1, followed by 5 daily doses of valganciclovir in week 2 in patients enrolled in this study who have been diagnosed with advanced primary or metastatic tumors to the liver.

[00246] Secondary objectives include: (i) evaluation of the plasma pharmacokinetics of Reximmune-C2; (ii) assessment of the surrogate of HSV-TK-m2 protein expression from Reximmune-C2 via serial [¹⁸F]FHBG PET and/or SPECT imaging; (iii) description and assessment of any preliminary evidence of anti-tumor activity of Reximmune-C2; and (iv) to provide clinical research testing for antibodies to retrovector gp70 env, replication-competent retrovirus in peripheral blood lymphocytes (PBLs); vector integration into genomic DNA of PBLs, and circulating hGM-CSF protein.

Methods

[00247] Study Design: Parallel group, open label dose escalation, three-center clinical trial.

[00248] Stratification: None.

[00249] Therapy: Reximmune-C2 will be administered as an intravenous infusion to separate patients. In Phase IA – investigating Reximmune-C2 - the dose will be escalated among cohorts of patients until DLT is observed. At the RP2D, additional patients will be recruited. In Phase IB patients will be pre-screened by [¹⁸F]FHBG PET for expression of the HSV-TK-m2. Those that express HSV-TK-m2 will receive additional doses of Reximmune-C2. Patients will not be pre-medicated unless hypersensitivity reactions occur.

[00250] Statistical Methods: Descriptive statistics will be used for statistical analysis.

[00251] Sample Size Determination: Precise sample size cannot be defined, as it is dependent on the observed toxicity. For each schedule, cohorts of three to six subjects will be treated at each dose level until the MTD is defined. Once the MTD is identified, this dose level will be expanded to a maximum of 12 patients who will be treated to better define the tolerability and pharmacokinetics of the dose and schedule. It is expected that 45-70 subjects will be enrolled, with 33 to 46 in the IA portion.

Enrollment Criteria

[00252] Subjects must meet all of the following inclusion criteria to be eligible for randomization into the study:

[00253] 1. Diagnosis of histologically documented, advanced stage, primary or metastatic adult solid tumors in the liver that are refractory to standard therapy or for which no curative standard therapy exists.

[00254] 2. Evidence of radiographically measurable or evaluable disease.

[00255] 3. All acute toxic effects of any prior radiotherapy, chemotherapy, or surgical procedures must have resolved to National Cancer Institute (NCI) Common Toxicity Criteria (CTC)(Version 4.0) Grade < 1.

[00256] 4. Age must be > 18 years.

[00257] 5. Last dose of antineoplastic therapy except for hormonal therapy must be > 21 days. External beam radiotherapy must have been < 25% bone marrow-containing skeleton.

[00258] 6. Patients may be Hepatitis B and C positive. (Patients may continue their antiviral medications).

[00259] 7. Patients may have intracranial metastases of any number if they have been brain irradiated and stable for 6 weeks. Patients may be taking anti-seizure medicines but must not be on steroids.

[00260] 8. Karnofsky performance status must be ≥ 70 .

[00261] 9. Life expectancy of at least 3 months.

[00262] 10. Patients must be able to travel to St. Luke's Medical Center for the PET scans.

[00263] 11. Required baseline laboratory data include:

Absolute neutrophil count (ANC)	$\geq 1,500/\text{mm}^3$ [SI units $10^9/\text{L}$]
Platelets	$\geq 75,000/\text{mm}^3$ [SI units $10^9/\text{L}$]
Hemoglobin	$\geq 8.0 \text{ gm/dL}$ [SI units mmol/L]
Serum Creatinine	$\leq 1.5 \times$ laboratory upper limit of normal (L-ULN)
Bilirubin	$\leq 2.0 \text{ mg/dL}$
Alkaline phosphatase	$\leq 5 \times$ L-ULN
AST, ALT	$\leq 5 \times$ L-ULN
LDH	$\leq 5 \times$ L-ULN
Pregnancy test (females of childbearing potential)	Negative within 7 days of starting Protocol

[00264] 12. Signed informed consent indicating that they are aware of the neoplastic nature of their disease and have been informed of the procedures to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts.

[00265] 13. Willing and able to comply with scheduled visits, treatment plan, and laboratory tests.

[00266] The presence of any of the following will exclude a subject from study enrollment

[00267] 1. Concurrent therapy with any anticancer therapy including any other investigational agent.

[00268] 2. Known intracranial edema or a CVA within 6 weeks of screening.

[00269] 3. Pregnant or breast-feeding women. Female subjects must agree to use effective contraception, must be surgically sterile, or must be postmenopausal. Male subjects must agree to use effective contraception or be surgically sterile. The definition of effective contraception will be based on the judgment of the Investigator or a designated associate. All at-risk female subjects must have a negative pregnancy test within 7 days prior to the start of study treatment.

[00270] 4. Clinically significant cardiac disease (New York Heart Association, Class III or IV).

[00271] 5. Dementia or altered mental status that would prohibit informed consent.

[00272] 6. Other severe, acute, or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration or may interfere with the interpretation of study results and, in the judgment of the Principal Investigator, would make the subject inappropriate for this study.

[00273] 7. Known side effects to antivirals in the ganciclovir class.

[00274] 8. Patients who are known to be HIV positive.

[00275] 9. Patient must not be taking steroids at the time of screening.

[00276] *Rationale for the Starting Dose and Schedule*

[00277] Reximmune-C has been dosed in 16 patients over a range of 1.0, 2.0 or 3.0 x10¹⁰ cfu (Dose Levels I, II, III respectively on day 3 of the cycle). There were no dose-limiting toxicities at any dose level. Unrelated adverse events were reported for the 16 patients in the study, but the number of events was low (in most cases 1 or 2 occurrences per preferred term), and most were Grade 1 or 2. Related nonserious adverse events occurred in 2 patients and both were Grade 2. Four patients experienced serious adverse events, all of which were deemed not related to the study drug. The trial was closed prior to determining the optimal dose and schedule of Reximmune-C. In this trial, the new Genevieve-2 Trial, initial dosing will be based on the 21 day toxicology and the HSV-TK-m1 study. Future dosing will proceed using total viral particles (TVP) which is a more accurate measure of titer than cfu per mL.

[00278] The schedule is based on the rationale that Reximmune-C2 exposure will not transduce all of the tumor cells. Therefore, patients will be dosed three times in a cycle over a period of 5 days.

[00279] The time between exposure to GDS and the expression of HSV-TK-m2 (and hGM-CSF) is estimated to be 48 to 72 hours. Therefore, 72 hours after the third dose of Reximmune-C2, valganciclovir will be initiated. The dose (which will be adjusted for renal function) will be given at conventional antiviral dose levels. Due to the potential toxicity of valganciclovir and the published observations that 5 days of ganciclovir should be sufficient to kill the majority of cells containing HSV-TK-m2, 5 days of therapy was chosen. Due to the potential toxicity of both Reximmune-C2 and valganciclovir, this will be followed by an approximately 9 day drug holiday. The hGM-CSF may be at sufficient concentrations at the time of valganciclovir addition to influence the presentation of any tumor associated antigens (TAAs) that may appear during tumor cell apoptosis.

[00280] Plasma samples will be taken after the first and third doses in Cycle One and after the first dose in Cycle Two for pharmacokinetics.

[00281] As distribution is primarily to the liver, toxicities will be carefully monitored there and because of the implications, the bone marrow.

[00282] This clinical protocol calls for the administration of Reximmune-C2 via intravenous infusion to patients with advanced malignancies, either primary hepatocellular or tumors metastatic to the liver. There will be two parts: Phase IA (dose escalation 3 doses/week every three weeks) and Phase IB (pre-screening after one dose of Reximmune-C2 and an [¹⁸F]FHBG scan). If the PET scan is positive, the patient will continue on study. If the PET scan is negative, the patient will receive 5 days of valganciclovir and will not continue in the trial. For Phase IA, dose escalation will follow an accelerated titration design, incorporating three patients per dose level until either one instance of DLT or two instances of NCI-CTC Grade 2 toxicities attributed to the study drug (except nausea/vomiting, fatigue, anorexia, alopecia or anemia) are observed. Thereafter, dosing in the clinical protocol will follow a modified Fibonacci schedule until dose-limiting toxicities are achieved.

[00283] Trial Design

[00284] This is a Phase 1, open-label, four center, dose-escalating trial. The dose will be increased until DLT is observed, and the MTD is defined.

[00285] Reximmune-C2 will be administered as an IV infusion over 15-60 minutes. It is anticipated that 33-70 patients will be treated during the course of the study.

[00286] For Phase IA, the dose of Reximmune-C2 will be escalated from 6.0×10^{11} TVP. In the accelerated dose escalation phase, cohorts of three patients will be enrolled at each dose level. The dose escalation increment will be 100% until a DLT or two CTC Grade 2 or greater toxicities are observed. When the accelerated dose escalation ends, the dose escalation for a new patient in the standard dose escalation will follow a modified Fibonacci scheme (i.e., dose

increments of 67%, 50%, 40%, 33% and 25%). A minimum of three patients per dose level will be enrolled. For Phase IB, the dose of Reximmune-C2 will be the RP2D. DLT will be assessed. If a DLT is observed in ≥ 2 out of six patients at a dose level, there will be no further dose escalation; this dose level will define the maximum administered dose (MAD).

[00287] The dose just below the MAD will be considered the MTD. Once the MTD is defined, this dose level can be expanded to a maximum of twelve patients to further characterize the pharmacokinetic and pharmacodynamic parameters and suitability as a recommended dose for Phase 2 clinical studies.

[00288] Treatment of Patients

[00289] Only qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of biotherapeutic agents in an appropriate environment.

[00290] Reximmune C2 is a Moloney Murine replication incompetent retrovector particle containing the genes encoding for a HSV-TK-m2 and hGM-CSF. The drug product contains DMEM (low glucose), RD-Retrovector Particles, L-glutamine, Sodium pyruvate, human serum albumin, n-butyric acid, Pulmozyme®, magnesium and other excipients.

[00291] Drug product is available in one vial size: 30 mL type 1 clear glass vials with a 20 mm finish (containing 25 mL of $\geq 1.0 \times 10^{10}$ TVP). The vials are closed with 20 mm Teflon coated serum stoppers and 20 mm flip-off lacquered flip tops.

[00292] Reximmune-C2 will be administered intravenously by infusion pump over 15 minutes up to a volume of 100 mL, from >100 mL to 200mL over 30 minutes, from >200 mL to 300 mL over 45 minutes, and from >300 mL to 400 mL over 60 minutes. Volumes over 400 mL will be administered at a rate determined by the Investigator and the Gleneagles Medical Monitor. Once the MTD has been identified for the schedule, the time of administration may be changed, if indicated (and as agreed between the Investigator and the Gleneagles Medical Monitor).

[00293] Valganciclovir is administered orally, and should be taken with food. Serum creatinine or creatinine clearance levels should be monitored carefully. Dosage adjustment is required based on creatinine clearance as shown in the Table below. Valganciclovir dosing may begin on day 7 to 9 of the cycle but must be given for 5 consecutive days.

[00294] Creatinine clearance can be calculated from serum creatinine by the following formula:

[00295] For males = $\{(140 - \text{age}[\text{years}]) \times (\text{body weight} [\text{kg}])\} / \{(72) \times (0.011 \times \text{serum creatinine} [\text{micromol/L}])\}$

[00296] For females = 0.85 x male value.

Table I. Valganciclovir Dosing for Renally Impaired Patients

Cr CL (ml/min)	Dose Day 1	Dose Days 2 - 5
≥60 ml/min	900 mg (two 450 mg tablets) bid	900 mg (two 450 mg tablets) qday
40-59 ml/min	450mg bid	450mg qday
25-39 ml/min	450mg	450 mg Day 3 and Day 5
10-24 ml/min	450mg	450 mg Day 4
<10 ml/min	Not recommended	Not recommended

[00297] The purpose of the Phase 1 study is to establish the MTD, DLT, safety and a RP2D of the investigational agent. Toxic effects are thus the primary study endpoint and will be assessed continuously. Response information will be obtained if patients have disease that can readily be measured and re-assessed. These assessments will be made with every cycle. Furthermore, a response must be noted between two examinations at least 6 weeks apart in order to be documented as a confirmed response to therapy.

- Evaluable for toxicity - All patients will be evaluable for toxicity if they receive any study drug.
- Evaluable for response - All patients who have received at least a single cycle of treatment and had tumor re-assessment will be considered evaluable for response. In addition, those patients who develop early progressive disease will also be considered evaluable for response. Patients on therapy for at least two cycles of treatment will have their response evaluated.

[00298] The determination of antitumor efficacy will be based on objective tumor assessments made according to the Immune-Related Response Criteria (irRC) system of evaluation and treatment decisions by the Investigator will be based on these assessments.

[00299] Given the presence of the GM-CSF transgene in Reximmune-C2 and the possibility of an immune response contributing to the tumor effect, the Immune response Criteria will be utilized for clinical response. The reasons for using The immune Response Criteria vs RECIST 1.1 are as follows: (1) the appearance of measurable anti-tumor activity may take longer for immune therapies than for cytotoxic therapies; (2) responses to immune therapy occur after conventional PD; (3) discontinuation of immune therapy may not be appropriate in some cases, unless PD is confirmed (as is usually done for response); (4) allowance for “clinically insufficient” PD (e.g. small new lesions in the presence of other responsive lesions) is recommended; and (5) durable SD may represent antitumor activity.

[00300] The comparisons between RECIST 1.1 and the Immune-Related Response Criteria are listed below:

Table II. Comparison of WHO RECIST and Immune-Related Response Criteria

	WHO	irRC
New measurable lesions (i.e., $\geq 5 \times 5$ mm)	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions (i.e., $< 5 \times 5$ mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	$\geq 50\%$ decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	$\geq 50\%$ decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (any any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

[00301] Timing and Type of Assessments

[00302] All baseline imaging-based tumor assessments are to be performed within 14 days prior to the start of treatment. For the purposes of this study, all patients' tumor assessments should be re-evaluated starting 9 weeks after initiation of treatment and every 6 weeks thereafter (e.g., Week 9, Week 15, Week 21, etc.) for both Phase IA and Phase IB. All patients with responding tumors (irCR or irPR) must have the response confirmed no less than 6 weeks after the first documentation of response. All patients with tumor progression must have progression confirmed no less than 6 weeks after the first documentation of progression.

[00303] The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of treatment. All measurements should be recorded in metric notation.

[00304] CT and CT/PET are the methods for tumor assessments. Conventional CT should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to the chest, abdomen, and pelvis.

[00305] Chest CT will be used for assessment of pulmonary lesions.

[00306] Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

[00307] [¹⁸F]FHBG PET-CT scans will be obtained after the patient receives the first three doses of Reximmune-C2 (cycle 1) in Phase IA and after the screening dose of Reximmune-C2 in Phase IB. In Phase IA additional [¹⁸F]FHBG PET-CT scans can be obtained in subsequent cycles at the discretion of the Investigator and with approval of the Medical Monitor.

[00308] Ultrasound should not be used to measure tumor lesions that are clinically not easily accessible for objective response evaluation, e.g., visceral lesions. It is a possible alternative to clinical measurements of superficial palpable nodes, SC lesions, and thyroid nodules. Ultrasound might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

[00309] Endoscopy, laparoscopy, and radionuclide scan should not be used for response assessment.

[00310] All patients' files and radiological images must be available for source verification and may be submitted for extramural review for final assessment of antitumor activity.

[00311] Measurability of Tumor Lesions

[00312] At baseline, tumor lesions will be categorized by the Investigator as measurable or non-measurable by the criteria as described below:

- Measurable: Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes).
- Non-Measurable: All other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and bone lesions, leptomeningeal disease, ascites, pleural or pericardial effusions, lymphangitis of the skin or lung, abdominal masses that are not confirmed and followed by imaging techniques, cystic lesions, previously irradiated lesions, and disease documented by indirect evidence only (e.g., by laboratory tests such as alkaline phosphatase).

[00313] **NOTE:** Cytology and histology: If measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

[00314] Response to therapy may also be assessed by independent, central, radiologic blinded review.

[00315] Recording Tumor Measurements

[00316] All measurable lesions up to a maximum of 10 lesions, representative of all involved organs, should be identified as target lesions and measured and recorded at baseline and at the stipulated intervals during treatment. Target lesions should be selected on the basis of their size (lesion with the longest diameters) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically).

[00317] The longest diameter will be recorded for each target lesion. The sum of the longest diameter for all target lesions will be calculated and recorded as the baseline. The sum of the longest diameters is to be used as reference to further characterize the objective tumor response of the measurable dimension of the disease during treatment. All measurements should be recorded in metric notation in centimeters.

[00318] All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as “present” or “absent.”

[00319] Definitions of Tumor Response

[00320] Immune-Related Response Criteria criteria will be followed for assessment of tumor response.

[00321] Determination of Overall Response by Immune-Related Response Criteria

[00322] Target Lesions for Solid Tumors

- Complete response (irCR) is defined as the disappearance of all lesions (whether measurable or not, and no new lesions); confirmation by a repeat, consecutive assessment no less than 6 weeks from the date first documented.
- Partial response (irPR) is defined as a $> 50\%$ decrease in tumor burden relative to baseline confirmed by a consecutive assessment at least 6 weeks after the first documentation.
- Progressive disease (irPD) is defined as a $> 25\%$ increase in tumor burden relative to nadir (minimum recorded tumor burden) confirmed by a repeat, consecutive assessment no less than 6 weeks from the date first documented lesions recorded since the treatment started, or the appearance of one or more new lesions.
- Stable Disease (irSD) is defined as not meeting the criteria for irCR or irPR, in absence of irPD.

[00323] Non-Target Lesions for Solid Tumors

[00324] The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or irSD is mandatory to differentiate between response or irSD and irPD.

[00325] Confirmation of Tumor Response

[00326] To be assigned a status of irPR or irCR, changes in tumor measurements in patients with responding tumors must be confirmed by repeat studies that should be performed ≥ 6 weeks after the criteria for response are first met. In the case of irSD, follow-up measurements must have met the irSD criteria at least once after study entry at a minimum interval of 6 weeks.

When both target and non-target lesions are present, individual assessments will be recorded separately. The overall assessment of response will involve all parameters as depicted in Table III.

[00327] The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as a reference for tumor progression the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

[00328] Patients will be defined as being not evaluable (NE) for response if there is no post-randomization oncologic assessment. These patients will be counted as failures in the analysis of tumor response data.

[00329] Clinical Efficacy Assessment: Performance Status.

[00330] Patients will be graded according to the Karnofsky performance status scale as described in Table IV.

Table IV. Karnofsky Performance Status Criteria

Grade	Criterion
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity, minor signs or symptoms of disease
80	Normal activity with effort, some signs or symptoms of disease
70	Care for self. Unable to carry on normal activities or to do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization is indicated although death not imminent
20	Hospitalization necessary, very sick, active supportive treatment necessary
10	Moribund, fatal processes progressing rapidly
0	Death

[00331] Tumor Marker Response

[00332] Method of Assessment

[00333] While not a fully validated measure of efficacy in many malignancies, serial determinations of tumor markers may allow evaluation of an easily performed, inexpensive, quantitative, clinical tool as a potential additional means for following the course of the illness during therapy.

[00334] A tumor marker decrease or increase will not be assessed as an objective measure of outcome. In particular, a rising tumor marker value will not be considered in the definition of tumor progression, but should prompt a repeat radiographic evaluation to document whether or not radiographic tumor progression has occurred.

[00335] Calculated Endpoint Definitions

[00336] Survival is defined as the time from date of first study drug treatment to date of death. In the absence of confirmation of death, survival time will be censored at the last date of follow-up.

[00337] Tumor response rate is defined as the proportion of patients who have any evidence of objective irCR or irPR.

[00338] TTP is defined as the time from treatment to first confirmed documentation of tumor progression or to death due to any cause. For patients who do not have objective evidence of tumor progression and who are either removed from study treatment or are given antitumor treatment other than the study treatment, TTP will be censored. A tumor marker increase meeting criteria for tumor marker progression does not constitute adequate objective evidence of tumor progression. However, such a tumor marker increase should prompt a repeat radiographic evaluation to document whether or not objective tumor progression has occurred.

[00339] TTF is defined as the time from treatment to first confirmed documentation of tumor progression, or to off-treatment date, or to death due to any cause, whichever comes first. Patients who are still on treatment at the time of the analysis and patients who are removed from therapy by their physicians during an objective response and who, at the off-treatment date, have no evidence for objective tumor progression will not be considered to have experienced treatment failure, unless the withdrawal is due to the occurrence of a medical event. For these patients, TTF will be censored at the off-study date. Censoring for TTF will also be performed in those patients who are given antitumor treatment, other than the study treatment, before the first of objective tumor progression, off-study date, or death. A tumor marker increase meeting criteria for tumor marker progression does not constitute adequate objective evidence of treatment failure. However, such a tumor marker increase should prompt a repeat radiographic

evaluation to document whether or not objective tumor progression (and thus treatment failure) has occurred.

[00340] Time to first definitive performance status worsening is the time from treatment until the last time the performance status was no worse than at baseline or to death, due to any cause, in the absence of previous documentation of definitive confirmed performance status worsening. For patients who do not have definitive performance status worsening and who are either removed from study or are given antitumor treatment other than the study treatment, definitive performance status worsening will be censored.

[00341] Time to first definitive weight loss is defined as the time from treatment until the last time the percent weight decrease from baseline was $< 5\%$ or to death due to any cause in the absence of previous documentation of definitive weight loss. For patients who do not have definitive weight loss and who are either removed from study or are given antitumor treatment other than study treatment, definitive weight loss will be censored.

[00342] Additional evaluations of the data may include best objective response, confirmed and unconfirmed objective response rate, duration of study treatment, time to first occurrence of new lesions, time to tumor response, stable disease at 24 weeks, and rate of progression free survival at 24 weeks. Data may be evaluated by RECIST 1.1 criteria, if needed.

[00343] Treatment Administration Assessment

[00344] For both Phase IA and IB: dose intensity is defined as the total dose/cycle times the number of weeks between start of treatment and last treatment plus 13 days.

[00345] Percent relative dose intensity is defined as the proportion of the actual dose intensity divided by the planned dose intensity for that same period of time.

ABBREVIATIONS

ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
BSA	Body surface area (m^2)
CL	Systemic plasma clearance
C_{max}	Peak plasma concentration
CR	Complete response
CRF	Case report form
CT	Computerized tomography
CTC	Common Toxicity Criteria
DLT	Dose Limiting Toxicities

EOI	End of infusion
FDA	Food and Drug Administration
G-CSF	Granulocyte-colony stimulating factor (filgrastim, Neupogen®)
GCP	Good clinical practice
GM-CSF	Granulocyte-macrophage colony-stimulating factor (sargramostim, Leukine®)
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
IEC	Independent Ethics Committee
i.p.	Intraperitoneal
IRB	Institutional Review Board
IV	Intravenous, intravenously
LD ₁₀ or LD ₅₀	Dose that is lethal to 10% or 50% of animals
LDH	Lactate dehydrogenase
MAD	Maximum Administered Dose
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NE	Not evaluable for tumor response
NOAEL	No Observed Adverse Effect Level
Non-CR	Non-complete response
Non-PD	Non-progressive disease
PBMC	Peripheral Blood Mononuclear Cells
PCE	Propylene Glycol: Cremophor® EL: Ethanol
PD	Progressive disease
PR	Partial response
SAER-S	Serious Adverse Event Report-Study
SC	Subcutaneous, subcutaneously
SD	Stable disease
STD ₁₀	Dose that is severely toxic to 10% of animals
TTP	Time to Progression
TTF	Time to Failure
T _½	Half-life
T _{max}	Time of maximum plasma concentration

V_{ss} Steady state volume of distribution

[00346] While preferred embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosed embodiments. It should be understood that various alternatives to the embodiments described herein may be employed in practicing the embodiments. It is intended that the following claims define the scope of the embodiments and that methods and structures within the scope of these claims and their equivalents be covered thereby.

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SEQUENCES**SEQ ID NO: 1: wild type HSV1-TK nucleotide sequence**

atggccttcgtacccccggccatcaacacgcgtctgcgttcgaccaggctgcgcggttctcgcggcc
 atagcaaccgacgtacggcggttgcgccctcgccggcagcaagaagccacggaagtccgcccgga
 gcagaaaatgcccacgctactgcgggtttatatagacgggtccccacgggatggggaaaaccacc
 accacgcaactgctggtggccctgggttcgcgcgcgacgatatcgtctacgtacccgagccgatga
 cttactggcggggtgctgggggcttccgagacaatcgcgaaacatctacaccacacaacaccgcct
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 ggcatgccttatgccgtgaccgacgccgttctggctcctcatatcgggggggaggctgggagct
 cacatgccccgcccccgccctcaccctcatcttcgaccgccatcccatcgccgccctcctgtg
 ctacccggccgcgcggtaccttatgggcagcatgacccccaggccgtgctggcggttcgtggcc
 ctcatcccgcgcgaccttgcccggcaccaacatcgtgcttggggcccttccggaggacagacaca
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 gaggactggggacagctttcggggacggccgtgccgccccagggtgccgagccccagagcaacg
 cgggcccacgaccccatatcggggacacgttatctaccctgtttcgggcccccgagttgctggc
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 tccatgcacgtctttatcctggattacgaccaatcgcccgccggctgccgggacgccctgctgc
 aacttacctccgggatgggtccagacccacgtcaccacccccggctccataccgacgatatgcga
 cctggcgcgcacgtttgcccgggagatgggggaggctaactga

SEQ ID NO: 2: wild type HSV1-TK amino acid sequence

MASYPGHQHASAFDQAARSRGHSNRRTALRPRRQQEATEVRPEQKMPTLLRVYIDGPHGMGKTT
 TTQLLVALGSRDDIVY
 VPEPMTYWRVLGASETIANIYTTQHRLDQGEISAGDAAVVM TSAQITMGMPYAVTDAVLAPHIG
 GEAGSSHAPPPALTLI
 FDRHPAALLCYPAARYLMGSMT PQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRP
 ERLDLAMLAAIRRVYG
 LLANTVRYLQCGGSWREDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTLFTLFRAPELLAPNGDL
 YNVFAWALDVLAKRLR
 SMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTPGSIPTICDLARTFAREMGEAN

SEQ ID NO: 3: HSV-TK in Reximmune-C HSV-TK; SR 39 mutant and R25G-R26S**Mutation of NLS**

atggccttcgtacccccggccatcaacacgcgtctgcgttcgaccaggctgcgcggttctcgcggcc
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gaggactggggacagcttttcggggacggccgtgccgccccaggggtgccgagccccagagcaacg
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SEQ ID NO: 4 (amino acid sequence encoded by SEQ ID NO: 3)

MASYPGHQHASAFDQAARSRGHSNGSTALRPRRQQEATEVRPEQKMPTLLRVYIDGPHGMGKTT
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 GMPYAVTDAVLAPHIGGEAGSSHAPPPALTIFLDRHPIAFMLCYPAARYLMGSMTQPQAVLAFVA
 LIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGERLDLAMLAAIRRVYGLLANTVRYLQCGGSWR
 EDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTLFTLFRAPPELLAPNGDLYNVFAWALDVLAKRLR
 SMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTTPGSIPTICDLARTFAREMGEAN

SEQ ID NO: 5: HSV-TK Sites to mutate are in bold, underlining (HSV-TK nuclear localization sequence, RR, and Substrate Binding Domain, LIF and AAL

atggcctcgtacccccggccatcaacacgcgtctgcgttcgaccaggctgcgcggttctcgc 60
 M A S Y P G H Q H A S A F D Q A A R S R
 ggccatagcaac**cgacgt**acggcggttgcgccct**cgccgg**cagcaagaagccacggaagtc
 120
 G H S N **R** **R** T A L R P **R** **R** Q Q E A T E V
 cgccccggagcagaaaatgcccacgctactgcgggtttatatagacgggtccccacgggatg
 180
 R P E Q K M P T L L R V Y I D G P H G M
 gggaaaaccaccaccacgcaactgctgggtggccctgggttcgcgcgacgatatcgtctac
 240
 G K T T T T Q L L V A L G S R D D I V Y
 gtacccgagccgatgacttactggcggggtgctgggggcttccgagacaatcgcgaaacatc
 300
 V P E P M T Y W R V L G A S E T I A N I
 tacaccacacaacaccgcctcgaccagggtgagatatcggccggggacgcggcggtggta
 360
 Y T T Q H R L D Q G E I S A G D A A V V
 atgacaagcgcccagataacaatgggcatgccttatgccgtgaccgacgccgttctggct
 420
 M T S A Q I T M G M P Y A V T D A V L A
 cctcatatcgggggggaggctgggagctcacatgccccgcccccgccctcacc**ctcatc**
 480
 P H I G G E A G S S H A P P P A L T **L** **I**
ttcgaccgccatcccatc**gccgccctc**ctgtgctacccggccgcgcggtaccttatgggc
 540
F D R H P I **A** **A** **L** L C Y P A A R Y L M G
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 600

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S M T P Q A V L A F V A L I P P T L P G
 accaacatcgtgcttggggcccttccggaggacagacacatcgaccgcctggccaaacgc
 660
 T N I V L G A L P E D R H I D R L A K R
 cagcgccccggcgagcggctggacctggctatgctggctgcgattcgccgcgtttacggg
 720
 Q R P G E R L D L A M L A A I R R V Y G
 ctacttgccaatacgggtgcggtatctgcagtgcgggcgggtcgtggcgaggaggactgggga
 780
 L L A N T V R Y L Q C G G S W R E D W G
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 840
 Q L S G T A V P P Q G A E P Q S N A G P
 cgaccccatatcggggacacggttatccctgtttcggggcccccgagttgctggcccc
 900
 R P H I G D T L F T L F R A P E L L A P
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 960
 N G D L Y N V F A W A L D V L A K R L R
 tccatgcacgtctttatcctggattacgaccaatcgcccgccggctgccgggacgcctg
 1020
 S M H V F I L D Y D Q S P A G C R D A L
 ctgcaacttacctccgggatgggtccagacccacgtcaccacccccggctccataccgacg
 1080
 L Q L T S G M V Q T H V T T P G S I P T
 atatgcgacctggcgcgcacgtttgccccgggagatgggggaggctaactga
 I C D L A R T F A R E M G E A N *

SEQ ID NOS: 6 and 7: Sac I-Kpn I (SR39) mutant region

GAGCTCACATGCCCCGCCCCCGGCCCTCACC**A**T**C**T**C**TCGACCGCCATCCCATCGCC-
CTCGAGTGTACGGGGCGGGGGCCGGGAGTGG**T**AG**A**AG**G**AGCTGGCGGTAGGGTAGCGG-
 Sac I

-**TT****A**T**G**CTGTGCTACCCGGCCGCGCGGTACC (SEQ ID NO: 6)

-**AA****G****T****A****C**GACACGATGGGCCGGCGCGC**CATGG** (SEQ ID NO: 7)

Kpn I

Kpn I	GGTACC	G		G		T		A		C	/	C	GTAC - 3'
		C	/	C		A		T		G		G	

Sac I	GAGCTC	G		A		G		C		T	/	C	AGCT - 3'
		C	/	T		C		G		A		G	

SEQ ID NOS: 8 and 9: Sac I-Kpn I (SR39) mutant region (cut)

CACATGCCCCGCCCCCGGCCCTCACCATCTTCTCGACCGCCATCCCATCGCCTTCATG
 TCGAGTGTACGGGGCGGGGGCCGGGAGTGGTAGAGAGCTGGCGGTAGGGTAGCGGAA
 Sac I (cut)

CTGTGCTACCCGGCCGCGCGGTAC (SEQ ID NO: 8)

GTACGACACGATGGGCCGGC (SEQ ID NO: 9)

Kpn I (cut)

Kpn I	GGTACC	G		G		T		A		C	/	C	GTAC - 3'
		C	/	C		A		T		G		G	

SEQ ID NOS: 10 and 11: Primers

SR39sackpn F1

5' CACATGCCCCGCCCCCGGCCCTCACCATCTTCTCGACCGCCATCCCATCGCCTTCATGCTG
 TGCTACCCGGCCGCGCGGTAC 3' (SEQ ID NO: 10)

SR39sackpn R1

5' CGCGCGGCCGGGTAGCACAGCATGAAGGCGATGGGATGGCGGTCGAGGAAGATGGTGAGGGC
 CGGGGGCGGGGCATGTGAGCT 3' (SEQ ID NO: 11)

SEQ ID NO: 12 Gene #3 mHSV-TK CO A168H(LIF...AHL): Length:1185

GTCAGCGGCCGCACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
 CTTCGACCAGGCCGCCCCGAGCCGCGGCCACAGCAACGGCAGCACCGCACTGCGGCCACGGCGC
 CAGCAGGAGGCCACCGAGGTGCGCCCCGAGCAGAAGATGCCACCCCTGCTGCGCGTGTACATCG
 ACGGACCACACGGCATGGGCAAGACCACCACCACCCAGCTGCTGGTGGCCCTGGGCAGCCGCGA
 CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGTGGGCGCCAGCGAGACCATC
 GCCAACATCTACACCACCCAGCACCGCCTGGACCAAGGCGAGATCAGCGCCGGCGACGCCGCCG
 TGGTGATGACCAGCGCCCAGATTACAATGGGCATGCCCTACGCCGTGACCGACGCCGTGCTGGC
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 GACCGGCACCCAATCGCACACCTGCTGTGCTACCCGGCAGCACGCTACCTGATGGGCTCCATGA
 CACCACAAGCCGTGCTGGCCTTCGTGGCCCTGATCCCACCAACACTGCCCGGCACCAACATCGT
 GCTGGGCGCCCTGCCCCGAGGACCGCCACATCGACCGCCTGGCCAAGCGCCAGCGCCCCGGCGAG
 CGCCTGGACCTGGCCATGCTGGCCGCCATCCGCCGCGTGTACGGCCTGCTGGCCAACACCGTGC
 GCTACCTGCAGTGCGGCGGCAGCTGGCGCGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCC
 ACCACAGGGCGCCGAGCCACAGAGCAACGCCGGACCACGACCACACATCGGCGACACCCTGTTC
 ACCCTGTTCCGGGCACCAGAGCTGCTGGCACCAAACGGCGACCTGTACAACGTGTTTCGCCTGGG
 CCCTGGACGTGCTGGCCAAGCGCCTGCGCTCCATGCACGTGTTTCATCCTGGACTACGACCAGTC
 ACCGGCCGGCTGCCGCGACGCCCTGCTGCAGCTGACCAGCGGCATGGTGCAGACCCACGTGACA
 ACACCCGGCAGCATCCCAACAATCTGCGACCTGGCCCGCACCTTCGCCCCGCGAGATGGGCGAGG
 CCAACTAATAGGGATCCCTCGAGAAGCTTGTC

SEQ ID NO: 13 Gene #4 mHSV-TK CO TK A167F(LIF...FAL): Length:1185

GTCAGCGGCCGCACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
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CAGCAGGAGGCCACCGAGGTGCGCCCCGAGCAGAAGATGCCCACCCTGCTGCGCGTGTACATCG
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 CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGCTGGGCGCCAGCGAGACCATC
 GCCAACATCTACACCACCCAGCACC GCCTGGACCAAGGCGAGATCAGCGCCGGCGACGCCGCCG
 TGGTGATGACCAGCGCCCAGATTACAATGGGCATGCCCTACGCCGTGACCGACGCCGTGCTGGC
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 CGCCTGGACCTGGCCATGCTGGCCGCCATCCGCCGCGTGTACGGCCTGCTGGCCAACACCGTGCC
 GCTACCTGCAGTGCGGCGGCAGCTGGCGCGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCC
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 ACACCCGGCAGCATCCCAACAATCTGCGACCTGGCCCCGCACCTTCGCCCCGCGAGATGGGCGAGG
 CCAACTAATAGGGATCCCTCGAGAAGCTTGTC

SEQ ID NO: 14 Gene #5 mHSV-TK CO dual mutant A167F-A168H (LIF...FHL):

Length:1185

GTCAGCGGCCGACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
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 CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGCTGGGCGCCAGCGAGACCATC
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 ACACCCGGCAGCATCCCAACAATCTGCGACCTGGCCCCGCACCTTCGCCCCGCGAGATGGGCGAGG
 CCAACTAATAGGGATCCCTCGAGAAGCTTGTC

SEQ ID NO: 15 Gene #6 mHSV-TK CO MB-IFL A168H(IFL...AHL): Length:1185

GTCAGCGGCCGACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
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 ACGGACCACACGGCATGGGCAAGACCACCACCACCCAGCTGCTGGTGGCCCTGGGCAGCCGCGA
 CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGCTGGGCGCCAGCGAGACCATC
 GCCAACATCTACACCACCCAGCACC GCCTGGACCAAGGCGAGATCAGCGCCGGCGACGCCGCCG

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TGGTGATGACCAGCGCCCAGATTACAATGGGCATGCCCTACGCCGTGACCGACGCCGTGCTGGC
 ACCACACATCGGCGGCGAGGCCGGCAGCAGCCACGCACCACCAGCACTGACCATCTTCCTG
 GACCGGCACCCAATCGCACACCTGCTGTGCTACCCGGCAGCACGCTACCTGATGGGCTCCATGA
 CACCACAAGCCGTGCTGGCCTTCGTGGCCCTGATCCCACCAACACTGCCCCGGCACCAACATCGT
 GCTGGGCGCCCTGCCCCGAGGACCGCCACATCGACCGCCTGGCCAAGCGCCAGCGCCCCGGCGAG
 CGCCTGGACCTGGCCATGCTGGCCGCCATCCGCCGCGTGTACGGCCTGCTGGCCAACACCGTGC
 GCTACCTGCAGTGCGGCGGCAGCTGGCGCGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCC
 ACCACAGGGCGCCGAGCCACAGAGCAACGCCGGACCACGACCACACATCGGCGACACCCTGTTC
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 ACCGGCCGGCTGCCGCGACGCCCTGCTGCAGCTGACCAGCGGCATGGTGCAGACCCACGTGACA
 ACACCCGGCAGCATCCCAACAATCTGCGACCTGGCCCCGCACCTTCGCCCCGCGAGATGGGCGAGG
 CCAACTAATAGGGATCCCTCGAGAAGCTTGTC

SEQ ID NO: 16 Gene #1 HSV-TK A168H dmNLS CO SC: Length:1185

GTCAGCGGCCGACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
 CTTTCGACCAGGCCGCCCGCAGCCGCGGCCACAGCAACGGCAGCACCGCACTGCGGCCAGGATCT
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 CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGTGGGCGCCAGCGAGACCATC
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 ACCACAGGGCGCCGAGCCACAGAGCAACGCCGGACCACGACCACACATCGGCGACACCCTGTTC
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SEQ ID NO: 17 Gene #2 HSV-TK A167F dmNLS CO SC: Length:1185

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CGCCTGGACCTGGCCATGCTGGCCGCCATCCGCCGCGTGTACGGCCTGCTGGCCAACACCGTGCG
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 ACCGGCCCGGCTGCCGCGACGCCCTGCTGCAGCTGACCAGCGGCATGGTGCAGACCCACGTGACA
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SEQ ID NO: 18 Gene #3 HSV-TK A168H NESdmNLS CO SC: Length:1221

GTCAGCGGCCGCGACCGGTACGCGTCCACCATGGCCCTGCAGAAAAAGCTGGAAGAGCTGGAAC
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 AGCAGAAGATGCCCCACCCTGCTGCGCGTGTACATCGACGGACCACACGGCATGGGCAAGACCAC
 CACCACCCAGCTGCTGGTGGCCCTGGGCAGCCGCGACGACATCGTGTACGTGCCCCGAGCCCATG
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 GCTACCCGGCAGCACGCTACCTGATGGGCTCCATGACACCACAAGCCGTGCTGGCCTTCGTGGC
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 CGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCCACCACAGGGCGCCGAGCCACAGAGCAAC
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 TGTCA

SEQ ID NO: 19 Gene #4 HSV-TK A167F NESdmNLS CO SC: Length:1221

GTCAGCGGCCGCGACCGGTACGCGTCCACCATGGCCCTGCAGAAAAAGCTGGAAGAGCTGGAAC
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 TGGACCAAGGCGAGATCAGCGCCGGCGACGCCGCCGTGGTGATGACCAGCGCCCAGATTACAAT
 GGGCATGCCCTACGCCGTGACCGACGCCGTGCTGGCACACACATCGGCGGGCGAGGCCGGCAGC
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CACCAAACGGCGACCTGTACAACGTGTTTCGCCTGGGCCCTGGACGTGCTGGCCAAGCGCCTGCG
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TGTCA

SEQ ID NO: 20 Gene #5 HSV-TK A168H NESdmNLS JCO SC: Length:1221

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AACAGAAAATGCCTACACTGCTGAGAGTGTATATTGATGGACCACATGGAATGGGAAAAACAAC
CACAACCCAGCTGCTGGTGGCTCTCGGATCTAGAGATGATATTGTGTATGTGCCTGAACCTATG
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GGGAATGCCTTATGCTGTGACAGATGCTGTGCTGGCACCACATATTGGAGGCGAAGCTGGAAGC
TCTCATGCACCACCACCAGCACTGACACTGATTTTTTGATCGGCATCCAATTGCACATCTGCTGT
GTTATCCGGCAGCAAGATATCTGATGGGAAGCATGACACCACAAGCCGTGCTGGCTTTTGTGGC
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ATCGATCGGCTGGCCAAACGGCAGAGACCTGGAGAACGGCTGGATCTGGCCATGCTGGCTGCCA
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CACCAAATGGAGACCTGTACAACGTGTTTGCCTGGGCTCTGGATGTGCTGGCTAAACGGCTGAG
ATCTATGCATGTGTTTATCCTGGACTATGATCAGTCACCGGCCGGATGTCGCGATGCCCTGCTG
CAGCTGACATCTGGGATGGTGCAGACACATGTGACAACACCTGGATCTATCCCAACAATCTGTG
ATCTGGCTAGAACATTCGCTAGGGAGATGGGAGAGGCCAACTAATGAGGATCCCTCGAGAAGCT
TGTCA

SEQ ID NO: 21 Gene #6 HSV-TK A167F NESdmNLS JCO SC: Length:1221

GTCAGCGGCCGACCCGGTACGCGTCCACCATGGCTCTGCAGAAAAAGCTGGAAGAGCTGGAAC
GGATGGCTCTTATCCTGGACATCAGCATGCTTCTGCTTTTGATCAGGCTGCCAGATCTAGAGGA
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TCTGATTCCACCAACACTGCCTGGAACAAACATCGTGCTGGGAGCTCTGCCTGAAGATAGACAT
ATCGATCGGCTGGCCAAACGGCAGAGACCTGGAGAACGGCTGGATCTGGCCATGCTGGCTGCCA
TTCGGAGAGTGTATGGCCTGCTGGCTAACACAGTGAGATATCTGCAGTGTGGAGGCTCTTGGAG
AGAGGATTGGGGACAGCTGTCTGGCACAGCTGTGCCACCACAGGGAGCCGAACCACAGAGCAAT
GCTGGACCACGACCACATATCGGAGACACACTGTTTACACTGTTTCGGGCACCAGAACTGCTGG
CACCAAATGGAGACCTGTACAACGTGTTTGCCTGGGCTCTGGATGTGCTGGCTAAACGGCTGAG
ATCTATGCATGTGTTTATCCTGGACTATGATCAGTCACCGGCCGGATGTCGCGATGCCCTGCTG
CAGCTGACATCTGGGATGGTGCAGACACATGTGACAACACCTGGATCTATCCCAACAATCTGTG

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ATCTGGCTAGAACATTCGCTAGGGAGATGGGAGAGGCCAACTAATGAGGATCCCTCGAGAAGCT
TGTCA

SEQ ID NO: 22

HSV-TK dmNLS A168H, CO & SC

dmNLS = double mutated Nuclear Localization Sequence

CO = codon optimized

SC = splice corrected at 327 and 555

Kozak Sequence, Underlined

gtcaGCGGCCGCAACCGGTACGCGTCCACCATGGCCAGCTACCCCGGCCACCAGCACGCCAGCGC
CTTCGACCAGGCCGCCCGCAGCCGCGGCCACAGCAACGGCAGCACCGCaCTGCGgCCaGGATCT
CAGCAGGAGGCCACCGAGGTGCGCCCCGAGCAGAAGATGCCCACCCTGCTGCGCGTGTACATCG
ACGGaCCaCACGGCATGGGCAAGACCACCACCACCCAGCTGCTGGTGGCCCTGGGCAGCCGCGA
CGACATCGTGTACGTGCCCCGAGCCCATGACCTACTGGCGCGTGCTGGGCGCCAGCGAGACCATC
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CaCCaCAaGCCGTGCTGGCCTTCGTGGCCCTGATCCCaCCaACaCTGCCCCGGCACCAACATCGT
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CGCCTGGACCTGGCCATGCTGGCCGCCATCCGCCGCGTGTACGGCCTGCTGGCCAACACCGTGCC
GCTACCTGCAGTGCGGCGGCAGCTGGCGCGAGGACTGGGGCCAGCTGAGCGGCACCGCCGTGCC
aCCaCAGGGCGCCGAGCCaCAGAGCAACGCCGGaCCaCGaCCaCACATCGGCGACACCCTGTTC
ACCCTGTTCCGgGCaCCaGAGCTGCTGGCaCCaAACGGCGACCTGTACAACGTGTTTCGCCTGGG
CCCTGGACGTGCTGGCCAAGCGCCTGCGCtccATGCACGTGTTTCATCCTGGACTACGACCAGtc
aCCgGCCGGCTGCCGCGACGCCCTGCTGCAGCTGACCAGCGGCATGGTGCAGACCCACGTGACa
ACaCCCGGCAGCATCCCaACaATCTGCGACCTGGCCCCGCACCTTCGCCCCGCGAGATGGGCGAGG
CCAATAATAGGGATCCCTCGAGAAGCTTgtca

SEQ ID NO: 23 – MAP Kinase Kinase Nuclear Export Polynucleotide Sequence

CTGCAGAAAAAGCTGGAAGAGCTGGAACCTGGATGGC

SEQ ID NO: 24 - MAP Kinase Kinase Nuclear Export Polypeptide Sequence

LQKKLEEELELDG

SEQ ID NO: 25 – Targeting Moiety

WREPSFMALS

CLAIMS

WHAT IS CLAIMED IS:

1. A method for identifying a patient capable of benefitting from gene therapy treatment comprising:
 - a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide to the patient;
 - b) administering to the patient a substrate of HSV-TK attached to a radioactive tracer;
 - c) measuring the relative amount and location of the radioactive signal present in the patient; and
 - d) determining the location of lesions in the patient, wherein patients with: radioactive signals above a certain threshold, and location of the radioactive signal correlating with lesions measured in step (d) of the patient, are identified as capable of benefitting from gene therapy treatment.
2. The method of claim 1, wherein the substrate of HSV-TK is chosen from the group consisting of FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, valganciclovir, acyclovir, valaciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-, ganciclovir and penciclovir-like side chains.
3. The method of claim 1, wherein the substrate of HSV-TK is FHBG (9-(4-fluoro-3-(hydroxymethyl)butyl]guanine).
4. The method of claim 1, wherein the radioactive tracer is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$.
5. The method of claim 1, wherein the radioactive tracer is ^{18}F .
6. The method of claim 1, wherein the HSV-TK substrate is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine).

7. The method of claim 1, wherein the radioactive tracer signal is measured using positron emission tomography (PET) scanning.
8. The method of claim 7, wherein the threshold level is at least above 2.0 SUV (standardized uptake value) or at least 20% above background on a PET scan.
9. The method of claim 7, wherein the threshold level is between about 1.0 SUV and about 3.0 SUV, or between about 20% to about 40% above background on a PET scan.
10. The method of claims 8 and 9, further comprising treating the patient with the HSV-TK retroviral particle.
11. The method of claim 1, wherein the viral nuclear localization sequence (NLS) of the encoded HSV-TK polynucleotide is mutated.
12. The method of claim 1, wherein the thymidine kinase polynucleotide is mutated to include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein.
13. The method of claim 1, wherein the thymidine kinase polynucleotide is mutated to increase substrate binding of the expressed thymidine kinase protein.
14. The method of claim 13, wherein the mutation is A168H.
15. The method of claim 13, further comprising mutating the thymidine kinase polynucleotide to remove the viral nuclear localization sequence (NLS) and include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein.
16. The method of claim 1, wherein the HSV-thymidine kinase polynucleotide is SEQ ID NO: 18.
17. The method of claim 16, further comprising a targeting protein expressed on the viral envelope.
18. The method of claim 17, wherein the targeting protein binds to collagen, laminin, fibronectin, elastin, glycosaminoglycans, proteoglycans or RGD.
19. The method of claim 17, wherein the targeting protein binds to collagen.

20. The method of claim 19, wherein the targeting protein is SEQ ID NO: 25.
21. A method for identifying a patient or subject in need of treatment for lesions and capable of benefitting from gene therapy treatment:
- a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells from the patient with the polynucleotide encoding HSV-thymidine kinase;
 - b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer;
 - c) measuring the relative amount of radioactive signal present in target tissue;
 - d) identifying patients wherein the level of radioactively-labelled HSV-TK substrate is above a threshold;
 - e) determining the location of lesions in the patient; and
 - e) treating said patient or subject with the gene therapy retroviral particle comprising an HSV-TK polynucleotide when the measured radioactive signal in the patient is above a certain threshold, and the location of the measured radioactive signal correlates with lesions measured in step (e) of the patient.
22. The method of claim 21, wherein the substrate of HSV-TK is chosen from the group consisting of FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, val-ganciclovir, acyclovir, val-aciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains.

23. The method of claim 21, wherein the substrate of HSV-TK is FHBG (3-(hydroxymethyl)butyl]guanine).
24. The method of claim 21, wherein the radioactive tracer is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$.
25. The method of claim 21, wherein the radioactive tracer is ^{18}F .
26. The method of claim 21, wherein the HSV-TK substrate is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine).
27. The method of claim 21, wherein the radioactive tracer signal is measured using positron emission tomography (PET) scanning.
28. The method of claim 27, wherein the threshold level is at least above 2.0 SUV (standardized uptake value) or at least 20% above background on a PET scan.
29. The method of claim 27, wherein the threshold level is between about 1.0 SUV and about 3.0 SUV.
30. The method of claims 28 and 29, wherein the HSV-TK retroviral vector particle comprises a second therapeutic polynucleotide.
31. The method of claim 21, wherein the viral nuclear localization sequence (NLS) of the encoded HSV-TK polynucleotide is mutated.
32. The method of claim 21, wherein the thymidine kinase polynucleotide is mutated to include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein.
33. The method of claim 21, wherein the thymidine kinase polynucleotide is mutated to increase substrate binding of the expressed thymidine kinase protein.
34. The method of claim 23, wherein the mutation is A168H.
35. The method of claim 23, further comprising mutating the thymidine kinase polynucleotide to remove the viral nuclear localization sequence (NLS) and include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein.

36. The method of claim 21, wherein the HSV-thymidine kinase polynucleotide is SEQ ID NO: 18.
37. The method of claim 36, further comprising a targeting protein expressed on the viral envelope.
38. The method of claim 37, wherein the targeting protein binds to collagen, laminin, fibronectin, elastin, glycosaminoglycans, proteoglycans or RGD.
39. The method of claim 37, wherein the targeting protein binds to collagen.
40. The method of claim 39, wherein the targeting protein is SEQ ID NO: 25.
41. A method for measuring the enzymatic activity of a transduced HSV-thymidine, the method comprising:
 - a) administering a gene therapy retroviral particle comprising an HSV-TK polynucleotide and transducing cells from the patient with the polynucleotide encoding HSV-thymidine kinase;
 - b) treating the cells with a substrate of HSV-TK attached to a radioactive tracer; and
 - c) measuring the relative amount of radioactive signal present in target tissue.
42. The method of claim 41, wherein the substrate of HSV-TK is chosen from the group consisting of FHBG (9-[4-fluoro-3-(hydroxymethyl)butyl]guanine), FHPG (9-([3-fluoro-1-hydroxy-2-propoxy]methyl)guanine), FGCV (fluoroganciclovir), FPCV (fluoropenciclovir), FIAU (1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-iodouracil), FEAU (fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), FMAU (fluoro-5-methyl-1-beta-D-arabinofuranosyluracil), FHOMP (6-((1-fluoro-3-hydroxypropan-2-yloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione), ganciclovir, val-ganciclovir, acyclovir, val-aciclovir, penciclovir, radiolabeled pyrimidine with 4-hydroxy-3-(hydroxymethyl)butyl side chain at N-1 (HHG-5-FEP) or 5-(2-)hydroxyethyl)- and 5-(3-hydroxypropyl)-substituted pyrimidine derivatives bearing 2,3-dihydroxypropyl, acyclovir-ganciclovir and penciclovir-like side chains.
43. The method of claim 41, wherein the substrate of HSV-TK is FHBG (3-(hydroxymethyl)butyl]guanine).
44. The method of claim 41, wherein the radioactive tracer is ^{18}F , ^{64}Cu , $^{99\text{m}}\text{Te}$, ^{11}C , ^{14}C , ^{124}I , ^{123}I , ^{131}I , ^{15}O , ^{13}N and/or $^{82}\text{RbCl}$.

45. The method of claim 41, wherein the radioactive tracer is ^{18}F .
46. The method of claim 41, wherein the HSV-TK substrate is [^{18}F]FHBG (9-(4- ^{18}F -fluoro-3-(hydroxymethyl)butyl]guanine).
47. The method of claim 41, wherein the radioactive tracer signal is measured using positron emission tomography (PET) scanning.
48. The method of claim 47, wherein the threshold level is at least above 2.0 SUV (standardized uptake value) or at least 20% above background on a PET scan.
49. The method of claim 47, wherein the threshold level is between about 1.0 SUV and about 3.0 SUV, or between about 20% to about 40% above background on a PET scan.
50. The method of claims 48 and 49, further comprising treating the patient with the HSV-TK retroviral particle.
51. The method of claim 41, wherein the viral nuclear localization sequence (NLS) of the encoded HSV-TK polynucleotide is mutated.
52. The method of claim 41, wherein the thymidine kinase polynucleotide is mutated to include a nuclear export sequence (NES) at or near the amino terminus of the expressed thymidine kinase protein.
53. The method of claim 41, wherein the thymidine kinase polynucleotide is mutated to increase substrate binding of the expressed thymidine kinase protein.
54. The method of claim 53, wherein the mutation is A168H.
55. The method of claim 53, further comprising mutating the thymidine kinase polynucleotide to remove the viral nuclear localization sequence (NLS) and include a nuclear export sequence (NES) at the amino terminus of the expressed thymidine kinase protein.
56. The method of claim 41, wherein the HSV-thymidine kinase polynucleotide is SEQ ID NO: 18.
57. The method of claim 56, further comprising a targeting protein expressed on the viral envelope.

58. The method of claim 57, wherein the targeting protein binds to collagen, laminin, fibronectin, elastin, glycosaminoglycans, proteoglycans or RGD.
59. The method of claim 57, wherein the targeting protein binds to collagen.
60. The method of claim 59, wherein the targeting protein is SEQ ID NO: 25.

FIG. 1

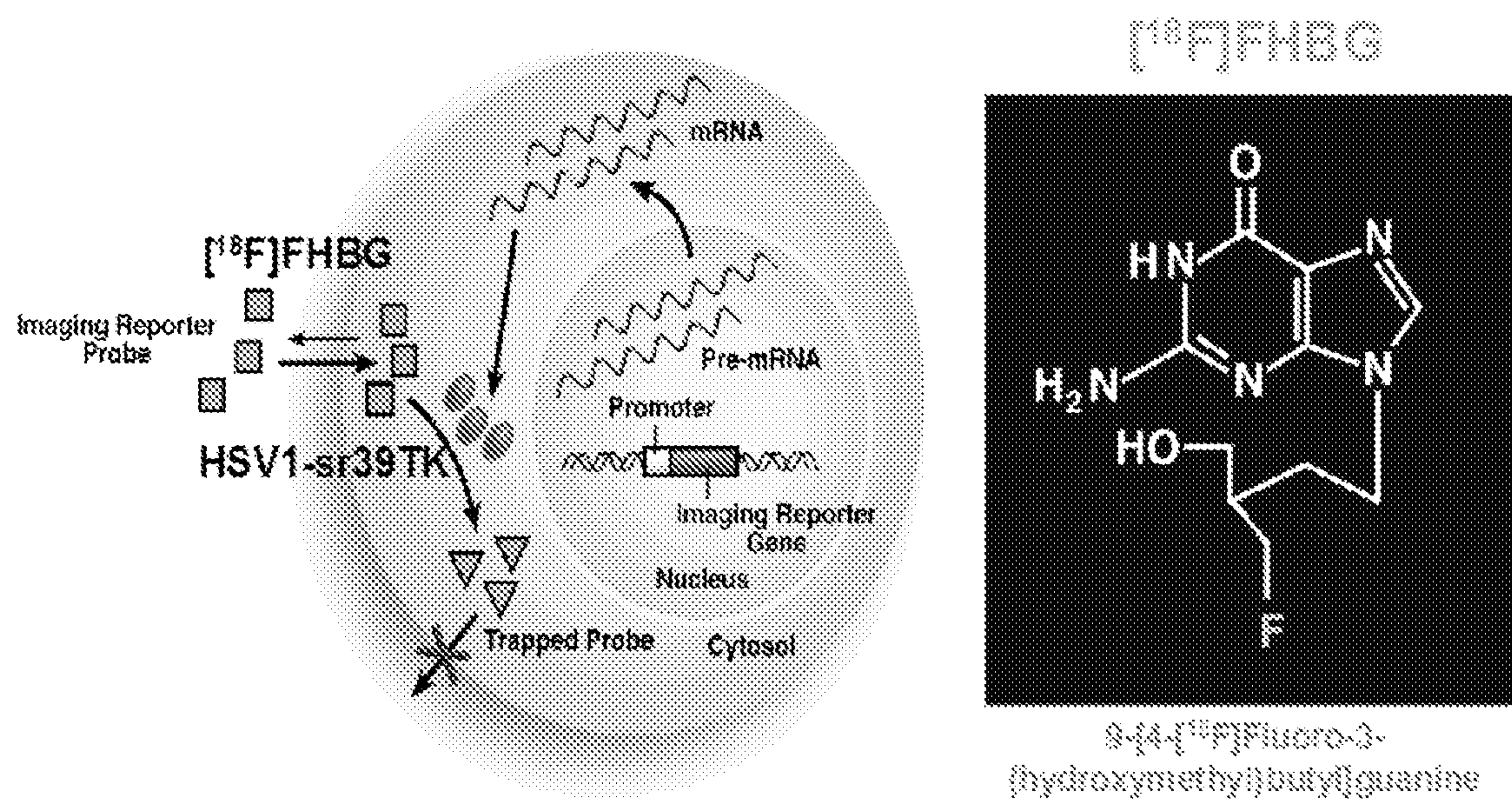


FIG. 2

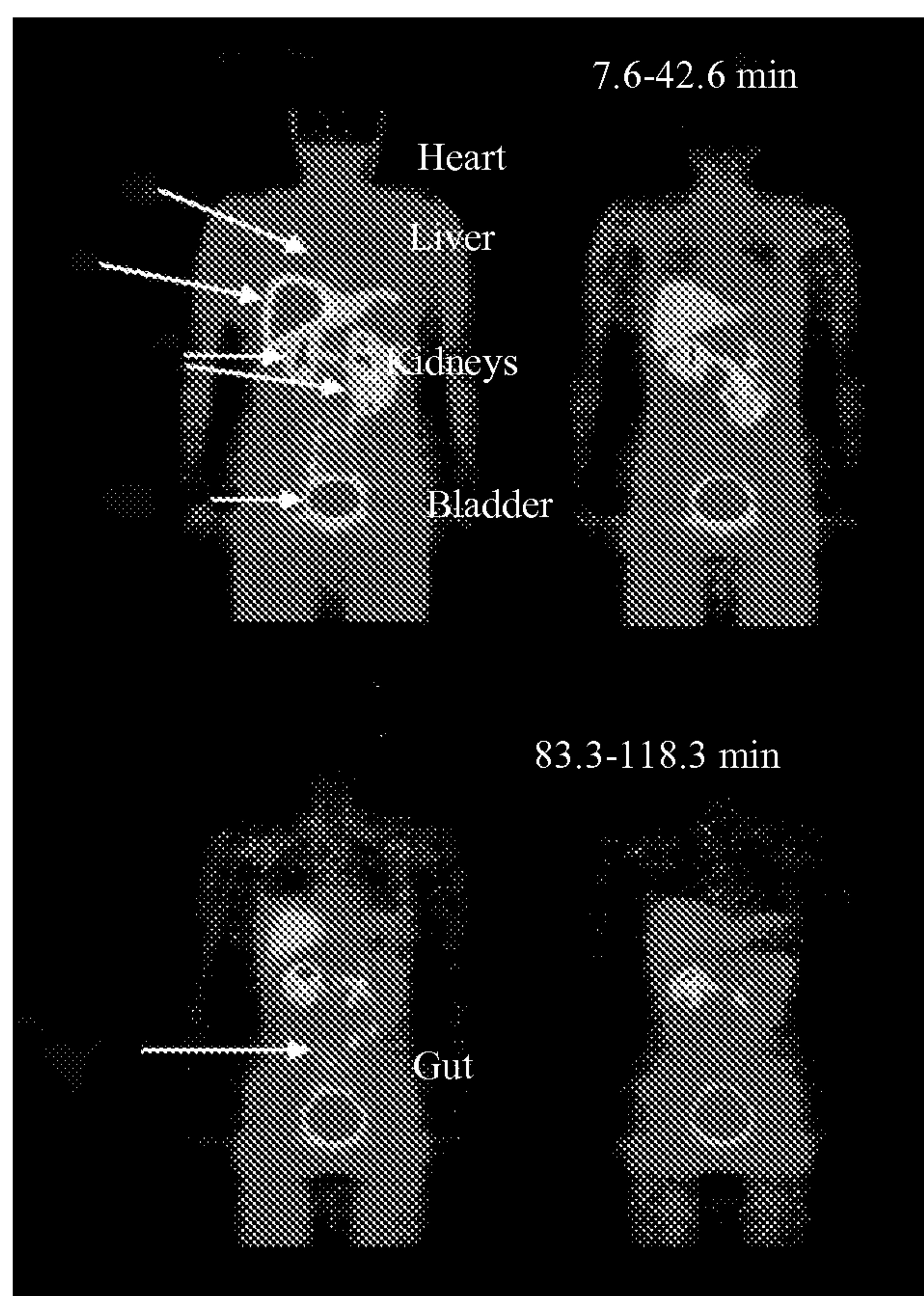


FIG. 3

SCHEMATIC for PHASE IA (q3week cycle)

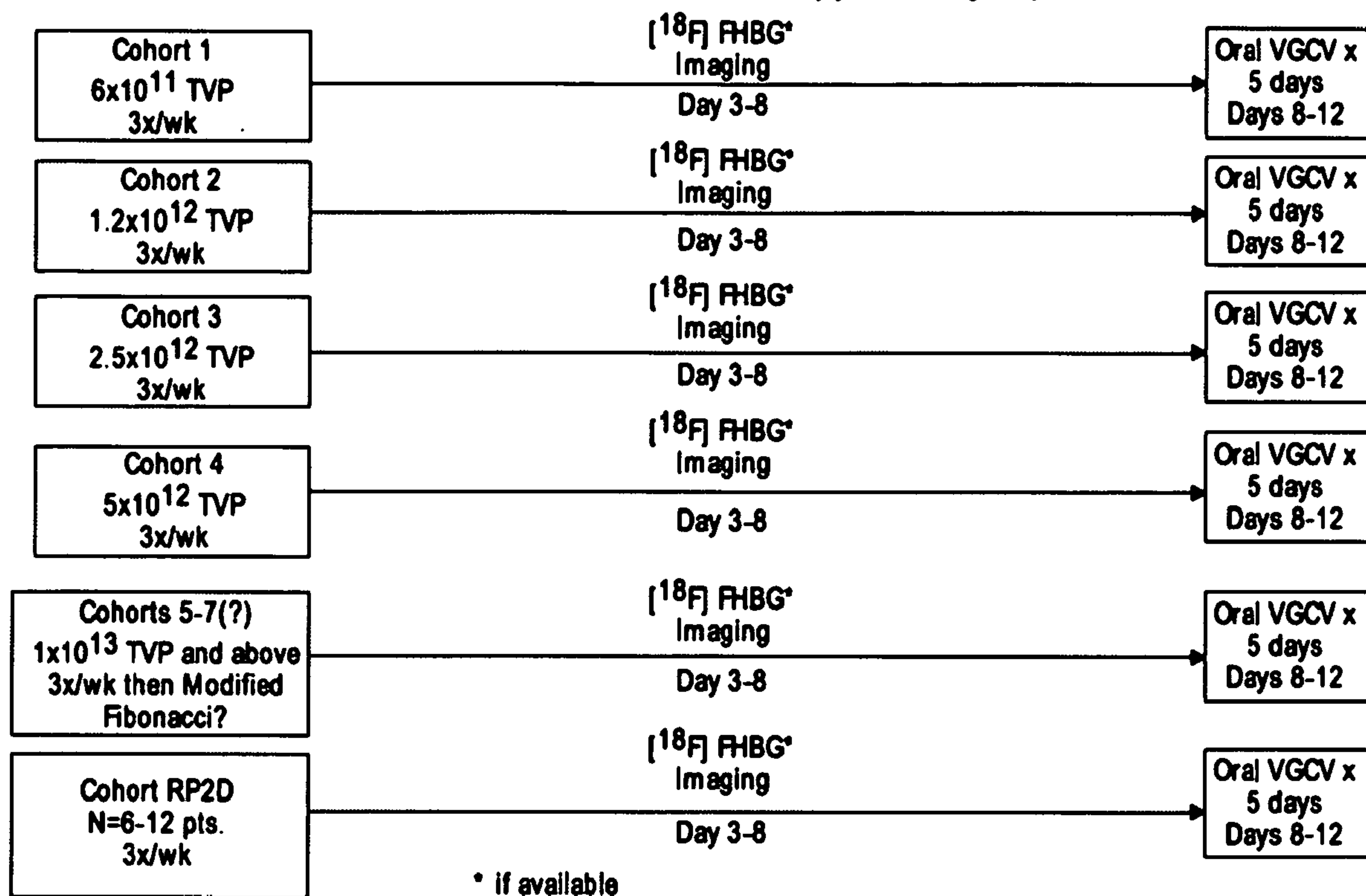


FIG. 4

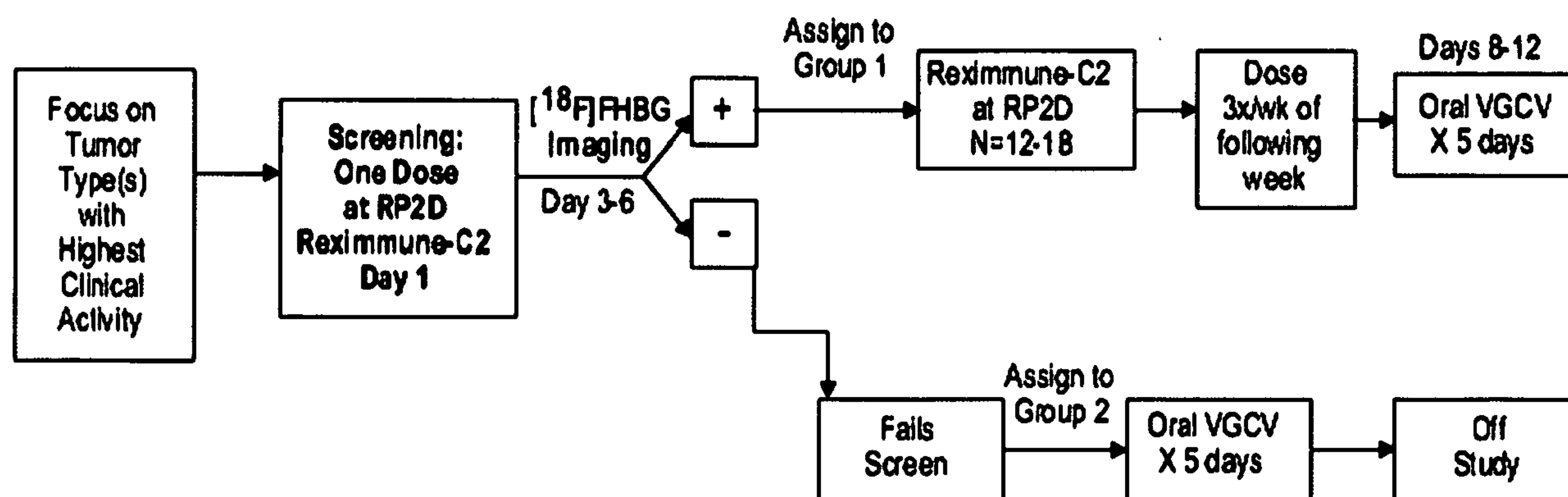


FIG. 5

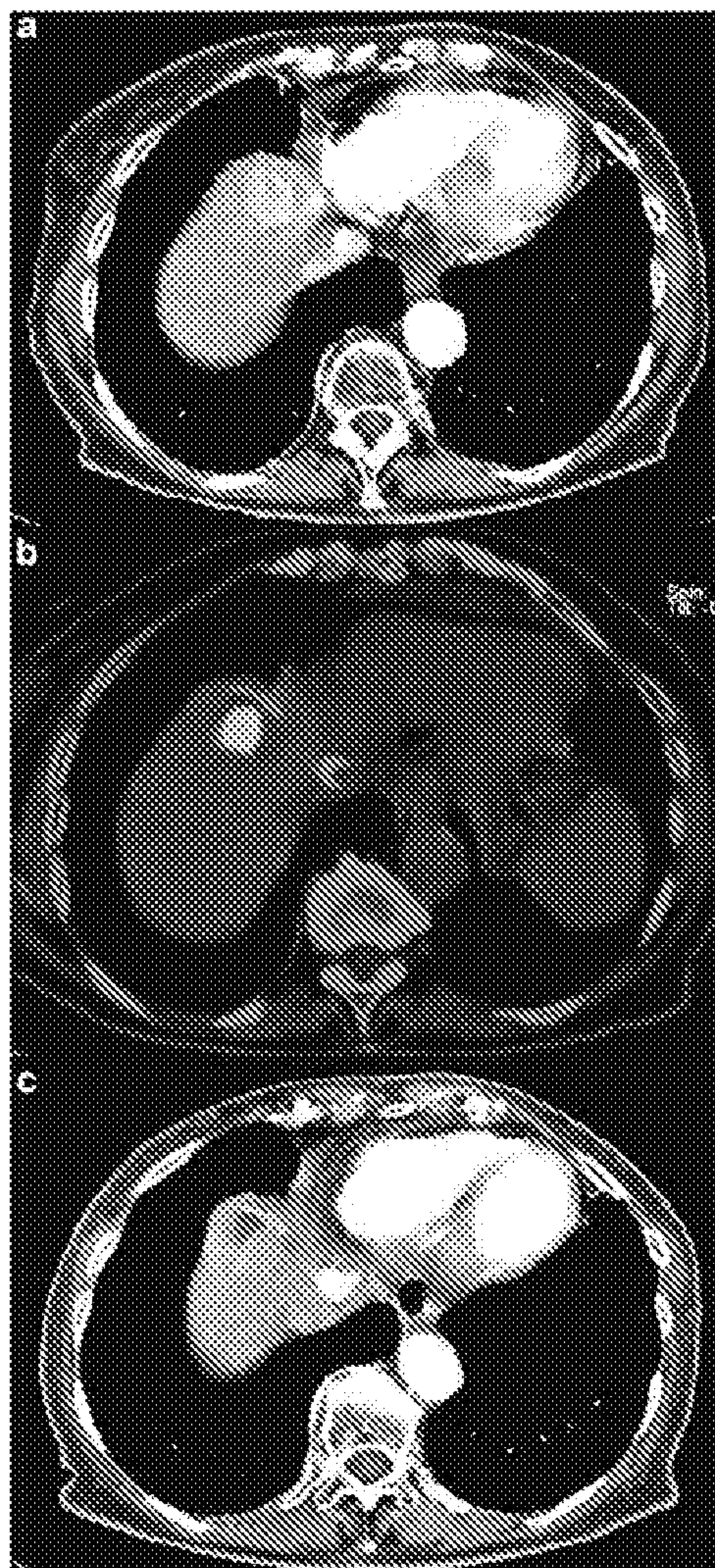


FIG. 6

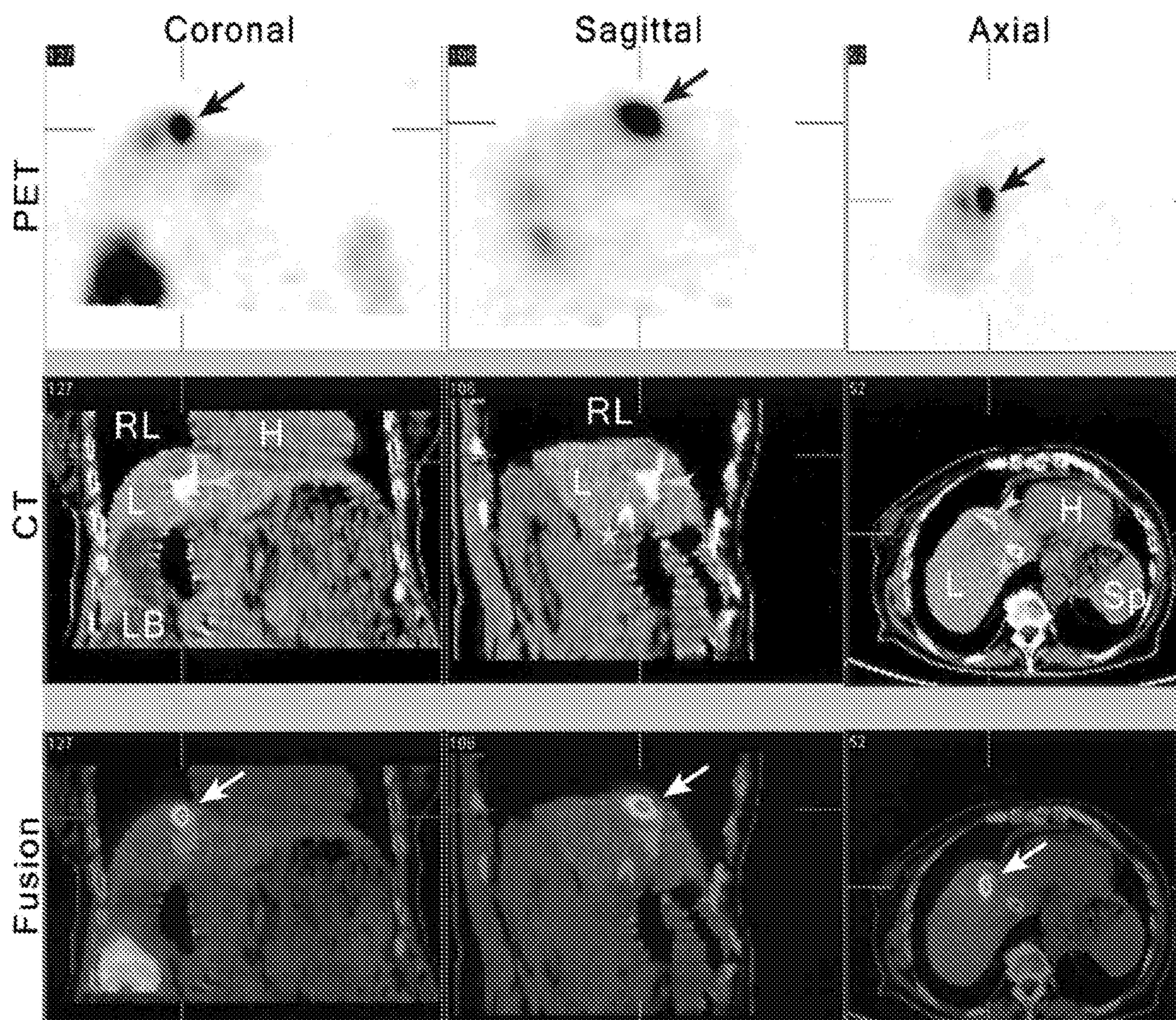


FIG. 7

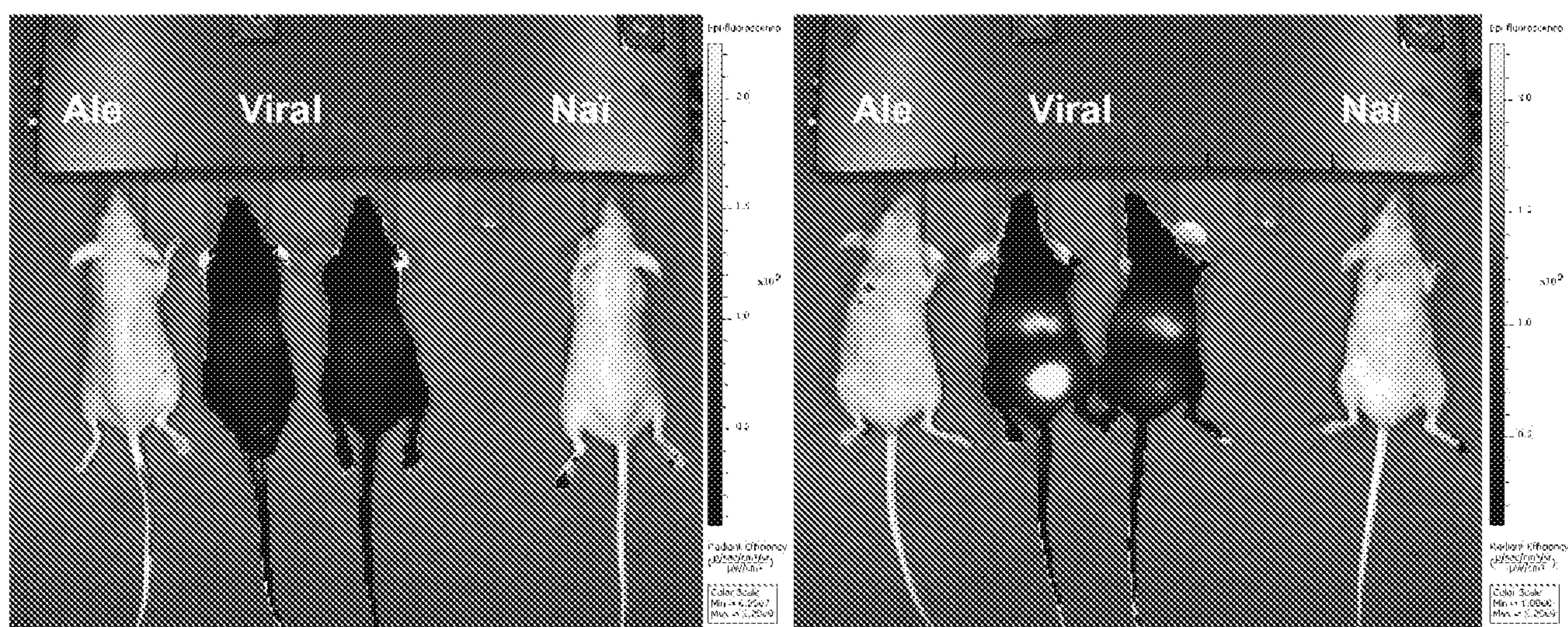


FIG. 8

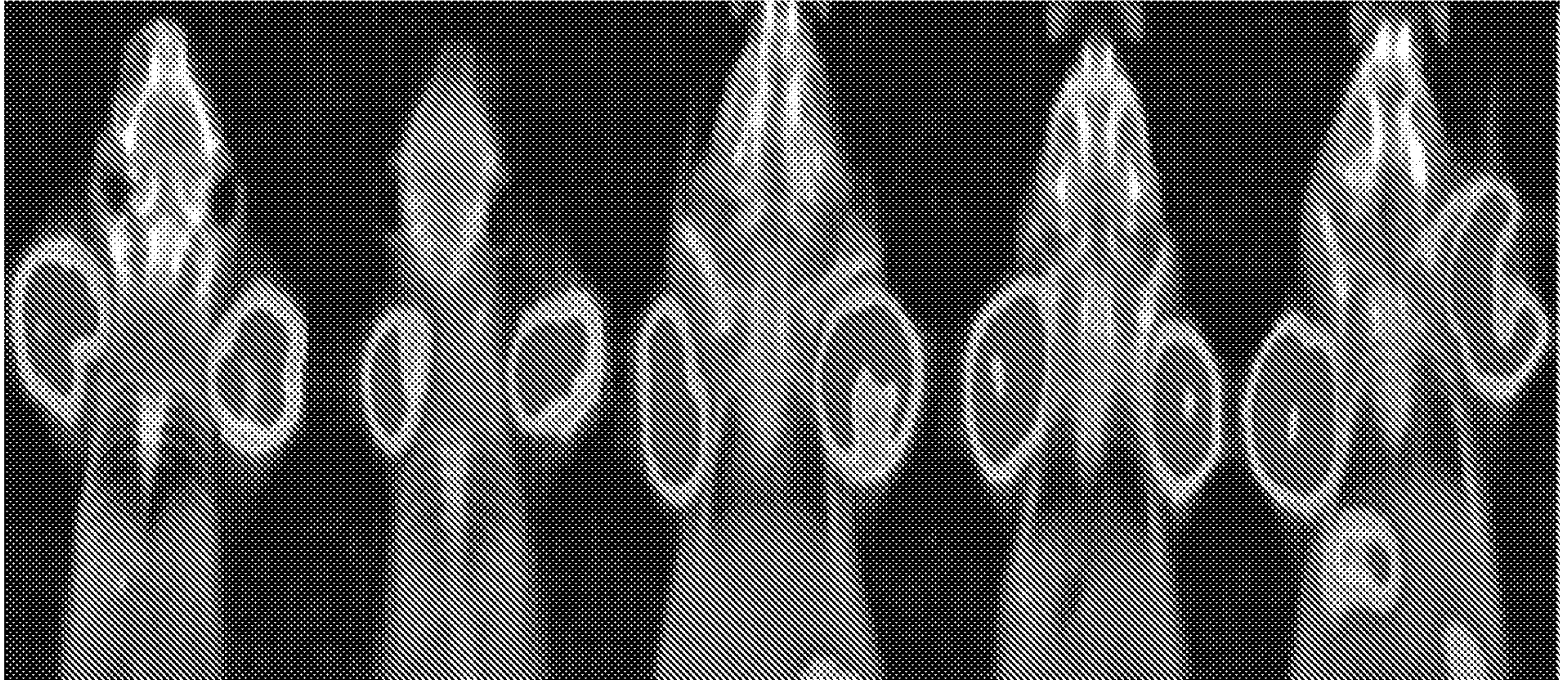


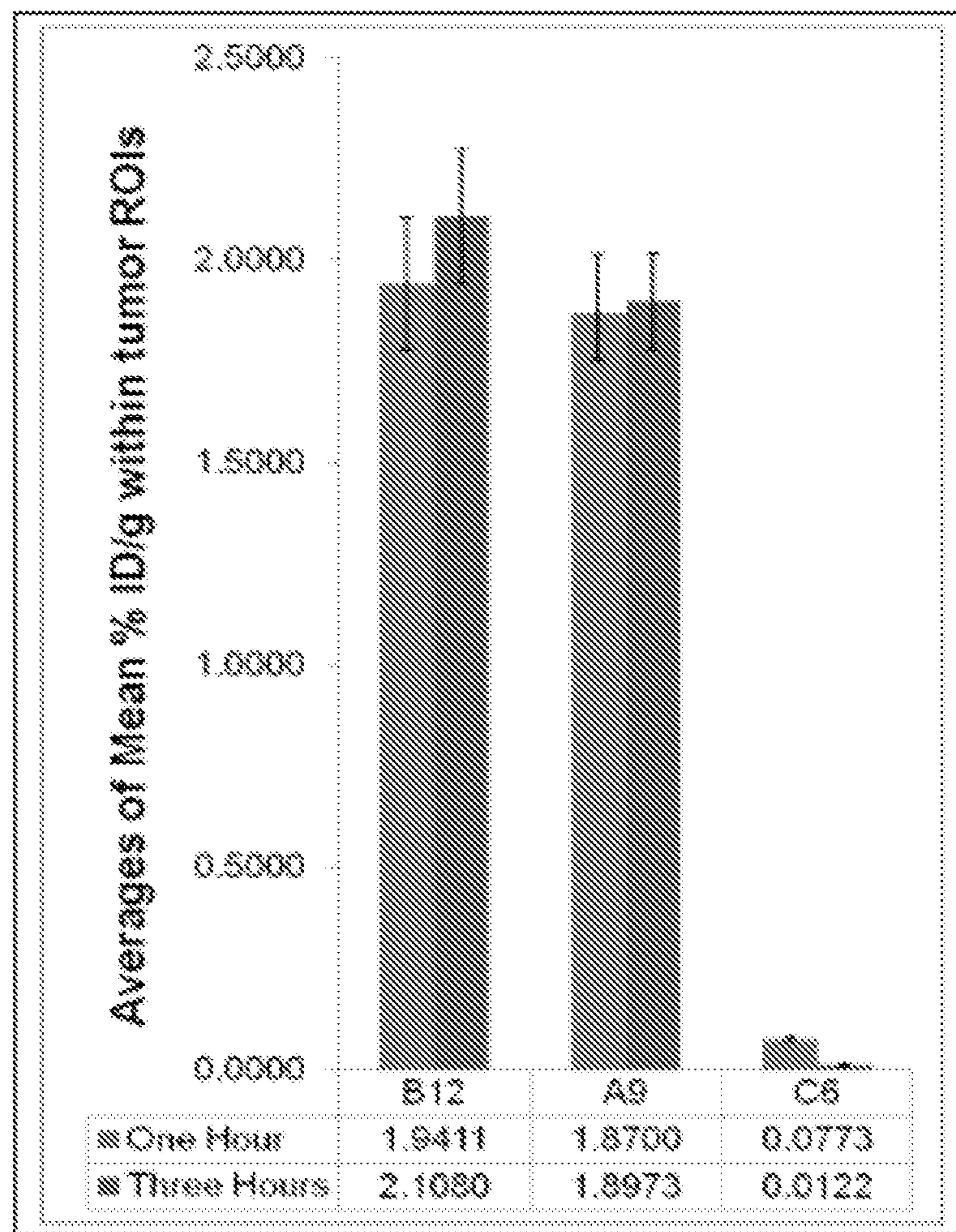
FIG. 9

FIG. 4

