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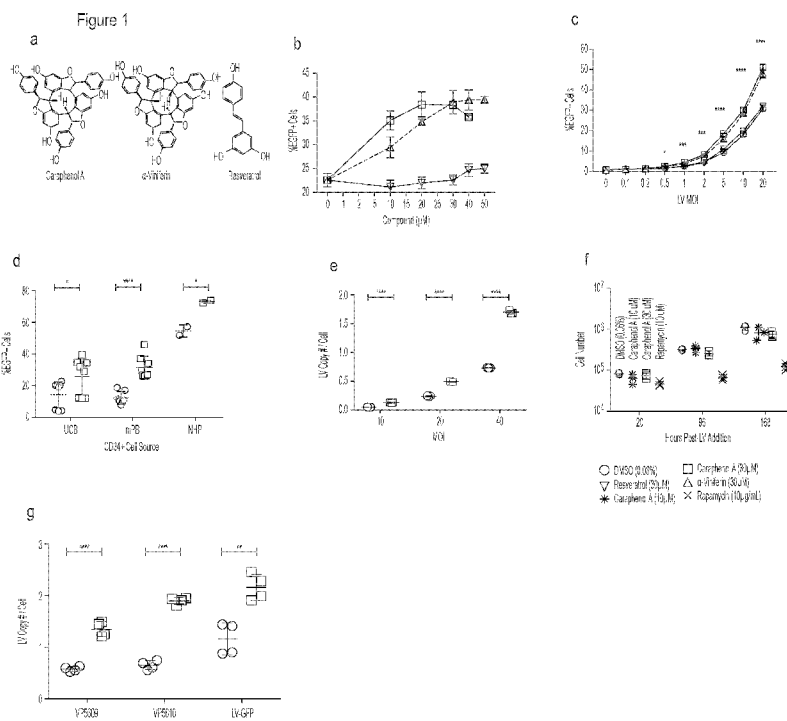
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(54) Title: METHODS AND COMPOSITIONS RELATED TO ENHANCING RETROVIRAL VECTOR ENTRY AND INTEGRATION IN HOST CELLS



(57) Abstract: The present invention provides methods for enhancing transduction efficiency of a viral vector into a host cell such as a stem cell. The methods involve transducing the host cell with the vector in the presence of a resveratrol oligomer compound (e.g., α -viniferin or caraphenol A or analog compound thereof). Also provided in the invention are kits or pharmaceutical combinations for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery. The kits or pharmaceutical combinations typically contain a viral vector encoding the therapeutic agent, and a resveratrol oligomer compound.

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Methods and Compositions Related to Enhancing Retroviral Vector Entry and Integration in Host Cells

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The subject patent application claims the benefit of priority to U.S. Provisional Patent Application Number 62/824,605 (filed March 27, 2019; now pending). The full disclosure of the priority application is incorporated herein by reference in its entirety and for all purposes.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with government support under grant numbers AI007354, GM103368 and HL116221 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Genetic modification of hematopoietic stem cells (HSCs) by γ -retroviral or lentiviral vectors (LVs) has demonstrated clinical efficacy in the treatment of several hematologic genetic disorders, including adenosine deaminase deficiency, Wiskott-Aldrich syndrome, metachromatic leukodystrophy, Fanconi anemia, β -thalassemia and sickle cell anemia. However, a critical factor in determining treatment efficacy and persistence remains the degree of modification and engraftment of true repopulating hematopoietic stem cells. LV restriction can be partially overcome through the addition of cytokines to hematopoietic stem cells before and during lentiviral vector transduction and by utilizing high amounts of LV. However, high levels and extended exposure to cytokines promotes cell cycling resulting in the loss of stem cell potency, thus decreasing the stem cell content and their function when transferred into humans during clinical studies and appropriate animal models for pre-clinical testing. While increasing the amount of LV per cell does increase transduction efficacy to some degree, the clinical procedure increases the cost for LV gene therapy for patients.

[0004] There is an unmet need in the art for more effective means for transducing retroviral vectors, esp. lentiviral vector such as HIV based vectors, into host cells (e.g., stem cells) in gene transfer. The present invention addresses this and other needs.

SUMMARY OF THE INVENTION

[0005] In one aspect, the invention provides methods for enhancing transduction efficiency of a retroviral vector into a host cell. The methods involve (a) contacting the cell with resveratrol or a resveratrol cyclotrimer compound of Formula I described herein, and (b) transducing the cell with the vector. In some of the methods, the enclosed compound has a structure shown in Formula II, Formula III or Formula IV described herein. In some methods, the employed resveratrol cyclotrimer compound is caraphenol A, α -viniferin or resveratrol, or an analog compound thereof.

[0006] In some embodiments, the host cell is contacted with the compound prior to, simultaneously with, or subsequent to being contacted with the vector. In some embodiments, the viral vector is a recombinant retroviral vector, an adenoviral vector or an adeno-associated viral vector. In some of these embodiments, the employed vector is a lentiviral vector. In some methods, the employed vector is a HIV-1 vector. In some embodiments, the employed host cell is a hematopoietic stem and progenitor cell (HSPC). In some of these embodiments, the employed host cell is human or non-human primate CD34⁺ cell. In some methods, the employed stem cell is isolated from umbilical cord blood, peripheral blood or bone marrow. In some embodiments, the employed host cell is a non-hematopoietic cell. In some embodiments, the employed compound is present during the entire transduction process. In some other embodiments, the employed compound is present during specific intervals of the transduction process. In some embodiments, the employed viral vector encodes a therapeutic agent. In some embodiments, the employed viral vector is a non-integrating lentiviral vector.

[0007] In another aspect, the invention provides kits for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery. The kits contain (a) a viral vector encoding the therapeutic agent and (b) resveratrol or a resveratrol cyclotrimer compound of Formula I disclosed herein. In some of these kits, the compound is caraphenol A, α -viniferin, resveratrol, or an analog compound thereof. Some kits of the invention are intended for a target cell that is a hematopoietic stem and progenitor cell (HSPC) or a non-hematopoietic cell. Some of these kits are used for delivering a therapeutic agent into a human or non-human primate CD34⁺ cell. In some kits of the invention, the employed viral vector is a recombinant retroviral vector, an adenoviral vector or an adeno-associated viral vector. In some of the kits, the employed viral vector is a lentiviral vector. Some kits of the invention are intended for delivering a therapeutic agent that is a

polynucleotide agent or a polypeptide agent. Some kits of the invention can additionally contain the target cell into which the therapeutic agent is to be delivered. For example, the kits can contain a human CD34⁺ hematopoietic stem and progenitor cell.

[0008] In another aspect, the invention provides methods for identifying a resveratrol cyclotrimer compound with improved properties in enhancing retroviral transduction into a host cell. The methods entail (a) synthesizing one or more structural analogs of a lead resveratrol cyclotrimer compound selected from the group consisting of caraphenol A or α -viniferin, and (b) performing a functional assay on the analogs to identify an analog that has an improved biological or pharmaceutical property relative to that of the lead compound. In some of these methods, the improved biological or pharmaceutical property to be monitored is a higher potency in enhancing retroviral transduction into CD34⁺ stem cells.

[0009] A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

DESCRIPTION OF THE DRAWINGS

[0010] Figure 1 shows that caraphenol A enhances lentiviral vector (the EGFP expressing pRRL-SIN-MND-EGFP lentiviral vector, termed LV) gene marking in a cell line and primary hematopoietic cells. (a) Chemical structure of caraphenol A, α -viniferin, and resveratrol. (b) HeLa cells were transduced with LV (multiplicity of infection, MOI = 10) in the presence of DMSO (diluent control, open circles) only or indicated concentrations of resveratrol (grey inverted triangle and grey line), α -viniferin (grey triangle and dashed grey line), or caraphenol A (black square and black line) over 8 hrs, before removal of caraphenol A and LV and *ex vivo* culture. Cells were analyzed 5 days later by flow cytometry for EGFP expression (n=3 independent experiments). For Panels (c)-(f), symbols for and concentrations of compound are shown at the bottom of the page. (c) HeLa cells were transduced as above with various MOIs of LV in the presence of 30 μ M of each compound for 8 h. Cells were analyzed 5 days later by flow cytometry for EGFP expression (n=3). Data are shown as linear plots (mean $\pm$ sd), *P< 0.032, **P< 0.0021, ***P<0.0002, ****P<0.0001 by two-tailed Student's t-test, comparing EGFP expression in caraphenol A and DMSO treated cells. (d) Human umbilical cord blood (UCB n=6 donors), human G-CSF mobilized peripheral blood (mPB, n=6 donors), and non-human primate bone marrow aspirate (NHP, n=2 donors) CD34⁺ cells were transduced with LV (MOI=8) in the presence of 30 μ M caraphenol A for 20 hrs before LV and compound removal and expansion. Cells were

analyzed 7 days later by flow cytometry for EGFP expression. Data presented as dot plots (mean $\pm$ sd) $*P<0.0406$, $****P<0.0001$ by two-tailed Student's t-test. (e) Average vector copy number (VCN) in UCB CD34⁺ (n=3 donors) at an increasing MOI of LV treated with either DMSO vehicle control or caraphenol A at 30 μ M. VCN was calculated as a ratio of copy number of integrated LV Gag sequences per RNase P copies. Data presented as dot plots (mean $\pm$ sd) $****P<0.0001$ by two-tailed Student's t-test. (f) Effects of caraphenol A (10 μ M or 30 μ M, see legend, bottom of figure) and rapamycin (10 μ g/mL) on proliferation of UCB CD34⁺ cells (n=3 donors) were evaluated. While rapamycin slowed proliferation of both cell types, resveratrol (not shown) and caraphenol A showed little to no effect in either cell type at multiple compound concentrations. No differences in viability were observed. Data presented as dot plots (mean $\pm$ sd). (g) VCN in mPB CD34⁺ cells (n=4 donors) of two separate batches of clinical-grade CL204i-EF1 α -h γ c-OPT SIN-lentiviral vectors (VP5609 and VP5610) developed for the treatment of SCID-X1, incorporating an internal EF1 α promoter to drive expression of a human IL-2R γ c. Cell were transduced with SCID-X1 or LV (control) at an MOI=15 in the presence of DMSO or 30 μ M caraphenol A. Data presented as dot plots (mean $\pm$ sd) $**P=0.0024$, $****P<0.0001$ by two-tailed Student's t-test.

[0011] Figure 2 shows improvement in gene delivery to human HSCs in mice. (a) Experimental set-up of mouse transplant experiments. NSG mice were irradiated with 2.40 Gy. UCB CD34⁺ cells from a pool of donors were thawed and pre-stimulated for 24h before a 4-h incubation of DMSO or caraphenol A (n=8 mice per treatment and MOI, 32 mice total), LV MOI 10 or MOI 25, was added to UCB for 20-h, after which, 3×10^5 cells per mouse were injected retro-orbitally and the remaining UCB CD34⁺ cells were grown out *ex vivo*. *Ex vivo* transgene expression was measured 7 and 14-days post-transduction. Peripheral blood samples were removed and evaluated every 3-5 weeks after an initial 6-7 week engraftment period. Mice were sacrificed at 22 weeks (terminal) and harvested for peripheral blood, bone marrow, and spleen. (b) Percent human CD45⁺ EGFP⁺ cells in peripheral blood of UCB CD34⁺ cell engrafted NSG mice transduced with LV at either MOI 10 (DMSO, open circles, Caraphenol A, solid black squares) (c) or 25 (DMSO, open circles, Caraphenol A, solid black squares) throughout indicated timepoints during the study period. Peripheral blood was removed from NSG transplanted mice retro-orbitally every 3-5 weeks. Human cells were gated from the total leukocyte population and analyzed for EGFP expression. Data presented as dot plots (mean $\pm$ sd) with the y-axis in log10 scale, $*P<0.028$, $**P<0.0042$, $***P<0.0006$ by two-tailed Mann-Whitney test. Percent human CD45⁺EGFP⁺

cells in spleen (d) and bone marrow (e) of UCB CD34⁺ cell engrafted NSG mice at 22-week terminal timepoints, comparing EGFP⁺ expression in human cells arising from NSG mice engrafted with caraphenol A and DMSO treated UCB CD34⁺ cells transduced at two different lentiviral vector MOIs. Data presented as dot plots (mean $\pm$ sd) comparing the two treatments from Spleen MOI 10 ***P=0.0003, MOI 25 *P=0.049 and bone marrow MOI 10 ***P=0.0006, MOI 25 (open circles) *P=0.042 by two-tailed Mann-Whitney test. (f)

Comparison of human cell engraftment in NSG mouse bone marrow at terminal timepoint in UCB CD34⁺ cells treated with caraphenol A and DMSO, as measured by total proportion of leukocytes that were mouse CD45⁻ human CD45⁺. Results presented as dot plots (mean $\pm$ sd), n.s. = not significant. (g) VCN of human cells in engrafted NSG mice bone marrow from caraphenol A and DMSO treated cohorts, 22 weeks after *ex vivo* LV transduction at 10 or 25 MOI and compound treatment. VCN was recorded as a ratio of integrated Gag sequences per RNaseP sequence. Data presented as dot plots (mean $\pm$ sd), comparing DMSO to caraphenol A treated mice at MOI 10 **P=0.0022, and MOI 25 *P=0.022 by two-tailed Mann-Whitney test. (h) Demonstration that long-term repopulating stem cells (Primary) give rise to increased levels of EGFP marked cells from CD34⁺ serially transplanted to secondary (Secondary) donor NSG mice. Percent human CD45⁺EGFP⁺ cells in bone marrow of NSG mice receiving UCB CD34⁺ cells at terminal timepoints of primary (left, 22 weeks) and secondary (right, 12 weeks) transplant, comparing EGFP⁺ expression in caraphenol A and DMSO mice at MOI 25. Data presented as dot plots, each representing individual mice and change from primary to secondary transplant.

[0012] Figure 3 shows that caraphenol A-treatment during CD34⁺ cell LV-transduction does not impact patterns of LV-integration observed in human cells relative to DMSO-treated controls. A composite of individual integration events obtained from bone marrow and spleen samples (n=8 mice per treatment, 10 and 25 MOIs, 32 mice total, see Fig. 2a) at the terminal endpoint of 22 weeks. (a) Frequency of unique integration events occurring relative to indicated genomic features, or within 30kb of known oncogenes as a function of caraphenol A- (black) or DMSO-treatment (grey). (b) Probability density function for integration to occur within 100 kb of transcription start sites (TSS) for DMSO-treated (grey line) compared to caraphenol A-treated (black dashed line) for human cells obtained and pooled from both bone marrow and spleen cells.

[0013] Figure 4 shows that concurrent caraphenol A treatment improves LV uptake into the cytoplasm. (a) Human UCB CD34⁺ cells (n=3 donors) were transduced in the presence

of DMSO (0.06%, open circles) or caraphenol A (Cara) (30 μ M, black squares) with LV-MND-NGFR, MOI 15, carrying the BLAM-Vpr protein. After a 6-h transduction, cells were loaded with the BLAM substrate CCF2-AM, and LV entry was quantified by flow cytometric detection of cells exhibiting cleaved CCF2. Transduction was measured by NGFR expression 7 days later with flow cytometry. Data presented as dot plots (mean $\pm$ sd). Fusion **P=0.0039, transduction *P=0.016 by two-tailed Student's t-test. (b) Human UCB CD34⁺ cells (n=4 donors) were transduced in the presence of DMSO (0.06%) or caraphenol A (30 μ M) using above BLAM-Vpr containing vector, then transferred to 4°C at indicated timepoints, before loading with BLAM substrate CCF2-AM at 12°C overnight. LV cytoplasmic fusion was quantified by flow cytometric analysis for cleaved CCF2. Data presented as linear plots (mean $\pm$ sd). Large plot (open circles) indicates mean $\pm$ sd of 3 donors combined. Small plots (closed circles) indicate mean $\pm$ sd of technical replicates (n=2 cultures) from each donor. Combination figure *P=0.0406, slopes of linear regression significantly different. (c) HeLa cells (n=5 cultures) were treated with LV at MOI 10 for 8 h and caraphenol A (30 μ M, black bar) was added at indicated timepoints after LV addition. DMSO (0.06%, clear bar) with LV only was added as a separate control. Compounds and LV were washed out 8 h after vector addition and cells were analyzed for EGFP expression by flow cytometry 5 days later. Data presented as bar graphs (mean $\pm$ sd). (d) Cells were pre-treated with caraphenol A (30 μ M, black bars) or DMSO as a control for indicated lengths of time before washout of compound and exposure to LV-GFP for an additional 8 h. After transduction, LV and any remaining compound were removed and cells were analyzed for EGFP expression by flow cytometry 5 days later. Data presented as bar graphs (mean $\pm$ sd).

[0014] Figure 5 shows structures of examples of resveratrol cyclotrimer compounds, including caraphenol A and α -viniferin.

[0015] Figure 6 shows that α -viniferin enhanced LV transduction of CD34⁺ stem and progenitor cells. Methods for this experiment were as discussed in Figure 1d.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[0016] The present invention is predicated in part on the discoveries by the present inventors that temporary co-application of one of several compounds with 9-membered ring containing resveratrol oligomers (e.g., the naturally occurring polyphenol caraphenol A)

significantly enhances lentiviral (LV) gene delivery efficiency to immortalized cell lines and to a range of human and non-human primate hematopoietic stem and progenitor cells (HSPCs) from various sources. Specifically, the inventors found that caraphenol A and α -viniferin, oligomeric forms of the natural product resveratrol, relieves hematopoietic CD34⁺ stem and progenitor cell lentiviral vector gene delivery restriction. It was found that these compounds enhance viral vector transduction of non-hematopoietic cells and hematopoietic cells. As exemplified herein (Figure 1), caraphenol A was able to enhance lentiviral vector transduction of HeLa cells and also CD34⁺ stem cells and progenitor cells (HSPCs). The inventors observed that the HSPC LV transduction enhancement was commensurate with increasing caraphenol A concentration, with an approximate 3-fold lentiviral transduction increase at 30 μ M. Treatment of human neonatal (cord blood derived) and rhesus non-human primate CD34⁺ HSPCs with caraphenol A also enhanced transduction 2-3-fold over DMSO treatment. Similarly, α -viniferin was also able to enhance lentiviral vector transduction efficiency in HeLa cells and adult CD34⁺ HSPCs (Fig. 1b and Fig. 5, respectively). It was demonstrated that α -viniferin has similar cellular LV transduction enhancement activity in HeLa cells and CD34⁺ HSPCs as caraphenol A, thus either compound can be used to enhance LV transduction. Thus, the LV enhancement activity of these compounds is broadly active on all developmental stages of CD34⁺ HSPC cells, neonate through adult, as well as other non-human primate. These exemplified studies further indicate that oligomers of resveratrol are more active as retroviral (e.g., lentiviral) vector transduction enhancers and would be consistent with the proposal that plant and chemically synthesized polyphenols within the same chemical family would have LV transduction enhancement activity.

[0017] Importantly, it was demonstrated by the inventors that the compounds exemplified herein (e.g., caraphenol A) improves clinical LV transduction efficacy. A limitation for the clinical use of lentiviral vector based CD34⁺ HSPC delivery is the vector length, which can be the result of genetic payload size and the addition of LV elements, such as insulator elements. Treatment of adult CD34⁺ HSPC with the clinical X-SCID LV vector CL20i4-EF1a-hyOPT was evaluated utilizing caraphenol A or Prostaglandin E2 (PGE2). It has been shown that it requires high amounts of this LV vector (100-150 MOI) and 2 treatments to obtain clinically useful viral copy number (VCN) per adult HSPC in the 0.5-1 range. PGE2 has been reported to be a transduction enhancer of clinical LV transduction. Surprisingly, it was found by the inventors that caraphenol A treatment enhanced VCN 2-3-

fold, when normalized to the DMSO control, with VCN in the 1.3-2.3 range, which was superior to PGE2 treatment. These findings indicate that the compounds exemplified herein (e.g., caraphenol A) improve LV transduction of clinical X-SCID vector in adult CD34⁺ HPSCs and is superior to PGE2.

[0018] It was further discovered by the inventors that treatment with the exemplified compounds (e.g., caraphenol A) enhances LV transduction of long-term repopulating human CD34⁺ stem cells. The “gold standard” for evaluating LV transduction of long-term repopulating CD34⁺ stem cells are transplant studies in immunodeficient mice. The rationale for the studies are that more differentiated progenitor and stem cells both engraft, but within 6 weeks the more differentiated cells start to die off and are replaced by progenitor cells arising from engrafted stem cells. This process is analogous to the hematopoietic developmental process in humans. The human cellular turnover can be followed in the peripheral blood of mice. If the LV transduction process preferentially targets differentiated hematopoietic cells, but not stem cells, over time the GFP marking of human cells will decrease in the peripheral blood given that GFP⁺ cells will not be replaced from stem cells. Alternatively, if stem cells are targeted then the peripheral blood cells will remain GFP⁺ over time given that stem cell derived progenitors will have the GFP gene. As exemplified in Figure 2, the inventors transplanted into irradiated NSG mice cord blood derived CD34⁺ HPSCs that were transduced with LVs containing a GFP gene. During LV transduction cells were treated with 30μM caraphenol A or DMSO control. It was found that mice receiving CD34⁺ HPSCs LV transduced in the presence of DMSO lost GFP expression over time in the blood as compared to CD34⁺ HPSCs LV transduced in the presence of caraphenol A.

[0019] To investigate the effect of caraphenol A on gene marking in long-term repopulating (LTR) HSCs, the inventors established a third cohort of humanized mice from high dose (MOI=25) LV-treated cells. Caraphenol A treatment again increased the EGFP marking considerably more *in vivo* after 22 weeks than from initial *ex vivo* observations. As before, caraphenol A engendered no significant effect on engraftment and lineage frequency. Subsequently, 1x10⁵ CD34⁺ cells isolated from the bone marrow of the lowest, middle and highest frequency EGFP marked mice from the caraphenol A- and DMSO-treated cells were engrafted into secondary recipient mice. Percent EGFP marking was observed to drop in cells originating from DMSO-treated CD34⁺ primary mice, but was dramatically increased in caraphenol A-treated CD34⁺ cell secondary mice (Fig. 2h).

[0020] These studies demonstrated that caraphenol A significantly enhanced LV transduction of long-term repopulating human stem cells as evidenced by continued and stable GFP expression in human cells in the blood of mice and a 2-fold greater VCN in human cells in the mouse bone marrow. These findings demonstrate that lower amounts of LV can be used to target human long-term repopulating stem cells if treated with caraphenol A during the transduction period.

[0021] Moreover, it was discovered that the exemplified compounds, e.g., caraphenol A and α -viniferin, regulate enhancement of retrovirus/lentiviral vector cellular transduction through the downregulation of IFITMs 2 and 3 proteins and alteration of late stage endosomes promoting increased capsid release from the endosome. It was found that IFITM3 is a major lentiviral vector restriction factor which can be downregulated with caraphenol A and α -viniferin cellular treatment.

[0022] The studies described herein showed that the resveratrol oligomer compounds exemplified herein (e.g., caraphenol A) relieve hematopoietic progenitor and stem cell resistance to LVs and enhances gene delivery efficiency 2-3-fold in HSCs, as compared to controls. Also, improving LV transduction efficiency evenly throughout HSPCs could improve the control of LV integration frequency per cell, thus reducing the integration variability found when the amount of LV dose is increased. Furthermore, the compounds can enhance hematopoietic stem cell transduction at low LV MOI, thus allowing less LV to be used for effective gene delivery. If translated to the clinic, the compounds should increase VSV-LV gene delivery efficacy to hematopoietic stem cells, reduce the time hematopoietic stem cells remain in tissue culture, decrease the amounts of LVs required for a desired therapeutic endpoint, while showing the same LV integration pattern in cells as without treatment. These improvements would improve gene replacement/modification at the cell level and reduce patient costs for gene replacement/modification therapy.

[0023] In accordance with these discoveries, the present invention provides methods for using the resveratrol oligomer compounds described herein to promote high frequency targeting and efficient payload delivery to a target host cell. The target host cell can be any human and non-human cells. For example, the host cell can be hematopoietic cells or non-hematopoietic cells. The resveratrol oligomer compound to be used in the invention can be any compound that has a structure as shown in Formula I, e.g., α -viniferin or caraphenol A. The compound can also be functional derivatives, variants or analogs of the compounds shown in Formula I, esp. analogs of the compounds shown in Fig. 5.

[0024] The methods described herein can have various clinical and industrial applications. Vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped lentiviral vectors (LVs) are the current clinical standard for gene delivery to cells of hematopoietic origin. Clinical gene therapy trials are underway to replace defective genes in hematopoietic CD34⁺ stem cells from patients with β -Thalassemia, X-linked adrenoleukodystrophy, Wiskott–Aldrich syndrome, X-linked Severe Combined Immunodeficiency and Metachromatic leukodystrophy utilizing lentiviral vectors. Moreover, clinical studies are proposed for gene modification for defective genes utilizing Zinc Fingers, TALENS, CRISPRs, etc., will utilize LV as well. LVs have the advantage of entry into and DNA integration within non-cycling cells, an advantage given that induction of cell cycling in hematopoietic CD34⁺ stem cells can result in the loss of stem cell qualities, which are detrimental to successful CD34⁺ stem cell transplants. The inherent resistance of hematopoietic CD34⁺ stem cells to LV gene delivery is a major impediment to successful gene delivery and gene modification for patient therapeutic use. For gene therapy to be effective in the clinic it will require efficient and predictable delivery of the desired payload for clinical endpoints. The generically regulated, intrinsic hematopoietic stem cell restriction of LV transduction can result in low numbers of cells containing LVs and would be predicted to reduce therapeutic gene correction. To overcome the inherent resistance of hematopoietic stem cells to LV gene delivery, those in the clinical field increase the amount of LV per unit of stem cells and perform repeated LV treatments. These clinical strategies can be effective. However, the drawbacks are the considerable costs of clinical grade LV which is required in high amounts and, in many clinical trials, provided during 2 treatments over a longer time period. Moreover, there is a concern that LV copy number among CD34⁺ cells, which includes stem and progenitor cells, may be unequally distributed with some less restrictive cells receiving higher LV copy number, which may increase the probability of LVs integrating within undesirable genes.

[0025] Application of the methods of the invention is not limited to viral vectors. Instead, the methods are also applicable to nanoparticles and viral-like vectors, containing viral envelopes, utilize similar cellular pathways as the virus sharing the same envelope. As the cargo capacity and cell compatible properties of nanoparticles and viral-like vectors improve at some point nanoparticles may replace viral derived vectors for clinical use. The studies described herein showed that treatment with the resveratrol oligomer compounds exemplified herein (e.g., caraphenol A) alters cellular pathways that enhance pH dependent viral envelopes, such as VSV-G. Such treatment could improve cellular entry and

endosomal escape of nanoparticles and viral-like vectors containing pH dependent viral envelopes.

[0026] There are various advantages associated with enhancing transduction efficiency via methods of the invention. As demonstrated herein, the resveratrol oligomer compounds such as caraphenol A significantly improve lentiviral vector transduction of both neonatal and adult CD34⁺ cells *ex vivo*. More importantly, the compound treatment enhances LV transduction of long-term hematopoietic repopulating stem cells as determined upon transplant in NSG immunodeficient mice, the accepted gold standard as a surrogate for non-human primates. This indicates that the compound treatment for LV gene delivery is more efficient in long-term repopulating stem cells than DMSO treatment and *ex vivo*. Several advantages are provided by the methods of the invention in enhancing retroviral transduction in gene delivery. For example, less LV can be used for CD34⁺ cell transduction when caraphenol A treatment was used. The implications for clinical translation are that less LV amount per patient CD34⁺ cell treatment could be used, thereby decreasing the cost of LV therapies / patient, the period of time for LV transduction and increasing therapeutic gene delivery efficacy (gene copies per cell) per HSC. Also, the exemplified resveratrol oligomer compounds such as caraphenol A and α -viniferin are not CD34⁺ cell cytotoxic. They do not affect cell proliferation as compared to some other known LV transduction enhancing compounds. In addition, the exemplified resveratrol oligomer compounds (e.g., caraphenol A) have a different LV efficacy mechanism(s) for LV CD34⁺ cell enhancement than PGE2, which does not alter expression of IFITM2/3, which is known as a LV enhancing agent for CD34⁺ cells. Since these compounds can be used together and each compound provides LV efficacy increase independently, the enhancing effect was additive. Further, it was demonstrated herein that, in addition to hematopoietic cells, the resveratrol oligomer compounds (e.g., caraphenol A) also enhances LV transduction of neural embryonic stem cells and non-hematopoietic cells.

II. Definition

[0027] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: *Academic Press Dictionary of Science and Technology*, Morris (Ed.), Academic Press (1st ed., 1992); *Oxford Dictionary of*

Biochemistry and Molecular Biology, Smith et al. (Eds.), Oxford University Press (revised ed., 2000); *Encyclopaedic Dictionary of Chemistry*, Kumar (Ed.), Anmol Publications Pvt. Ltd. (2002); *Dictionary of Microbiology and Molecular Biology*, Singleton et al. (Eds.), John Wiley & Sons (3rd ed., 2002); *Dictionary of Chemistry*, Hunt (Ed.), Routledge (1st ed., 1999); *Dictionary of Pharmaceutical Medicine*, Nahler (Ed.), Springer-Verlag Telos (1994); *Dictionary of Organic Chemistry*, Kumar and Anandand (Eds.), Anmol Publications Pvt. Ltd. (2002); and *A Dictionary of Biology (Oxford Paperback Reference)*, Martin and Hine (Eds.), Oxford University Press (4th ed., 2000). In addition, the following definitions are provided to assist the reader in the practice of the invention.

[0028] The term "analog" is used herein to refer to a molecule that structurally resembles a reference molecule but which has been modified in a targeted and controlled manner, by replacing a specific substituent of the reference molecule with an alternate substituent. Compared to the reference molecule (e.g., α -viniferin or caraphenol A), an analog can exhibit the same, similar, or improved utility. Methods for synthesizing and screening candidate analog compounds of a reference molecule to identify analogs having altered or improved traits (e.g., an α -viniferin or caraphenol A analog compound with better potency in enhancing viral transduction) are well known in the art.

[0029] The term "contacting" has its normal meaning and refers to combining two or more agents (e.g., two compounds or a compound and a cell) or combining agents and cells. Contacting can occur in vitro, e.g., mixing a compound and a cultured cell in a test tube or other container. It can also occur in vivo (contacting a compound with a cell within a subject) or ex vivo (contacting the cell with compound outside the body of a subject and followed by introducing the treated cell back into the subject).

[0030] Host cell restriction refers to resistance or defense of cells against viral infections. Mammalian cells can resist viral infections by a variety of mechanisms. Viruses must overcome host cell restrictions to successfully reproduce their genetic material.

[0031] Hematopoietic stem and progenitor cells (HSPCs) are a rare population of precursor cells that possess the capacity for self-renewal and multilineage differentiation. Blood cells are produced by the proliferation and differentiation of a very small population of pluripotent hematopoietic stem cells (HSCs) that also have the ability to replenish themselves by self-renewal. During differentiation, the progeny of HSCs progress through various intermediate maturational stages, generating multi-potential and lineage-committed progenitor cells prior to reaching maturity. Bone marrow (BM) is the major site of

hematopoiesis in humans and, under normal conditions, only small numbers of hematopoietic stem and progenitor cells (HSPCs) can be found in the peripheral blood (PB).

[0032] Hematopoietic stem cells (HSCs) are a heterogeneous population of multipotent stem cells that can give rise to all the blood cell types from the myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells). These cells are found in the bone marrow of adults; within femurs, pelvis, ribs, sternum, and other bones. The cells can usually be obtained directly from the iliac crest part of the pelvic bone, using a special needle and a syringe. They are also collected from the peripheral blood following pre-treatment with cytokines, such as G-CSF (granulocyte colony-stimulating factors) or other reagents that induce cells to be released from the bone marrow compartment. Other sources for clinical and scientific use include umbilical cord blood, as well as peripheral blood.

[0033] Retroviruses are enveloped viruses that belong to the viral family Retroviridae. The virus itself stores its nucleic acid, in the form of a +mRNA (including the 5'-cap and 3'-PolyA inside the virion) genome and serves as a means of delivery of that genome into host cells it targets as an obligate parasite, and constitutes the infection. Once in a host's cell, the virus replicates by using a viral reverse transcriptase enzyme to transcribe its RNA into DNA. The DNA is then integrated into the host's genome by an integrase enzyme. The retroviral DNA replicates as part of the host genome, and is referred to as a provirus. Retroviruses include the genus of Alpharetrovirus (e.g., avian leukosis virus), the genus of Betaretrovirus; (e.g., mouse mammary tumor virus), the genus of Gammaretrovirus (e.g., murine leukemia virus or MLV), the genus of Deltaretrovirus (e.g., bovine leukemia virus and human T-lymphotropic virus), the genus of Epsilonretrovirus (e.g., Walleye dermal sarcoma virus), and the genus of Lentivirus.

[0034] Lentivirus is a genus of viruses of the Retroviridae family, characterized by a long incubation period. Lentiviruses can infect most non-dividing cells nearly as well as dividing cells. They can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. Examples of lentiviruses include human immunodeficiency viruses (HIV-1 and HIV-2), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV). Additional examples include BLV, EIAV and CEV.

[0035] The term "operably linked" when referring to a nucleic acid, means a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked"

when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

[0036] The term "polynucleotide" or "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, that comprise purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Polynucleotides of the embodiments of the invention include sequences of deoxyribopolynucleotide (DNA), ribopolynucleotide (RNA), or DNA copies of ribopolynucleotide (cDNA) which may be isolated from natural sources, recombinantly produced, or artificially synthesized. A further example of a polynucleotide is polyamide polynucleotide (PNA). The polynucleotides and nucleic acids may exist as single-stranded or double-stranded. The backbone of the polynucleotide can comprise sugars and phosphate groups, as may typically be found in RNA or DNA, or modified or substituted sugar or phosphate groups. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. The polymers made of nucleotides such as nucleic acids, polynucleotides and polynucleotides may also be referred to herein as nucleotide polymers.

[0037] Polypeptides are polymer chains comprised of amino acid residue monomers which are joined together through amide bonds (peptide bonds). The amino acids may be the L-optical isomer or the D-optical isomer. In general, polypeptides refer to long polymers of amino acid residues, e.g., those consisting of at least more than 10, 20, 50, 100, 200, 500, or more amino acid residue monomers. However, unless otherwise noted, the term polypeptide as used herein also encompass short peptides which typically contain two or more amino acid monomers, but usually not more than 10, 15, or 20 amino acid monomers.

[0038] Proteins are long polymers of amino acids linked via peptide bonds and which may be composed of two or more polypeptide chains. More specifically, the term "protein" refers to a molecule composed of one or more chains of amino acids in a specific order; for example, the order as determined by the base sequence of nucleotides in the gene coding for the protein. Proteins are essential for the structure, function, and regulation of the body's cells, tissues, and organs, and each protein has unique functions. Examples are hormones,

enzymes, and antibodies. In some embodiments, the terms polypeptide and protein may be used interchangeably.

[0039] Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene, C₁₄H₁₂O₃) is a stilbenoid, a type of natural phenol, and produced by certain plants in response to injury or upon attack by pathogens. Both caraphenol A (C₄₂H₂₈O₉) and α -viniferin (C₄₂H₃₀O₉) are resveratrol cyclotrimers from plants produced in response to injury or upon attack by pathogens and both compounds have diverse biological activities. Chemical structures of resveratrol, caraphenol A and α -viniferin are shown in Figure 1.

[0040] Stem cells are biological cells found in all multicellular organisms, and can divide (through mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells (these are called pluripotent cells), but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues. There are three accessible sources of autologous adult stem cells in humans: bone marrow, adipose tissue (lipid cells) and blood. Stem cells can also be taken from umbilical cord blood just after birth.

[0041] A cell has been "transformed" or "transfected" by exogenous or heterologous polynucleotide when such polynucleotide has been introduced inside the cell. The transforming polynucleotide may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming polynucleotide may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming polynucleotide has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming polynucleotide. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

[0042] A "variant" of a reference molecule (e.g., α -viniferin or caraphenol A) refers to a molecule which has a structure that is derived from or similar to that of the reference

molecule. Typically, the variant is obtained by modification of the reference molecule in a controlled or random manner. As detailed herein, methods for modifying a reference molecule to obtain functional derivative compounds that have similar or improved properties relative to that of the reference molecule are well known in the art.

[0043] A "vector" is a replicon, such as plasmid, phage or cosmid, to which another polynucleotide segment may be attached so as to bring about the replication of the attached segment. Vectors capable of directing the expression of genes encoding for one or more polypeptides are referred to as "expression vectors".

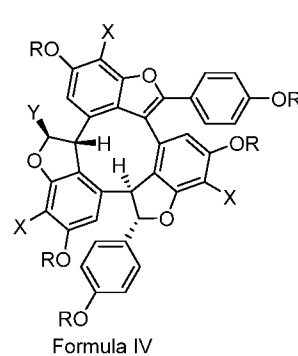
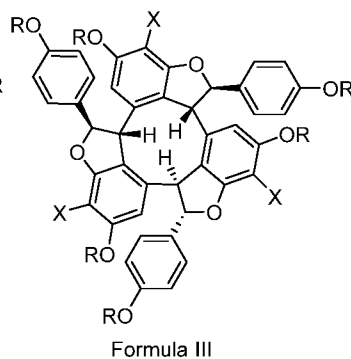
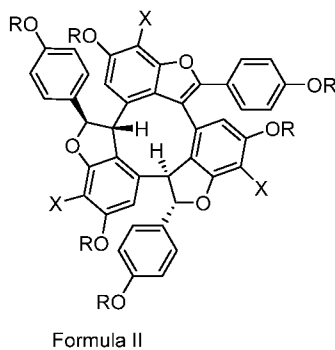
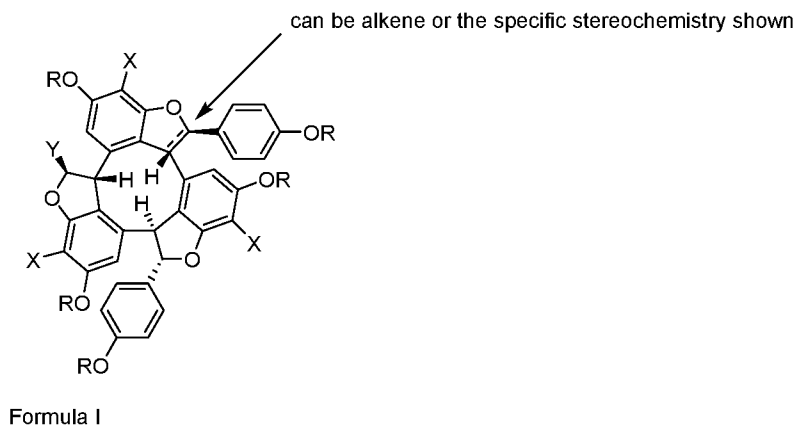
[0044] A retrovirus (e.g., a lentivirus) based vector or retroviral vector means that genome of the vector comprises components from the virus as a backbone. The viral particle generated from the vector contains essential vector components compatible with the RNA genome, including reverse transcription and integration systems. Usually these will include the gag and pol proteins derived from the virus. If the vector is derived from a lentivirus, the viral particles are capable of infecting and transducing non-dividing cells. Recombinant retroviral particles are able to deliver a selected exogenous gene or polynucleotide sequence such as therapeutically active genes, to the genome of a target cell.

III. Resveratrol oligomer compounds suitable for the invention

[0045] The present invention relates to novel methods and compositions for high frequency targeting and efficient payload delivery of viral vectors to host cells. The invention is based on the discovery by the present inventors that several resveratrol oligomer compounds are able to enhance transduction of retroviral vectors into both hematopoietic cells and non-hematopoietic cells. As used herein, resveratrol oligomer compounds refer to 9-membered ring containing compounds that are derived from resveratrol. In particular, they refer to cyclic resveratrol trimers derived from resveratrol. In general, cyclic resveratrol trimers suitable for methods of the invention have a structure as shown in Formula I. In the formula, each R at the five places in the structure is independently H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate. In some embodiments, R is the same at all 5 places in the structure. X is H, halogen, alkyl, aryl, and etc. using Pd- or Sn-based couplings as well as metallation (lithiation/Grignard formation) from the halogenated intermediate made by electrophilic aromatic substitution. In some embodiments, X is the same at all places in the structure. Y is any aromatic ring including heteroaromatic. In various embodiments, Y is installed as a nucleophile.

[0046] The arrow next to Formula I below points to a possible double bond in the structure, reflecting variation in the stereochemistry of the encompassed compounds. In some embodiments, the stereochemistry of the dihydrobenzofurans is trans when there is not a double bond at that position. Both a-viniferin and caraphenol A can have such a stereochemistry. In some of these embodiments, the compound is a-viniferin or a related derivative, which does not contain the double bond as shown in Formula III. In some other embodiments, the compound (e.g., caraphenol A) can have a double bond at that location as shown in Formula II.

[0047] In various embodiments, the compound to be employed in the invention has a structure that falls under any one of Formulae II-IV. In these formulae, R is H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate. In some embodiments, R is the same at all places in the structures. X is H, halogen, alkyl, aryl, and etc. from the halogenated intermediate made by electrophilic aromatic substitution. In some embodiments, X is the same at all places in the structures. Y is any aromatic ring including heteroaromatic. In various embodiments, Y is installed as a nucleophile.



[0048] While α -viniferin (Formula III), and caraphenol A (Formula II) were exemplified herein, any compound with substantially similar structure can also be used in the methods of the invention. As demonstrated herein, any compound that is able to downregulate IFITM 2 and/or IFITM 3 proteins, or to promote increased capsid release from the endosome, can be used in the practice of the invention. In some preferred embodiments, the compound to be used in the invention is any of the compounds shown in Fig. 5. For example, some methods of the invention can use caraphenol A. Some other methods of the invention can use α -viniferin. As detailed below, α -viniferin and caraphenol A are both derivative compounds of resveratrol. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi.

[0049] α -Viniferin (PubChem CID 196402) is a trimer of resveratrol, and has several biological activities, including anti-inflammatory, anti-oxidant, anti-arthritis, and anti-tumor activities. It exhibits significant inhibitory effect towards the enzyme acetylcholinesterase, which helps breaking down of acetylcholine into choline and acetic acid. α -Viniferin has a chemical structure of (2R,2aR,7R,7aR,12S,12aS)-2,7,12-tris(4-hydroxyphenyl)-2,2a,7,7a,12,12a-hexahydrobis[1]benzofuro[3',4':4,5,6;3'',4'':7,8,9]cyclonona[1,2,3-cd][1]benzofuran-4,9,14-triol (C₄₂H₃₀O₉). It can be isolated from *Caragana chamlagu* and from *Caragana sinica* and from the stem bark of *Dryobalanops aromatica*. Caraphenol A (PubChem CID 484751) is also biologically active trimeric form of the natural product resveratrol. It has a chemical structure of 3,4-[[6-Hydroxy-2 α -(4-hydroxyphenyl)-2,3-dihydrobenzofuran-4,3 β -diyl][6-hydroxy-2 β -(4-hydroxyphenyl)-2,3-dihydrobenzofuran-4,3 α -diyl]-6-hydroxy-2-(4-hydroxyphenyl)benzofuran (C₄₂H₂₈O₉). Chemical synthesis of caraphenol A can be performed as described in Wright et al., *Angew. Chem. Int. Ed.* 53:3409-3413, 2014. It can be also isolated from the roots of *Caragana sinica* as described in Luo et al, *Tetrahedron* 57:4849-4854, 2001

[0050] Other than α -viniferin or caraphenol A, any variant or derivatives of these two compounds may also be used in the practice of the invention. For example, α -viniferin is an inhibitor of the enzyme acetylcholinesterase. Thus, a structural analog or variant of α -viniferin with similar or improved inhibitory activity on acetylcholinesterase may be suitable for the present invention. These include α -viniferin or caraphenol A analog compounds known in the art. Suitable compounds for the invention also include novel compounds that can be identified in accordance with screening assays routinely practiced in the art or the

screening methods described herein. For example, a library of candidate compounds (e.g., analogs generated from α -viniferin or caraphenol A) can be screened in vitro for activities in downregulating IFITM 2 and/or IFITM 23 proteins, or for activities in promoting increased capsid release from the endosome. The candidate compounds (e.g., α -viniferin variants) can also be screened for ability in inhibiting the enzymatic activity of acetylcholinesterase. The screening can be performed via methods that can be readily adapted from well-known screening formats as described in the art, e.g., Yu et al., *Cancer Res.* 69: 6232–40, 2009; Livingstone et al., *Chem Biol.* 2009, 16:1240-9; Chen et al., *ACS Chem Biol.* 2012, 7:715-22; and Bhagwat et al., *Assay Drug Dev Technol.* 2009, 7:471-8. In various embodiments, the candidate compounds can be randomly synthesized chemical compounds, peptide compounds or compounds of other chemical nature. The candidate compounds can also comprise molecules that are derived structurally from the resveratrol oligomer compounds described herein (e.g., α -viniferin or caraphenol A or analogs).

[0051] The resveratrol oligomer compounds or analogs that are suitable for the invention can be readily obtained from commercial sources or de novo synthesized. For example, α -viniferin or caraphenol A can be purchased commercially, e.g., from BOC Sciences (Shirley, NY). The compounds can be further purified with protocols as described herein, e.g., by preparative reverse-phase HPLC. Caraphenol A can also be synthesized via published protocols, e.g., as described in Wright et al., *Angewandte Chemie (International ed. in English)* 53, 3409-3413, 2014. Structures and chemical synthesis of various other resveratrol oligomer compounds described herein are also well characterized in the art.

IV. Screening for novel analog compounds with improved properties

[0052] In addition to the resveratrol oligomer compounds exemplified herein and variants discussed above, the invention also provides methods of screening for novel resveratrol oligomer compounds. Some of the screening methods of the present invention are directed to identifying analogs or derivatives of the exemplified resveratrol oligomers (e.g., compounds shown in Figure 5) with improved properties. An important step in the drug discovery process is the selection of a suitable lead chemical template upon which to base a chemistry analog program. The process of identifying a lead chemical template for a given molecular target typically involves screening a large number of compounds (often more than 100,000) in a functional assay, selecting a subset based on some arbitrary activity

threshold for testing in a secondary assay to confirm activity, and then assessing the remaining active compounds for suitability of chemical elaboration.

[0053] The resveratrol oligomers exemplified herein, e.g., caraphenol A and α -viniferin, provide lead compounds to search for related compounds that have improved biological or pharmaceutical properties. For example, analogs or derivatives of these resveratrol oligomers can be screened for to identify compounds that are more potent in enhancing retroviral transduction into CD34+ HSPCs. Compounds with such improved properties can be more suitable for various pharmaceutical applications.

[0054] The screening methods typically involve synthesizing analogs, derivatives or variants of a resveratrol oligomer (e.g., caraphenol A and α -viniferin). Often, a library of structural analogs of a given resveratrol oligomer is prepared for the screening. A functional assay (e.g., lentiviral transduction of adult CD34+ stem cells as described herein) is then performed to identify one or more of the analogs or derivatives that have an improved biological property relative to that of the resveratrol oligomer from which the analogs or variants are derived. As noted above, the analogs can be screened for improved potency in enhancing retroviral transduction of HSPCs. Alternatively, they can be assayed to identify compounds with better pharmaceutical properties, e.g., stability, toxicity, or other pharmacokinetic characters.

[0055] Structures and chemical properties of the lead resveratrol oligomers (e.g., caraphenol A and α -viniferin) are all well-known and characterized in the art. To synthesize analogs or derivatives based from the chemical backbones of these resveratrol oligomers, only routinely practiced methods of organic chemistry that are well known to one of ordinary skill in the art are required. For example, combinatorial libraries of chemical analogs of a known compound can be produced using methods described herein. Exemplary methods for synthesizing analogs of various compounds are described in, e.g., Overman, *Organic Reactions*, Volumes 1-62, Wiley-Interscience (2003); Broom et al., *Fed Proc.* 45: 2779-83, 1986; Ben-Menahem et al., *Recent Prog Horm Res.* 54:271-88, 1999; Schramm et al., *Annu. Rev. Biochem.* 67: 693-720, 1998; Bolin et al., *Biopolymers* 37: 57-66, 1995; Karten et al., *Endocr Rev.* 7: 44-66, 1986; Ho et al., *Tactics of Organic Synthesis*, Wiley-Interscience; (1994); and Scheit et al., *Nucleotide Analogs: Synthesis and Biological Function*, John Wiley & Sons (1980).

[0056] Once a library of candidate structural analogs of a lead resveratrol oligomer compounds are synthesized, a functional assay is then performed to identify one or more of

the analogs or derivatives that have an improved biological property relative to that of the lead compound. The desired compound may have an improved property that is at least 10%, 25%, 50%, 75%, 100%, 200%, or 500% better than that of the lead compound. Any assays described herein or known in the art for assessing retroviral transduction can be used to identify an improved property in analogs or derivatives of a given resveratrol oligomer. These include the assay of lentiviral transduction of adult CD34⁺ stem cells as exemplified herein. In the assay, the candidate analogs can be screened for an ability to achieve an increased percentage of transduced cells under a given assay condition. In some embodiments, the candidate analog compounds can be screened for other biological activities noted above. These include, e.g., activities in downregulating IFITM 2, IFITM 3 and/or IFITM 23 proteins, activities in promoting increased capsid release from the endosome, and activities in inhibiting the enzymatic activity of acetylcholinesterase (for α -viniferin analogs). In some other embodiments, the structural analog compounds can be screened for improved pharmacokinetic properties, e.g., in vivo half-life. Compounds with such improved properties can be more suitable for various therapeutic applications. Improved pharmaceutical properties of a resveratrol oligomer analog can be assayed using methods such as those described in, e.g., Remington: *The Science and Practice of Pharmacy*, Mack Publishing Co., 20th ed., 2000.

V. Enhancing viral transduction with resveratrol oligomer compounds

[0057] The invention provides methods and compositions for enhancing viral transduction into the host cell. By enhancing vector entry and/or integration, the methods of the present invention can be used to enhance transduction efficiency of recombinant retroviruses or retroviral vectors expressing various exogenous genes. For example, recombinant retroviruses expressing an exogenous gene or heterologous polynucleotide sequence can be transduced into host cells with enhanced transduction efficiency in various gene therapy and agricultural bioengineering applications. In some preferred embodiments, the methods are intended for enhanced viral transduction in gene therapy. For example, a current problem with clinical stem cell based therapy is that viral vector entry and payload delivery does not occur without some form of stem cell proliferation. This potentially can result in differentiation of stem cells and loss of stem cell function when placed back into the host. Methods of the invention allow high frequency targeting to stem cells, and high efficiency delivery, without overt stem cell engraftment and growth problems.

[0058] Typically, methods of the invention involve transfecting a retroviral vector into host cells (e.g., stem cell or progenitor cells such as human HSPCs) that has been treated with a suitable amount of a resveratrol oligomer compound described (e.g., α -viniferin or caraphenol A), the invention provides methods for enhancing transduction of recombinant vectors, esp. retroviral vectors. In the practice of the methods of the invention, resveratrol or a resveratrol oligomer compound can be contacted with the cell prior to, simultaneously with, or subsequent to addition of the retroviral vector or recombinant retrovirus. This is followed by culturing the host cells under suitable conditions so that the viral vector or virus can be transduced into the cells.

[0059] Methods of the invention can be employed for enhancing transduction efficiency of various recombinant viruses or viral vectors used for gene transfer in many settings. In some embodiments, methods of the invention are used for promoting transduction of retroviruses or retroviral vectors, e.g., lentiviral vectors. Retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription. The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These elements contain strong promoter and enhancer sequences and are also required for integration in the host cell genome.

[0060] Retroviral vectors or recombinant retroviruses are widely employed in gene transfer in various therapeutic or industrial applications. For example, gene therapy procedures have been used to correct acquired and inherited genetic defects, and to treat cancer or viral infection in a number of contexts. The ability to express artificial genes in humans facilitates the prevention and/or cure of many important human diseases, including many diseases which are not amenable to treatment by other therapies. For a review of gene therapy procedures, see Anderson, *Science* 256:808-813, 1992; Nabel & Felgner, *TIBTECH* 11:211-217, 1993; Mitani & Caskey, *TIBTECH* 11:162-166, 1993; Mulligan, *Science* 926-932, 1993; Dillon, *TIBTECH* 11:167-175, 1993; Miller, *Nature* 357:455-460, 1992; Van Brunt, *Biotechnology* 6:1149-1154, 1998; Vigne, *Restorative Neurology and Neuroscience*

8:35-36, 1995; Kremer & Perricaudet, *British Medical Bulletin* 51:31-44, 1995; Haddada *et al.*, in *Current Topics in Microbiology and Immunology* (Doerfler & Böhm eds., 1995); and Yu *et al.*, *Gene Therapy* 1:13-26, 1994.

[0061] In order to construct a retroviral vector for gene transfer, a nucleic acid encoding a gene of interest is inserted into the viral genome in the place of certain viral sequences to produce a viral construct that is replication-defective. In order to produce virions, a producer host cell or packaging cell line is employed. The host cell usually expresses the gag, pol, and env genes but without the LTR and packaging components. When the recombinant viral vector containing the gene of interest together with the retroviral LTR and packaging sequences is introduced into this cell line (e.g., by calcium phosphate precipitation), the packaging sequences allow the RNA transcript of the recombinant vector to be packaged into viral particles, which are then secreted into the culture media. The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for transducing host cells (e.g., stem cells) in gene transfer applications.

[0062] Suitable host or producer cells for producing recombinant retroviruses or retroviral vectors according to the invention are well known in the art (e.g., 293T cells exemplified herein). Many retroviruses have already been split into replication defective genomes and packaging components. For other retroviruses, vectors and corresponding packaging cell lines can be generated with methods routinely practiced in the art. The producer cell typically encodes the viral components not encoded by the vector genome such as the gag, pol and env proteins. The gag, pol and env genes may be introduced into the producer cell and stably integrated into the cell genome to create a packaging cell line. The retroviral vector genome is then introduced-into the packaging cell line by transfection or transduction to create a stable cell line that has all of the DNA sequences required to produce a retroviral vector particle. Another approach is to introduce the different DNA sequences that are required to produce a retroviral vector particle, e.g. the env coding sequence, the gag-pol coding sequence and the defective retroviral genome into the cell simultaneously by transient triple transfection. Alternatively, both the structural components and the vector genome can all be encoded by DNA stably integrated into a host cell genome.

[0063] The methods of the invention can be practiced with various retroviral vectors and packaging cell lines well known in the art. Retroviral vectors are comprised of *cis*-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum *cis*-acting LTRs are sufficient for replication and packaging of the vectors, which

are then used to integrate the therapeutic gene into the target cell or host cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), simian immunodeficiency virus (SIV), human immunodeficiency virus (HIV), and combinations thereof (*see, e.g., Buchscher et al., J. Virol.* 66:2731-2739, 1992; Johann *et al., J. Virol.* 66:1635-1640, 1992; Sommerfelt *et al., Virol.* 176:58-59, 1990; Wilson *et al., J. Virol.* 63:2374-2378, 1989; Miller *et al., J. Virol.* 65:2220-2224, 1991; and PCT/US94/05700). Particularly suitable for the present invention are lentiviral vectors. Lentiviral vectors are retroviral vector that are able to transducer or infect non-dividing cells and typically produce high viral titers. Lentiviral vectors have been employed in gene therapy for a number of diseases. For example, hematopoietic gene therapies using lentiviral vectors or gamma retroviral vectors have been used for x-linked adrenoleukodystrophy and beta thalassaemia. *See, e.g., Kohn et al., Clin. Immunol.* 135:247-54, 2010; Cartier *et al., Methods Enzymol.* 507:187-198, 2012; and Cavazzana-Calvo *et al., M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. Nature* 467:318-322, 2010. Methods of the invention can be readily applied in gene therapy or gene transfer with such vectors. In some other embodiments, other retroviral vectors can be used in the practice of the methods of the invention. These include, *e.g.,* vectors based on human foamy virus (HFV) or other viruses in the Spumavirus genera.

[0064] In particular, a number of viral vector approaches are currently available for gene transfer in clinical trials, with retroviral vectors by far the most frequently used system. All of these viral vectors utilize approaches that involve complementation of defective vectors by genes inserted into helper cell lines to generate the transducing agent. pLASN and MFG-S are examples are retroviral vectors that have been used in clinical trials (Dunbar *et al., Blood* 85:3048-305 (1995); Kohn *et al., Nat. Med.* 1:1017-102 (1995); Malech *et al., Proc. Natl. Acad. Sci. U.S.A.* 94:22 12133-12138 (1997)). PA317/pLASN was the first therapeutic vector used in a gene therapy trial. (Blaese *et al., Science* 270:475-480, 1995). Transduction efficiencies of 50% or greater have been observed for MFG-S packaged vectors (Ellem *et al., Immunol Immunother.* 44:10-20, 1997; Dranoff *et al., Hum. Gene Ther.* 1:111-2, 1997). Many producer cell line or packaging cell line for transfecting retroviral vectors and producing viral particles are also known in the art. The producer cell to be used in the invention needs not to be derived from the same species as that of the target cell (*e.g.,* human target cell). Instead, producer or packaging cell lines suitable for the present

invention include cell lines derived from human (e.g., HEK 292 cell), monkey (e.g., COS-1 cell), mouse (e.g., NIH 3T3 cell) or other species (e.g., canine). Additional examples of retroviral vectors and compatible packaging cell lines for producing recombinant retroviruses in gene transfers are reported in, e.g., Markowitz et al., *Virology* 167:400-6, 1988; Meyers et al., *Arch. Virol.* 119:257-64, 1991 (for spleen necrosis virus (SNV)-based vectors such as vSNO21); Davis et al., *Hum. Gene Ther.* 8:1459-67, 1997 (the "293-SPA" cell line); Povey et al., *Blood* 92:4080-9, 1998 (the "1MI-SCF" cell line); Bauer et al., *Biol. Blood Marrow Transplant.* 4:119-27, 1998 (canine packaging cell line "DA"); Gerin et al., *Hum. Gene Ther.* 10:1965-74, 1999; Sehgal et al., *Gene Ther.* 6:1084-91, 1999; Gerin et al., *Biotechnol. Prog.* 15:941-8, 1999; McTaggart et al., *Biotechnol. Prog.* 16:859-65, 2000; Reeves et al., *Hum. Gene Ther.* 11:2093-103, 2000; Chan et al., *Gene Ther.* 8:697-703, 2001; Thaler et al., *Mol. Ther.* 4:273-9, 2001; Martinet et al., *Eur. J. Surg. Oncol.* 29:351-7, 2003; and Lemoine et al., *J. Gene Med.* 6:374-86, 2004. Any of these and other retroviral vectors and packaging producer cell lines can be used in the practice of the present invention.

[0065] Many of the retroviral vectors and packaging cell lines used for gene transfer in the art can be obtained commercially. For example, a number of retroviral vectors and compatible packaging cell lines are available from Clontech (Mountain View, CA). Examples of lentiviral based vectors include, e.g., pLVX-Puro, pLVX-IRES-Neo, pLVX-IRES-Hyg, and pLVX-IRES-Puro. Corresponding packaging cell lines are also available, e.g., Lenti-X 293T cell line. In addition to lentiviral based vectors and packaging system, other retroviral based vectors and packaging systems are also commercially available. These include MMLV based vectors pQCXIN, pQCXIQ and pQCXIH, and compatible producer cell lines such as HEK 293 based packaging cell lines GP2-293, EcoPack 2-293 and AmphoPack 293, as well as NIH/3T3-based packaging cell line RetroPack PT67. Any of these and other retroviral vectors and producer cell lines may be employed in the practice of the present invention.

[0066] The methods of the invention can be employed in the transfer and recombinant expression of various exogenous genes or heterologous polynucleotide sequences. Typically, the gene or heterologous polynucleotide sequence is derived from a source other than the retroviral genome which provides the backbone of the vector used in the gene transfer. The gene may be derived from a prokaryotic or eukaryotic source such as a bacterium, a virus, a yeast, a parasite, a plant, or an animal. The exogenous gene or heterologous polynucleotide sequence expressed by the recombinant retroviruses can also be

derived from more than one source, i.e., a multigene construct or a fusion protein. In addition, the exogenous gene or heterologous polynucleotide sequence may also include a regulatory sequence which may be derived from one source and the gene from a different source. For any given gene to be transferred via the viral vectors, a recombinant retroviral vector can be readily constructed by inserting the gene operably into the vector, replicating the vector in an appropriate packaging cell as described above, obtaining viral particles produced therefrom, and then infecting the target cells (e.g., stem cells) with the recombinant viruses.

[0067] In some preferred embodiments, the exogenous gene or heterologous polynucleotide sequence harbored by the recombinant retrovirus is a therapeutic gene. The therapeutic gene can be transferred, for example to treat cancer cells, to express immunomodulatory genes to fight viral infections, or to replace a gene's function as a result of a genetic defect. The exogenous gene expressed by the recombinant retrovirus can also encode an antigen of interest for the production of antibodies. In some exemplary embodiments, the exogenous gene to be transferred with the methods of the present invention is a gene that encodes a therapeutic polypeptide. For example, transfection of tumor suppressor gene p53 into human breast cancer cell lines has led to restored growth suppression in the cells (Casey et al., *Oncogene* 6:1791-7, 1991). In some other embodiments, the exogenous gene to be transferred with methods of the present invention encodes an enzyme. For example, the gene can encode a cyclin-dependent kinase (CDK). It was shown that restoration of the function of a wild-type cyclin-dependent kinase, p16INK4, by transfection with a p16INK4-expressing vector reduced colony formation by some human cancer cell lines (Okamoto, *Proc. Natl. Acad. Sci. U.S.A.* 91:11045-9, 1994). Additional embodiments of the invention encompass transferring into target cells exogenous genes that encode cell adhesion molecules, other tumor suppressors such as p21 and BRCA2, inducers of apoptosis such as Bax and Bak, other enzymes such as cytosine deaminases and thymidine kinases, hormones such as growth hormone and insulin, and interleukins and cytokines.

[0068] The recombinant retroviruses or retroviral vectors expressing an exogenous gene can be transduced into any host or target cells in the presence of resveratrol or a resveratrol cyclic trimer compound (e.g., α -viniferin or caraphenol A) for recombinant expression of the exogenous gene. As exemplified herein, host cells or target cells for the present invention can be are stem cells or non-stem cells. Suitable stem cells can be hematopoietic stem cells

and progenitor cells. Other than hematopoietic stem cells and progenitor cells, other types of stem cells and non-hematopoietic cells can also be used in the invention. Thus, stem cells suitable for practicing the invention include and are not limited to hematopoietic stem cells (HSC), embryonic stem cells or mesenchymal stem cells. They include stem cells obtained from both human and non-human animals including vertebrates and mammals. Other specific examples of target cells include cells that originate from bovine, ovine, porcine, canine, feline, avian, bony and cartilaginous fish, rodents including mice and rats, primates including human and monkeys, as well as other animals such as ferrets, sheep, rabbits and guinea pigs.

[0069] Transducing a recombinant retroviral vector into the target cell in the presence of resveratrol or a resveratrol cyclic trimer compound (e.g., α -viniferin or caraphenol A) can be carried out in accordance with protocols well known in the art or that exemplified in the Examples below. For example, the host cell (e.g., HSPCs) may be pre-treated with the resveratrol compound prior to transfection with the retroviral vector. Alternatively, the target host cell can be transfected with the viral vector in the presence of resveratrol or a resveratrol cyclic trimer compound complexes described herein (e.g., α -viniferin or caraphenol A or an analog compound). The concentration of the compound to be used can be easily determined and optimized by the skilled artisans, depending on the nature of the compound, the recombinant vector or virus used, as well as when the cell is contacted with the compound (prior to or simultaneously with transfection with the vector). Typically, the compound (α -viniferin or caraphenol A or an analog) should present in a range from about 10 nM to about 2 mM. Preferably, the compound used in the methods is at a concentration of from about 50 nM to about 500 μ M, from about 100 nM to 100 μ M, or from about 0.5 μ M to about 50 μ M. More preferably, the compound is contacted with the producer cell at a concentration of from about 1 μ M to about 20 μ M, e.g., 1 μ M, 2 μ M, 5 μ M or 10 μ M.

[0070] The invention also provides pharmaceutical combinations, e.g. kits, that can be employed to carry out the various methods disclosed herein. Such pharmaceutical combinations typically contain a resveratrol oligomer compound described herein (e.g., α -viniferin, caraphenol A or analog described herein), in free form or in a composition with one or more inactive agents, and other components. The pharmaceutical combinations can also contain one or more appropriate retroviral vectors (e.g., a lentiviral vector described herein) for cloning a target gene of interest. The pharmaceutical combinations can additionally contain a packaging or producer cell line (e.g., 293T cell line) for producing a

recombinant retroviral vector that expresses an inserted target gene or polynucleotide of interest. In some embodiments, the pharmaceutical combinations contain a host cell or target cell into which an exogenous gene harbored by the recombinant retroviral vector or virus is to be delivered. In various embodiments, the pharmaceutical combinations or kits of the invention can optionally further contain instructions or an instruction sheet detailing how to use the resveratrol oligomer compounds (e.g., α -viniferin or caraphenol A) to transduce recombinant retroviruses or retroviral vectors with enhanced efficiency.

EXAMPLES

[0071] The following examples are provided to further illustrate the invention but not to limit its scope.

Example 1 Caraphenol A and α -viniferin enhance gene delivery ex vitro

[0072] We first examined resveratrol, α -viniferin, and caraphenol A (Fig. 1a), a closely related resveratrol cyclotrimer of higher oxidation state, for their capacity to augment LV transduction in cells. Caraphenol A and α -viniferin, but not resveratrol, showed an ability to enhance LV gene delivery to HeLa cells in a compound dose dependent manner at a range of MOIs, achieving ~2-fold greater frequency of EGFP expression at doses between 30-50 μ M (Fig. 1b,c). Caraphenol A treatment also enhanced LV gene delivery in several HSPC types. 2-fold or greater enhancement in gene marking frequency was observed in primary umbilical cord blood-derived (UCB), G-CSF-mobilized peripheral blood-derived (mPB), and non-human primate (NHP) bone marrow aspirate CD34⁺ HSPCs (Fig. 1d). While the degree of initial marking with DMSO-treated cells was variable between donors, caraphenol A treatment consistently improved gene delivery frequency. Improvement in transduction was most significant in mPB CD34⁺ HSPCs, a clinically important cell type that has been shown to be more resistant to transduction than UCB CD34⁺ HSPCs. Caraphenol A improved integrated vector copy number (VCN) at a range of MOIs in UCB CD34⁺ HSPCs (Fig. 1e), indicating treatment may improve transgene delivery even at high LV doses used in clinical trial protocols. While synthetic caraphenol A served as the initial point for our investigations, we observed similar transduction enhancement with plant-derived, HPLC-purified compounds. Enhanced transduction of CD34⁺ cells was also observed with α -viniferin (Fig. 6).

[0073] Our group and others have previously identified that treatment with the mTOR inhibitor rapamycin relieves LV transduction resistance in mouse and human HSPCs. Critically though, rapamycin also slows the proliferation of treated cells *ex vivo*, which might consequently slow progenitor expansion required for rapid leukocyte recovery in clinical transplant settings. By contrast, caraphenol A treatment of either UCB or mPB CD34⁺ HSPCs did not slow proliferation *ex vivo* when used at a range of concentrations (Fig. 1f). Furthermore, caraphenol A treatment showed no effect on cell viability in culture or plating efficiency and lineage differentiation by colony-forming unit assay (not shown). Finally, VCN analysis of transduction of two separate vectors recently used for correction of X-linked severe combined immunodeficiency (X-SCID) demonstrated that caraphenol A application improves therapeutic gene delivery to mPB CD34⁺ HSPCs (Fig. 1g), within a therapeutic range. These findings confirm that caraphenol A treatment enhances transduction in a range hematopoietic tissue types with a favorable toxicity profile for *ex vivo* clinical gene therapy applications.

Example 2 Caraphenol A treated HSCs maintain improved gene marking *in vivo* without altering lentiviral integration profiles

[0074] To evaluate if gene delivery enhancement *ex vivo* by caraphenol A extends to long-term repopulating HSCs (LTR-HSCs), we transduced pooled human UCB-derived CD34⁺ HSPCs pre-treated for 4 hours (h) (total transduction period = 24 h) with 30μM caraphenol A or 0.06% DMSO (vehicle control), and transplanted 3×10⁵ cells each into irradiated NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ mice (Fig. 2a). Two separate cohorts (n=8 mice per treatment) were established with CD34⁺ HSPCs transduced with either low (MOI=10) or high (MOI=25) LV doses, and the resulting human engraftment was evaluated by EGFP expression using flow cytometric analysis of peripheral blood sampled approximately every 3 weeks (Fig. 2a-c). All mice were healthy throughout the treatment period and no adverse events were apparent following caraphenol A treatment. Significant enhancement of EGFP marking levels in human CD45⁺ (huCD45⁺) cells in the peripheral blood after caraphenol A treatment was observed in both low (10 MOI) (Fig. 2b) and modest (25 MOI) MOI cohorts (Fig. 2c), although enhancement was more pronounced at low MOI. Of interest, while the mean %EGFP expression in huCD45⁺ cells was consistent throughout measured timepoints in both studies, the persistence of marking differed between mice receiving caraphenol A- and DMSO-treated cells. In both cohorts, several mice transplanted with DMSO-treated cells

showed early EGFP-expression that waned over time, while gene marking in mice receiving caraphenol A-treated cells was stable throughout the study (Fig. 2b-c). This finding is consistent with caraphenol A improving transduction of long-term repopulating (LTR) human hematopoietic stem cells in NSG mice, in addition to hematopoietic progenitor cells observed in *ex vivo* cultures presented in Fig. 1d-g.

[0075] Mice were sacrificed after 22 weeks and EGFP-expression in peripheral blood, bone marrow and spleen were assessed (Fig. 2b-e). Human cell present in spleen and engraftment and VCN in bone marrow were also determined (Fig. 2f,g). One mouse in the low MOI DMSO treated group had final human bone marrow engraftment below 2% and was excluded from further analyses. Consistent with terminal peripheral bleeds and *ex vivo* observations, caraphenol A treatment significantly increased EGFP gene marking frequency of human CD45⁺ cells resident in spleen and bone marrow cells (Fig. 2d,e). No significant difference in human cell engraftment was observed in caraphenol A-versus DMSO-treated cells in either cohort (Fig. 2f), consistent with the absence of Caraphenol A toxicity on hematopoietic progenitors and stem cells. At low MOI, VCN values from the bone marrow of mice transplanted with caraphenol A-treated CD34⁺ HSPCs showed ~10-fold higher levels compared to DMSO-treated controls *ex vivo* (MOI 10 VCN = 0.05 *ex vivo* vs. 0.5 *in vivo*, Fig. 1e vs. 2g), consistent with the interpretation that caraphenol A treatment enhances lentiviral vector efficacy of a normally low efficiency cellular event. Importantly, transfer of CD34⁺ cells obtained from NSG mice originally transplanted with Caraphenol A LV transduced CD34⁺ cells to second recipient NSG mice demonstrated increased EGFP gene marking of cells arising from long-term repopulating hematopoietic stem cells, as compared to the DMSO controls (Fig. 2h). These findings are consistent with increased EGFP gene marking of hematopoietic long-term engrafting stem cells that are fully revealed in the secondary transplanted NSG mice. These findings further indicate that caraphenol A treatment has even more potent transduction enhancement effects in long-term repopulating hematopoietic stem cells in mice after transplants, above and beyond that seen *ex vivo* in HSPCs (Fig. 1d-g). The lineage analysis of bone marrow subsets showed increased EGFP marking in myeloid, T and B cell subsets in mice receiving caraphenol A-treated cells. No skewing of lineage frequency was observed in myeloid, T or B subsets in either cohort, providing evidence that enhanced EGFP marking was not due to dysregulated hematopoiesis in caraphenol A treated cells.

[0076] With the observation that VCN was increased per cell both *ex vivo* and *in vivo* after caraphenol A treatment, and given the known effects of resveratrol and other polyphenols on global patterns of gene regulation, we investigated whether caraphenol A treatment altered patterns of LV integration in host cells. High-throughput retroviral integration site (RIS) analysis was performed on human cells from bone marrow and spleen samples harvested from both the low and high MOI cohorts. Tens of thousands of unique integrations were identified across all samples. No significant differences were observed in the frequency of chromosomal insertion between treatment groups relative to genomic features or known oncogenes, although caraphenol A appeared to slightly reduce integrations in close proximity to transcription start sites (Fig. 3a,b). Together, these findings suggest that caraphenol A treatment enhances gene delivery frequency *ex vivo*, and that enhancement is maintained and potentially elevated in long-term repopulating HSCs without biasing integration of LV in treated cells.

Example 3 Caraphenol A treatment facilitates lentiviral escape from endosomes

[0077] We next sought to investigate the step in the vector entry process whereby caraphenol A mediates transduction enhancement in immortalized cell lines and hematopoietic primary cells. As has been well established, lentiviruses encounter physical barriers and cellular restriction factors throughout the entry process, including at receptor binding, membrane fusion, core uncoating, reverse transcription, nuclear entry and integration. We observed no effect of caraphenol A treatment on either the frequency or density of the VSV-G receptor, LDL-R, in UCB-derived CD34⁺ HSPCs. Unlike VSV-G-pseudotyped LVs, transduction by LVs pseudotyped with the measles virus hemagglutinin and fusion proteins was not significantly enhanced by caraphenol A treatment in UCB CD34⁺ HSPC. Measles virus has been proposed to enter at the cell surface by inducing macropinocytosis, which is distinct from the clathrin-mediated and pH-dependent endocytosis mechanism used by VSV-G. These results imply that caraphenol A enhancement affects some but not all vector entry routes.

[0078] In agreement with these findings, treatment of UCB CD34⁺ HSPCs with caraphenol A increased LV-endosomal membrane fusion and escape of LV cores to the cytoplasm, as measured by the BLAM-Vpr assay (Fig. 4a, left panel). Moreover, the relative fold increase observed at fusion is within the range of transduction enhancement measured 7 days later (Fig. 4a, right panel). To evaluate the kinetics of fusion enhancement, we used a

modified BLaM assay protocol where vector fusion is inhibited by cold temperature at various timepoints after LV addition (Miyanchi et al., Cell 137, 433-444, 2009). UCB-derived CD34⁺ HSPCs pre-treated with caraphenol A showed immediate 2-fold greater fusion that was maintained at all timepoints, indicating that the rate of LV cytoplasmic entry is increased by caraphenol A treatment (Fig. 4b). Some variability in rate, but not response to treatment, was observed between donors, indicating that inherent vector restriction of fusion may dictate response to gene therapy in some patients. mPB-derived CD34⁺ HSPCs also showed an enhanced rate of LV fusion with caraphenol A treatment by the BLaM assay, which translated to increased transduction 7 days later. A recent small molecule screen identified prostaglandin-E2 (PGE-2) as an enhancer of transduction that improved gene delivery at a post-fusion step (Heffner et al., Mol. Ther. 26, 320-328, 2018). We observed similar findings, and in addition saw additive transduction enhancement of UCB CD34⁺ HSPCs when cells were co-incubated with PGE-2 and caraphenol A, suggesting that they function at different stages of vector transduction. Additionally, measurements of both early and late stage reverse transcription (RT) products by quantitative polymerase chain reaction (qPCR) showed 2 to 3-fold increases upon LV and caraphenol A addition, while the ratio of early to late products remained the same per UCB-donor. These results indicated that while caraphenol A increases the total pool of reverse transcribed viral DNA, it has no effect on RT efficiency in UCB-derived CD34⁺ HSPCs.

[0079] Having determined the point in vector entry where caraphenol A acts, we next evaluated the timing of the observed effect. Caraphenol A treatment showed maximal transduction enhancement in HeLa cells when LV and caraphenol A were added simultaneously, though caraphenol A could be added up to 4h after LV and still result in some enhancement (Fig. 4c). Washout studies in HeLa cells showed some residual enhancement when caraphenol A was added to cells before washout and vector addition, although maximal enhancement occurs with continuous treatment in the presence of LV (Fig. 4d). In summary, these findings localize the effect of caraphenol A to enhancing LV escape from the endosome, with maximal effect observed with simultaneous addition of caraphenol A and vector.

Example 4 LV restriction by IFITM2/3 proteins is relieved by caraphenol A treatment

[0074] Having observed an increase in cytoplasmic entry of LV after caraphenol A treatment, we sought to identify cellular restriction factors that mediate this process. Recent

studies have implicated the interferon-induced transmembrane (IFITM) family of proteins as potent restrictors of viruses that utilize endosomes and pH-dependent fusion for endosomal escape. Furthermore, these proteins are intrinsically expressed in pluripotent and multipotent tissue types. To evaluate the effect of IFITM proteins on transduction, we ectopically expressed IFITM1, IFITM2 or IFITM3 in HEK 293T cells and compared transduction efficiency with the LV previously used for *ex vivo* and *in vivo* experiments. While IFITM1 ectopic expression induced slight enhancement in transduction, LV gene delivery was somewhat reduced in IFITM2-expressing and greatly reduced in IFITM3-expressing cells. Consequently, all further analysis was performed with antibodies that detect both IFITM2 and IFITM3 equivalently. Additionally, after introducing the $\Delta 17-20$ mutation into IFITM3, which has been reported to redirect IFITM3 localization from the endosomal compartment to the cell periphery (Jia et al., J. Virol. 86, 13697-13707, 2012), we observed a complete loss of restriction. These findings indicate that IFITM3, and to a lesser extent IFITM2, which are localized to endosomes has a significant role in restricting VSV-G-mediated LV transduction (Shi et al., Retrovirology, 14, 1-10, 2017).

[0075] Next, we evaluated the effect of caraphenol A treatment on IFITM2/3 expression in HeLa cells. We observed a high level of IFITM2/3 protein expression by western blot (WB) in these cells that was reduced by >50% after 4 h of caraphenol A treatment, an effect that was also observed with an antibody more specific for IFITM3. Longer incubation of cells with caraphenol A showed a recovery of IFITM2/3 protein levels with a return to baseline after 24 h during continuous incubation with compound. Consequently, we sought to determine whether IFITM3 expression was required for caraphenol A transduction enhancement. We generated an IFITM3 knockout in the HeLa-derived TZM-bl cell line using transient CRISPR/Cas9 expression and homologous recombination for selection. These cells showed a complete loss of IFITM3 protein expression with no apparent effect on IFITM2 levels. Caraphenol A treatment of wild-type (WT) TZM-bl cells exhibited a 1.5-fold increase in transduction while no effect was observed with resveratrol treatment. By contrast, IFITM3 knockout cells demonstrated an increased baseline level of transduction and caraphenol A treatment led to only a modest additional enhancement in these cells, indicating that reducing levels of IFITM3 reduces the enhancement effect following treatment with the compound. The residual effect of caraphenol A in IFITM3 KO cells may result from downregulation of IFITM2.

[0076] After observing the effect of caraphenol A on IFITM protein expression in cell lines, mPB-derived CD34⁺ HSPCs from several donors were evaluated by western blot for IFITM1, IFITM2 and IFITM3 expression. No appreciable IFITM1 was observed, but IFITM2/3 were seen in all tested donors. Flow cytometry analysis of UCB-derived CD34⁺ HSPCs treated for 4 h with caraphenol A showed a 20% reduction in IFITM2/3 expressing cells, as well as a 50% reduction in median fluorescence intensity in the population of IFITM2/3⁺ cells. These findings support the conclusion that caraphenol A induces a reduction in IFITM2/3 expression in therapeutically relevant primary cells.

[0077] To evaluate the direct effect of caraphenol A and resveratrol on IFITM2/3 protein localization in the endosomal compartment, HeLa cells and mPB derived CD34⁺ HSPCs were examined by confocal microscopy. HeLa cells treated with caraphenol A and LV showed an overall reduction of the IFITM2/3 signal, with most signal relocated from the periphery of the cell to a peri-nuclear localization, an effect that was not observed with DMSO or resveratrol treatment. 3D analysis and quantification of IFITM2/3-containing endosomes demonstrated a significant reduction in both the number and staining intensity, compared to DMSO- or resveratrol-treated cells at 30 minutes (min) and 2 h following LV addition. In contrast to HeLa cells, mPB CD34⁺ HSPCs exhibited limited endosome activity without LV addition. Within 30 min of LV addition lysosomal-associated membrane protein-1 (LAMP1⁺) endosomes were evident and the IFITM2/3 signal was re-localized to the endosomal compartment from peri-nuclear locations. As seen in HeLa cells, a reduction and relocalization of the IFITM2/3 signal in caraphenol A-treated mPB CD34⁺ HSPCs was also observed. Of interest, the kinetics of the effect was different than HeLa cells, as a significant reduction in IFITM2/3⁺ vesicle number was not observed until 2 h after LV addition, although a significant reduction in staining intensity per endosome was observed at both timepoints. In support of the above findings from the BLAM-Vpr assay, we observed increased loss of HIV p24⁺ signal from endosomes with caraphenol A treatment in CD34⁺ cells and in HeLa cells. Additionally, we noted differing effects of caraphenol A on various endosomal compartments. We observed a small but significant reduction in the number and staining intensity of late endosomes carrying LAMP1 after caraphenol A treatment, in both HeLa and mPB-derived CD34⁺ HSPCs, but no effect on the number of early endosomal antigen-1 (EEA1) staining vesicles and in HeLa cells, indicating that transduction enhancement is likely not mediated by an increase in the early endosomal compartment.

[0078] Given the observation that caraphenol A reduced the number of LAMP1⁺ vesicles in both cell types tested, we next investigated the effect of caraphenol A on pH of the endosomal compartment in HeLa cells using LysoSensor pH-sensitive dextran. Interestingly, caraphenol A treatment over 4 h showed a decrease in 525/405nm OD ratio that is indicative of overall cellular increase in endosomal pH⁶⁶. This effect was even greater than that observed after treatment with the endosomal acidification inhibitor chloroquine. Together with data from confocal analysis, our assessment of endosomal pH supports the conclusion that caraphenol A causes significant changes to both the function and frequency of the late endosomal compartment. Along with the dramatic downregulation of IFITM2/3 expression, this data supports the finding that alterations to endosomal composition may be influencing transduction efficiency of multiple cell types.

[0079] The observation that caraphenol A reduced the number of LAMP1⁺ vesicles in both cell types tested which also corresponded with loss of endosomally-localized p24 might suggest that this effect was dependent on IFITM3. To address this as a possible mechanism of endosomal control, both *IFITM3* knockout and WT TZM-bl cells were evaluated as above in the presence of LV and caraphenol A or DMSO. Caraphenol A reduced the number and intensity of IFITM2/3 containing endosomes in WT TZM-bl cells and reduced remaining IFITM2 number and intensity in *IFITM3* knockout cells. Interestingly, the reduction in LAMP1⁺ vesicle number and staining intensity was not observed in the *IFITM3* knockout cells after caraphenol A treatment, in contrast to wild-type cells. Therefore, these results support the finding that effects of caraphenol A on the late endosome compartment are dependent upon the presence of IFITM3 proteins.

Example 5 Materials and methods

[0080] *Chemicals and Reagents:* Resveratrol and rapamycin were commercially purchased (Calbiochem, Millipore-Sigma, CAT# 554325, CAT# 553210). Caraphenol A was originally synthesized at Columbia University via published protocols (Wright et al., *Angewandte Chemie* 53, 3409-3413, 2014). Naturally derived caraphenol A and α -viniferin were purchased (caraphenol A CAT# 354553-35-8, α -Viniferin CAT# 78690-98-9, BOC Sciences, Shirley, NY) and purified by preparative reverse-phase HPLC (CH₃CN/H₂O-0.07% TFA 10:90 to 90:10 over 30 min, 3 mL/min, R_t = 21.4 min) to provide pure caraphenol A and α -viniferin, possessing NMR spectral data consistent with prior reports. Chloroquine was purchased from Sigma-Aldrich (CAT# 50-63-5). Cytokines IL-3 (CAT#

10779-594), IL-6 (CAT# 200-06), TPO (CAT# 300-18), SCF (CAT# 300-25), and Flt3-L (CAT# 300-19) were all ordered from Peprotech. BIT 9500 was purchased from Stem Cell Technologies (CAT# 09500). Prostaglandin-E2 was a kind gift from Gabor Veres, PhD (Bluebird Bio, Boston, MA.).

[0081] *HPLC:* HPLC was conducted using a Waters 600 pump/controller, a Waters 996 photodiode array detector, and a Cosmosil 5C18-AR-II column. ^1H and ^{13}C NMR spectra were obtained using a Bruker Avance III HD 600 MHz spectrometer equipped with either a 5 mm QCI or 5 mm CPDCH probe.

[0082] *Lentiviral Vector:* Third generation pRRL-SIN-MND-EGFP lentiviral vector (LV) was produced, concentrated, and titered as previously described (Swan et al., Gene Ther. 13, 1480-1492, 2006). In short, transgene plasmid and accessory packaging plasmids were transfected with calcium-phosphate onto minimal passage 293T cells. 36 h after transfection, supernatant was collected and concentrated by ultracentrifugation on a sucrose gradient. MOI was calculated by a dilution series on 293T lines and analysis of EGFP expression.

[0083] *LV Transduction:* All HeLa cells were grown in DMEM (Corning CAT# 15-013-CV) containing 10% FBS (Omega Scientific, CAT# FB-01), 1% Pen/Strep (Invitrogen CAT#15140122), and 1% L-Glutamine (Invitrogen CAT#25030081). HeLa cells were split at 2×10^4 cells/well of a 48-well plate and grown overnight. Cells were incubated over 8 h with LV and indicated compounds, after which, both were removed and the cells were cultured for 6-7 days before flow cytometry analysis. Cells were removed with trypsin and neutralized with FACs buffer (PBS+2%FBS), pelleted at 300xg, and resuspended in FACs buffer. Flow cytometry was performed on a BD LSR-II flow cytometer.

[0084] *CD34⁺ cell isolation and use:* Umbilical cord blood (UCB) was generously donated from the Cleveland Cord Blood Center (Cleveland, OH). CD34⁺ UCB cells, under approved institutional protocol (information and approval is available upon request) and in accordance with the Declaration of Helsinki, were isolated using the EasySep Human Cord Blood CD34 Positive Selection Kit (STEMCELL Technologies, Vancouver, BC, Canada). Frozen G-CSF mobilized peripheral blood (mPB) CD34⁺ cells were purchased from the Cooperative Center for Excellence in Hematology at the Fred Hutchinson Cancer Research Center (Seattle, WA). CD34⁺ non-human primate cells were isolated by bone marrow aspiration from rhesus macaques at the Wisconsin National Primate Research Center, Madison, WI, as per institutional protocols (information and approval is available upon

request). mPBs CD34⁺ cells were grown in SCGM (CellGenix CAT# 20802-0500), while UCB CD34⁺ cells were grown in IMDM, with supplements, as previously reported (Swan et al., *supra*). mPBs CD34⁺ cells were pre-stimulated for 48 h with 0.1 µg/mL TPO, 0.1 µg/mL SCF, and 0.1 µg/mL Flt3-L before LV addition. UCB CD34⁺ cells were pre-stimulated for 24 h as previously reported (Swan et al., *Gene Ther.* 13, 1480-1492, 2006). During LV transduction, mPB and UCB CD34⁺ cells were cultured under identical conditions as pre-stimulation, with the addition of 4 µg/mL Polybrene. CD34⁺ cells were incubated for 4 h with either DMSO vehicle control, caraphenol A, or rapamycin, after which they were transduced with LV for another 20 h in the absence or presence of selected LV transduction enhancers. After LV transduction, washed CD34⁺ cells were cultured with: 0.1 µg/mL TPO, 0.1 µg/mL SCF, 0.1 µg/mL FLT-3, 0.06 µg/mL IL-3, 0.06 µg/mL IL-6 for mPB cells, and 0.1 µg/mL SCF, 0.05 µg/mL IL-3, 0.05 µg/mL IL-6, and 10%FBS for UCB cells. All CD34⁺ cells were cultured for 7-14 days and then analyzed by flow cytometry (BD LSR-II).

[0085] *qPCR*: VCN was established through qPCR from the genomic DNA of total cell populations. Cells were collected and processed by the Qiagen DNeasy Blood and Tissue Kit (CAT# 69506). qPCR for LV targeted the late product U5Ψ with primers MH531 and MH352 with probe LRT-P, while early product RU5 used primers hRU5-F2 and hRU5-R with probe hRU5-P (Mbisa et al., *Methods Mol. Biol.* 485, 55-72, 2009). Genomic loading was standardized with Taqman RNaseP (CAT# 4401631). A Roche LightCycler 480 was used for genomic product amplification and analyses.

[0086] *Transplantation*: NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ mice were obtained from Jackson Laboratory and maintained at The Scripps Research Institute under approved institutional protocols (available upon request). For human CD34⁺ HSPC transplantation studies, 4-6 week old female mice were randomly assigned to experimental groups and irradiated at 240 cGy using a cesium source. 3x10⁵ UCB CD34⁺ cells, LV transduced as discussed above, per mouse were injected retro-orbitally and the remaining cells were cultured *ex vivo* for transgene expression analyses 7 and 14-days post-transduction. Peripheral blood was sampled every 3-5 weeks after an initial 6-7 week engraftment period, with red cells removal before flow cytometry analyses, as previously reported (Swan et al., *supra*). Mice were sacrificed at 22 weeks (terminal) and harvested for peripheral blood, bone marrow, and spleen. Engraftment was determined with BD BioSciences antibodies BUV395-mCD45 (CAT# 564279) and APC-hCD45 (CAT# 555485). Lineage was determined with antibodies PerCP-Cy5.5-hCD3 (CAT# 560835), V450-hCD19 (CAT#

560353), and PE-hCD33 (CAT# 555450). Gating was established using fluorescence minus one controls (FMOC) and EGFP expression was gated using human CD34⁺ engrafted but non-transduced mice. All flow cytometry was performed on a BD LSR-II.

[0087] *CD34⁺ Cell Viability, Proliferation, and Colony Forming Unit Assessment:* UCB CD34⁺ cells were pre-treated for 4 h with the indicated dose of compounds before transduction with LV for 20 h. Cells were then washed and seeded, in duplicate or triplicate, as described above, for 14 days. At the indicated times aliquots of LV transduced cells were analyzed for viability, as previously reported (Swan et al., Gene Ther. 13, 1480-1492, 2006). Colony-forming unit (CFU) assessment of CD34⁺ progenitor cell differentiation was completed as previously described (Miyoshi et al., Science 283, 682-686, 1999).

[0088] *Integration Site Analysis:* Processing of gDNA to amplify integration loci included modified genomic sequencing (MGS)-PCR, followed by Illumina paired-end Miseq next generation sequencing. Integration sites were identified by a method similar to that described by Hocum *et al.* (BMC Bioinformatics 16, 212, 2015) using a published pipeline which includes custom scripts available upon request (Radtke et al., Sci. Transl. Med. 9, 2017). The *Homo sapiens* reference genome (GRCh38/hg38, GCA_000001405.15, Dec. 2013) provided by the Genome Reference Consortium was downloaded from the UCSC genome browser. Resulting files were parsed for multiple possible alignments for each sequence read, such that any sequence read with a secondary alignment with percent identity up to 95% of the best alignment was discarded. Sequence reads were then grouped based on their genomic alignment positions and orientation (sense (+) vs. antisense (-)). Any alignments within 5 base pairs of one another with identical orientations were considered to originate from the same integration event; the genomic position with the greatest number of contributing sequence reads was defined as the integration locus. A custom Python script was used to localize integration sites to genomic features using Refseq gene lists available from the UCSC genome browser and to known oncogenes using COSMIC: catalog of somatic mutations in cancer.

[0089] *BLaM Assay:* UCB- or mPB-derived CD34⁺ cells were pre-treated for 4 h with DMSO or caraphenol A, then transduced with BLaM-Vpr-LV (MOI=15) for an additional 6 h in the presence of DMSO or caraphenol A. Cells were then washed and resuspended in 125 µl loading medium (IMDM containing 20% BIT9500 or SCGM, no antibiotics). BLAM substrate loading and cellular analyses were completed, as previously reported (Swan et al., supra). The BLaM Vpr kinetic assay was developed from methods previously described

(Miyanchi et al., supra). Briefly, cells were treated as per the standard method, then transitioned to 4°C at indicated timepoints; compounds and vector were then washed out and samples were loaded as above for 8 h at 12°C, before processing for flow cytometry.

[0090] *IFITM overexpression lines:* pQCXIP-FLAG-IFITM1, -IFITM2, -IFITM3, and -IFITM3 Δ 17-20 (gifts from C. Liang), were transfected into 293T cells with Mirus TransIT-LT1. Stably expressing cells were created following selection with puromycin for two weeks.

[0091] *Western Blotting:* 5×10^4 LV transduced or control HeLa cells were lysed directly in wells on ice with 75 μ L of RIPA buffer (CAT# 89900) plus Proteinase Inhibitor (CAT# 87785). Lysates were measured for protein concentration with Pierce BCA Protein Assay Kit (CAT# 23227) and normalized. Samples were processed with LDS Sample Buffer (CAT# B0007) and Sample Reducing Agent (CAT# B0004). Lysates were run on 4-12% Bis-Tris Bolt Precast Gel from Invitrogen (Cat# NW04120BOX) and transferred to an activated PVDF FL membrane. Membranes were incubated with dilutions of the following primary antibodies: anti-IFITM2 (Proteintech, 66137-1-Ig), anti-IFITM3 (Abcam, ab109429), anti-IFITM2/3 (Proteintech, 66081-1-Ig). Membranes were then incubated with an HRP secondary and exposed to film.

[0092] *TZM-bl IFITM3 KO Cell Line:* IFITM3 KO TZM-bl cells (Platt et al., J. Virol. 72, 2855-2864, 1998) were created by transfection with a set of plasmids encoding Cas9 and three IFITM3-specific guide RNAs (sc-403281, Santa Cruz Biotechnology) and a set of three plasmids providing templates for homology-directed repair (sc-403281-HDR, Santa Cruz Biotechnology). A population of modified cells was selected following puromycin treatment for three weeks.

[0093] *IFITM Intracellular Analysis:* Cells were fixed/permeabilized with Cytofix/CytoPerm reagent from BD (CAT#554714) for 20 min and washed in Perm/Wash buffer from BD (CAT#554723). Cells were pelleted and resuspended in anti-IFITM2/3 antibody diluted in Perm/Wash buffer, incubated at room temperature for 30 min, and washed in Perm/Wash buffer. Cells were acquired and analyzed on a LSRFortessa (BD).

[0094] *Confocal microscopy of HeLa:* 1×10^5 HeLa cells were plated on poly-L lysine coated coverslips, 1 day after plating, cells were pre-treated for 4 h with the indicated doses of compounds, before addition of LV at a MOI of 15. Cells were treated with LV for 30 min or 2 h in 48 well plates, before washing 3 times in 2%FBS + PBS buffer to remove attached surface bound LV. Cells were then fixed in 3.7% confocal grade paraformaldehyde

(ThermoFisher Scientific, CAT# 50-980-487,) for 8 min and washed 3 times with confocal wash buffer (PBS + 0.1% saponin and 1% BSA). After fixing, cells were treated overnight at 4 °C with mouse anti-IFITM2/3, rabbit anti-LAMP1 Antibody (ThermoFisher Scientific, CAT# PA1-654A) both diluted 1:400 and goat anti-HIV p24 (Abcam, CAT# ab53841) diluted 1:100 in confocal wash buffer. After incubation, slides were again washed 3 times with wash buffer and incubated for 1hr with Alexa Fluor® 488 donkey anti-mouse antibody (ThermoFisher Scientific, CAT# A-21202), Alexa Fluor® 568 donkey anti-goat antibody (ThermoFisher Scientific, CAT# A-11057) and Alexa Fluor® 647 Donkey anti-rabbit IgG (minimal x-reactivity) Antibody (BioLegend, CAT# 406414) at 1:200 at room temperature. After antibody treatment, slides were again washed 3 times with wash buffer then treated for 30 min with Hoechst 33342 nuclei stain (ThermoFisher Scientific, H3570) at 1:2000 dilution, washed again 3 times and mounted on slides for 3 d with ProlongGold Antifade Reagent (ThermoFisher, CAT# P36930). Confocal microscopy analysis of vector and endosomal markers was performed using a Zeiss 710 laser scanning confocal microscope at 64× oil-immersion magnification at a 2-fold digital zoom with a 1024×1024 field size. For establishing voltage and aperture for confocal microscopy, snap images were captured for each channel using control slides treated only with secondary antibody or no vector added. Z-stack depth was set using the Hoechst channel, with images collected in 16 slice intervals, 0.2µm per image, collected at ~1 image per min. At least 50 cells total were imaged for each condition. Z-stacks were analyzed with Imaris Software (Bitplane, Zurich, Switzerland) using the ImarisCell analysis module, quantifying number and intensity of IFITM2/3 and LAMP-1⁺ vesicles. Cell boundaries were identified using the 647 channel, with thresholding set by untreated or secondary antibody treated control images. Statistical analysis of vesicles number and average staining intensity was conducted using GraphPad Prism 7 software (GraphPad, San Diego, CA), and evaluated with the Kruskal-Wallis test using Dunn's multiple comparison correction.

[0095] *Confocal Microscopy of mPB CD34⁺ Cells:* Mobilized peripheral blood CD34⁺ cells were thawed and pre-stimulated, then plated in 48 well plates and treated with compound as described above. 1×10⁵ CD34⁺ cells were transduced per vector and compound condition. After washing, cells were spun via cytospin onto positively charged confocal grade microscopy slides at 450xg for 5 min. After cytospin, cells were fixed, treated with appropriate antibodies, and imaged as described above for HeLa cells, excluding nuclei stain.

[0096] *LysoSensor*: HeLa cells were split at 5×10^4 cells per well in a 96-well plate. Cells were cultured overnight then treated with LysoSensor Yellow-Blue Dextran (Life Technologies L-22460) at 1mg/mL for 2 h. At time point 0, LysoSensor and molecule were added to wells to make a final concentration of 1mg/mL LysoSensor. Cells were trypsinized and transferred directly to FACs buffer at various time points. Flow cytometry was performed on a BD LSR-II.

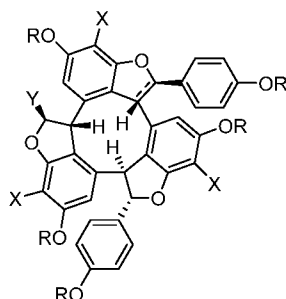
[0097] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

[0098] All publications, databases, GenBank sequences, patents, and patent applications cited in this specification are herein incorporated by reference as if each was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for enhancing transduction efficiency of a retroviral vector into a host cell, comprising:

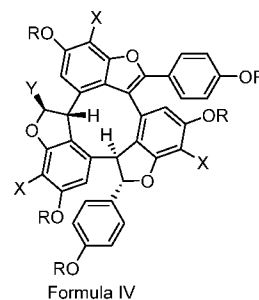
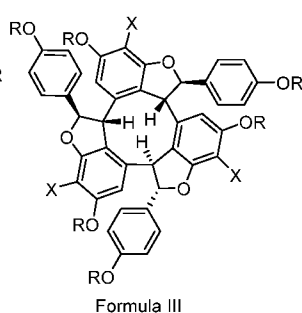
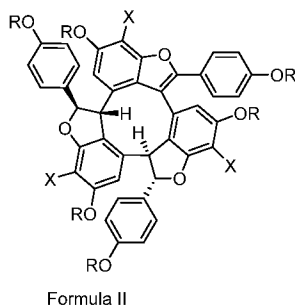
(a) contacting the cell with resveratrol or a resveratrol cyclotrimer compound of Formula I,



wherein each R is independently H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate; each X is independently H, halogen, alkyl, aryl, or a halogenated intermediate made by electrophilic aromatic substitution; Y is any aromatic ring including heteroaromatic; and

(b) transducing the cell with the vector; thereby enhancing transduction efficiency of the retroviral vector into the host cell.

2. The method of claim 1, wherein the resveratrol cyclotrimer compound has a structure shown in Formula II, Formula III or Formula IV below, wherein each R is independently H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate; each X is independently H, halogen, alkyl, aryl, or a halogenated intermediate made by electrophilic aromatic substitution; and Y is any aromatic ring including heteroaromatic.



3. The method of claim 1, wherein R is the same at all places in the formula.

4. The method of claim 1, wherein X is the same at all places in the formula.
5. The method of claim 1, wherein Y is installed as a nucleophile.
6. The method of claim 1, wherein the resveratrol cyclotrimer compound is caraphenol A, α -viniferin or resveratrol, or an analog compound thereof.
7. The method of claim 1, wherein the host cell is contacted with the compound prior to, simultaneously with, or subsequent to being contacted with the vector.
8. The method of claim 1, wherein the viral vector is a recombinant retroviral vector, an adenoviral vector or an adeno-associated viral vector.
9. The method of claim 1, wherein the vector is a lentiviral vector.
10. The method of claim 1, wherein the vector is a HIV-1 vector.
11. The method of claim 1, wherein the host cell is a hematopoietic stem and progenitor cell (HSPC).
12. The method of claim 11, wherein the host cell is human or non-human primate CD34⁺ cell.
13. The method of claim 11, wherein the stem cell is isolated from umbilical cord blood, peripheral blood or bone marrow.
14. The method of claim 1, wherein the host cell is a non-hematopoietic cell.
15. The method of claim 1, wherein the compound is present during the entire transduction process or at specific intervals.
16. The method of claim 1, wherein the viral vector encodes a therapeutic agent.
17. The method of claim 1, wherein the viral vector is a non-integrating lentiviral vector.

- 18.** A kit for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery, comprising (a) a viral vector encoding the therapeutic agent, and (b) resveratrol or a resveratrol cyclotrimer compound of Formula I.
- 19.** The kit of claim 18, wherein the compound is caraphenol A, α -viniferin, resveratrol, or an analog compound thereof.
- 20.** The kit of claim 18, wherein the target cell is a hematopoietic stem and progenitor cell (HSPC) or a non-hematopoietic cell.
- 21.** The kit of claim 20, wherein the HSPC is human or non-human primate CD34⁺ cell.
- 22.** The kit of claim 18, wherein the viral vector is a recombinant retroviral vector, an adenoviral vector or an adeno-associated viral vector.
- 23.** The kit of claim 18, wherein the viral vector is a lentiviral vector.
- 24.** The kit of claim 18, wherein therapeutic agent is a polynucleotide agent or a polypeptide agent.
- 25.** The kit of claim 18, further comprising a target cell into which the therapeutic agent is to be delivered.
- 26.** The kit of claim 25, wherein the target cell is human CD34⁺ hematopoietic stem and progenitor cell.
- 27.** A method for identifying a resveratrol cyclotrimer compound with improved properties in enhancing retroviral transduction into a host cell, comprising (a) synthesizing one or more structural analogs of a lead resveratrol cyclotrimer compound selected from the group consisting of caraphenol A or α -viniferin, and (b) performing a functional assay on the analogs to identify an analog that has an improved biological or pharmaceutical property relative to that of the lead compound; thereby identifying a resveratrol cyclotrimer compound with improved properties in enhancing retroviral transduction into a host cell.

28. The method of claim 27, wherein the improved biological or pharmaceutical property is higher potency in enhancing retroviral transduction into CD34⁺ stem cells.

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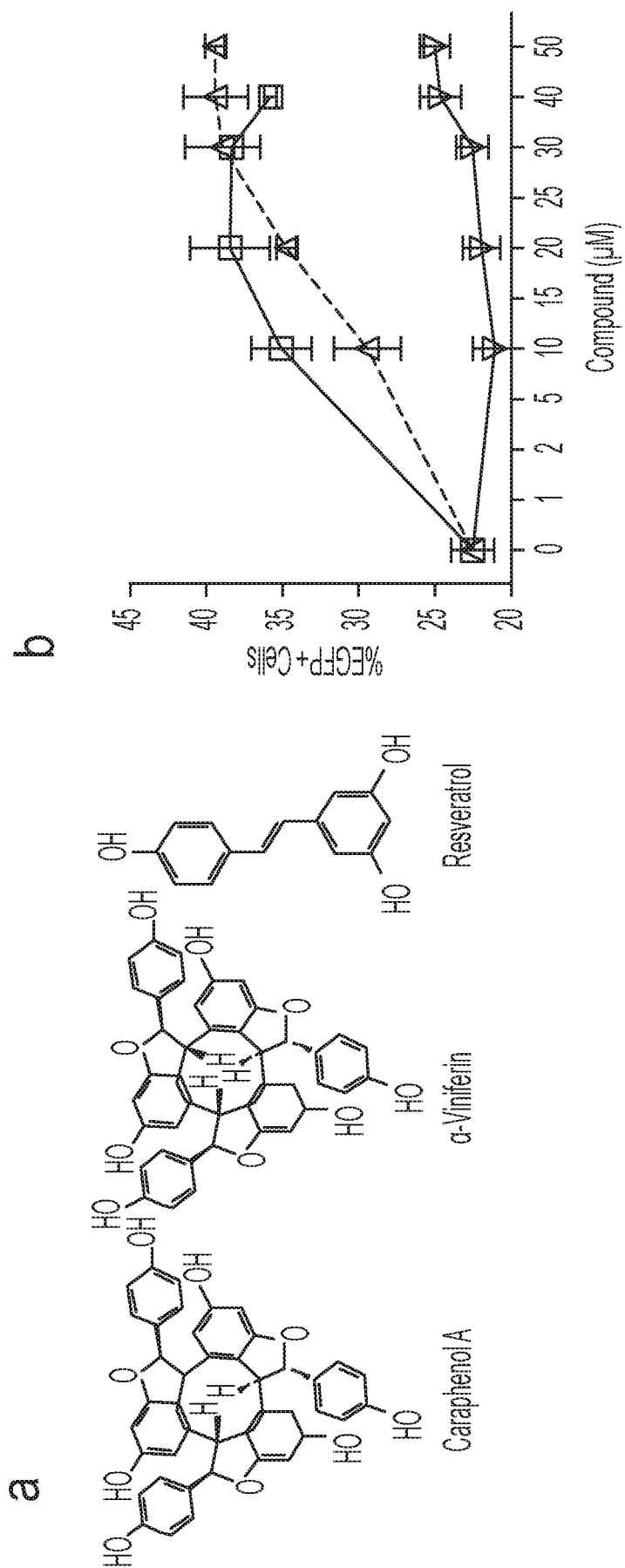


Figure 1

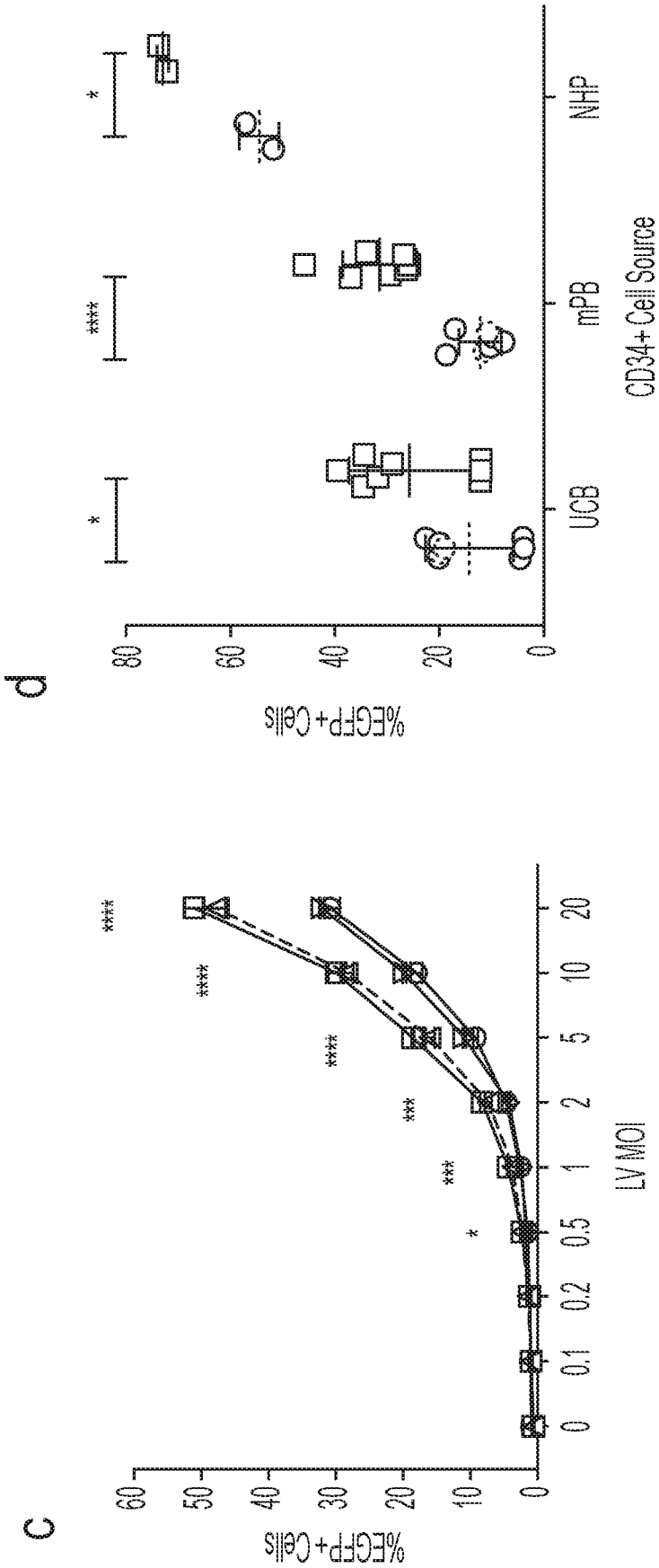


Figure 1
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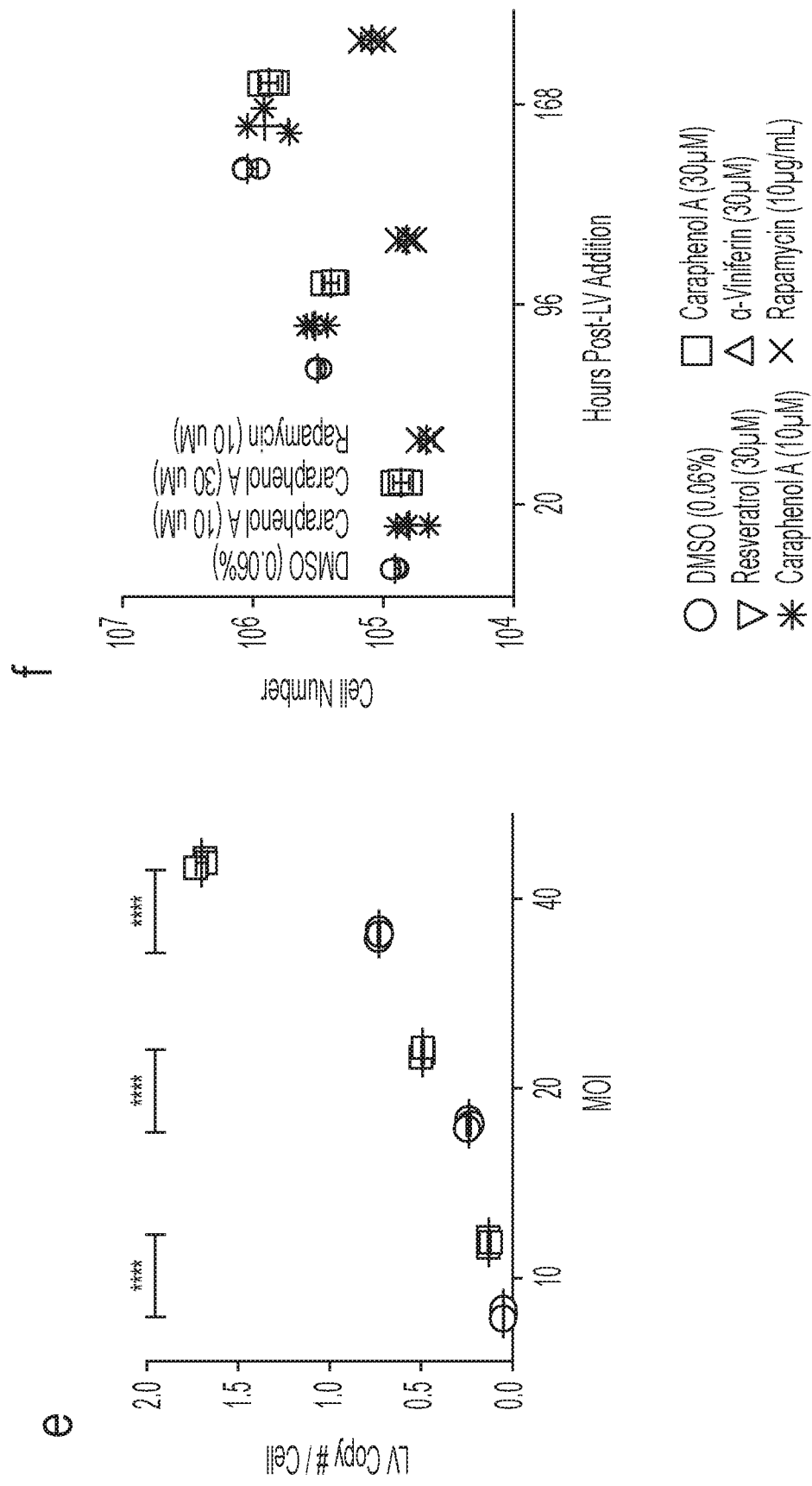


Figure 1
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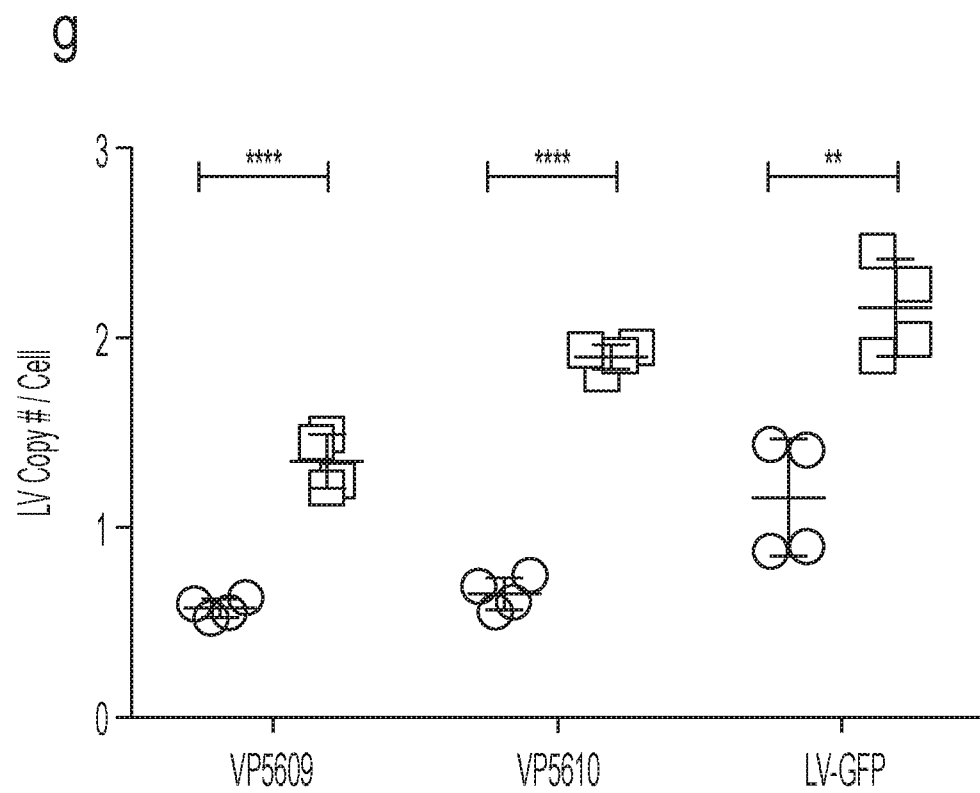


Figure 1
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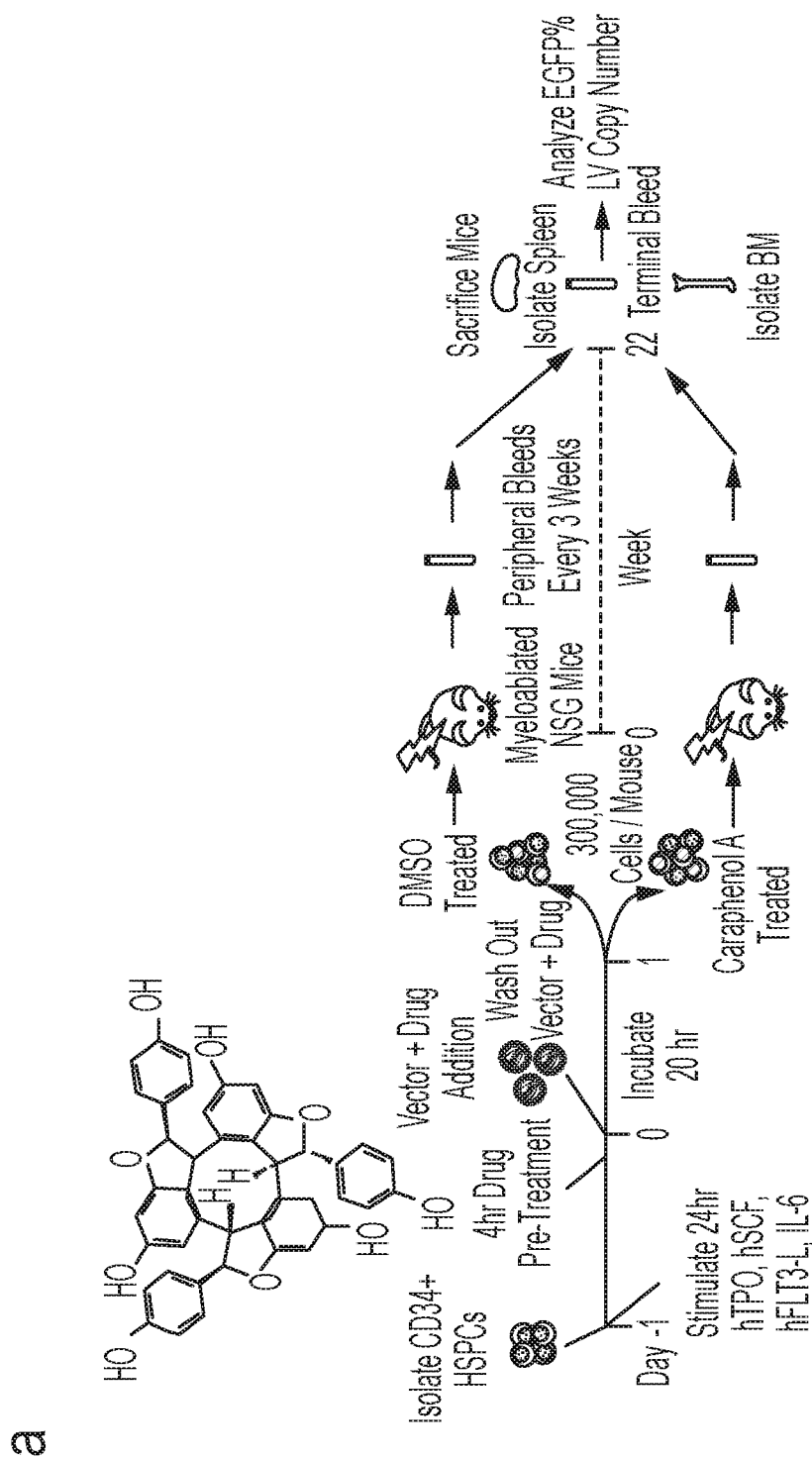


Figure 2

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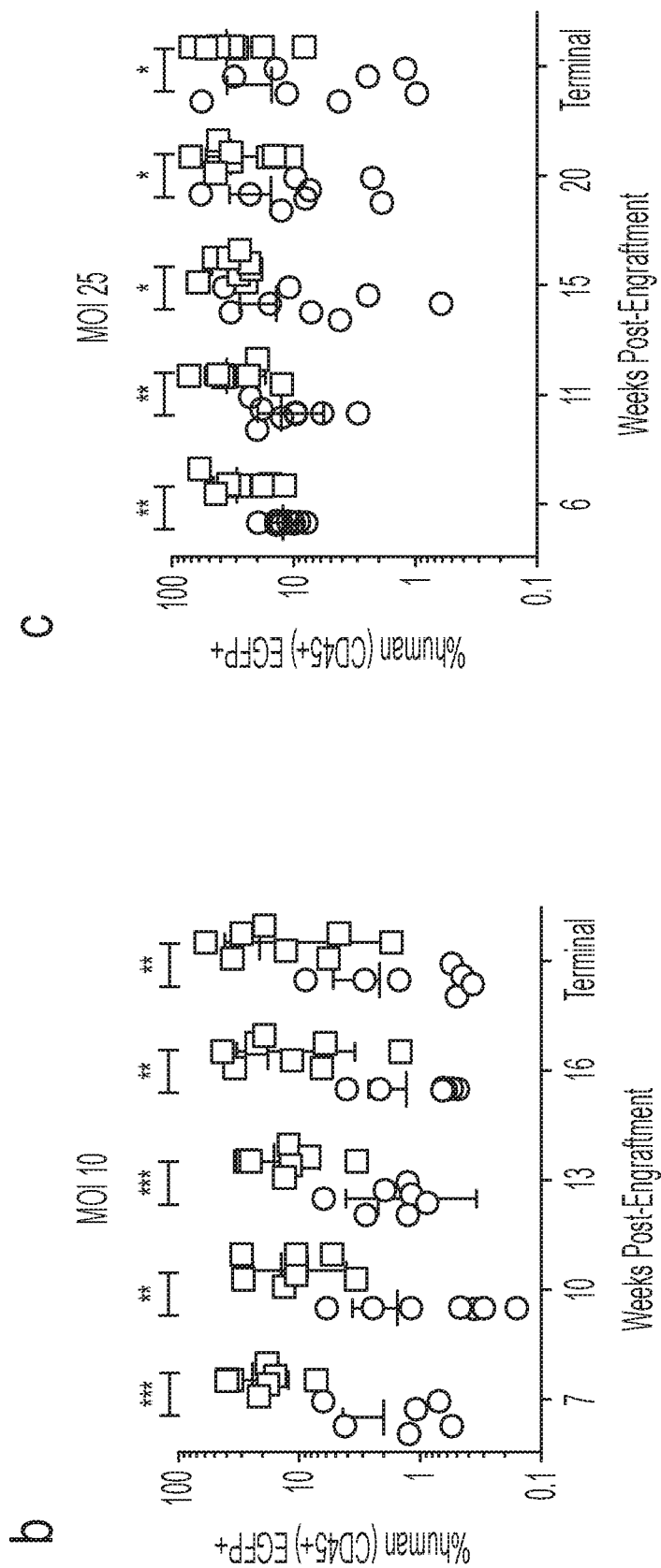


Figure 2
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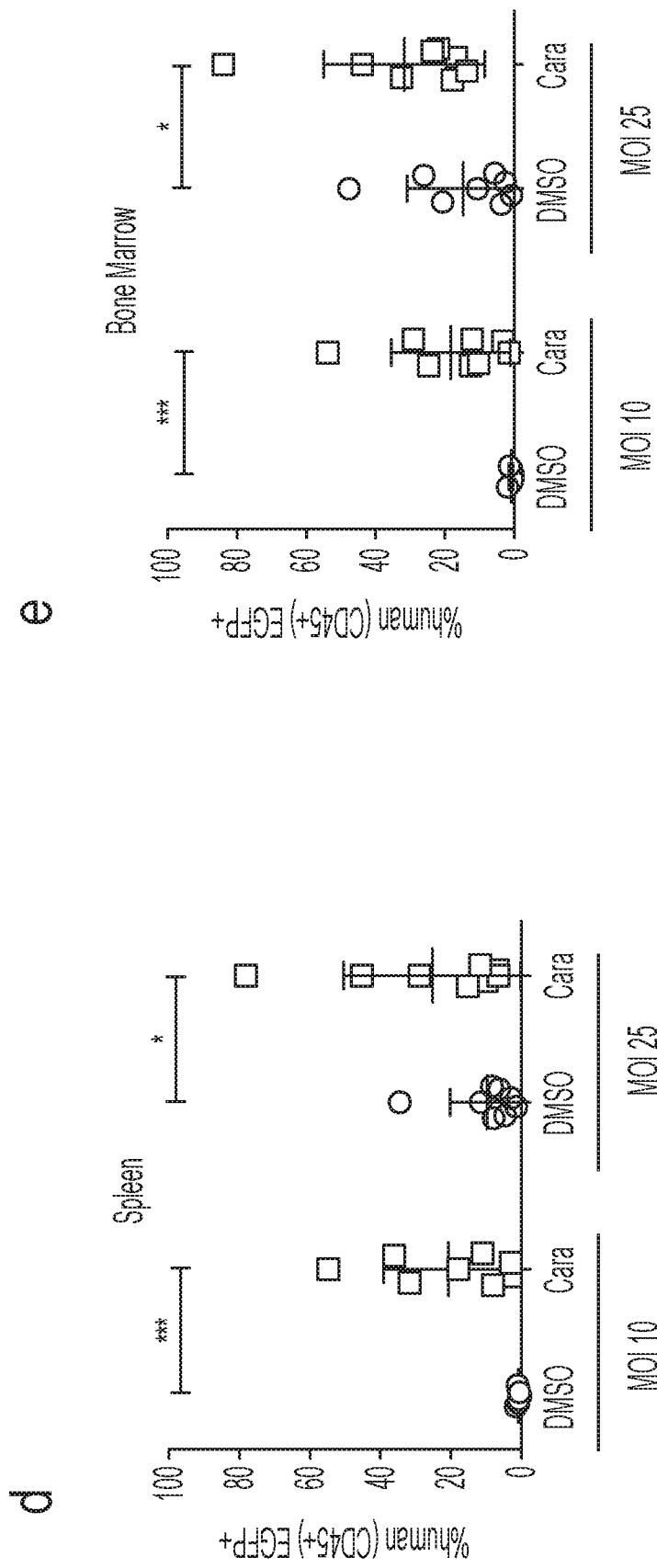


Figure 2
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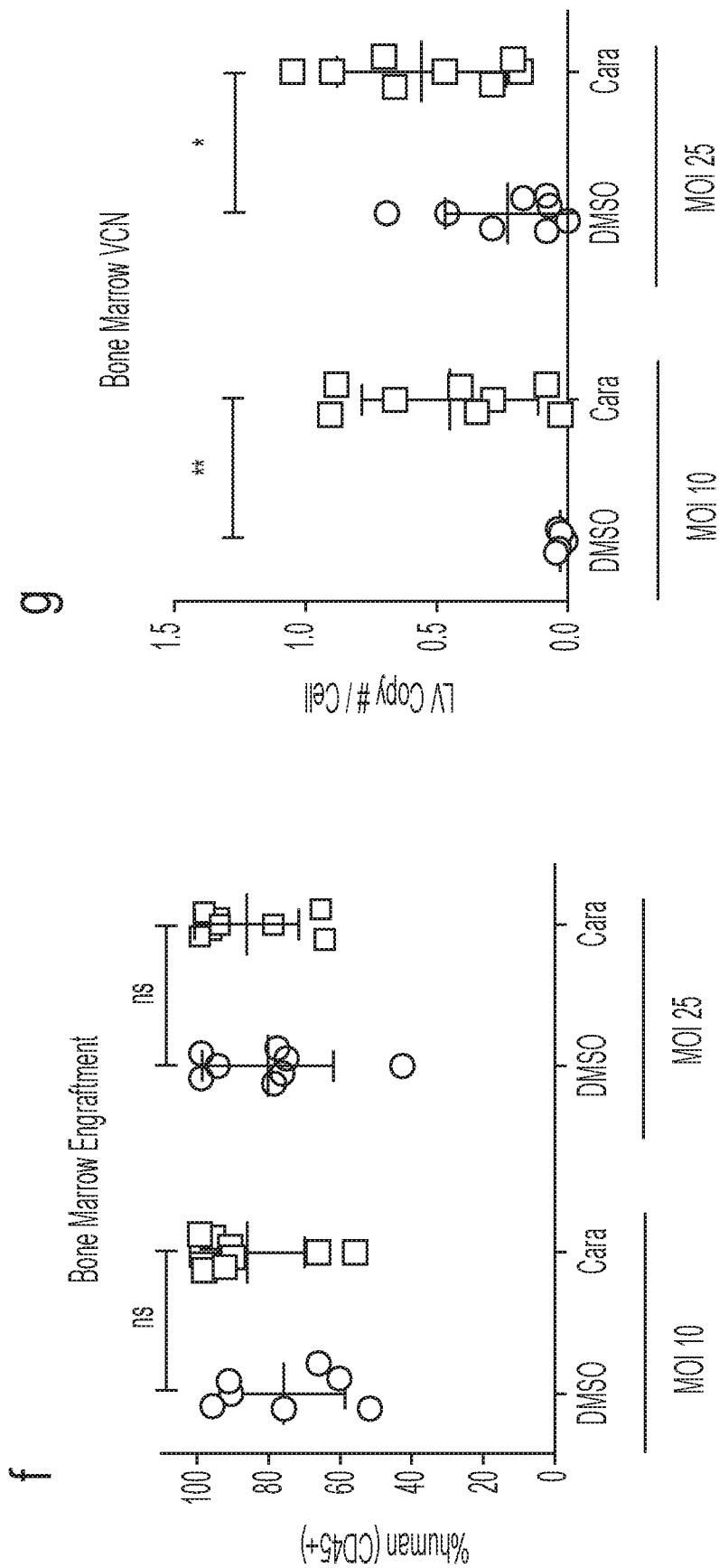


Figure 2
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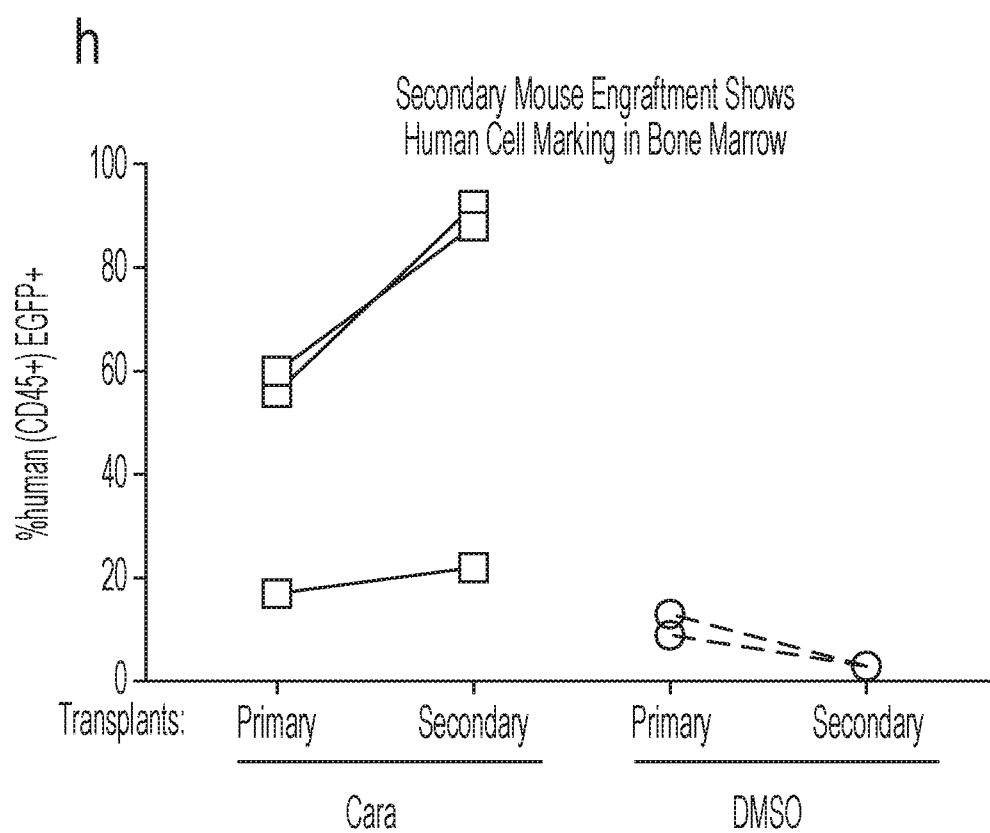


Figure 2
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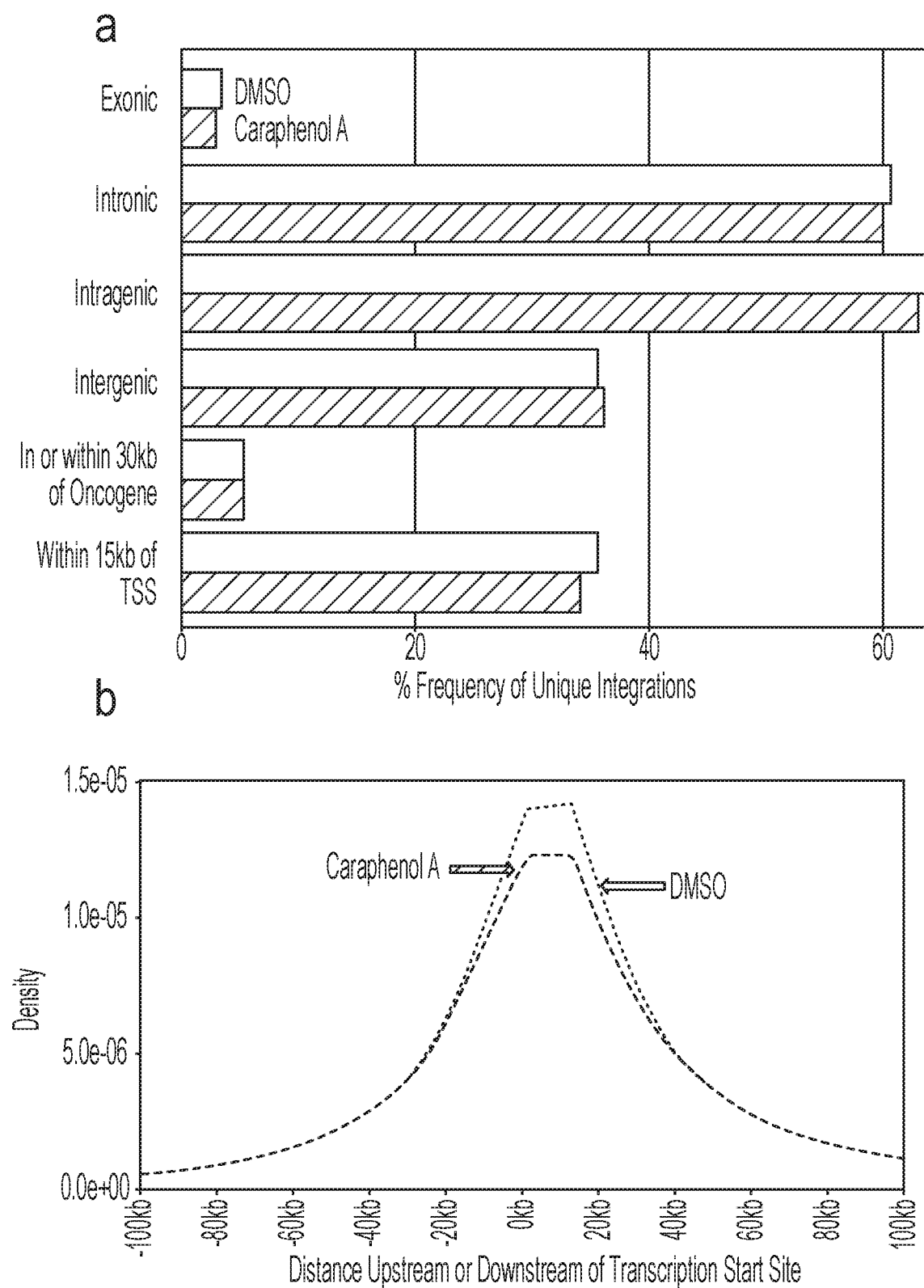


Figure 3

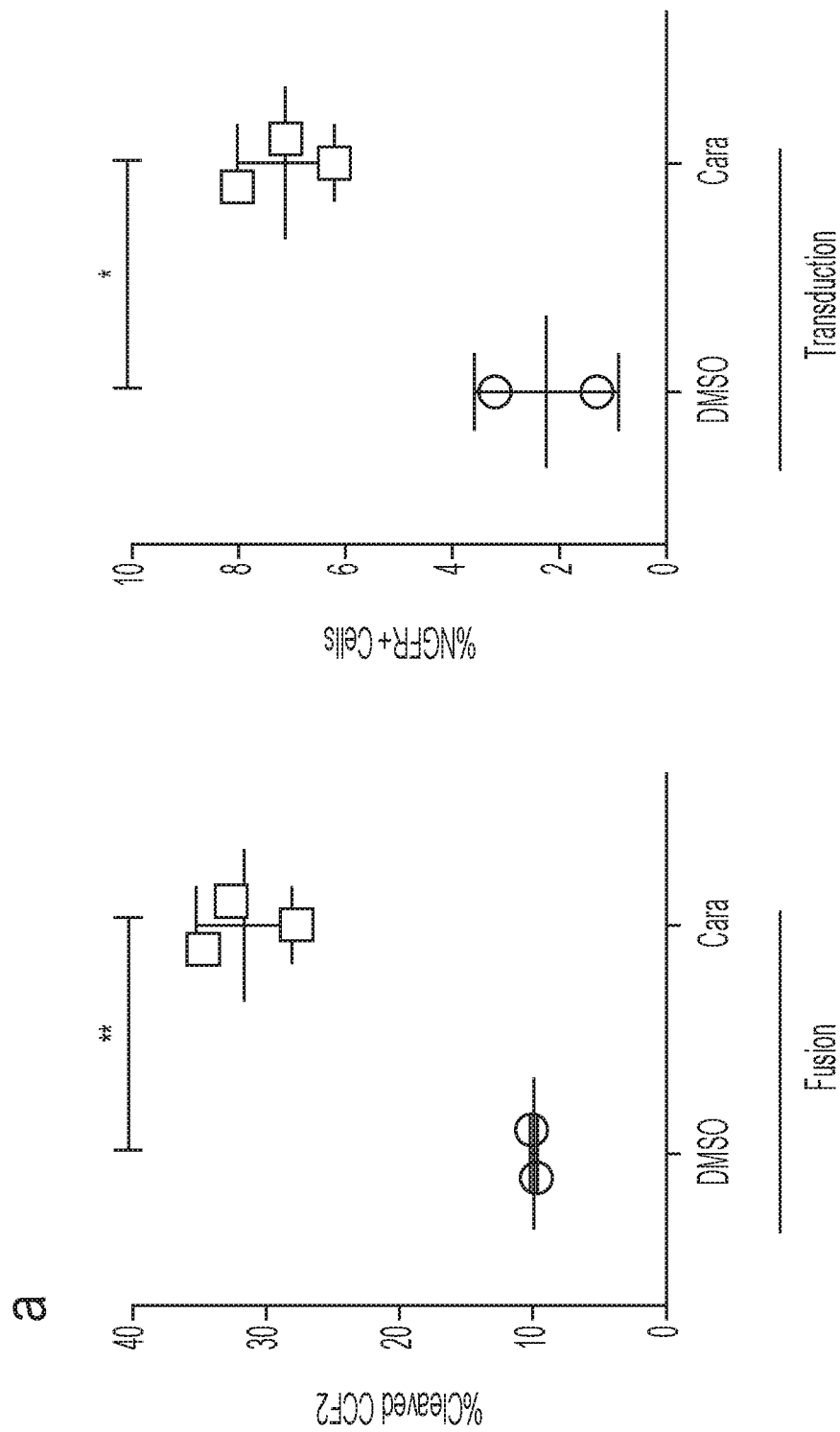


Figure 4

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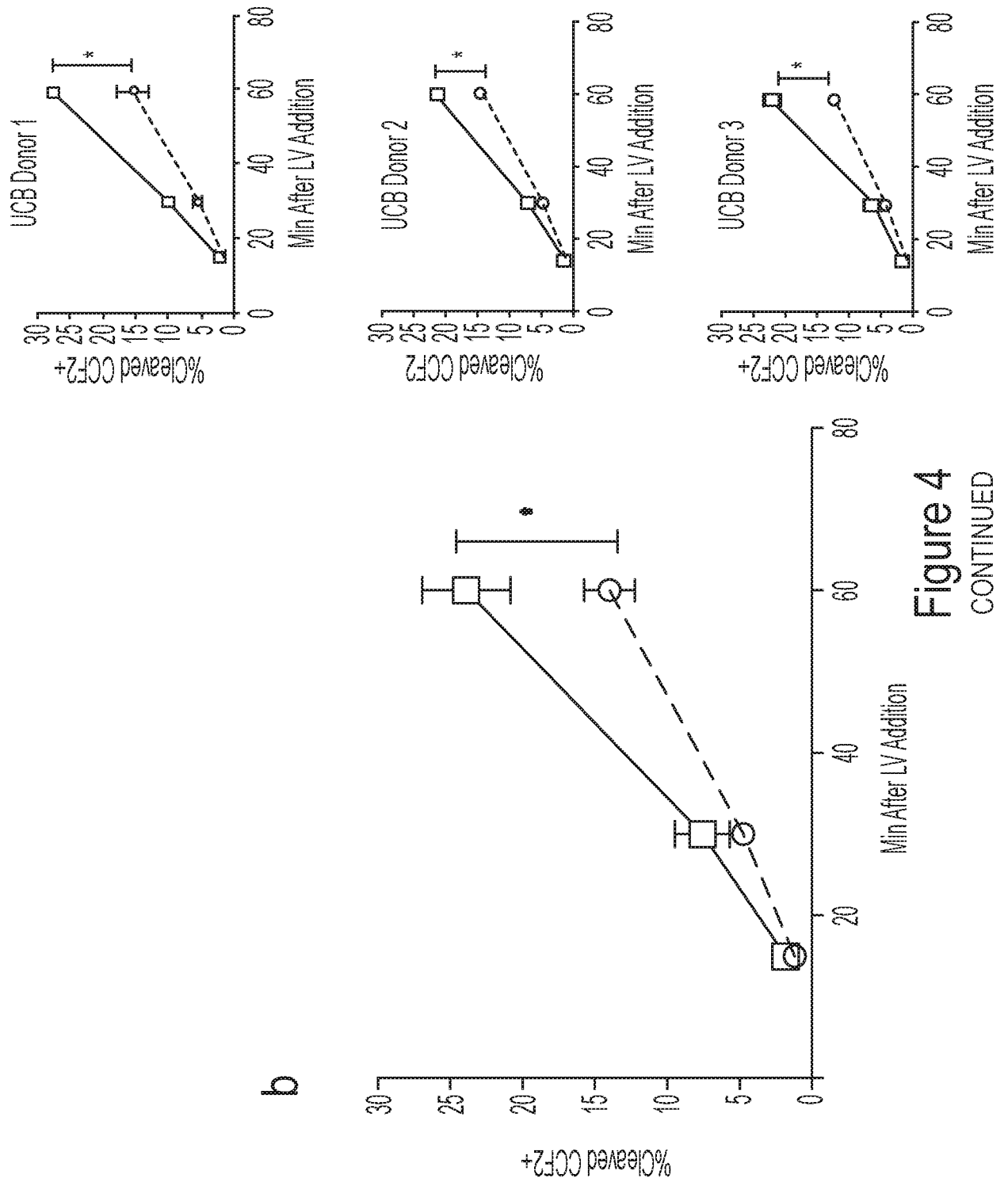


Figure 4
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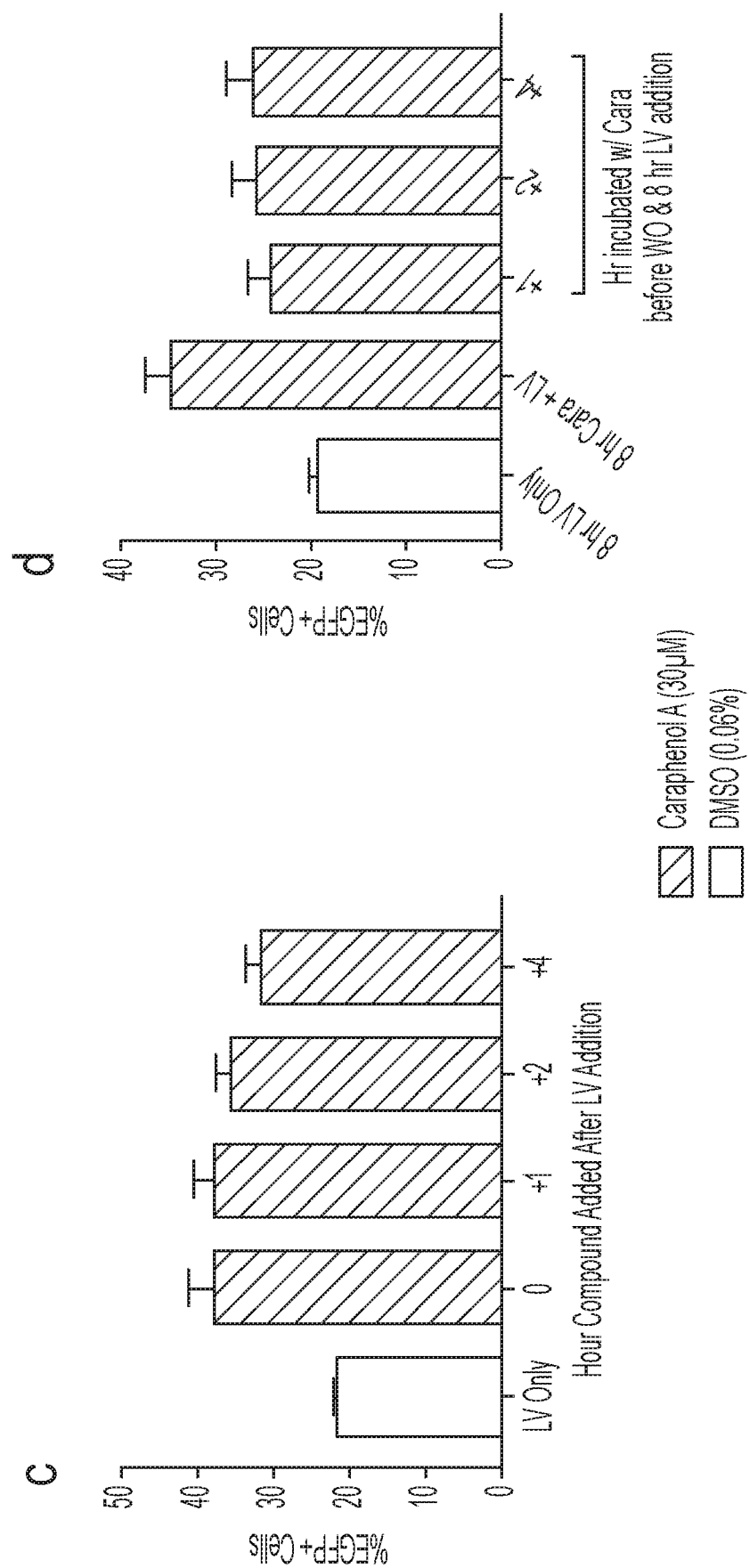
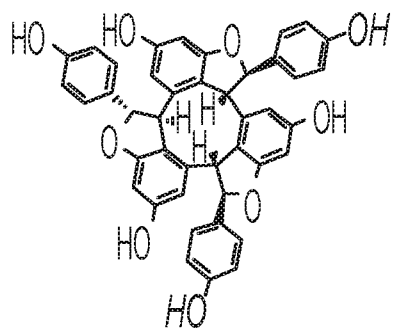


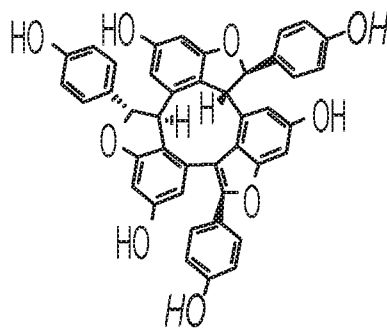
Figure 4
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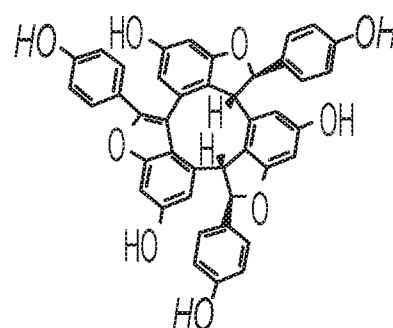
9-Membered Ring Containing Resveratrol Oligomers



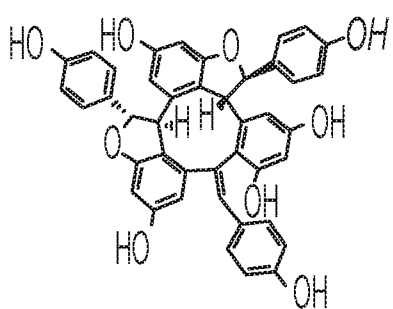
1: a-viniferin



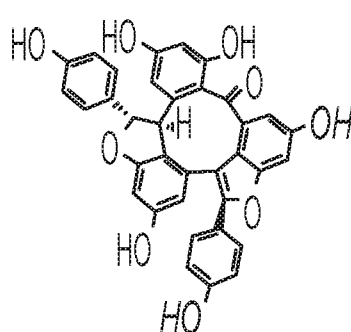
2: caraphenol A



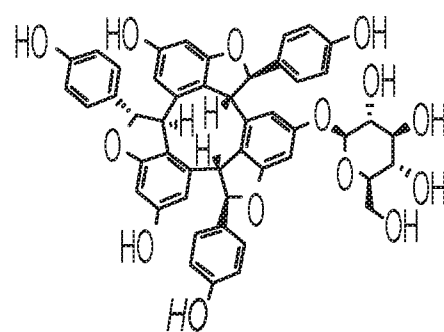
3: sophorastilbene A



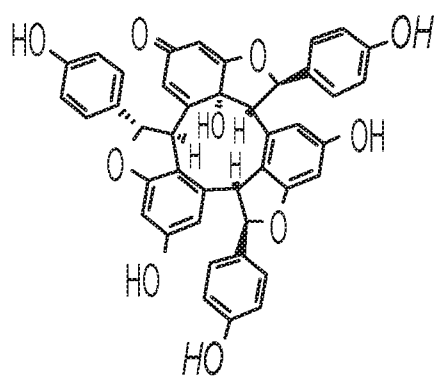
4: caragaphenol A



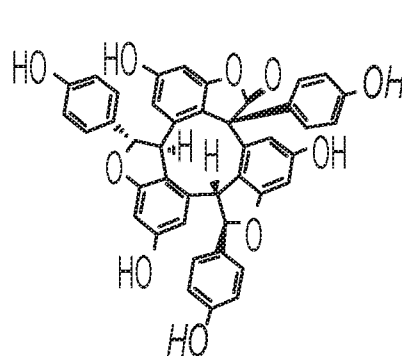
5: carasiphenol D



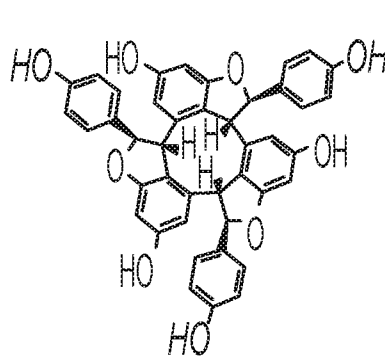
6: a-viniferin 13b-O-f3-glucopyranoside



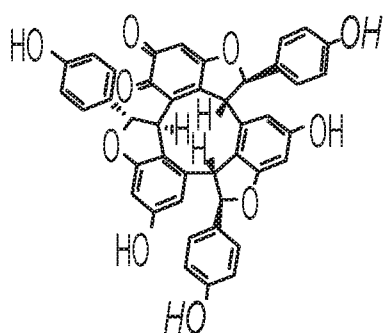
7: grandiphenol C



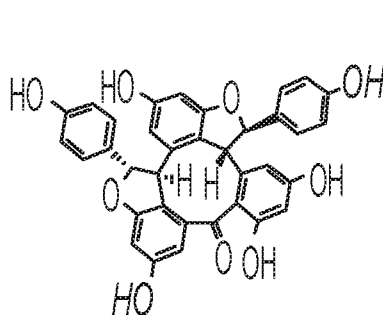
8: grandiphenol D



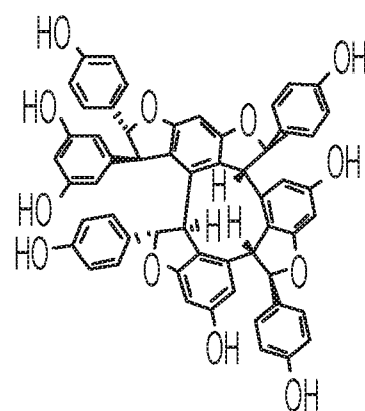
9: curcusinol



10: hopeanolin



11: hopeachinol



12: miyabenol B

Figure 5

α -V-iniferin enhances pRRL-SIN-MND-EGFP lentiviral vector transduction of adult CD34⁺ stem and progenitor cells

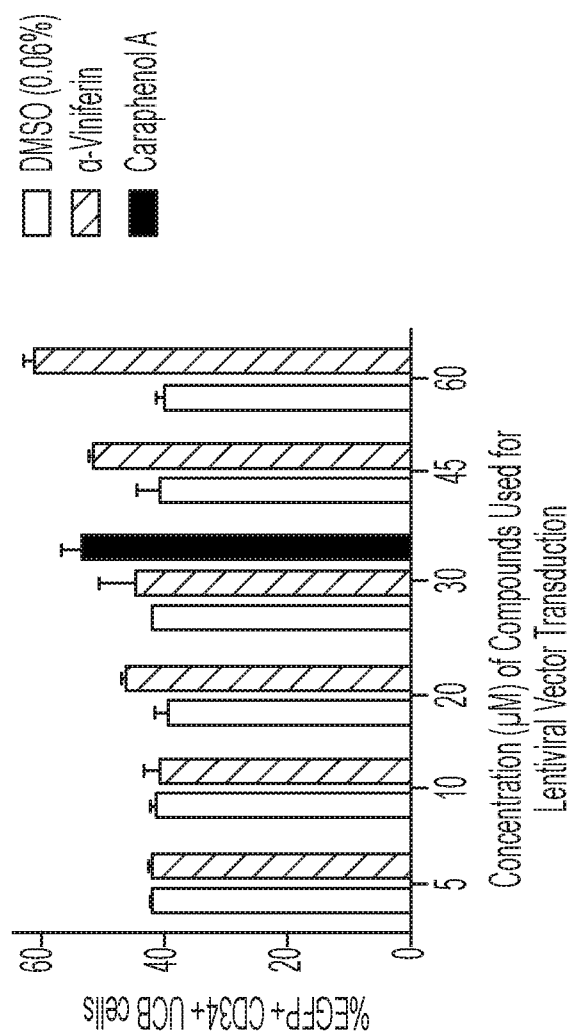


Figure 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/24651

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/86; C07C 15/52 (2020.01)

CPC - C12N 15/86; C07C 15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018/0339066 A1 (THE SCRIPPS RESEARCH INSTITUTE) 29 November 2018; paragraphs [0004], [0006]-[0009], [0055], [0065]	1, 6-26
Y	(JIN, HG et al.) 'Combining the mammalian target of rapamycin inhibitor, rapamycin, with resveratrol has a synergistic effect in multiple myeloma'; 05 March 2018, Oncology Letters; Volume 15, Issue 5, pages 6257-6264; abstract	1, 6-26
A	WO 2013/049364 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 04 April 2013; entire document	1, 6-26
A	US 2016/0201085 A1 (TORBETT, B et al.) 14 July 2016; entire document	1, 6-26
P, X	(OZOG, S et al.) 'Resveratrol trimer enhances gene delivery to hematopoietic stem cells by reducing antiviral restriction at endosomes'; 17 October 2019, Blood; Volume 134, Issue 16, pages 1298-1311; abstract only	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 May 2020 (08.05.2020)

Date of mailing of the international search report

16 JUL 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/24651

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---Continued Within the Next Supplemental Box---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 6-26

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US20/24651

-Continued from Box No. III Observations where unity of invention is lacking -

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid. Groups I+, Claims 1-26; and resveratrol (compound structure; are directed toward methods and kits for enhancing transduction efficiency of a retroviral vector into a host cell.

The methods and kits will be searched to the extent they encompass resveratrol (first exemplary compound structure). Applicant is invited to elect additional compound(s), with fully specified structure(s) thereof (e.g. no optional or variable atoms, bonds, or substituents) for each, to be searched. Additional compound(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part) and 6-36 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass resveratrol (compound structure). Applicants must specify the claims that encompass any additionally elected compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a compound of Formula I, wherein each R is H; each H is H; and Y is an aromatic ring (compound structure).

Group II, Claims 27-28 are directed toward a method for identifying a resveratrol cyclotrimer compound with improved properties in enhancing retroviral transduction into a host cell.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Groups I+ include resveratrol, not present in Group II; the special technical features of Group II include a method comprising performing a functional assay on analogs to identify an analog that has an improved biological or pharmaceutical property relative to that of a lead compound, not present in Groups I+.

Groups I+ and II share the technical features including: a resveratrol cyclotrimer compound.

However, these shared technical features are previously disclosed by the article entitled "Chemistry and Biology of Resveratrol-Derived Natural Products" by Keylor, et al. (hereinafter 'Keylor').

Keylor discloses a resveratrol cyclotrimer compound (resveratrol cyclotrimer shown as compound 6 in figure 3; page 8980, figure 3; page 8980, first column, first paragraph).

Groups I+ share the technical features including: a method for enhancing transduction efficiency of a retroviral vector into a host cell, comprising: (a) contacting the cell with resveratrol or a resveratrol cyclotrimer compound of Formula I, as shown, wherein each R is independently H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate; each X is independently H, halogen, alkyl, aryl, or a halogenated intermediate made by electrophilic aromatic substitution; Y is any aromatic ring including heteroaromatic; and (b) transducing the cell with the vector; thereby enhancing transduction efficiency of the retroviral vector into the host cell; a kit for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery, comprising (a) a viral vector encoding the therapeutic agent, and (b) resveratrol or a resveratrol cyclotrimer compound of Formula I.

However, these shared technical features are previously disclosed by US 2018/0339066 A1 (THE SCRIPPS RESEARCH INSTITUTE) (hereinafter 'Scripps') in view of the article entitled "Combining the mammalian target of rapamycin inhibitor, rapamycin, with resveratrol has a synergistic effect in multiple myeloma" by Jin, et al. (hereinafter 'Jin').

Scripps discloses a method for enhancing transduction efficiency of a retroviral vector into a host cell (methods for enhancing transduction efficiency of a retroviral vector into a stem cell (host cell); paragraph [0006]), comprising: (a) contacting the cell with a compound; and (b) transducing the cell with the vector; thereby enhancing transduction efficiency of the retroviral vector into the host cell (method comprises transducing the stem cell with the retroviral vector in the presence of a compound (contacting cell with a compound); paragraph [0006]), but Scripps does not disclose wherein the compound is resveratrol or a resveratrol cyclotrimer compound of Formula I, wherein each R is independently H, alkyl, acyl, silyl, carbonate, carbamate, sulfonate, or phosphonate; each X is independently H, halogen, alkyl, aryl, or a halogenated intermediate made by electrophilic aromatic substitution; Y is any aromatic ring including heteroaromatic. However, Jin discloses wherein a compound is resveratrol (method of using the compound resveratrol in cancer therapy; abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method, as previously disclosed by Scripps, in order to have provided wherein a compound is resveratrol, as previously disclosed by Jin, for providing pharmaceutical compositions and compounds useful in the inhibitor of mTOR complexes for treating cancer (Jin; abstract; Scripps; paragraphs [0006], [0057]). Further, Scripps discloses a kit for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery (kits for delivering a therapeutic agent into a target cell with enhanced targeting frequency and payload delivery; paragraph [0009]), comprising (a) a viral vector encoding the therapeutic agent (kit comprises a viral vector encoding the therapeutic agent; paragraph [0009]), and (b) a compound, but Scripps does not disclose wherein the compound is resveratrol or a resveratrol cyclotrimer compound of Formula I. However, Jin discloses wherein a compound is resveratrol (method of using the compound resveratrol in cancer therapy; abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the kit, as previously disclosed by Scripps, in order to have provided wherein a compound is resveratrol, as previously disclosed by Jin, for providing pharmaceutical compositions and compounds useful in the inhibitor of mTOR complexes for treating cancer (Jin; abstract; Scripps; paragraphs [0006], [0057]).

Since none of the special technical features of the Groups I+ and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Keylor reference, unity of invention is lacking.