GENE EDITING FOR LATENT HERPES SIMPLEX VIRUS INFECTION REDUCES VIRAL LOAD AND SHEDDING IN VIVO

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/568996, filed March 22, 2024, the disclosure of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing XML associated with this application is provided in XML format and is hereby incorporated by reference into the specification. The name of the XML file containing the sequence listing is 1896-P97WO_Sequence_Listing.xml. The XML file is 78,240 bytes; was created on March 19, 2025; and is being submitted electronically via Patent Center with the filing of the specification.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

[0003] This invention was made with Government support under AI132599 awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

[0004] HSV infections can cause recurrent orofacial, corneal, anogenital, or other lesions, and infections of newborns can lead to disseminated disease and devastating neurological sequelae. Genital infection with HSV-2 increases the risk of acquisition of HIV and is a major driver of the global HIV pandemic. Current antiviral therapy can treat acute episodes and suppress outbreaks but does not cure established infection. Recurrent outbreaks result from the ability of HSV to establish latent infection within ganglionic neurons innervating the affected sites. Latent HSV in ganglia is unaffected by traditional antivirals, explaining the inability of antivirals to cure, and reactivations typically commence again once therapy is stopped.

[0005] A promising potentially curative strategy involves gene editing directed at latent HSV itself. In a recent study, AAV-delivered meganucleases eliminated over 90% of HSV-1 genomes from the superior cervical ganglia (SCV) of latently infected mice. Despite this impressive reduction in ganglionic HSV loads after gene editing, the relevance that such reduction would have for human HSV infection is uncertain. Infected persons are typically not concerned with ganglionic viral loads *per se*,

but instead about symptomatic disease and/or viral shedding, and the associated risk of transmission to others. Existing mouse models are limited in their ability to address these issues, since latently infected mice rarely spontaneously reactivate HSV or show viral shedding at peripheral tissues.

[0006] In view of the limitations of the present art, a need remains for antiviral therapies that reduce or eliminate the latent virus that causes recurrent disease. The present disclosure addresses these and related needs.

SUMMARY

[0007] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[0008] In one aspect, the present disclosure provides a method of treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type I(HSV-1) reactivation in a subject.

[0009] In some embodiments, the method comprises administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

[0010] In some embodiments, the at least one HSV-l-specific meganuclease is configured to induce one or more DNA double strand breaks (DSB) in one or more genes in the genome of the HSV. In some embodiments, the at least one HSV-1 specific meganuclease is configured to target a duplicated region of the HSV-1 genome. In some embodiments, the one or more adeno-associated viral vector further comprises a neuron specific promoter. In an embodiment, the one or more adeno-associated viral vector (AAV) serotypes is selected from AAV7, AAV9, AAV-DJ, AAV-DJ/8, AAV-Rh10, or a combination thereof.

[0011] In some embodiments, the HSV-1-specific meganuclease is selected from HSV-1m4, HSV-1m5, HSV-1m8, or a combination thereof.

[0012] In an embodiment, the latent HSV-1 infection is an ocular or genital HSV-1 infection.

[0013] In some embodiments, the one or more adeno-associated viral vector (AAV) serotype is AAV9 or AAV-DJ/8, and the at least one HSV-1-specific meganuclease is HSV-1m5.

[0014] In some embodiments, the composition comprises of a plurality of a single adeno-associated viral vector (AAV) serotype, each of the plurality of single AAV comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In an embodiment, the AAV serotype is AAV9, and the HSV-1-specific meganuclease is HSV-1m4.

[0015] In yet another embodiment, the method comprises administering to the subject a composition comprising a plurality of at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each viral vector serotype comprises a nucleic acid sequence encoding the same or a different HSV-1-specific meganuclease. In an embodiment, the at least three adeno-associated viral vector (AAV) serotypes comprise AAV9, AAV-DJ/8, and AAV-Rh10 and each comprising a nucleic acid sequence encoding an HSV-1-specific meganuclease. In some embodiments, the HSV-1-specific meganuclease is HSV-1m4, HSV-1m5, or a combination thereof.

[0016] In an embodiment, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining HSV. In an embodiment, the reduction in ganglionic HSV load in SCG is about 73% to about 95% and in TG is about 43% to about 48% as compared to an untreated subject.

[0017] In yet another embodiment, the recurrent latent HSV infection is a genital infection. In an embodiment, the composition is effective in reducing ganglionic HSV load in Dorsal root ganglion (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0018] In some embodiments, the method comprises administering to the subject a composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1

specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are HSV-1m5 and HSV-1m8, respectively. In some embodiments, the composition is administered intravenously or transdermally.

[0019] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV. In an embodiment, the reduction in ganglionic HSV load in SCG is about 89% to about 98% and in TG is about 42% to about 61%, as compared to an untreated subject.

[0020] In yet another embodiment, the recurrent latent HSV infection is a genital infection and the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV. In an embodiment, the reduction in ganglionic HSV load in DRG is about 97% to about 98% as compared to an untreated subject.

[0021] In some embodiments, the composition is administered at a total dose selected from about 1.8 to about 5.4×10^{13} vg/kg to the subject. In some embodiments, the administered total dose is effective in a dose-dependent (i) reduction in ganglionic HSV loads; and (ii) reduced peripheral viral shedding in the subject as compared to an untreated subject.

[0022] In some embodiments, the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject. In some embodiments, the composition is associated with improved tolerability with no hepatotoxicity or neurotoxicity in the subject. In yet another embodiment, the method is effective in preventing transmission of HSV-1 to a new subject.

[0023] In some embodiments, the subject is a mammalian subject. In some embodiments, the mammalian subject is human.

[0024] In some embodiments, the reactivation of latent HSV-1 is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is a BET protein inhibitor. In some embodiments, the BET protein inhibitor is administered prior to, concomitant with, or after administration of the compositions disclosed herein. In some embodiments, the BET protein inhibitor is JQ1.

[0025] In yet another aspect, the present disclosure provided a method for reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in a subject. In some embodiments, the method comprises administering to the subject a composition comprising at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each of the at least three different adeno-associated viral vector serotypes comprise a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

[0026] In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In an embodiment, the first and the second HSV-l-specific meganucleases are HSV-1m5 and HSV-1m8, respectively. In some embodiments, the adeno-associated vector further comprises a neuron specific promoter.

[0027] In some embodiments, the composition is administered intravenously or transdermally.

[0028] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In an embodiment, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0029] In an embodiment, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral

load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0030] In some embodiments, the method is effective in reducing or eliminating potential transmission to a new host/subject.

[0031] In some embodiments, the reactivation is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is a BET protein inhibitor. In some embodiments, the BET protein inhibitor is administered prior to, concomitant with, or after administration of the compositions disclosed herein.

[0032] In another aspect, the present disclosure pertains to a method for reducing or preventing hepato- and neurotoxicity associated with the administration of AAV-delivered gene editing for treatment of a recurrent infection associated with latent HSV-1 reactivation. In some embodiments, the method comprises administering to the subject a composition comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding an HSV-1-specific meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the adeno-associated viral vector comprising a nucleic acid sequence encoding the HSV-1-specific meganuclease targeting a duplicated region of the HSV-1 genome further comprises a neuron specific promoter. In some embodiments, the one or more adeno-associated viral vector (AAV) serotype is AAV9. In some embodiments, the HSV-1-specific meganuclease is HSV-1m4.

[0033] In some embodiments, the recurrent infection associated with latent HSV reactivation is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0034] In some embodiments, recurrent infection associated with latent HSV reactivation is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0035] In some embodiments, the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject. In some embodiments, the composition is effective in

reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in the subject.

[0036] In some embodiments, the reactivation of the latent HSV-1 is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is administered prior to, concomitant with, or after administration of the composition comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the plurality of single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the latency reversing agent is a BET protein inhibitor.

[0037] In another aspect, the present disclosure provides a method for reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in a subject. In some embodiments, the method comprises administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

[0038] In an embodiment, the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each of the at least three different adeno-associated viral vector serotypes comprise a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In some embodiments, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

[0039] In some embodiments, the adeno-associated vector further comprises a neuron specific promoter.

[0040] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0041] In some embodiments, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0042] In some embodiments, the method is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject.

[0043] In some embodiments, the reactivation is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is administered prior to, concomitant with, or after administration of the compositions disclosed herein. In some embodiments, the latency reversing agent is a BET protein inhibitor.

[0044] In yet another aspect, the present disclosure provides a composition comprising a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each adeno-associated viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In some embodiments, the adeno-associated viral vector further comprises a neuron specific promoter. In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

[0045] In some embodiments, the composition is effective for reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1

(HSV-1) reactivation in a subject. In some embodiments, the composition is effective in reducing or eliminating potential transmission to a new host/subject.

[0046] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0047] In some embodiments, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV

[0048] In some embodiments, the composition is administered intravenously or transdermally.

[0049] In some embodiments, the composition is administered prior to, concomitantly with, or after administration to the subject of an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is a BET protein inhibitor.

[0050] In another aspect, provided herein is a composition, comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the adeno-associated viral vector serotype further comprises a neuron specific promoter. In some embodiments, the plurality of the single adeno-associated viral vector (AAV) serotype consists of AAV9, and the HSV-1-specific meganuclease encoded by the at least one nucleic acid sequence is HSV-1m4.

[0051] The compositions disclosed herein are effective in treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type 1(HSV-1) reactivation in a subject. In some embodiments, the compositions are effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation.

[0052] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0053] In some embodiments, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0054] In some embodiments, the compositions of the present disclosure are effective in reducing or eliminating potential transmission to a new host/subject. In yet another embodiment, the compositions of the present disclosure are associated with improved tolerability and reduced hepato- and neurotoxicity.

DESCRIPTION OF THE DRAWINGS

[0055] The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

[0056] **FIGS. 1A-1E** show a decrease of ganglionic HSV loads from genital and ocular infection after meganuclease therapy using various AAV serotypes. Experimental timeline of vaginal (**FIG. 1A**) or ocular infection (**FIG. 1B**) and meganuclease therapy. RO, retroorbital; TV, tail vein. HSV loads in dorsal root ganglia (DRGs) from control (n = 7) and dual meganuclease treated (n = 4) mice vaginally infected with HSV-1; p = 0.001 (**FIG. 1C**). HSV loads in SCGs and TGs from control (n = 10) and dual meganuclease treated (n = 10) mice ocularly infected with HSV-1; p = 0.0046 and 0.0034 for SCG and TG, respectively (**FIG. 1D**). Gene editing at the m5 target site of residual virus quantified by T7E1 assay in SCG and TG from dual meganuclease treated mice (n =10) (**FIG. 1E**). Each graph shows individual and mean values with standard deviation, percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (unpaired one-tailed Mann-Whitney test with **: $p < 0.01$). AAV loads are shown in **FIGS. 18A-18B**.

[0057] **FIGS. 2A-2G** show decrease of peripheral virus shedding in meganuclease-treated mice. Experimental timeline of ocular infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 2A**), Experiment 1 (n = 10 per group). HSV loads in SCGs (**FIG. 2B**) (p = 0.0057) and TGs (**FIG. 2C**). Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (unpaired one-tailed Mann-Whitney test with ns: not significant, **: $p < 0.01$) are indicated. Gene editing at the HSV-1m5 (m5) target site of residual virus quantified by T7E1 assay in SCG and TG from dual meganuclease treated mice (**FIG. 2D**). HSV titers in eye swabs collected daily

from day 1 to 4 post JQ1 reactivation from control (**FIG. 2E**) and dual meganuclease-treated (**FIG. 2F**) infected mice. Panels 2i-k show data for both SCG and both TG from each mouse. Area under the curve (AUC) analysis (**FIG. 2G**) with *p* value (unpaired one-tailed Mann-Whitney test). AAV loads are shown in **FIGS18A-18D.** Each graph shows individual and mean values with standard deviation.

[0058] **FIGS. 3A-3G** show decrease of peripheral virus shedding in meganuclease-treated mice. Experimental timeline of ocular infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 3A**), Experiment 2 (n = 8 per group). HSV loads in SCGs (**FIG. 3B**) and TGs (**FIG. 3C**). Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (unpaired one-tailed Mann-Whitney test with ns: not significant, **: $p < 0.01$) are indicated. Gene editing at the m5 target site of residual virus quantified by T7E1 assay in SCG and TG from dual meganuclease treated mice (**FIG. 3D**). HSV titers in eye swabs collected daily from day 1 to 4 post JQ1 reactivation from control (**FIG. 3E**) and dual meganuclease-treated (**FIG. 3F**) infected mice. FIGS. 2B-2D show data for both SCG and both TG from each mouse. Area under the curve (AUC) analysis (g) with *p* value (unpaired one-tailed Mann-Whitney test). AAV loads are shown in **FIGS. 18E-18F**. Each graph shows individual and mean values with standard deviation.

[0059] **FIGS. 4A-4L** show decrease of peripheral virus shedding in meganuclease-treated mice. Experimental timeline of ocular infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 4A**). HSV loads (**FIG. 4A**) and (**FIGS. 4D-4E**), AAV loads in SCGs (**FIG. 4B**); p = 0.0016, 0.0012 and <0.0001 for 0.6, 1.2 and 1.8 $x10^{12}$, respectively, **FIG. 4D** and TGs (**FIG. 4C**; p = 0.068, 0.0025 and 0.0016 for 0.6, 1.2 and 1.8 $x10^{12}$, respectively, in control infected mice (n = 11) and infected mice treated with dual therapy delivered with 0.6 (n = 12), 1.2 (n = 12) or 1.8 (n = 12) $x10^{12}$ total vg AAV dose (**FIG. 4E**). Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (ordinary one-way Anova, multiple comparisons with ns: not significant, **: $p < 0.01$, ****: $p < 0.0001$) are indicated. Virus titers in eye swabs collected daily from day 1 to 4 after each weekly JQ1 reactivation from control infected mice (**FIG. 4F**) and infected mice treated with dual therapy delivered with 0.6 (**FIG. 4G**), 1.2 (**FIG. 4H**) or 1.8 (**FIG. 4I**) $x10^{12}$ total vg AAV dose. Area under the curve (AUC) analysis of virus shedding after first (**FIG. 4J**), second (**FIG. 4K**), and third (**FIG. 4L**) JQ1 reactivation from control infected mice (n = 11) and infected mice treated with dual therapy

delivered with 0.6 (n = 12), 1.2 (n = 12) or 1.8 (n = 12) x10^{12} total vg AAV dose. *p* values (unpaired, ordinary one-way Anova, with multiple comparisons) compared virus shedding between treatment groups and the control group. Each graph shows individual and mean values with standard deviation.

[0060] FIGS. 5A-5G show peripheral virus shedding decreases in dual meganuclease-treated mice. Experimental timeline of ocular infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 5A**). b, HSV loads in SCGs (b; p = 0.0012, and <0.0001 for CTRL vs AAV/MN no JQ1 and for CTRL vs AAV/MN 2x JQ1, respectively) (**FIG. 5B**), and TGs (c; p = 0.0089, and 0.0293 for CTRL vs AAV/MN no JQ1 and for CTRL vs AAV/MN 2x JQ1, respectively) (**FIG. 5C**) of control infected mice either unreactivated (CTRL no JQ1) or reactivated (CTRL 2x JQ1) and infected mice treated with dual therapy delivered with 1.8 x10^{12} total AAV dose either unreactivated (AAV/MN no JQ1) or reactivated (AAV/MN 2x JQ1), with n =12 per group. Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (unpaired one-tailed Mann-Whitney test with *: $p < 0.05$; **: $p < 0.01$, ****: $p < 0.0001$) are indicated. Virus titers in eye swabs collected daily from day 1 to 4 after two weekly JQ1 reactivations (red arrows) from control infected mice (**FIG. 5D**) and infected mice treated with dual therapy delivered with 1.8 x10^{12} total AAV dose (**FIG. 5E**). Area under the curve (AUC) analysis after the first (**FIG. 5F**), and the second (**FIG. 5G**) JQ1 reactivation from control infected mice either unreactivated (CTRL no JQ1) or reactivated (CTRL 2x JQ1) and infected mice treated with dual therapy delivered with 1.8 x10^{12} total AAV dose either unreactivated (AAV/MN no JQ1) or reactivated (AAV/MN 2x JQ1), with n =12 per group. *p* values (unpaired one-tailed Mann-Whitney test) are indicated. Each graph shows individual and mean values with standard deviation. AAV loads are shown in **FIGS. 18G-18H**.

[0061] FIGS. 6A-6G show peripheral virus shedding decreases in dual meganuclease-treated mice. Experimental timeline of intravaginal HSV-1 infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 6A**). HSV loads in DRGs from control infected mice reactivated with 3 weekly JQ1 injections and infected mice treated with dual therapy unreactivated or reactivated with 3 weekly JQ1 injections with n = 8 per group; p = 0.0055, and 0.0198 (ordinary one-way Anova, multiple comparisons) for CTRL+JQ1 vs AAV/MN no JQ1 and for CTRL +JQ1 vs AAV/MN+JQ1, respectively (**FIG. 6B**). HSV titers in vaginal swabs collected daily from day 1 to 4 post JQ1 injections

(red arrows) from control (**FIG. 6C**) and dual meganuclease-treated (**FIG. 6D**) infected mice. Area under the curve (AUC) analysis after the first (**FIG. 6E**), second (**FIG. 6F**), and third (**FIG. 6G**) JQ1 reactivation from control infected mice (n = 8) and infected mice treated with dual therapy, both reactivated with 3 weekly JQ1 injections (n = 8). *p* values (unpaired one-tailed Mann-Whitney test) are indicated. Each graph shows individual and mean values with standard deviation. The AAV viral loads are shown in **FIG. 18I**.

[0062] **FIGS. 7A-7F** show simplification of the meganuclease/AAV regimen. Experimental timeline of ocular infection and meganuclease therapy (**FIG. 7A**). HSV loads in SCGs (b; $p < 0.0001$) and TGs (c; $p = 0.0046$ for AAV9 and 0.0142 for 9-Dj/8-Rh10) from infected control and infected mice treated with HSV-1m4 (m4) delivered by retro-orbital (RO)) injections of $5x10^{11}$ vg total of the single or triple combinations of AAV9, -DJ/8 and -Rh10. Percent decrease of HSV loads in treated mice (n =10 per group) compared to control mice (n = 10) and statistical analysis (Ordinary one-way Anova, multiple comparisons with *: $p < 0.05$; **: $p < 0.01$, ****: $p < 0.0001$; ns: not significant) (**FIGS. 7B-7C**). Inflammatory cell foci (ICF) in liver sections from either HSV infected control mice (n = 10), or mice treated with m4 delivered using AAV single or triple combinations of AAV9, -DJ/8 and -Rh10 (n = 10 per group); $p = 0.0009$ for Rh10 (**FIG. 7D**). Severity scores of axonopathy (**FIG. 7E**) and inflammation (**FIG. 7F**) in TG from infected control mice (n = 3 TG) and infected mice treated with m4 delivered using single or triple combinations of AAV9, -DJ/8 and -Rh10 (n = 3 TG per group) and statistical analysis (Ordinary one-way Anova, multiple comparisons with ns: not significant; ***: $p < 0.001$). Each graph shows individual and mean values with standard deviation. The AAV viral loads are shown in **FIGS. 18J-18I**.

FIGS 8A-8L show simplified meganuclease therapy decreases peripheral shedding in infected mice. Experimental timeline of ocular HSV-1 infection, meganuclease treatment and viral reactivations with JQ1 (**FIG. 8A**). Schematic of active m4 and inactive m4i meganuclease (**FIG. 8B**). HSV loads in both SCGs (c; $p < 0.0001$ for m4) and both TGs (d; $p = 0.003$ for m4) from control infected mice and infected mice treated the active m4 or inactive m4i (n = 10 per group) (**FIGS. 8C-8D**). Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (unpaired one-tailed Mann-Whitney test with *: $p < 0.05$; ****: $p < 0.0001$; ns: not significant) are indicated. Virus titers in eye swabs collected at day 1 to 3 after each JQ1 reactivation from control infected mice (**FIG. 8E**) and infected mice treated with active m4 (**FIG. 8F**) or inactive m4i (**FIG.**

8G). Area under the curve (AUC) analysis of virus shedding after first (**FIG. 8H**), and second (**FIG. 8I**) JQ1 reactivation from control infected mice and infected mice treated with active m4 or inactive m4i (n = 10 per group). p values (unpaired one-tailed Mann-Whitney test) are indicated. j. Inflammatory cell foci (ICF) in liver sections from either HSV infected control mice, mice treated with active m4 or inactive m4i (n = 10 per group); p = 0.0234 for m4. k-l. Severity scores of axonopathy (k; p = 0.0007 for m4) and inflammation (l) in TG from HSV infected control mice, mice treated with active m4 or inactive m4i (n = 10 per group) with statistical analysis (Ordinary one-way Anova, multiple comparisons with ns: not significant; *: $p < 0.05$; ***: $p < 0.001$). Each graph shows individual and mean values with standard deviation. AAV viral loads are shown in **FIGS. 18M-18O**.

FIGS. 9A-9G show tissue restriction of meganuclease expression improves tolerability. Experimental timeline of ocular infection and meganuclease therapy (**FIG. 9A**). Average weight change of infected control mice (n = 13) or HSV infected mice treated with m4 expressed from either the ubiquitous CBh promoter, or the neuronal promoters E/CamKII or E/hSyn (n =12 per group) (**FIG. 9B**). Inflammatory cell foci (ICF) in liver sections from either HSV infected control mice (n =13), or mice treated with m4 expressed from either the CBh, E/CamKII or E/hSyn promoter (n =12 per group); p = 0.00455 for CBh-m4 (**FIG. 9C**). Severity scores of inflammation (**FIG. 9D**; p = <0.0001 for CBh-m4) and axonopathy (**FIG. 9E**; p = <0.0001 for CBh-m4) in TG from infected control mice (n = 10) and infected mice treated with m4 expressed from either the CBh, E/CamKII or E/hSyn promoter (n =12 per group) with statistical analysis (Ordinary one-way Anova, multiple comparisons with ns: not significant; *: $p < 0.05$; ****: $p < 0.0001$). HSV loads in SCGs (**FIG. 9F**) and TGs (**FIG. 9G**) from infected control (n = 10) and infected mice treated with m4 expressed from either the CBh, E/CamKII or E/hSyn promoter (n =12 per group). Percent decrease of HSV loads in treated mice compared to control mice and statistical analysis (Ordinary one-way Anova, multiple comparisons with *p* values; ns: not significant). Each graph shows individual and mean values with standard deviation. The AAV viral loads are shown in **FIGS. 19A-19C**.

FIGS. 10A-10G show reduction of ganglionic latent HSV loads after meganuclease therapy delivered using various AAV serotypes does not depend on the route of administration. Experimental timeline of ocular infection and meganuclease therapy (**FIG. 10A**). HSV loads in SCGs and TGs from infected control and infected mice treated with

HSV-1m5 (m5) delivered by retro-orbital (RO) or whisker pad (WP) injections of 10^{12} vg of various AAV (**FIG. 10B**). Percent decrease of HSV loads in treated mice compared to control mice and significant statistical analysis (Ordinary one-way Anova, multiple comparisons with *: $p < 0.05$; **: $p < 0.01$, ****: $p < 0.0001$). AAV loads in SCGs and TGs from infected control (CTRL, circles) and infected mice treated with m5 delivered using AAV serotype 7 (squares), 9 (upward triangles), DJ (downward triangles), DJ/8 (diamonds) administered by either RO or WP injections (see FIG. 1A) (**FIG. 10C**). Percent mutation quantified by T7 assay in latent HSV genomes present in SCG and TG collected from infected control (CTRL, circles) and infected mice treated with m5 delivered using AAV serotype 7 (squares), 9 (upward triangles), DJ (downward triangles), DJ/8 (diamonds) administered by either RO or WP injections (see FIG. 1A) (**FIG. 10D**). **FIGS. 10E-10G**, same data as above in panels **FIGS. 10A-10D** presented per route of administration of the AAV delivery vectors.

FIGS. 11A-11G show a reduction of ganglionic HSV loads after meganuclease therapy delivered using various AAV serotypes combinations. Experimental timeline of ocular infection and meganuclease therapy (**FIG. 11A**). HSV loads in SCGs (**FIG. 11B**) and TGs (**FIG. 11C**) from infected control and infected mice treated with m5 delivered by RO injection of 10^{12} vg of either single AAV serotype, dual AAV serotype combinations or triple AAV serotype combination. Each graph shows individual and mean values with standard deviation, percent decrease of HSV loads and significant statistical difference between treated and control mice (ordinary one-way Anova test with multiple comparisons *: $p < 0.05$; **: $p < 0.01$; ****: $p < 0.0001$). AAV loads in SCG (**FIG. 11D**) and TG (**FIG. 11E**) from infected control (CTRL, circles) and infected mice treated with m5 delivered using AAV combination of serotype 9, DJ/8 and Rh10 administered by RO injections (see FIG. 1C). Percent mutation quantified by T7 assay in latent HSV genomes present in SCG (**FIG. 11F**) and TG (**FIG. 11G**).

FIGS. 12A-12F show JQ1 reactivation leads to peripheral virus shedding**.** Experimental timeline of ocular infection and HSV reactivation (**FIG. 12A**). Latently infected mice were administered one IP injection of either (**FIG. 12B**) vehicle n = 9, or (**FIG. 12C**) JQ1 n = 9, black arrow, 50 mg/kg. HSV titers in eye swabs collected from day 0 to 3 post-JQ1. Experimental timeline of ocular infection and HSV reactivation using

hyperthermic stress (31 dpi) or JQ1 injection (38 dpi) (**FIG. 12D**). Latently infected mice were reactivated at day 0 (black arrow) by either (**FIG. 12E**) hyperthermic stress n = 12, or (**FIG. 12F**) JQ1 IP injection (50 mg/kg) n 10. HSV titers in eye swabs collected from day 1 to 4 post-JQ1 are plotted for each mouse.

FIGS. 13A-13H show JQ1 reactivation leads to peripheral virus shedding. Experimental timeline of ocular infection and sequential HSV reactivation with JQ1 injections (**FIG 13A**). HSV titers in eye swabs collected daily for 3 days after the 1st (day 32 p.i. in **FIG. 13A** and day 0 in **FIGS. 13B-13E**), 2nd (day 39 p.i. in **FIG. 13A** and day 7 on graph **FIGS. 13B-13E**) or 3rd (day 46 p.i. in **FIG. 13A** and day 14 in **FIGS. 13B-13E**) IP injection of either vehicle (black arrows) or JQ1 (grey arrows, 50 mg/kg) **FIG. 13B**, mice (n = 12) received 3 sequential IP injections of vehicle, **FIG. 13C**, mice (n = 12) received 1 IP injection of JQ1 followed by 2 sequential IP injections of vehicle, **FIG. 13D**, mice (n = 12) received 2 sequential IP injections of JQ1 followed by 1 IP injection of vehicle, and **FIG. 13E**, mice received 3 sequential IP injections of JQ1. Experimental timeline of ocular infection and sequential HSV reactivation with JQ1 injections (**FIG. 13F**). HSV viral loads in SCG (**FIG. 13G**) and TG (**FIG. 13H**) collected from mice after either 3 sequential injections of vehicle (0, n = 12), 1 JQ1 injection followed by 2 sequential injections of vehicle (1, n = 12), 2 sequential JQ1 injections followed by 1 injection of vehicle (2, n = 12), 3 sequential JQ1 injections (3, n = 12) or 7 sequential JQ1 injections (7, n = 4). Each graph shows individual and mean values with standard deviation.

FIGS. 14A-14F show histopathology of liver from dual meganuclease-treated mice. H&E staining of liver section from naive mouse (**FIG. 14A,**10x and **FIG. 14B**, 40x), HSV-infected mouse administered $3x10^{12}$ vg AAV (**FIG. 14C**, 10x and **FIG. 14D**, 40x) and mouse administered $3x10^{12}$ vg AAV only (**FIG. 14E**, 10x and **FIG. 14F**, 60x).

FIGS. 15A-15B show inflammatory cell foci in liver of meganuclease-treated mice. ICF in liver sections from either HSV infected control mice (control, black open circles, n = 11), or treated with dual meganuclease therapy at a dose of $0.6x10^{12}$ vg AAV (grey thick open circles, n= 12), $1.2x10^{12}$ vg AAV (medium grey open circles, n = 12) and $1.8x10^{12}$ vg AAV (dark grey open circles n =12) from experiment described in FIGS. 4A-4L (**FIG. 15A**). Statistical analysis using ordinary one-way Anova with multiple comparisons test, ns: not significant; ****: $p < 0.0001$. ICF in liver sections from either

HSV infected control mice unreactivated (control no JQ1, black circles, n = 12), control mice reactivated with JQ1 (control 2x JQ1, black squares, n =12), HSV infected mice treated with dual meganuclease therapy unreactivated (AAV/MN no JQ1, open circles n =12), or reactivated with JQ1 (AAV/MN 2x JQ1, open squares n = 12) from experiment described in FIGS. 5A-5G (**FIG. 15B**). Statistical analysis using unpaired one-tailed *t* test. ns: not significant; **: $p < 0.01$; ***: $p < 0.001$.

FIGS. 16A-16H show histopathology of TG from dual meganuclease-treated mice. Images of H&E stained trigeminal ganglia sections from either latently infected control mice not reactivated (CTRL no JQ1 (slide 10 in Table 3): **FIG. 16A**, 2.5x and FIG. **16B,** 20x), and reactivated with JQ1 (CTRL 2xJQ1 (slide 11 in Table 3): FIG. 16**C**, 2.5x and FIG. 16**D**, 20x) or $1.8x10^{12}$ vg AAV/dual meganuclease treated mice not reactivated (AAV/MN no JQ1 (slide 1 in Table 3): **FIG. 16E**, 2.5x and **FIG. 16F**, 20x) and reactivated (AAV/MN 2xJQ1 (slide 4 in Table 3): **FIG. 16G**, 2.5x and **FIG. 16H**, 20x). Black arrows indicate signs of axonopathy, grey arrows indicate neurons with central chromatolysis.

FIG. 17 shows viral shedding after a double dose of JQ1 in 67% (6 /9) of reactivated mice. Latently infected mice were administered 2 IP injections of JQ1 (50 mg/kg) separated by 12h, n = 9. HSV titers in eye swabs collected from day 0 to 3 post-JQ1 are plotted for each mouse.

FIGS. 18A-18O show ddPCR quantification of AAV viral loads. **FIG. 18A** shows AAV loads in DRGs from latently infected mice following intravaginal administration of HSV-1 either control untreated (CTRL, open circles, n = 7) or treated with AAV-delivered meganuclease dual therapy (AAV, open squares n = 4) in the experiment presented in FIGS. 1A, 1C. **FIG. 18B** shows AAV loads in SCG and TG from latently infected mice following ocular administration of HSV-1 either control untreated (CTRL, open circles, n = 10) or treated with AAV-delivered meganuclease dual therapy (AAV, open squares, n = 10) in the experiment presented in FIGS. 1B, 1D. **FIGS. 18C-18D** show AAV loads in SCGs (**FIG. 18C**) and TGs (**FIG. 18D**) from latently infected mice following ocular administration of HSV-1 either control untreated (CTRL, black circles, n = 10) or treated with AAV-delivered meganuclease dual therapy (AAV, open squares, n = 10) in the experiment presented in Figure 2a-f. **FIGS. 18E-18F**

show AAV loads in SCGs (**FIG. 18E**) and TGs (**FIG. 18F**) from latently infected mice following ocular administration of HSV-1 either control untreated (CTRL, black circles, n = 8) or treated with AAV-delivered meganuclease dual therapy (AAV, open squares, n = 8) in the experiment presented in FIGS.2G-2l. **FIGS. 18G-18H** show AAV loads in SCGs (**FIG. 18G**) and TGs (**FIG. 18H**) from latently infected mice following ocular administration of HSV-1 either control untreated no reactivated (CTRL no JQ1, black circles, n =12), treated with AAV-delivered meganuclease dual therapy not reactivated (AAV/MN no JQ1, black squares, n =12), control untreated reactivated twice with JQ1 (CTRL 2x JQ1, open circles, n =12) or treated with AAV- delivered meganuclease dual therapy reactivated twice with JQ1 (AAV/MN 2x JQ1, open squares, n =12) in the experiment presented in FIGS. 4A-4G. AAV loads in DRGs from latently infected mice following intravaginal administration of HSV-1 either control untreated reactivated with JQ1 (CTRL+JQ1, circles, n = 8), treated with AAV-delivered meganuclease dual therapy not reactivated (AAV/MN no JQ1, open squares, n = 8), or treated with AAV-delivered meganuclease dual therapy reactivated with JQ1 (AAV/MN+JQ1, open triangles, n = 8) in the experiment presented in FIGS. 4H-4K **(FIG. 18I)**. **FIGS. 18J-18L** show AAV loads in SCGs (**FIG. 18J**), TGs (**FIG. 18K**) and livers (**FIG. 18L**) from HSV- 1 infected control (CTRL, open circles, n= 10) and HSV-1 infected mice treated with m4 delivered with either AAV single serotype 9 (light grey circles, n = 10), DJ/8 (dark grey circles, n= 10), Rh10 (light grey circles, n= 10), or triple serotypes 9, DJ/8 and Rh10 (medium grey circles, n= 10) administered by either RO in the experiment presented in FIGS. 5A-5G. **FIGS. 18M-18O** show AAV loads in SCGs (**FIG. 18M**), TGs (**FIG, 18N**) and livers (**FIG. 18O**) from HSV-1 infected control (CTRL, black circles, n= 10) and HSV-1 infected mice treated with either m4 (light grey circles, n= 10), or m4i (dark grey circles, n= 10) administered by RO in the experiment presented in FIGS. 6A-6G.

FIGS. 19A-19C show ddPCR quantification of AAV viral loads. AAV loads in SCGs (**FIG. 19A**), TGs (**FIG. 19B**) and livers (**FIG. 19C**) from HSV-1 infected control (CTRL, black circles) and HSV-1 infected mice treated with AAV9-CBh-m4 (dark grey circles), AAV9-E/CamKII-m4 (medium grey circles), or AAV9-E/hSyn-m4 (light grey circles) administered by either RO in the experiment presented in FIGS. 7A-7F.

FIGS. 20A-20E show detection of m4 expression in TG from treated mice. Western blot detection of m4 expression (anti-HA) in TG collected from uninfected mice at 1 (mice ID# 1- 3 and 12-14), 4 (mice ID# 4-6 and 15-17), and 8 (mice ID# 7-10, 11, and 18-21) weeks after RO administration of $1x10^{12}$ vg either AAV9-E/CamKII-m4 (**FIG. 20A**), or AAV9-CBh-m4 (**FIG. 20B**). Immune cell foci (ICF) in liver sections (**FIG. 20C**) and AAV loads in liver (**FIG. 20D**), and TG (**FIG. 20E**) from uninfected mice treated with either AAV9-CBh-m4), or AAV9-E/CamKII-m4 collected at 1, 4, and 8 weeks post AAV administration. $ Mouse ID#11 did not receive any AAV. *Samples from mice 16 and 18 are from week 4 and 8, respectively. The protein molecular weight markers were run on the same gel, but the data was acquired as a separate colorimetric data image from the chemiluminescent data image for the anti-HA (**FIG. 20A**) or anti-β-actin signals (**FIG. 20B**).

FIG. 21 shows schematics of pscAAV plasmids. Plasmids used for the production of scAAV delivery vectors. ITR: inverted terminal repeat, mutated ITR: ITR with deletion of the D region, HA: HA-tag, NLS: nuclear localization signal.

DETAILED DESCRIPTION

[0063] Human infection with HSV is lifelong, and while current antiviral approaches can reduce symptoms and transmission, they do not cure. As such, there is a strong unmet desire for new and potentially curative approaches for HSV. The present disclosure provides gene editing as a potential curative therapy for HSV in three important ways. First, the present disclosure establishes a model of HSV reactivation in mice using a small molecule, to show that a reduction in ganglionic HSV loads via gene editing results in a significant reduction in viral shedding in a mouse model with established orofacial infection. Second, the present disclosure demonstrates a high efficacy of gene editing of latent HSV in DRG after genital HSV infection. Third, the hepato- and neurotoxicity associated with the administration of adeno-associated viral vector gene therapy/gene editing , was shown to be reduced or eliminated by modulating factors including but not limited to the AAV dose, simplifying the therapy regimen, and using a cell type-specific promoter. Together, these findings address several of the major drivers of interest in HSV cure and support the use of gene editing for HSV infection.

[0064] Mice are easily infected with HSV and have been critical in defining many aspects of HSV infection, latency, and immune control. A major drawback, however, has been the fact that latent HSV infection in mice exhibits minimal to no spontaneous reactivation or peripheral virus shedding. Thus, mice have been of limited utility in studying HSV therapeutics or vaccines that are directed at control of latent infection and reactivation. HSV reactivation in mice can be induced by various stimuli, such as immunosuppression, hyperthermic stress, or ultraviolet B irradiation, but these approaches induce minimal shedding, can be cumbersome, and may be applicable to only certain specific mouse strains or HSV isolates.

[0065] The present disclosure provides a model of small-molecule induction of HSV-1 reactivation and peripheral shedding in latently-infected mice and demonstrates that gene editing mediates a dramatic reduction not only in ganglionic viral loads, but importantly also in induced viral shedding. Optimization of the therapeutic approach through regimen simplification, dose reduction, and tissue restriction of meganuclease expression results in almost complete elimination of undesired effects on liver and ganglia, supporting the continued clinical development of this strategy.

[0066] Specifically, the present disclosure provides for the reactivation of HSV by JQ1, a bromodomain inhibitor/BET protein inhibitor that has been proposed as a latency-reversing agent for human immunodeficiency virus (HIV). Previously, JQ1 was reported to reactivate HSV in cell culture models of latency and induce shedding from the eyes of latently infected mice, although the amount and timing of shedding was not fully defined. The present disclosure demonstrates that JQ1 reproducibly induces detectable viral shedding at the periphery from a substantial subset of latently-infected mice, at quantitative levels (10^2 to 10^6 copies/mL) that are similar to those observed in human observational studies. Additional studies in C57BL/6 mice latently infected with HSV-1 showed that virus shedding could be also induced with JQ1 administration (**TABLE 4**), suggesting that JQ1 induced virus shedding is not limited to the Swiss Webster strain. Thus, the JQ1 reactivation model as provided herein is useful for addressing the mechanisms and determinants of HSV reactivation and peripheral shedding, and vaccines or therapeutics aiming to reduce such shedding.

[0067] Among people infected with HSV, a major concern and driver of the desire for cure is the risk of transmission of the virus to others. While an up to 90% reduction of latent HSV within ganglia after gene editing has been demonstrated, it

remained unclear what effect such reduction would have on viral shedding at the periphery. Using the JQ1 reactivation model, the present disclosure demonstrates that reduction of ganglionic load via gene editing has a profound effect on viral shedding, both in terms of the fraction of samples with detectable virus, and in the amount of virus shed. In humans, the relationship between HSV shedding quantity and the likelihood of viral transmission remains incompletely understood, but previous mathematical modeling suggests that reduction of shedding to levels below 10^4 viral copies (as observed in most treated animals that exhibited residual shedding) would be expected to greatly reduce, if not fully eliminate, the risk of viral transmission.

[0068] Advantageously, much like in humans, the JQ1 reactivation model in mice exhibits stochastic nature of the induced viral reactivation and shedding. Within a given cohort, only a subset of JQ1-treated mice shed detectable HSV, and shedding in one episode was not predictive of subsequent shedding after repeated JQ1 reactivations. Similarly, individual mice often shed unilaterally from a single eye; again, this was not predictive of the laterality of subsequent shedding episodes. These findings are similar to observations in humans, in whom shedding is episodic and stochastic, and can occur at distinct anatomical locations during different shedding episodes.

[0069] The present disclosure demonstrates a similar trend towards reduction of HSV shedding after AAV/meganuclease therapy. A lower latent viral load was observed in DRG after genital HSV infection resulting in lower reactivation rates after JQ1 administration. Meta-analysis of the combined data from all experiments confirmed a highly significant reduction in HSV shedding from AAV/meganuclease-treated animals compared with controls in both oral and genital HSV infection. This reduction in shedding proved to be both dose- and duration-of-therapy-dependent. The latter observation is particularly encouraging regarding the potential clinical translation of the work. Mice were evaluated for ganglionic load and shedding approximately one month after AAV/meganuclease administration, but data suggest that HSV gene editing efficacy likely continues past this point, which may lead to more complete reduction or elimination of viral shedding at later time points.

[0070] Importantly, the present disclosure provides that AAV-delivered meganucleases can readily enter neurons and edit HSV within DRG, the site of HSV latency in genital disease. Superior HSV viral load reduction in SCG (which are autonomic ganglia; typically ~90% reduction) have been observed compared to TG (sensory ganglia;

typically ~50-60% reduction). This raised the possibility that DRG (also sensory ganglia) might also show similar modest rates of genome reduction as TG. However, gene editing of latent HSV genomes proved to be highly efficient within DRG (about 97% to about 98% reduction), suggesting that the differing efficiencies are not intrinsic to the type of ganglion (sensory vs. autonomic). The relative efficiencies of AAV transduction in the various ganglia may potentially be driven mainly by the relative permeability of the blood/ganglionic barrier for each ganglionic type.

[0071] HSV-1 target sequences of the meganucleases used in this study are not well conserved in HSV-2. Therefore, vaginal/genital infection experiments using HSV-1 were performed to make the point that there appears to be no barrier to treating genital vs. orofacial disease; that is, the latently infected neurons in both sites are readily accessible to AAV vectors as well as meganuclease-mediated editing of latent HSV genomes. The successful treatment of genital HSV-1 infection is not a trivial result – over half of new cases of genital herpes in the US are now due to HSV-1.

[0072] AAV vectors generally have been considered safe, particularly in comparison with other gene therapy vectors. However, at high doses AAV vectors can induce liver toxicity, manifesting initially as transaminase elevation. At AAV doses higher than those used in the present disclosure, liver toxicity can be severe and has led to liver failure in several animal models. The present disclosure demonstrates strong anti-HSV activity at AAV doses that were well tolerated.

[0073] More recently, histological evidence of neuronal injury after AAV administration has been described in mice, rats, piglets, dogs, and non-human primates, and in at least two human trial participants at autopsy. The causative mechanism of such injury remains unclear; among the current leading hypotheses are saturation of neural protein-folding capacity and TLR9-mediated recognition of vector or transgene RNA. Despite histological evidence of neuronal injury, clinical signs in experimental animals have been rare, consisting mainly of mild gait or balance disturbance. Such signs have only been reported in a single patient among several thousand human participants in trials of AAV-delivered gene therapies. Consistent with these other studies, subtle evidence of neuronal injury in experimental mice, manifesting as neuronal degeneration, necrosis, and axonopathy, were observed but without any associated behavioral alterations. Specifically, using a neuronal-specific promoter to drive the meganuclease expression demonstrated surprising effects, in that they show an absence of the neuronal toxicity readily detected

with use of a ubiquitous promoter. These data support consideration of an alternative hypothesis, that ganglionic neurotoxicity is mediated indirectly through AAV effects on non-neuronal cells, rather than on the neurons themselves. In any event, the data associated with regimen simplification, dose reduction, and tissue restriction of transgene expression demonstrates that mitigation avenues can be designed to eliminate both hepato- and neurotoxicity.

[0074] Unless specifically defined herein, all terms used herein have the same meaning as they would to one skilled in the art of the present invention.

[0075] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0076] Following long-standing patent law, the words "a" and "an," when used in conjunction with the word "comprising" in the claims or specification, denotes one or more, unless specifically noted.

[0077] Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise," "comprising," and the like, are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to indicate, in the sense of "including, but not limited to." Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words "herein," "above," and "below," and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application. The word "about" indicates a number within range of minor variations above or below the stated reference number. For example, "about" can refer to a number within a range of 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% above or below the indicated reference number.

[0078] The term "subject" or "host" herein refers to a mammal being assessed for reducing or eliminating HSV-1 infection associated with latent HSV-1 reactivation. In certain embodiments, the mammal is a human. The term "subject" encompasses, without limitation, individuals having HSV-1. In some embodiments, the subject is one who is diagnosed and currently being treated for, or seeking treatment, monitoring, adjustment or modification of an existing therapeutic treatment, or is at a risk of developing an HSV infection. In one embodiment, the HSV infection is HSV-1infection. The term "HSV-1 infection" refers to the undesired proliferation or presence of invasion of HSV-1 in a host

organism. In some embodiments, the infection can be caused by actively replicating lytic HSV-1 and can be referred to as lytic infection. Such an infection is usually symptomatic. In some embodiments, the infection can be caused by quiescent or latent HSV-1 and can be referred to as latent HSV-1 infection. Such an infection is usually asymptomatic. A latent viral infection can reactivate to become a lytic viral infection or recurrent HSV-1 infection and can result in recurrence of active symptomatic HSV-1 related disease.

[0079] As used herein, the term "protein" refers to designating a series of amino acid residues connected to each other by peptide bonds between the alpha- amino and carboxy groups of adjacent residues. The term "protein" can also refer to a polymer of amino acids, including modified amino acids (e.g., phosphorylated, glycated, glycosylated, etc.) and amino acid analogs, regardless of its size or function. The term "protein" can also be used to refer to a gene product and fragments thereof.

[0080] As used here, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0081] As used here, the term "pharmaceutically acceptable carrier" means a pharmaceutically- acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid or solvent encapsulating material necessary or used in formulating an active ingredient or agent for delivery to a subject. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

[0082] The terms, "decrease", "reduce", "lower", "eliminate", or "inhibit" are all used herein generally to mean a decrease by a statistically significant amount. For example, "decrease", "reduce", "lower", or "inhibit" means a decrease by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (e.g. absent level or non- detectable level as compared to a reference level), or any decrease between 10-100% as compared to a reference level. "Eliminate" means removal of up to about 90% as compared to a reference level, i.e., detection is not possible.

[0083] Herpes simplex virus (HSV) comprises at least HSV type 1. As used herein HSV-1 and HSV are used interchangeably to refer to HSV type 1 (HSV-1). HSV-1 belongs to the Herpesviridae family of DNA viruses that cause infections in humans. HSV-1, once acquired, remains with the host for life, and typically remains latent in the form of stable dsDNA episome in the nuclei of sensory neurons. HSV-1 is a highly adapted human pathogen with a rapid lytic replication cycle and exhibits the ability to invade sensory neurons and establish latent or quiescent infection without showing any cytopathology. Latent infections are subject to reactivation whereby infectious viruses can be recovered in peripheral tissue enervated by the latently infected neurons following a specific physiological stress. A major factor in these switches from lytic to latent infection and back involves changes in transcription patterns, mainly as a result of the interaction between viral promoters, the viral genome and cellular transcriptional machinery.

[0084] The primary infection site for HSV-1 is at the mucosal surfaces. In some embodiments, HSV-1 can access sensory nerve endings and through retrograde transport migrate from the site of infection to the trigeminal ganglion (TG) and superior cervical ganglion (SCG). There, HSV-1 can infect the TG and SCG, and the TG and SCG remain the site of latency until HSV-1 is reactivated by, among other things stress, where HSV-1 migrates from the TG or SCG through retrograde transport to the primary site of infection.

[0085] The HSV-1 genome is a linear, double stranded DNA duplex 152,261 base pairs (bp) in length, and with a base composition of 68% G+C which circularizes upon infection. The HSV-1 genome is divided into six important regions. One, the ends of the linear molecules, the "a" sequences: these are important in both circularization of the viral DNA, and in packaging the DNA in the virion. Two, the 9,000 bp long repeat (RL), which encode both an important immediate early regulatory protein (aO) and the promoter of most of the "gene" for the latency associated transcript (LAT). Three, the long unique region (UL), which is 108,000 bp long, encodes at least 56 distinct proteins; it contains, for example, genes for the DNA replication enzymes and the capsid proteins. Four, the 6,600 bp short repeats (Rs) encode the very important "a" immediate early protein; this is a very powerful transcriptional activator which acts along with aO ICPO and a27 (ICP27/UL54) (in the UL) to stimulate the infected cell for all viral gene expression that leads to viral DNA replication. Five, the origins of replication: the OHL is in the middle of the UL region; the oris is in the Rs and thus, is present in two copies. All sets of ori's operate during infection to give a very complicated replication complex, very similar to that seen

in the replication of phage T4. Six, the 13,000 bp unique short region (Us) encodes 12 ORFs, several of which are glycoproteins important in viral host range and response to host defense.

[0086] The virus encodes nearly 100 transcripts and more than 70 open translational reading frames (ORFs). Most ORFs are expressed by a single transcript. About 40 genes are considered as essential for virus replication in culture, including UL19 (SEQ ID NO: 11), UL30 (SEQ ID NO: 12), and UL54(SEQ ID NO: 23). UL19 is expressed in the late stages of the infection cycle and codes for the major capsid protein, VPR. UL30 (SEQ ID NO: 12) is expressed in the early stages of the infection cycle and codes for the catalytic subunit of the viral DNA polymerase. UL54 is expressed in the inter- mediate stages of the infection cycle and codes for the immediate early regulatory protein ICP27. ICP0 (SEQ ID NO: 13) is expressed and functions at the earliest stages of the productive infection cycle and is important to initiate early tran-scription and replication.

Meganucleases

[0087] Meganucleases are essentially represented by homing endonucleases. Homing Endonucleases (HEs) are a widespread family of natural meganucleases including hundreds of protein families (Chevalier, B. S. and B. L. Stoddard, *Nucleic Acids Res.*, 2001, 29, 3757-3774). These proteins are encoded by mobile genetic elements which propagate by a process called "homing": the endonuclease cleaves a cognate allele from which the mobile element is absent, thereby stimulating a homologous recombination event that duplicates the mobile DNA into the recipient locus.

[0088] As used here, "meganuclease" is a double-stranded endonuclease having a large polynucleotide recognition site, at least 12 bp, preferably from 12 by to 60 bp. Meganucleases are also called rare-cutting or very rare-cutting endo- nucleases. In some embodiments, meganucleases can be either monomeric or dimeric. In some embodiments, the meganuclease can be any natural meganuclease such as a homing endonuclease. In some embodiments, the meganuclease can be any artificial or man-made meganuclease endowed with such high specificity, either derived from homing endonucleases of group I introns and inteins, or other proteins such as Zinc-Finger proteins or group II intron proteins, or compounds such as nucleic acid fused with chemical compounds.

[0089] The detailed three-dimensional structures of several homing endonucleases are known, namely I-Dmo I, PI-See I, PI-Pfu I, I-Cre I, I-Ppo I, and a hybrid homing endonuclease I-Dmo I/I-CreI called E-DreI (Chevalier et al., 2001, *Nat Struct Biol,* 8, 312-316; Dunn et al., 1997, *Cell,* 89, 555-564; Heath et al., 1997, *Nat Struct Biol,* 4, 468-476; Hu et al., 2000, *J Biol Chem,* 275, 2705-2712; Ichiyanagi et al., 2000, *J Mol Biol,* 300, 889-901; Jurica et al., 1998, *Mal Cell,* 2, 469-476; Poland et al., 2000, *J Biol Chem,* 275, 16408-16413; Silva et al., 1999, *J Mol Biol,* 286, 1123-1136; Chevalier et al., 2002, *Molecular Cell,* 10, 895-905).

[0090] The LAGLIDADG homing endonucleases (LHEs) family is the largest family of proteins clustered by their most general conserved sequence motif: one or two copies of a twelve-residue sequence: the di-dodecapeptide, also called LAGLIDADG motif. Homing endonucleases with one dodecapeptide (D) are around 20 kDa in molecular mass and act as homodimer. Those with two copies (DD) range from 25 kDa (230 AA) to 50 kDa (HO, 545 AA) with 70 to 150 residues between each motif and act as monomer. Cleavage is inside the recognition site, leaving 4 nt staggered cut with 3'OH overhangs. I-CeuI, and I-CreI illustrate the homodimeric homing endonucleases with one Dodecapeptide motif (mono-dodecapeptide). There is a growing number of LHEs with well-characterized activities and structures. The choice of the most appropriate LHE will be readily apparent to one of skill in the art depending on the target gene. The initial LAGLIDADG homing endonuclease can be selected from the group comprising: I-DmoI, I-CreI, PI-See I, and PI-PfuI.

[0091] In accordance with the foregoing, in one aspect the disclosure provides a method of treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type I(HSV-1) reactivation in a subject.

[0092] In some embodiments, the method comprises administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease. In some embodiments, the at least one HSV-1-specific meganuclease is effective in disrupting at least one gene in the genome of HSV-1.

[0093] In some embodiments, the at least one HSV-l-specific meganuclease is configured to induce one or more DNA double strand breaks (DSB) in one or more genes in the genome of the HSV. In some embodiments, the adeno-associated viral

vector further comprises a neuron specific promoter. In an embodiment, the one or more adeno-associated viral vector (AAV) serotypes is selected from AAV7, AAV9, AAV-DJ, AAV-DJ/8, AAV-Rh10, or a combination thereof. In some embodiments, the meganuclease is selected from HSV-1m4, HSV-1m5, HSV-1m8, or a combination thereof.

[0094] In an embodiment, the latent HSV-1 infection is an ocular or genital HSV-1 infection.

[0095] In some embodiments, the one or more adeno-associated viral vector (AAV) serotype is AAV9 or AAV-DJ/8, and the at least one HSV-1-specific meganuclease is m5.

[0096] In some embodiments, the composition comprises of a plurality of a single adeno-associated viral vector (AAV) serotype, comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In an embodiment, the AAV serotype is AAV9, and the meganuclease is m4.

[0097] In yet another embodiment, the method comprises administering to the subject a composition comprising a plurality of at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each viral vector serotype comprises a nucleic acid sequence encoding the same or a different HSV-specific meganuclease, separately. In an embodiment, the at least three adeno-associated viral vector (AAV) serotypes comprise AAV9, AAV-DJ/8, and AAV-Rh10 and each viral vector serotype comprises a nucleic acid sequence encoding an HSV-1-specific meganuclease. In some embodiments, the HSV-1-specific meganuclease is m4, m5, or a combination thereof.

[0098] In an embodiment, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining HSV. In an embodiment, the reduction in ganglionic HSV load in SCG is about 73% to about 95% and in TG is about 43% to about 48% as compared to an untreated subject.

[0099] In yet another embodiment, the recurrent latent HSV infection is a genital infection. In an embodiment, the composition is effective in reducing ganglionic HSV load in Dorsal root ganglion (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0100] In some embodiments, the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are m5 and m8, respectively. In some embodiments, the composition is administered intravenously or transdermally.

[0101] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV. In an embodiment, the reduction in ganglionic HSV load in SCG is about 89% to about 98% and in TG is about 42% to about 61%, as compared to an untreated subject.

[0102] In yet another embodiment, the recurrent latent HSV infection is a genital infection and the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV. In an embodiment, the reduction in ganglionic HSV load in DRG is about 97% to about 98% as compared to an untreated subject.

[0103] In some embodiments, the composition is administered at a total dose selected from about 1.8 to about 5.4×1013 vg/kg to the subject. In some embodiments, the administered total dose is effective in a dose-dependent (i) reduction in ganglionic HSV loads; and (ii) reduced peripheral viral shedding in the subject as compared to an untreated subject.

[0104] In some embodiments, the method is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject. In some embodiments, the method is associated with improved tolerability with no hepatotoxicity or neurotoxicity in the subject. In yet another embodiment, the method is effective in preventing the transmission of HSV-1 to a new subject.

[0105] In some embodiments, the subject is a mammalian subject. In some embodiments, the mammalian subject is human.

[0106] In some embodiments, the reactivation of latent HSV-1 is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is a BET protein inhibitor. In some embodiments, the BET protein inhibitor is administered prior to, concomitant with, or after administration of the viral vector compositions disclosed herein. In some embodiments, the BET protein inhibitor is JQ1.

[0107] In yet another aspect, the present disclosure provided a method for reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in a subject. In some embodiments, the method comprises administering to the subject a composition comprising at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each of the at least three viral vector serotypes comprise a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

[0108] In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In an embodiment, the first and the second HSV-l-specific meganucleases are m5 and m8, respectively. In some embodiments, the adeno-associated vector further comprises a neuron specific promoter.

[0109] Methods of generating the different adeno-associated viral vector serotypes expressing the meganuclease gene of interest (GOI) are known to one of skill in the art. Each of the individual AAV vector serotypes are made with a mixture of three plasmids; the plasmid carrying the meganuclease gene of interest (GOI) and expression regulator (e.g., pscAAV-CBh-HSV1m4), the plasmid with the specific AAV capsid serotype (e.g. for an AAV9 serotype it is pAAV9) and the pHelper that carry function for the generation and assembly of the AAV vector (pHelper which is used for all the different AAV regardless of the serotype and GOI). The pHelper plasmid provided the helper genes for AAV packaging. For example, to generate AAV9-CBh-m4, a mixture of pscAAV-CBh-m4, pHelper and pAAV9, was utilized.

[0110] In some embodiments, the composition is administered intravenously or transdermally.

[0111] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In an embodiment, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0112] In an embodiment, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0113] In some embodiments, the method is effective in reducing or eliminating potential transmission to a new host.

[0114] In some embodiments, the reactivation is by administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is a BET protein inhibitor. In some embodiments, the BET protein inhibitor is administered prior to, concomitant with, or after administration of the compositions disclosed herein.

[0115] In another aspect, the present disclosure pertains to a method for reducing or preventing hepato- and neurotoxicity associated with the administration of AAV-delivered gene editing for treatment of a recurrent infection associated with latent HSV-1 reactivation. In some embodiments, the method comprises administering to the subject a composition comprising a plurality of a single adeno-associated viral

vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the adeno-associated viral vector comprising a nucleic acid sequence encoding the meganuclease targeting a duplicated region of the HSV-1 genome further comprises a neuron specific promoter. In some embodiments, the one or more adeno-associated viral vector (AAV) serotype is AAV9. In some embodiments, the meganuclease is m4.

[0116] In some embodiments, the recurrent infection associated with latent HSV reactivation is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0117] In some embodiments, recurrent infection associated with latent HSV reactivation is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0118] In some embodiments, the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject. In some embodiments, the composition is effective in reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in the subject.

[0119] In some embodiments, the reactivation of the latent HSV-1 in a subject comprises administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is administered prior to, concomitant with, or after administration of the composition comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the plurality of single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the latency reversing agent is a BET protein inhibitor.

[0120] In another aspect, the present disclosure provides a method for reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in a subject. In

some embodiments, the method comprises administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

[0121] In an embodiment, the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes. In some embodiments, each viral vector serotype comprises a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In some embodiments, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are m5 and m8, respectively.

[0122] In some embodiments, the adeno-associated vector further comprises a neuron specific promoter.

[0123] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0124] In some embodiments, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0125] In some embodiments, the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject.

[0126] In some embodiments, the reactivation comprises administering to the subject an effective amount of a latency reversing agent. In some embodiments, the latency reversing agent is administered prior to, concomitant with, or after administration of the composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease. In some embodiments, the latency reversing agent is a BET protein inhibitor.

[0127] In yet another aspect, the present disclosure provides a composition comprising a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately. In some embodiments, the adeno-associated viral vector further comprises a neuron specific promoter. In an embodiment, the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease. In some embodiments, the first and the second HSV-l-specific meganucleases are m5 and m8, respectively.

[0128] As used herein, "administering" refers to administering the one or more viral vectors comprising a sequence encoding an HSV-1-specific meganuclease into a subject by a method or route which is effective in reducing or eliminating latent HSV-1 in the subject, and results in complete or partial inoculation of the subject at the desired site. Complete inoculation means no amount of HSV-1 is detectable by the methods known or used by a person having skill in the art. Partial inoculation means the amount of HSV-1 in the subject is decreased relative to the amount of HSV-1 present in the subject prior to the administering. For example, the amount of HSV-1 in the subject is decreased by about 10% to about 99%, about 15% to about 99%, about 20% to about 99%, about 30% to about 99%, about 40% to about 99%, about 50% to

about 99%, about 60% to about 99%, about 70% to about 99%, about 80% to about 99%, about 90% to about 99%, about 10% to about 98%, or about 20% to about 95%.

[0129] In some embodiments, the composition is administered intravenously or transdermally.

[0130] In another aspect, provided herein is a composition, comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome. In some embodiments, the adeno-associated vector serotype further comprises a neuron specific promoter. In some embodiments, the plurality of the single adeno-associated viral vector (AAV) serotype consists of AAV9, and the HSV-1-specific meganuclease encoded by the at least one nucleic acid sequence is m4.

[0131] The compositions disclosed herein are effective in treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type 1(HSV-1) reactivation in a subject. In some embodiments, the compositions are effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation. In some embodiments, the compositions of the present disclosure are effective in reducing or eliminating potential transmission to a new host/subject. In some embodiments, the compositions are effective in reducing or eliminating potential transmission to a new host/subject. In yet another embodiment, the compositions of the present disclosure are associated with improved tolerability and reduced hepato- and neurotoxicity.

[0132] The compositions disclosed herein may be formulated with at least one pharmaceutically acceptable carrier for administration.

[0133] In some embodiments, the compositions disclosed herein are administered to the subject prior to, concomitantly with, or after administration of an effective amount of an HSV-1 latency reversing agent. In some embodiments, the HSV-1 latency reversing agent is a BET protein inhibitor.

[0134] In some embodiments, the recurrent latent HSV infection is an orofacial infection. In some embodiments, the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG). In some embodiments, the composition is effective in editing the genome of remaining latent HSV.

[0135] In some embodiments, the recurrent latent HSV infection is a genital infection. In some embodiments, the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG). In some embodiments, the composition is effective in editing the genome of remaining HSV.

[0136] As used herein, "viral vectors" refer to the use of adeno-associated virus vectors (AAV) or any viral vector engineered from an AVV to deliver one or more HSV-1- specific meganuclease to the desired target. In some embodiments, the viral vector is a neurotropic adeno-associated viral vector. In some embodiments, the engineered AAV can comprise a self-complementary adeno-associated virus (scAAV). In some embodiments, the engineered AAV can comprise a single-stranded adeno-associated virus (ssAAV). The viral vectors, e.g., AAVs, scAAVs, ssAAVs, and the like, comprising one or more sequences encoding one or more HSV-I-specific meganucleases were generated according to the method of Choi et al., (Choi, V. W., Asokan, A., Haberman, R. A. & Samulski, R. J. Production of recombinant adeno-associated viral vectors for in vitro and in vivo use. *Curr Protoc Mal Biol* Chapter 16, Unit 16 25, doi:10.1002/0471142727. mb1625s78 (2007)) the contents of which are herein incorporated by reference.

[0137] In some embodiments, the one or more viral vectors are an AAV. In some embodiments, the AAV can comprise any serotype well known to those with ordinary skill in the art. In some embodiments, the one or more AAVs can comprise AAVl, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVl0, AAV11, AAV12, AAV-Rh8, AAV-RHl0 serotype adeno-associated virus, or a combination thereof. In some embodiments, the one or more AAVs can comprise AAV-Rh10, AAV8, AAV1 serotype adeno- associated virus, or a combination thereof. In still other embodiments, the one or more AAVs is AAV-Rh10, and/or AAV8 serotype adeno-associated virus.

[0138] In some embodiments, the one or more viral vectors is a self-complementary AAV (scAAV0. In some embodiments, the scAAV can comprise any serotype well known to those with ordinary skill in the art. In some embodiments, the one or more scAAVs can comprise AAVl, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVl0, AAV11, AAV12, AAV-Rh8, AAV-RHl0 serotype adeno-associated virus, or a combination thereof. In some embodiments, the one or more scAAVs can comprise AAV-Rh10, AAV8, AAV1 serotype adeno-associated

virus, or a combination thereof. In still other embodiments, the one or more scAAVs is AAV-Rhl0 and/or AAV8 serotype adeno-associated virus.

[0139] In some embodiments, the one or more viral vectors are an ssAAV. In some embodiments, the ssAAV can comprise any serotype well known to those with ordinary skill in the art. In some embodiments, the one or more ssAAVs can comprise AAVl, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVl0, AAV11, AAV12, AAV-Rh8, AAV-RH-10 serotype adeno-associated virus, or a combination thereof. In some embodiments, the one or more ssAAV can comprise AAV-Rh10, AAV8, AAV1 serotype adeno-associated virus, or a combination thereof. In still other embodiments, the one or more ssAAVs is AAV-Rhl0 and/or AAV8 serotype adeno-associated virus.

[0140] In some embodiments, the one or more HSV-1- specific meganucleases can be configured to induce one or more DNA double strand breaks (DSB). DNA DSBs are created in HSV-1 genomes upon expression of homing endonucleases that target specific sequences in essential HSV-1 genes. DSBs are repaired by non-homologous end joining which is error prone so that continual cleavage of HSV-1 target sites leads to disruption/mutation of HSV-1 genes. As used herein, the phrase "configured to induce one or more DNA DSBs" refers to the use of meganucleases that target specific HSV-1 target site(s) to cause DSBs. As used herein, "target sequence" or "target site" refers to a nucleic acid sequence within the viral genome that comprises a sequence to which the specific meganuclease targets resulting in gene editing of HSV-1 target sites.

[0141] In some embodiments, the one or more HSV-1-specific meganucleases are derived from the I-CreI enzyme. In some embodiments, HSV-1-specific meganucleases can be configured to induce one or more DNA DSBs in any HSV-1 gene that is well known to one of ordinary skill in the art. For example, in some embodiments, the one or more HSV-1-specific meganucleases is a meganuclease that can be configured to target U_L19(SEQ ID NO: 11), U_L30(SEQ ID NO:12), U_L54(SEQ ID NO. 23), ICP0(SEQ ID NO:13), and the like. In some embodiments, the one or more HSV-1-specific meganucleases is a meganuclease that can be configured to target any combination of U_L19, U_L30, ICP0, U_L54, and/or any HSV-1 gene well known to one of ordinary skill in the art.

[0142] In still other embodiments, the HSV-1-specific meganuclease is HSV1m5 (m5) (SEQ ID NO: 19) that targets a 24 bp sequence in U_L19 (SEQ ID NO:11). In some

embodiments, the HSV-1-specific meganuclease is HSV1m8 (m8) (SEQ ID NO:20) that targets a 24 bp sequence in U_L30 (SEQ ID NO:12). In some embodiments, the HSV-1-specific meganuclease is HSV1m4 (m4) (SEQ ID NO: 21) that targets the duplicated gene ICP0 (SEQ ID NO:13). In still other embodiments, the HSV-1 specific meganuclease is any combination of HSV1m5 (SEQ ID NO:19), HSV1m8 (SEQ ID NO:20), and/or HSV1m4 (SEQ ID NO: 21).

[0143] In some embodiments, the one or more meganuclease can comprise a sequence as set forth in SEQ ID NOs: 19, 20, and 21. In some embodiments, one or more meganuclease is a meganuclease that can be configured to target one or more sequences as set forth in SEQ ID NOs:11, 12, 13.

[0144] As used herein, "delivering" or "administering" refers to administering the compositions disclosed herein comprising the one or more viral vectors comprising one or more sequences encoding one or more HSV-1-specific meganuclease to a subject by a method or route which results in at least partial inoculation of the subject at the desired site.

[0145] In some embodiments, the one or more viral vectors of the present disclosure and the compositions disclosed herein can be delivered into the cell and/or administered to a subject according to methods generally well known to one of ordinary skill in the art which are appropriate for the particular viral vector, cell type, and/or subject. Exemplary methods of administration to a subject include, but are not limited to, administering the composition and/or the viral vector to the subject via intravenous injection, subcutaneous injection, intramuscular injection, autologous cell transfer, or allogeneic cell transfer. In still other embodiments, the viral vector is combined with one or more pharmaceutically acceptable carrier for administration. The pharmaceutically acceptable carriers can include those well known to one of ordinary skill in the art.

[0146] In still other embodiments, an "effective amount" of the viral vector is delivered to edit HSV-1 genome. As used herein, the term "effective" refers to any amount that induces a desired response while not inducing significant toxicity in the subject, e.g., edit the specific HSV-1 genome.

[0147] In some embodiments, the viral vectors and/or the compositions disclosed herein are administered or delivered into a cell. In some embodiments, the cell can be in a mammalian subject. In some embodiments, the mammalian subject can be human. In still other embodiments, the cell can be a superior cervical ganglia (SCG) cell. In still other embodiments, the cell can be a trigeminal ganglia (TG) cell. In still other embodiments,

the cell can be a combination of a SCG cell and/or a TG cell. In some embodiments, the cell can be a dorsal root ganglion (DRG) cell. In still other embodiments, the cell can be a combination of a SCG cell, TG cell, and/or a DRG cell.

[0148] In some embodiments, the viral vectors and/or compositions disclosed herein are administered or delivered to a cell and/or a subject prior to or after reactivation of a latent HSV-1 infection in the cell and/or in the subject. In some embodiments, the reactivation of latent HSV-1 is mediated by a bromo- and extra-terminal domain protein inhibitor (BET bromodomain inhibitor or BET inhibitor). As used herein, "BET inhibitor" or "BET protein inhibitor" refers to a compound that binds to BET and inhibits and/or reduces the biological activity of BET. In some embodiments, the BET inhibitor substantially or completely inhibits the biological activity of BET. In some embodiments, the biological activity is binding of BET to chromatin (e.g., histones associated with DNA) and/or another acetylated protein. In some embodiments, the BET inhibitor can inhibit one or more of BRD2, BRD3, BRD4, and BRDT. In some embodiments, the BET protein inhibitor can be selected from any of those BET protein inhibitors well known to one of ordinary skill in the art. In still other embodiments, the BET protein inhibitor can be selected from the group consisting of JQ1, birabresib, molibresib, apabetalone, ZEN-3694, BMS-986158, INC-B057643, and the like.

[0149] As used herein, the BET protein inhibitor JQ1, also known as (+)-JQ1 has the following chemical name: (tert-butyl (S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate).

[0150] Thus, in some embodiments, the method comprises delivering or administering to the cell and/or subject an effective amount of a BET inhibitor prior to delivering or administering an effective amount of one or more compositions of the present disclosure. In some embodiments, the method comprises delivering or administering to the cell and/or subject an effective amount of a BET inhibitor following the delivery or administration of an effective amount of one or more compositions of the present disclosure. In some embodiments, the method comprises delivering or administering to the cell and/or subject an effective amount of a BET inhibitor concomitantly with the delivery or administration of an effective amount of one or more compositions of the present disclosure. In some embodiments, the BET inhibitor is JQ1.

[0151] In some embodiments, the BET inhibitor can be delivered or administered at an "effective amount" of the BET inhibitor into the HSV-1-infected cell or administered

to an HSV-1 infected subject. As used herein, "effective amount" refers to the amount of a BET protein inhibitor that reduces the biological activity of BET, and thus, achieves the desired response without inducing significant toxicity in the cell or subject. In some embodiments, the desired response is induction of viral shedding. In some embodiments, the dose or effective amount of BET protein inhibitor is administered in mg/kg. In other embodiments, the dose of BET protein inhibitor is administered as a quantity to achieve a particular concentration within the HSV-1-infected cell or as a dose based on body weight of a subject. Determination of the effective amount of the dose and the type of dose (e.g., a dose based on body weight of the subject or a dose to achieve a particular concentration with the HSV-1-infected cell) is well within the capability of those skilled in the art.

[0152] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention. Publications cited herein and the subject matter for which they are cited are hereby specifically incorporated by reference in their entireties.

[0153] The following examples are set forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed.

EXAMPLE 1

[0154] *Meganuclease therapy reduces ganglionic viral load after ocular or genital HSV infection.* Several AAV serotypes for delivery of meganucleases to latently-infected mice were previously evaluated, and best results were found with AAV-Rh10, followed by AAV8 and AAV1. To further improve efficacy, additional neurotropic AAV serotypes, including AAV7, AAV9, AAV-DJ, and AAV-DJ/8, were tested for delivery of the anti-HSV1 meganuclease, m5, at a dose of 10^{12} AAV genomes (vg) per mouse (**FIG. 10A**), using the model of orofacial HSV disease previously described. Both AAV9 and AAV-DJ/8 were superior to 10^{12} vg AAV-Rh10, showing HSV reductions in superior cervical ganglia (SCG) of 95% ($p = 10^{-5}$) and 90% ($p = 0.018$), respectively, relative to untreated controls (**FIG. 10B**), comparing favorably with the 65% reduction previously obtained with m5 alone delivered with AAV-Rh10. Similarly, AAV9 and AAV-DJ/8 showed better activity than AAV-Rh10 in trigeminal ganglia (TG), with HSV load reductions of 48% ($p = 0.07$) and 41% ($p = 0.5$), respectively (**FIG. 10B**), compared with

prior observations of no detectable reduction using AAV-Rh10 delivering m5. The route of AAV administration (retro-orbital vein vs. intradermally into the whisker pad) did not have any detectable impact on either AAV transduction or gene editing efficiencies (**FIGS. 10E-10G**).

[0155] It has been previously demonstrated that gene editing of HSV could be increased by using combinations of AAV serotypes for meganuclease delivery, rather than a single AAV serotype, a finding ascribed to the heterogeneity of neuronal subsets within HSV-infected ganglia. Therefore, gene editing with the anti-HSV1 meganuclease m5, which cleaves a sequence in the *UL19* (SEQ ID NO: 11) gene coding for the major capsid protein VP5, when delivered using single AAV serotypes vs. combinations of AAV9, AAV-DJ/8, and AAV-Rh10 (**FIG. 11A**) administered as a total dose of 10^{12} vg per mouse, was evaluated. The combinations of AAV serotypes led to robust HSV gene editing, with the triple combination of AAV9, AAV-DJ/8, and AAV-Rh10 showing especially strong reductions in HSV loads and mutagenesis of residual HSV across both SCG and TG (**FIGS. 11B-11G**), as was previously demonstrated.

EXAMPLE 2

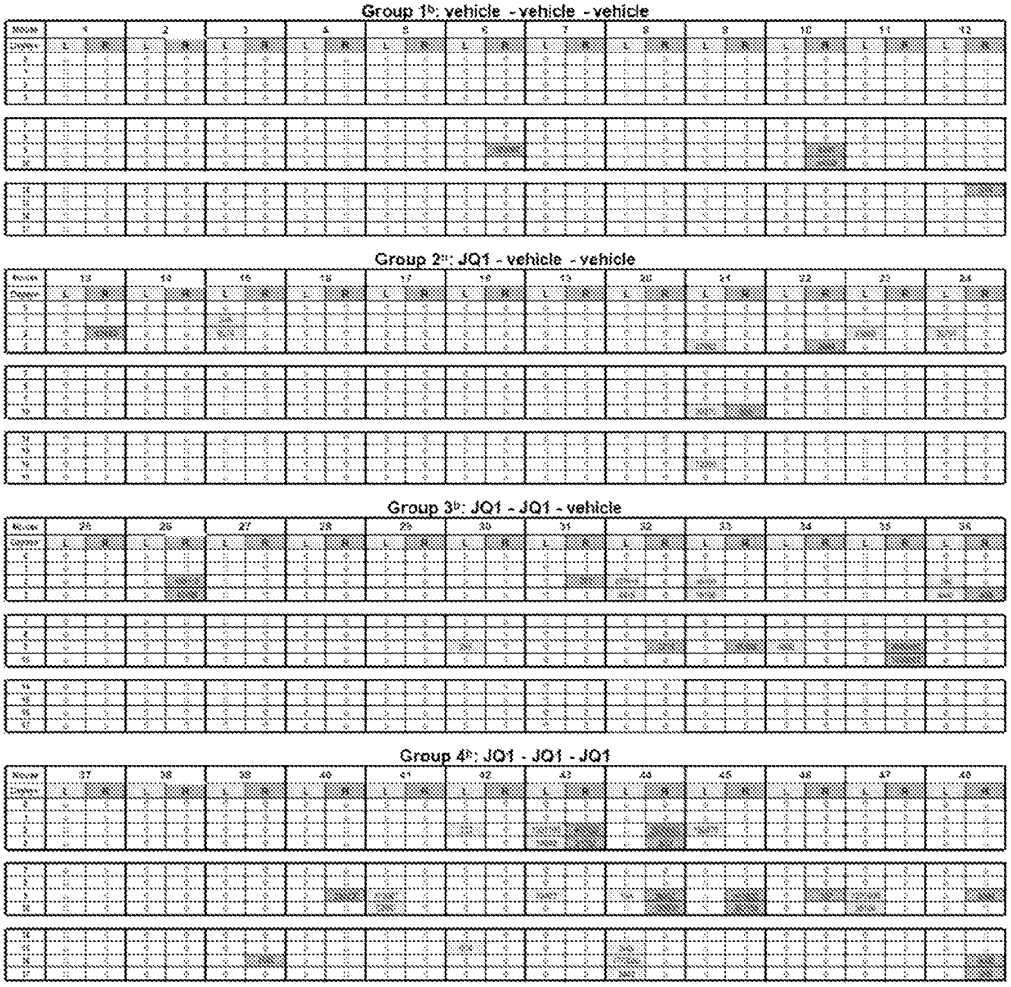
[0156] While orofacial infections with HSV are extremely common, genital infections, which lead to latent infection of dorsal root ganglia (DRG), also represent a major cause of morbidity. Latent genital infections in mice were established by intravaginal inoculation with HSV-1 after treatment with Depo Provera, which synchronizes the estrus cycle and increases HSV infection. Infected mice were treated with a total dose of $3x10^{12}$ vg of the AAV9, AAV-DJ/8, and AAV-Rh10 combination delivering two HSV1-specific meganucleases simultaneously (m5 along with m8, which targets a sequence in the *UL30* (SEQ ID NO: 12) gene coding for the catalytic subunit of the viral DNA polymerase. In parallel, the same AAV combination was tested against latent orofacial HSV infection as described above (FIGS. 1A-1B). Remarkably, the efficacy in the vaginal model of infection was the highest observed to date, with a 97.7% reduction in HSV viral load in DRG (**FIG. 1C**). This compared favorably with the orofacial infection group treated in parallel, in which (in agreement with previous studies) robust gene editing was observed with significant reductions of ganglionic HSV loads of 89% in SCG and 61% in TG (**FIGS. 1D-1E**).

EXAMPLE 3

[0157] *Induction of HSV shedding using the BET bromodomain inhibitor JQ1.* Mice generally show little if any spontaneous HSV reactivation, with minimal to no viral shedding at peripheral sites, limiting their utility in cure studies. The BET (Bromo and Extra-Terminal domain) bromodomain inhibitor JQ1 was reported to reactivate latent HSV *in vitro* in primary neuronal cultures, and HSV could be detected in the eyes of about one-third of latently-infected mice treated with JQ1. To evaluate the utility of JQ1 these studies were extended to determine the quantitative kinetics of HSV shedding after JQ1 therapy.

[0158] A single intraperitoneal (IP) injection of JQ1 (50 mg/kg) given to latently-infected mice (**FIG. 12A**) led to detectable shedding from the eyes of 56% (5/9) of animals, compared with 0/9 animals treated with vehicle alone (**FIGS. 12B-12C**). Viral shedding was transient, peaking at 2 days post JQ1, with maximal viral loads ranging from about 10^2 to almost 10^6 copies/swab (**FIG. 12C**). A direct comparison suggested that JQ1 may be a more powerful reactivation stimulus for HSV than hyperthermic stress (HS), which led to detectable virus shedding in less than 20% of animals (2/12 HS vs 4/10 JQ1), with peak shedding viral loads two logs lower than after JQ1 treatment (**FIGS. 12D-12F**). Sequential treatment with JQ1 at one-week intervals led to repeated shedding episodes with similar kinetics as observed above (**FIGS. 13A-13E**). Over the course of three sequential JQ1 reactivations (**FIG. 13E**), shedding from individual mice was stochastic; 10/12 (83%) mice shed detectable virus at least once, but only 1/12 (8%) shed after all three treatments, while 4/12 (33%) and 5/12 (42%) shed only after two or one of the three treatments, respectively (**FIG. 13E**Fig. S4 and **Table 1**). Shedding was typically unilateral (only detected in one eye), despite the initial inoculation being to both eyes (unilateral shedding was observed in 33/37 (89%) of events). The side of shedding in one episode was not predictive of the side of future shedding events (**Table 1**). Importantly for cure studies, repeated weekly reactivation of viruses with JQ1 up to 7 weekly injections did not change ganglionic viral loads compared with control animals (**FIGS. 13G-13H**).

TABLE 1 Virus titers in eye swabs collected after mock and JQ1 reactivations.



[a] Virus titers are expressed in copies/ml.

[b] Mice were subjected to 3 weekly reactivation: mock reactivation (vehicle) for Group 1, JQ1 reactivation (50 mg/kg) for Group 4 or combinations of mock (vehicle) and JQ1 (50 mg/kg) reactivations for groups 2 and 3.

[c] At each time point, daily swabs were collected from day 0 to day 3 after each reactivation, from the left eye and right eye and analyzed separately.

EXAMPLE 4

[0159] *Reduction of ganglionic HSV load is associated with reduced peripheral shedding.* The ability to reproducibly induce HSV reactivation and shedding with JQ1 allowed the investigation of the relationship between ganglionic viral load reduction using meganucleases and subsequent viral shedding at peripheral sites. Latently-infected mice were treated as above using the AAV9, AAV-DJ/8, and AAV-Rh10 combination delivering HSV1-specific meganucleases m5 and m8 at a total dose of $3x10^{12}$ vg or left untreated as controls. One month later, mice were administered JQ1, and eye swabs were collected daily for 4 days (**FIG. 2A**). Consistent with previous results, ganglionic tissues from treated mice showed a 98% and 42% reduction in mean viral loads in SCG and TG, respectively, when compared to control untreated animals (**FIGS. 2B-2C**) and detection of gene editing in the remaining viral genomes (**FIG. 2D**). After JQ1 administration, only 3/10 (30%) of the dual meganuclease-treated mice had detectable virus in eye swabs, compared with 5/10 (50%) of control untreated animals (**FIGS. 2E-2F**). The mean titer of HSV in positive eye swabs was $3x10^4$ copies/ml in the meganuclease-treated animals, compared with $1.2x10^5$ copies/ml in the control animals. Area under the curve analysis (AUC) demonstrated a 95% reduction ($p = 0.15$) in total viral shedding in treated vs. control animals (**FIG. 2G**).

[0160] In a separate experiment performed similarly (**FIG. 3A**), ganglionic tissues from treated mice showed 97% reduction in mean latent HSV genomes in both SCG and TG when compared to control untreated animals (**FIGS. 3B-3C**) and gene editing in the remaining genomes (**FIG. 3D**). While 3/8 mice from the control group shed virus with a mean viral titer of 8.2 $x10^5$ copies/ml, 0/8 meganuclease-treated mice had detectable shedding, representing a 100% decrease in total virus shed ($p = 0.10$, **FIGS. 3E-3G**).

EXAMPLE 5

[0161] *Safety of AAV/meganuclease therapy.* AAV-vectored therapies are generally considered safe. Nevertheless, dose-limiting liver toxicity has been observed after AAV administration in humans, non-human primates, and mice, typically at doses of $2x10^{14}$ vg/kg or greater. The $3x10^{12}$ vg/animal dose (approximately $1x10^{14}$ vg/kg) used in the experiments described in **FIGS. 1A-1E** and **FIGS. 3A-3G** approached the level associated with liver toxicity in previous studies. Across multiple studies 7/70 (10%) animals treated with the $3x10^{12}$ vg/animal dose were observed to exhibit clinical signs consistent with hepatotoxicity, including weight change, bloating, and general health

decline. Hepatotoxicity was confirmed in these animals by subsequent histopathological evaluation (**Table 2 and FIGS. 14A-14F**).

TABLE 2. Summary of Histologic Findings in the Liver.

	Naive[c,e]	HSV + AAV/MN[d,e,f]	AAV/MN only[c,e,f]
No. animals examined	2	1	2
Hepatocellular phenotypic alteration	(0)[a]	(1)	(2)
Minimal	-	-	-
Mild	-	1[b]	-
Marked	-	-	2
Inflammation mononuclear cell; Periportal and parenchymal	(0)	(1)	(2)
Minimal	-	-	-
Mild	-	1	-
Moderate	-	-	2
Hepatocellular necrosis, single cell	(0)	(1)	(2)
Minimal	-	1	-
Moderate	-	-	2
Hyperplasia; Kupffer and oval cell	(0)	(1)	(2)
Minimal	-	-	-
Moderate	-	1	2
Biliary cholestasis	(0)	(1)	(2)
Minimal	-	-	-
Mild	-	-	2

[a] Total number of animals with finding.

[b] Number of animals with the indicated severity for a specific finding.

[c] The liver was collected from naïve and AAV/MN only mice at 20 post AAV/MN administration.

[d] The liver was collected from HSV+AAV/MN mice at 33 post AAV/MN administration.

[e] All the mice were age-matched.

[f] AAV/MN was administered at a dose of 3.2×10^{12} vg AAV.

[0162] Therefore, lower total doses of triple AAV serotype/dual meganuclease therapy (0.6, 1.2 or 1.8×10^{12} vg/animal or 1.8, 3.6, or 5.4×10^{13} vg/kg) were evaluated for their tolerability and effects on viral load and JQ1-induced HSV shedding (**FIG. 4A**). These doses showed substantially improved tolerability, both clinically and upon histopathological examination and quantification of the number of inflammatory cell foci (ICF) in livers (**FIG. 15A**). Dose-dependent reductions in ganglionic HSV loads were observed across the three treatment groups compared to controls, ranging from 69% and

47% in SCG and TG, respectively, at the $0.6x10^{12}$ dose to 94% and 73% at the $1.8x10^{12}$ dose (**FIGS. 4B-4C**). **FIGS. 4D-4E** show corresponding AAV loads in SCG and in TG, respectively.

[0163] To evaluate the effect of these reduced doses on HSV shedding, treated mice were subjected to three weekly rounds of JQ1 administration, followed by eye swabbing as described above. While the percentage of dual meganuclease treated animals shedding virus after the first JQ1 reactivation was not reduced compared with the control mice, it was substantially lower than controls at all doses by the third JQ1 reactivation (0% (0/12), 8% (1/12) and 0% (0/12) for 0.6, 1.2 and $1.8x10^{12}$ vg/mouse groups, respectively, versus 18% (2/11) in the control group) (**FIGS. 4F-4I**). This finding may relate to the two additional weeks available for meganuclease expression and gene editing activity by the time of the third JQ1 reactivation. Consistent with this interpretation, the reduction in total viral shedding, as determined by AUC analysis, appeared to become more complete over time, with up to a 97-100% reduction in all three groups by the final JQ1 reactivation (**FIGS. 4J-4l**).

[0164] The efficacy of reduced-dose dual meganuclease therapy ($1.8x10^{12}$ vg) was confirmed in a separate experiment (**FIG. 5A**), showing a significant decrease in ganglionic viral loads in both SCG and TG (**FIGS. 5B-5C**). In this experiment, 7 of 12 control animals showed detectable viral shedding after JQ1 reactivation, compared with only 1 of 12 animals treated with AAV-meganuclease therapy, (**FIGS. 5D-5E**) and reduction in total viral shedding, as determined by AUC analysis (**FIGS. 5F-5G**). While none of the treated mice exhibited any clinical signs of hepatotoxicity, higher numbers of ICF in liver of treated animals receiving the $1.8x10^{12}$ vg dose compared to control mice were observed (**FIG. 15B**). Histologic analysis of H&E-stained TG sections from both control and treated animals revealed subtle evidence of neuronal injury, manifesting as neuronal degeneration, necrosis, and axonopathy. The scores grading prevalence and severity of the microscopic changes were higher in treated animals compared to control mice (**FIGS. 16A-16H** and Table 3). However, no mice in either the control or experimental group showed detectable signs of neuropathy.

TABLE 3. Summary of Histologic Findings in trigeminal ganglia.

	CTRL no JQ1, n = 1[a,b]			CTRL 2x JQ1, n = 1[a,b]			AAVMB[c] no JQ1, n = 3[a,b]			AAVMB[c] 2x JQ1, n = 3[a,b]		
1- Trigeminal ganglia	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]
Inflammation, LP/E	1	1	1	1	1	1	3 1 1	1.6	1.6	2 1 2	1.6	1.6
Neuronal degeneration	0	0	0	0	0	0	2 0 0	0.7	2	1 0 1	0.7	2
Neuronal necrosis	0	0	0	0	0	0	1 0 1	0.7	1	1 0 1	0.7	2
Perivascular infiltrates	0	0	0	0	0	0	1 0 0	0.3	1	0 0 0	0	0
Sum - Scores:	**1**	**1**	**1**	**1**	**1**	**1**	**7 1 2**	**3.3**	**5.6**	**4 1 4**	**3**	**5.6**
2- Associated nerve fiber	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]	Score	Mean score[d]	Mean severity[e]
Gliated myelin sheath	2	2	2	2	2	2	3 3 3	3	3	2 1 2	1.7	1.7
Digestion chamber	0	0	0	1	1	1	2 2 0	1.3	2	1 0 1	0.7	1
Perivascular infiltrates	0	0	0	0	0	0	2 1 2	1.7	1.7	1 0 1	0.7	1
Inflammation, LP/E	1	1	1	1	1	1	1 1 3	1.7	1.7	0 0 0	0	0
Sum - Scores:	**3**	**3**	**3**	**4**	**4**	**4**	**8 7 8**	**7.7**	**8.4**	**4 1 4**	**3.1**	**3.7**

[a] Number of TG per slide
[b] Each slide had 3 sections from one TG
[c] AAV dose administered was 1.8×10^{12} vg/mouse
[d] Mean score = Sum of scores from TG in the group/number of TG in the group
[e] Mean severity = Sum of scores from TG in the group/number of TG with a score > 0 in the group

TABLE 4. JQ1-induced virus shedding in C57BL/6 mice latently infected with HSV-1 17+ (lab strain) or HSV-1 clinical isolates.

HSV Copies/10ul DNA						
day 0 post JQ1	Day 1 post JQ1	Day 2 post JQ1	Day 3 post JQ1	Eye	Mouse ID	HSV-1 Strain dose
0	0	0	0	R	1	HSV-1 17+ 2E5 PFU per eye
0	0	0	0	L		
0	0	0	0	R	2	
0	0	0	0	L		
0	0	0	0	R	3	
0	82	279	0	L		
0	0	0	0	R	7	6676 2E5 PFU per eye
0	0	0	0	L		
0	0	0	0	R	8	
0	0	0	0	L		
0	0	0	0	R	9	
0	29	5123	1209	L		
0	0	0	0	R	10	7258 2E5 PFU per eye
0	0	0	0	L		
0	0	0	408	R	11	
0	0	0	0	L		
0	0	34315	0	R	12	
0	0	28	0	L		
0	2	0	0	R	13	7577 2E5 PFU per eye
0	0	0	0	L		
0	0	0	0	R	14	
0	0	1079	0	L		
0	0	0	0	R	15	
3	0	0	0	L		
0	0	0	0	R	16	7261 2E5 PFU per eye
0	0	0	0	L		
0	0	0	0	R	17	
0	0	24	0	L		
0	0	1222	0	R	18	
0	0	0	0	L		

EXAMPLE 6

[0165] *Reduced dose of AAV/meganuclease treatment of genitally infected animals decreases DRG latent viral load and may reduce genital HSV shedding.* As noted above, genital HSV infection is a major cause of morbidity in humans. The effect of reduced-dose dual meganuclease therapy (total dose of $1.8x10^{12}$ vg/animal) in vaginally-infected mice was evaluated (**FIG. 6A**). In agreement with previous results, the reduced-dose therapy led to a 78.8% ($p = 0.02$) to 95.6% ($p = 0.006$) reduction in latent virus genomes in DRGs (**FIG. 6B**).

[0166] The ability of JQI to induce HSV shedding in the genital infection model, as observed in the ocular infection model, was evaluated. Over 3 sequential JQ1 reactivations, only 2 of 8 control animals (and 1 of 8 AAV/meganuclease treated animals) shed detectable virus, a rate lower than the 40-50% reactivation we typically observe after ocular infection (**FIGS. 6C-6D**). The apparently lower rate of reactivation seen in the vaginal model compared to the ocular model may be due to lower levels of ganglionic viral loads in the DRG (10^2-10^3 vg/10^6 cells in DRG, **FIG. 6B** vs 10^4-10^5 vg/10^6 cells in SCG or TG, **FIG. 5B-5C**). While this lower reactivation rate prohibited meaningful statistical analysis, the observation that 2 out of the 8 control mice shed virus over 2 to 3 sequential days, while only 1 of the 8 AAV-treated mice shed virus, on a single day and at a substantially lower level, is qualitatively in agreement with observations after ocular infection (**FIGS. 6C-6G**).

EXAMPLE 7

[0167] *Meta-analysis of the effect of AAV/meganuclease therapy on HSV shedding.* The stochastic nature of clinical HSV reactivation, recapitulated when induced by JQ1 in mice (**FIGS. 13A-13H** and **Table 1**), makes evaluation of viral shedding extremely resource intensive. Practical constraints, including the number of animals that can be housed and studied at the same time, along with the extended duration of each study (~3 months), limited the statistical power of individual experiments. Therefore, a meta-analysis of data from all experiments presented above (Examples 1-5), combining evidence from infection sites (orofacial or genital), thus comparing 174 swabs from AAV/meganuclease-treated animals to 99 swabs from experimentally-matched controls, was performed. The primary endpoint was viral shedding, expressed either as a binary variable (equal to 1 for samples in which HSV was detected and 0 otherwise) or the log10-transformed AUC for quantitative viral shedding. The experiments depicted in FIGS. 1-6 represent all the shedding studies with the dual meganuclease/triple AAV therapy performed, and each suggests a strong and consistent trend toward a substantial reduction in viral shedding after AAV/meganuclease therapy. Across all studies, the proportion of swabs with detectable HSV was 48% lower among AAV/meganuclease-treated animals compared to controls. The meta-analysis confirmed that animals receiving AAV/meganuclease therapy had a statistically significant reduction in viral shedding (OR = 0.41, $p = 0.010$, by generalized linear mixed models, GLMM).

[0168] Next, the effect of dose or duration of meganuclease therapy and the probability of viral shedding (expressed as a binary variable) or the quantity of viral shedding (expressed as the log10-transformed AUC), was evaluated. Overall, the probability of viral shedding significantly decreased with the dose of AAV/meganuclease (OR = 0.66; $p = 0.023$, GLMM), and also with the duration of meganuclease therapy (OR = 0.42; $p < 0.001$, GLMM) in treated animals compared to controls. The data further indicate that overall, the quantity of virus shed (AUC) significantly decreased with the AAV/meganuclease dose at a rate of -0.36 $\log_{10}$ copy-days per 10^{12} increase in dose (LMM; $p = 0.028$), and also with the duration of meganuclease therapy, at a rate of -0.48 $\log_{10}$ copy-days per additional week after treatment (LMM; $p = 0.017$). No significant association was detected between the log10-transformed AUC and the interaction between time and dose (LMM; $p = 0.59$).

EXAMPLE 8

[0169] *Simplification of the AAV-meganuclease regimen.* The studies described above were performed using a triple AAV serotype/dual meganuclease approach, resulting in each animal receiving a total of 6 unique vectors (3 serotypes x 2 meganucleases). Clinical translation of such a complex regimen could raise manufacturing and quality control issues. To avoid the foregoing issues, simplification of AAV/meganuclease therapy to reduce the complexity of therapeutic regimen was sought.

[0170] The dual cutting meganuclease m4 (SEQ ID NO: 21), recognizes a sequence in the duplicated gene *ICP0* (SEQ ID NO:13) in the HSV-1 genome and has been previously shown to induce significant decrease of latent viral loads in ganglia of latently infected mice. Latently infected mice were administered a total dose of $5x10^{11}$ vg of either the combination of AAV9, AAV-DJ/8, and AAV-Rh10, or each single AAV serotype delivering the HSV1-specific meganuclease m4 (**FIG. 7A**). Consistent with the results using the lower dose of 6 x 10^{11} of the triple AAV-dual meganuclease therapy (**FIGS. 4A-4L**), ganglionic tissues from treated mice with the triple AAV combination delivering m4 showed a 73.9% ($p < 0.0001$) and 43.7% ($p = 0.014$) reduction in mean viral loads in SCG and TG, respectively, when compared to control untreated animals. When m4 was delivered using single AAV serotypes, the data confirmed that AAV9 on its own could recapitulate the viral load decrease seen with the triple AAV serotype combination, with 77.8% ($p < 0.0001$) and 49% ($p = 0.0046$) reduction in mean viral loads in SCG and TG, respectively (**FIGS. 7B-7C**). Furthermore, mice having received AAV9 alone showed the

lowest levels of liver inflammation of any of the groups, similar to those in the control liver (**FIG. 7D**). At this reduced dose, regardless of the AAV serotype combination used, no detectable neurotoxicity was observed compared to the control animals (**FIGS. 7E-7F**).

[0171] To confirm that a simplified regimen composed of AAV9-m4 was also able to reduce peripheral virus shedding, latently infected mice were treated as above using AAV9 delivering either HSV1-specific meganuclease m4 (SEQ ID NO: 21) or a catalytically inactive version (m4i) (SEQ ID NO: 22) at a dose of $1x10^{12}$ vg. One month later, mice were subjected to two weekly rounds of JQ1 administration, followed by daily eye swabbing for 3 days (**FIGS. 8A-8B**). A decrease of ganglionic viral loads of 89.6% ($p < 0.0001$) and 69% ($p = 0.03$) in SCG and TG respectively, was observed in m4-treated mice but not in mice treated with the inactive form of the meganuclease m4i (**FIGS. 8C-8D**). Furthermore, 6 out of 9 control mice and 6 out of 10 m4i-treated mice shed virus after JQ1 reactivations, while only 3 out of 10 m4-treated mice had detectable virus shedding after reactivation (**FIGS. 8E-8I**). These data demonstrate that a simplified regimen can substantially reduce ganglionic viral loads, with an associated decrease in virus shedding after reactivation, and that these effects are dependent on an active enzyme and not on AAV itself. In this experiment, mice treated with m4 had slightly higher levels of liver ICF and TG axonopathy, but not more TG inflammation, compared to control mice (FIGS. 8J-8L).

EXAMPLE 9

[0172] *Tissue restriction of meganuclease expression improves tolerability.* Across studies, it was observed that ~10% of the animals treated with a high dose of AAV/meganuclease ($2\text{-}3x10^{12}$ vg/animal, or approximately $6\text{-}9x10^{13}$ vg/kg) exhibited clinical signs consistent with hepatotoxicity, including weight change, bloating, and general health decline. When lower doses were evaluated, substantially improved tolerability, both clinically and upon histopathological examination and ICF quantification, was observed. To further reduce hepatoxicity, the use of neuron-specific promoters (Calmodulin Kinase II (CamKII) and human synapsin (hSyn)) combined with the CMV enhancer, was evaluated to test the hypothesis that limiting enzyme expression to neuronal tissues would decrease or perhaps prevent liver toxicity (**FIG. 9A**). Latently infected mice treated with a high dose ($2x10^{12}$ vg) of AAV9-E/CamKII-m4 (SEQ ID NO: 8) or AAV9-E/hSyn-m4 (SEQ ID NO: 10) did not show any clinical signs of hepatotoxicity (weight change, general health decline, or ICF), in contrast to mice treated with $2x10^{12}$ vg AAV9-CBh-m4 (SEQ ID NO: 6) (**FIGS. 9B-9C**). Moreover, while liver inflammation increased

over time in AAV9-CBh-m4-treated mice, it remained low in AAV9-E/CamKII-m4 (**FIG. 20D**).

[0173] Intriguingly, histopathologic signs of neurotoxicity in TG from AAV9-E/CamKII-m4 or AAV9-E/hSyn-m4 treated mice were also similar to those in control mice, while they were significantly higher in TG from AAV9-CBh-m4 treated mice (FIGS. 9D-9E). A decrease of ganglionic viral loads of 67.9% ($p = 0.07$) and 70.4% ($p = 0.05$) in SCG and TG respectively, was observed in AAV9-E/CamKII-m4-treated mice but not in mice treated with the AAV9-E/hSyn-m4 (**FIGS. 9F-9G**). Assessment of m4 expression in neuronal tissues at different times post administration of either AAV9-CBh-m4 or AAV9-E/CamKII-m4 showed that the m4 expression increased over time but was in general slightly lower in tissues from AAV9-E/CamKII-m4-treated mice compared with AAV9-CBh-m4-treated mice (**FIGS. 20A-20B**). This may explain the slightly lesser degree of viral load reduction in AAV9- E/CamKII-m4-treated mice compared with AAV9- CBh-m4-treated mice (**FIGS. 9F-9G**). In conclusion, AAV9-E/CamKII-m4 regimen retains efficacy and shows improved tolerability compared to AAV9-CBh-m4.

EXAMPLE 10

[0174] *Mice.* Five- to eight-week-old female Swiss Webster mice were purchased from either Taconic or Charles River and housed in accordance with the Fred Hutch Cancer Center and NIH guidelines on the care and use of animals in research. Experimental procedures performed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Fred Hutch Cancer Center. Standard housing, diet, bedding, enrichment, and light/dark cycles were implemented under animal biosafety level 2 (ABSL2) containment.

[0175] A limited set of preliminary experiments was performed in C57BL/6 mice (Charles River), and we observed similar rates of viral load reduction and gene editing by T7 assay, suggesting that results were not strain-specific.

EXAMPLE 11

Ocular HSV infection.

[0176] Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (12 mg/kg). Mice were infected in both eyes by dispensing 10^5 PFU of HSV1 syn17+ contained in 4 ul following corneal scarification using a 28-gauge needle.

Vaginal HSV infection.

[0177] Mice were treated with 2 mg of Depo-Provera injected subcutaneously. Five to seven days later, they were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (12 mg/kg) and intravaginally infected with either $5x10^2$ or 10^3 PFU of HSV1 syn17+ contained in 4 ul using a pipette after clearing the vaginal lumen with a Calginate swab.

EXAMPLE 12

AAV inoculation.

[0178] Mice anesthetized with isoflurane were administered the indicated AAV vector dose by either unilateral intradermal whisker pad (WP), or intravenous injection: unilateral retro-orbital (RO; ocular HSV infected mice) or tail vein (TV; ocular and vaginal HSV infected mice) injection. Tissues were collected at the indicated time.

EXAMPLE 13

Study approval.

[0179] All animal procedures were approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Center. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health ("The Guide").

EXAMPLE 14

HSV reactivation.

[0180] JQ1 reactivation was performed by intraperitoneal (IP) injection of (+)-JQ-1 (JQ1, MedChemExpress) at a dose of 50 mg/kg at the indicated time. JQ1 was prepared from a stock solution (50mg/ml in DMSO, Sigma) by 1:10 dilution in a vehicle solution 10% w/v 2-hydroxypropyl-β-cyclodextrin in PBS (Sigma). Alternatively, JQ1 reactivation was performed with 2 IP injections separated by 12h, which resulted in the detection of virus shedding in the eyes of 6 out of 9 (66.6%) mice (Supplemental figure 5). A limited set of experiments was performed in C57BL/6 mice (Charles River), and we observed similar rates and kinetics of peripheral viral load shedding, suggesting that the results of JQ1 reactivation were not mouse strain specific. Hyperthermic stress (HS) reactivation was performed as previously described.

EXAMPLE 15

Cells, herpes simplex viruses, and AAV stocks.

[0181] HEK293 and Vero cell lines (ATCC # CCL-81) were propagated in Dubelcco's modified Eagle Medium supplemented with 10% fetal bovine serum. HSV-1 strain *syn*17+ (kindly provided by Dr. Sawtell) was propagated and tittered on Vero cells.

AAV production and tittering.

[0182] AAV vector plasmids pscAAV-CBh-m5 ((SEQ ID NO:4), pscAAV-CBh-m8 (SEQ ID NO:5), pscAAV-CBh-m4(SEQ ID NO:6), pscAAV-CBh -m4i (SEQ ID NO:7) and pscAAV-E/CamKII-m4 (SEQ ID NO:8) were used to generate the AAV stocks in this study (**FIG. 21**). All AAV stocks were generated from transfected HEK293 cells and culture media produced by the Viral Vector Core of the Wellstone Muscular Dystrophy Specialized Research Center (Seattle). AAV stocks were generated by PEG-precipitation of virus from cell lysates and culture media, followed by iodixanol gradient separation and concentration into PBS using an Amicon Ultra-15 column (EMD Millipore). AAV stocks were aliquoted and stored at -80°C. All AAV vector stocks were quantified by qPCR using primers/probe against the AAV ITR, with linearized plasmid DNA as a standard, according to the method of Aurnhammer et al. AAV stocks were treated with DNase I and Proteinase K prior to quantification.

EXAMPLE 16

Quantification of viral loads in tissues.

[0183] Total genomic DNA was isolated from ganglionic tissues using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD) per the manufacturer's protocol. Viral genomes were quantified by ddPCR in tissue DNA samples using an AAV ITR primer/probe set for AAV, and a gB primer/probe set (SEQ ID NOs: 14-16) for HSV as described previously. Cell numbers in tissues were quantified by ddPCR using mouse-specific RPP30 primer/probe set: Forward 5'-GGCGTTCGCAGATTTGGA (SEQ ID NO:1), Reverse 5'- TCCCAGGTGAGCAGCAGTCT (SEQ ID NO:2), probe 5'-ACCTGAAGGCTCTGCGCGGACTC (SEQ ID NO:3). In some control ganglia, sporadic samples showed positivity for AAV genomes, although the levels were typically >2-3 logs lower than in ganglia from treated mice having received AAV. This was attributed to low-level contamination of occasional tissue samples. The ganglionic AAV loads for experiments presented in **FIGS. 1A-1E** to **FIGS. 8A-8L** are shown in **FIGS. 18A-18O** and those for the experiment presented in **FIGS. 9A-9G** are shown in **FIGS. 19A-19C**. The

statistical analysis was performed using GraphPad Prism version 9.4.1. The test used for each data set is indicated in the figure legends.

EXAMPLE 17

Quantification of HSV in eye swabs.

[0184] Swab samples were collected into vials containing 1 ml of digestion buffer (KCL, Tris HCl pH8.0, EDTA, IGEPAL CA-630). DNA was extracted from 200 ml of digestion buffer using QIAamp 96 DNA Blood Kits (Qiagen, Germantown, MD) and eluted into 100 ml AE buffer (Qiagen). Then, 10 ml of DNA was used to set up 30 ml real-time TaqMan quantitative PCR reactions. The primers and probes were as described previously. QuantiTect Multiplex PCR mix (Qiagen) was used for PCR assays. The PCR cycling conditions were as follows: 1 cycle at 50°C for 2 minutes, 1 cycle at 95°C for 15 minutes, and 45 cycles of 94°C for 1 minute and 60°C for 1 minute. Exo internal control was spiked into each PCR reaction to monitor inhibition. A negative result was accepted only if the internal control was positive with a cycle threshold (CT) within 3 cycles of the Exo CT of no template controls.

EXAMPLE 18

Western blot detection.

[0185] Tissues lysates were obtained from 1 TG per mouse collected in 200 ul RIPA buffer (PIERCE, Thermo Fisher Scientific) with protease inhibitor cocktail (Roche) and disrupted by sonication on ice. Thirteen microliters of tissue lysates were loaded onto 4-12% NuPAGE gel, transfer onto nitrocellulose membrane and probe for m4 expression using rabbit anti-HA antibody (1:1000 mAb clone C29F, Cell signaling) and β-actin (1: 1000 mAb clone13E5, cell signaling) for protein loading. Membrane hybridization and detection were performed using PIERCE Fast Western blotting kit Super signal, West Pico Rabbit (Thermo Fisher Scientific) per manufacturer protocol and imaged using ChemiDoc Imaging System (BIO-RAD)

EXAMPLE 19

Inflammatory cell foci (ICF) quantification.

[0186] Liver tissues were paraffin-embedded, sectioned and H&E stained by the Experimental histopathology shared resources of the Fred Hutchinson Cancer Center. ICF were counted by a blinded observer and expressed as the number of ICF per surface area which was determined using Fiji.

Grading of neuronal changes within trigeminal ganglia.

[0187] Trigeminal ganglia were paraffin-embedded, sectioned, and H&E stained by the Experimental histopathology shared resources of the Fred Hutchinson Cancer Center. Microscopic changes were graded as to severity by a veterinarian pathologist using a standard grading system whereby 0 = no significant change, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe.

EXAMPLE 20

Statistical analysis.

[0188] Statistical analyses for each individual experiment were performed using GraphPad Prism version 9.4.1 and R. Tests were two-sided and p-values smaller than 0.05 considered significant. The specific test used for each analysis is indicated in the corresponding figure legend.

[0189] Meta-analyses were performed on combined data from all experiments (Figures 1-6). To assess whether AAV/MN-treated mice shed virus less often than control animals and whether the frequency of viral shedding decreased with the duration of meganuclease therapy, a generalized linear mixed models (GLMM) describing the probability of viral shedding (with viral shedding defined as an AUC > 0) as a function of therapy duration and dose treated as continuous variables, while adjusting for experiment, was used. Animal-specific random intercepts were included in the model to capture intra-mice dependencies between observations. Including an interaction term between dose and duration to evaluate whether change in the probability of viral shedding over time was affected by dose (in particular, whether it decreased faster with dose) was also considered. Association between viral shedding and covariates (dose, therapy duration) are reported as odds ratios (OR). The significance of the association between the probability of viral shedding and dose, therapy duration, and their interactions was evaluated using two-sided Wald tests.

[0190] To study the relationship between the quantity of virus shedding and therapy dose and duration, a linear mixed models (LMM) describing the log10-transformed AUC as a function of therapy dose and duration, treated as continuous variables, while adjusting for experiment, was used. The model included an interaction between dose and therapy duration to assess whether change in AUC over time was impacted by dose (e.g., whether the log10-transformed AUC decreased faster with dose). Animal-specific random intercepts were also included to capture intra-mice dependencies between observations.

The standard errors of regression coefficients were estimated using a robust, sandwich-type estimator. Association between viral shedding and covariates (dose, therapy duration) are reported as odds ratios (OR). The significance of the association between the log10-transformed AUC and dose, therapy duration, and their interactions was evaluated using Wald tests. Both sets of analyses also evaluated models that included dose squared and square root of dose to perform sensitivity analysis and assess whether the relationship between the probability of viral shedding (or the log10-transformed AUC) and dose were nonlinear. Likewise, models were considered that included squared therapy duration or square root of therapy duration to perform sensitivity analysis and assess whether relationships between the probability of shedding (or the log10-transformed AUC) and therapy duration were nonlinear.

Table 5 Sequences of the Disclosure

SEQ ID NO.	Sequence Name	Sequence
1	Mouse-specific RPP30 primer (forward)	GGCGTTCGCAGATTTGGA
2	Mouse-specific RPP30 primer (reverse)	TCCCAGGTGAGCAGCAGTCT
3	Mouse-specific RPP30 probe	ACCTGAAGGCTCTGCGCGGACTC

4	pscAAV-CBh-m5	ttatagggccgccgccgccgccgcctcgccataa aaggaaactttcggagcgcgccgctctgattggc tgccgccgcacctctccgcctcgccccgccccgc ccctcgccccgccccgcctggcgcgcgccccccc ccccccccgcccccatcgctgcacaaaataatta aaaaataaataaatacaaaattgggggtggggag ggggggagatggggagagtgaagcagaacgtggg gctcacctcgaccatggtaatagcgatgactaat acgtagatgtactgccaagtaggaaagtcccata aggtcatgtactgggcacaatgccaggcgggcca tttaccgtcattgacgtcaatagggggcgtactt ggcatatgatacacttgatgtactgccaagtggg cagtttaccgtaaatactccacccattgacgtca atggaaagtccctattggcgttactattgacgtc aatgggcgggggtcgttgggcggtcagccaggcg ggccatttaccgtaagttatgtaacggtaccgaa ttggcgcgccgataaccccactccctctctgcgc gctcgctcgctcactgaggccgggcgaccaaagg tcgcccgacgcccgggctttgcccgggcggcctc agtgagcgagcgagcgcgcagctgcattaatgaa tcggccaacgcgcggggagaggcggtttgcgtat tgggcgctcttccgcttcctcgctcactgactcg ctgcgctcggtcgttcggctgcggcgagcggtat cagctcactcaaaggcggtaatacggttatccac agaatcaggggataacgcaggaaagaacatgtga gcaaaaggccagcaaaaggccaggaaccgtaaaa aggccgcgttgctggcgtttttccataggctccg cccccctgacgagcatcacaaaaatcgacgctca agtcagaggtggcgaaacccgacaggactataaa gataccaggcgtttcccccctggaagctccctcgt gcgctctcctgttccgaccctgccgcttaccgga tacctgtccgcctttctcccttcgggaagcgtgg cgctttctcatagctcacgctgtaggtatctcag

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SEQ ID NO.	Sequence Name	Sequence
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SEQ ID NO.	Sequence Name	Sequence
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11	UL19 targeted by HSV1m5 (sequence targeted by HSV-1 specific meganuclease **underlined and bolded**)	ttacagagccagtcccttgagcggggatgcgtca tagacgagatactgcgcgaagtgggtctcccgcg cgtgggcttccccgttgcgggcactgcggaggag ggcggggtcgctggcgcaggtgagcgggtaggcc tcctgaaacaggccacacgggtcctccacgagtt cgcggcaccccgggggcgcttaaactgtacgtc gctggcggcggtggccgtggacaccgccgaaccc gtctccacgatcaggcgctccaggcagcgatgtt tggcggcgatgtcggccgacgtaaagaacttaaa gcaggggctgagcaccggcgaggccccgttgagg tggtaggccccgttatagagcaggtccccgtacg aaaatcgctgcgacgcccacgggttggccgtggc cgcgaaggcccgggacgggtcgctctggccgtgg tcgtacatgagggcggtgacatccccctccttgt cccccgcgtaaacgcccccggcggcgcgtccccg ggggttgcagggccggcggaagtagttgacgtcg gtcgacacgggggtggcg**ataaactcacacacgg** **cgtcctgg**ccgtggtccatccctgcgcgccgcgg cacctgggcgcacccgaacacggggacgggctgg gccggccccaggcggtttcccgccacgaccgcgt tccgcaggtacacggctgccgcgttgtccaggag aggggagccccgcggcccaggtaaaagttttgg ggaaggttgcccatgtcggtgacggggttgcgga cggttgccgtggccacgacggcggtgtagcccac gcccaggtccacgttcgcgcgcggctgggtgagc gtgaagtttacccccccgccagtttcgtgccggg ccacctggagctggcccaggaagtacgcctccga cgcgcgctccgagaacagcacgttctcagtcaca aagcggtcctgtcggacgacggtgaacccaaacc cgggatggaggcccgtcttgagctgatgatgcaa ggccacgggactgatcttgaagtaccccgccatg agcgcgtaggtcagcgcgttctccccggccgcgc tctcgcggacgtgctgcacgacgggctgtcggat

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12	UL30 targeted by HSV1m8 (sequence targeted by HSV-1 specific meganuclease **underlined and bolded**)	atgttttccggtggcggcggcccgctgtcccccg gaggaaagtcggcggccagggcggcgtccgggtt ttttgcgcccgccggccctcgcggagccagccgg ggacccccgccttgtttgaggcaaaacttttaca acccctacctcgccccagtcgggacgcaacagaa gccgaccgggccaacccagcgccatacgtactat agcgaatgcgatgaatttcgattcatcgccccgc gggtgctggacgaggatgcccccccggagaagcg cgccggggtgcacgacggtcacctcaagcgcgcc cccaaggtgtactgcgggggggacgagcgcgacg tcctccgcgtcgggtcgggcggcttctggccgcg gcgctcgcgcctgtggggcggcgtggaccacgcc ccggcggggttcaaccccaccgtcaccgtctttc acgtgtacgacatcctggagaacgtggagcacgc gtacggcatgcgcgcggcccagttccacgcgcgg tttatggacgccatcacaccgacggggaccgtca tcacgctcctgggcctgactccggaaggccaccg ggtggccgttcacgtttacggcacgcggcagtac ttttacatgaacaaggaggaggtcgacaggcacc tacaatgccgcgccccacgagatctctgcgagcg catggccgcggccctgcgcgagtccccgggcgcg tcgttccgcggcatctccgcggaccacttcgagg cggaggtggtggagcgcaccgacgtgtactacta cgagacgcgcc**ccgctctgttttaccgcgtctac** **g**tccgaagcgggcgtgtgctgtcgtacctgtgcg acaacttctgcccggccatcaagaagtacgaggg tggggtcgacgccaccacccggttcatcctggac aaccccgggttcgtcaccttcggctggtaccgtc tcaaaccgggccggaacaacacgctagcccagcc ggcggccccgatggccttcgggacatccagcgac gtcgagtttaactgtacggcggacaacctggcca tcgaggggggcatgagcgacctaccggcatacaa gctcatgtgcttcgatatcgaatgcaaggcgggg

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SEQ ID NO.	Sequence Name	Sequence
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13	ICP0 (RL2) targeted by HSV1m4 (sequence targeted by HSV-1 specific meganuclease **underlined and bolded**)	tcgcatttgcacctcggcactcggagcgagacgc agcagccaggcagactcgggccgccccctctccg catcaccacagaagccccgcctacgttgcgaccc ccagggaccctccgtccgcgaccctccagccgca tacgacccccatggagccccgccccggagcgagt acccgccggcctgagggccgcccccagcgcgagg tgaggggccgggcgccatgtctggggcgccatat tgggggcgccatattgggggcgccatgttggg ggaccccgacccttacactggaaccggccgcca tgttgggggaccccccactcatacacgggagccgg gcgccatgttggggcgccatgttaggggcgtgg aaccccgtgacactatatatacagggaccggggg cgccatgttaggggtgcggaaccccctgaccct atatatacagggaccggggtcgccctgttggggg tcgccatgtgacccccctgactttatatatacaga cccccaacacatacacatggcccctttgactcag acgcagggcccggggtcgccgtgggacccctga ctcatacacagagacacgcccccacaacaaacac acaaggaccggggtcgccgtgttgggggcgtggt ccccactgactcatacgcaggcccccttactca cacgcatctaggggggtggggaggagccgcccgc catatttgggggacgccgtgggacccccgactcc ggtgcgtctggagggcgggagaagagggaagaag aggggtcgggatccaaaggacggacccagaccac ctttggttgcagacccctttctcccccctcttcc gaggccagcaggggggcaggactttgtgaggcgg gggggggagaggggggaactcgtgggtgctgattg acgcgggaaatcccccccccattcttacccgcccc ccttttttcccccttagcccgccccggatgtctgg gtgtttccctgcgaccgagacctgccggacagca gcgactctgaggcggagaccgaagtgggggggcg gggggacgccgaccaccatgacgacgactccgcc tccgaggcggacagcacggacacggaactgttcg

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SEQ ID NO.	Sequence Name	Sequence
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14	gB primer for ddPCR and qPCR quantification of HSV (forward)	CCGTCAGCACCTTCATCGA
15	gB primer for ddPCR and qPCR quantification of HSV (reverse)	CGCTGGACCTCCGTGTAGTC
16	gB probe for ddPCR and qPCR quantification of HSV	CCACGAGATCAAGGACAGCGGCC
17	UL19 primer (forward)	CTGGCCGTGGTCGTACATGA
18	UL19 primer (reverse)	TCACCGACATGGGCAACCTT

SEQ ID NO.	Sequence Name	Sequence
19	Meganuclease HSV1m5	MANTKYNKEFLLYLAGFVDGDGSIIAQIKPNQSY KFKHQLSLTFDVTQKTQRRWFLDKLVDEIGVGYV TDSGSVSRYRLSEIKPLHNFLTQLQPFLKLKQKQ ANLVLKIIEQLPSAKESPDKFLEVCTWVDQIAAL NDSKTRKTTSETVRAVLDSLSEKKKSSPAAGDSS VSNSEHIAPLSLPSSPPSVGSNKEFLLYLAGFVD SDGSIIAQIEPNQSYKFKHRLKLTFKVTQKTQRR WILDKLVDEIGVGYVRDSGSVSNYILSEIKPLHN FLTQLQPFLKLKQKQANLALKIIEQLPSAKESPD KFLEVCTWVDQVAALNDSKTRKTTSETVRAVLDS LSEKKKSSP
20	Meganuclease HSV1m8	MANTKYNEEFLLYLAGFVDGDGSIIAQIKPKQSA KFKHQLSLTFQVTQKTQRRWFLDKLVDEIGVGYV RDSGSVSHYYLSEIKPLHNFLTQLQPFLELKQKQ ANLVLKIIEQLPSAKESPDKFLEVCTWVDQVAAL NDSKTRKTTSETVRAVLDSLSEKKKSSPAAGGSD KYNQALSKYNQALSKYNQALSGGGGSNKKFLLYL AGFVDSDGSIIAQIKPNQHCKFKHQLALTFQVTQ KTQRRWFLDKLVDRIGVGYVRDSGSVSNYKLSEI KPLHNFLTQLQPFLKLKQKQANLVLKIIEQLPSA KESPDKFLEVCTWVDQVAALNDSKTRKTTSETVR AVLDSLSEKKKSSP

SEQ ID NO.	Sequence Name	Sequence
21	Meganuclease HSV1m4	MANTKYNEEFLLYLAGFVDGDGSIIAQIKPNQSY KFKHQLSLTFMVTQKTQRRWFLDKLVDEIGVGYV RDAGSVSNYILSKIKPLHNFLTQLQPFLELKQKQ ANLALKIIEQLPSAKESPDKFLEVCTWVDQVAAL NDSKTRKTTSKTVRAVLDSLGEKKKSSPAAGGSD KYNQALSKYNQALSKYNQALSGGGGSNKKFLLYL AGFVDSDGSIIAQIKPNQEYKFKHYLSLTFAVTQ KTQRRWFLDKLVDRIGVGYVYDSGSVSYYKLSKI KPLHNFLTQLQPFLKLKQKQANLALKIIEQLPSA KESPDKFLEVCTWVDQVAALNDSKTRKTTSETVR AVLDSLSEKKKSSP
22	Meganuclease HSV1m4i	MANTKYNEEFLLYLAGFVDGDGSIIAQIKPNQSY KFKHQLSLTFMVTEKTQRRWFLDKLVDEIGVGYV RDAGSVSNYILSKIKPLHNFLTQLQPFLELKQKQ ANLALKIIEQLPSAKESPDKFLEVCTWVDQVAAL NDSKTRKTTSKTVRAVLDSLGEKKKSSPAAGGSD KYNQALSKYNQALSKYNQALSGGGGSNKKFLLYL AGFVDSDGSIIAQIKPNQEYKFKHYLSLTFAVTE KTQRRWFLDKLVDRIGVGYVYDSGSVSYYKLSKI KPLHNFLTQLQPFLKLKQKQANLALKIIEQLPSA KESPDKFLEVCTWVDQVAALNDSKTRKTTSETVR AVLDSLSEKKKSSP

23	UL54 DNA sequence	ATGgcgactgacattgatatgctaattgacctcg gcctggacctctccgacagcgatctggacgagga cccacccgagccggcggagagccgccgcgacgac ctggaatcggacagcagcggggagtgttcctcgt cggacgaggacatggaagacccccacggagagga cggaccggagccgatactcgacgccgctcgcccg gcggtccgcccgtctcgtccagaagaccccggcg tacccagcacccagacgcctcgtccgacggagcg gcagggccccaacgatcctcaaccagcgccccac agtgtgtggtcgcgcctcggggcccggcgaccgt cttgctcccccgagcagcacgggggcaaggtggc ccgcctccaaccccccaccgaccaaagcccagcct gcccgcggcggacgccgcgggcgtcgcaggggtc ggggtcgcggtggtcccggggccgccgatggttt gtcggacccccgccggcgtgcccccagaaccaat cgcaacccgggggggacccccgccccggggcggggt ggacggacggccccggcgcccccccatggcgaggc gtggcgcggaagtgagcagcccgacccacccgga ggcccgcggacacggggcgtgcgccaagcacccc ccccgctaatgacgctggcgattgcccccccgcc cgcggacccccgcgccccggccccggagcgaaag gcgcccgccgccgacaccatcgacgccaccacgc ggttggtcctgcgctccatctccgagcgcgcggc ggtcgaccgcatcagcgagagctttggccgcagc gcacaggtcatgcacgacccctttgggggggcagc cgtttcccgccgcgaatagcccctgggccccggt gttggcgggccaaggagggccctttgacgccgag accagacgggtctcctgggaaaccttggtcgccc acggcccgagcctctatcgcacttttgccggcaa tcctcgggccgcatcgaccgccaaggccatgcgc gactgcgtgctgcgccaagaaaatttcatcgagg cgctggcctccgccgacgagacgctggcgtggtg caagatgtgcatccaccacaacctgccgctgcgc

SEQ ID NO.	Sequence Name	Sequence
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[0191] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type I(HSV-1) reactivation in a subject, the method comprising:

administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

2. The method of claim 1, wherein the at least one HSV-l-specific meganuclease is configured to induce one or more DNA double strand breaks (DSB) in one or more genes in the genome of the HSV or targets a duplicated region in the HSV-1 genome.

3. The method of claim 1 or claim 2, wherein the adeno-associated vector further comprises a neuron specific promoter.

4. The method of any one of claims 1-3, wherein the one or more adeno-associated viral vector (AAV) serotypes is selected from AAV7, AAV9, AAV-DJ, AAV-DJ/8, AAV-Rh10, or a combination thereof.

5. The method of any one of claims 1-4, wherein the at least one HSV-1-specific meganuclease is effective in disrupting at least one gene in the genome of HSV-1, and wherein the at least one HSV-1-specific meganuclease is selected from HSV-1m4, HSV-1m5, HSV-1m8, or a combination thereof.

6. The method of any one of claims 1-5, wherein the latent HSV-1 infection is an ocular or genital HSV-1 infection.

7. The method of any one of claims 1-5, wherein the one or more adeno-associated viral vector (AAV) serotype is AAV9 or AAV-DJ/8, and wherein the at least one HSV-1-specific meganuclease is HSV-1m5.

8. The method of any one of claims 1-6 wherein the composition comprises of a plurality of a single adeno-associated viral vector (AAV) serotype, comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome.

9. The method of claim 8, wherein the AAV serotype is AAV9, and wherein the HSV-1-specific meganuclease is HSV-1m4.

10. The method of claims 1-6, wherein the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes, and wherein each viral vector serotype comprises a nucleic acid sequence encoding the same or a different HSV-specific meganuclease.

11. The method of claim 10, wherein the at least three adeno-associated viral vector (AAV) serotypes comprise AAV9, AAV-DJ/8, and AAV-Rh10, and wherein each viral vector comprises a nucleic acid sequence encoding an HSV-1-specific meganuclease.

12. The method of claim 11, wherein the HSV-1-specific meganuclease is HSV-1m5.

13. The method of claim 11, wherein the HSV-1-specific meganuclease is HSV-1m4.

14. The method of any one of claims 1-13, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCV) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining HSV.

15. The method of claim 14, wherein the reduction in ganglionic HSV load in SCG is about 73% to about 95% and in TG is about 43% to about 48% as compared to an untreated subject.

16. The method of any one of claims 1-13, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic HSV load in Dorsal root ganglion (DRG).

17. The method of any one of claims 1-6, wherein the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

18. The method of claim 17 wherein the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease.

19. The method of claim 17 or claim 18, wherein the first and the second HSV-1-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

20. The method of any one of claims 17-19, wherein the composition is administered intravenously or transdermally.

21. The method of any one of claims 17-20, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

22. The method of claim 21, wherein the reduction in ganglionic HSV load in SCG is about 89% to about 98% and in TG is about 42% to about 61%, as compared to an untreated subject.

23. The method of any one of claims 17-20, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

24. The method of claim 23, wherein the reduction in ganglionic HSV load in DRG is about 97% to about 98% as compared to an untreated subject.

25. The method of any one of claims 17-24, wherein the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject.

26. The method of any one of claims 17-25, wherein the composition is administered at a total dose selected from about 1.8 to about 5.4×10^{13} vg/kg to the subject.

27. The method of claim 26, wherein the administered total dose is effective in a dose-dependent (i) reduction in ganglionic HSV loads; and (ii) reduced peripheral viral shedding in the subject as compared to an untreated subject.

28. The method of claim 8 or claim 9, wherein the composition is associated with improved tolerability with no hepatotoxicity or neurotoxicity in the subject.

29. The method of any one of Claims 1-28, wherein the subject is a mammalian subject.

30. The method of Claim 29, wherein the mammalian subject is human.

31. The method of any one of claims 1-30, wherein the method is effective in preventing transmission of HSV-1 to a new host/subject.

32. The method of any one of claims 1-31, wherein the latent Herpes simplex virus type I(HSV-1) reactivation is by administering of an effective amount of a latency reversing agent to the subject, wherein the latency reversing agent is a BET protein inhibitor.

33. The method of claim 31, wherein the BET protein inhibitor is administered prior to, concomitant with, or after administration of the composition administered in claim 1, claim 17, or claim 18.

34. A method for reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in a subject, the method comprising: administering to the subject a composition comprising: at least three different

adeno-associated viral vector (AAV) serotypes, each viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

35. The method of claim 34, wherein the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease.

36. The method of claim 34 or claim 35, wherein the first and the second HSV-1-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

37. The method of any one of claims 34-36, wherein the adeno-associated vector further comprises a neuron specific promoter.

38. The method of any one of claims 34-37, wherein the composition is administered intravenously or transdermally.

39. The method of any one of claims 34-38, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

40. The method of any one of claims 34-38, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

41. The method of any one of claims 34-40, wherein the method is effective in reducing or eliminating potential transmission to a new host/subject.

42. The method of any one of claims 34-41, wherein the latent Herpes simplex virus type I(HSV-1) reactivation is by administering of an effective amount of a latency reversing agent to the subject, wherein the latency reversing agent is a BET protein inhibitor.

43. The method of claim 42, wherein the BET protein inhibitor is administered prior to, concomitant with, or after administration of the composition of claim 34 or claim 35.

44. A method for reducing or preventing hepato- and neurotoxicity associated with the administration of AAV-delivered gene editing for treatment of a recurrent infection associated with latent HSV-1 reactivation, the method comprising:

administering to the subject a composition comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome.

45. The method of claim 44, wherein the adeno-associated viral vector serotype comprising a nucleic acid sequence encoding an HSV-l-specific meganuclease further comprises a neuron specific promoter.

46. The method of any one of claim 44 or 45, wherein the one or more adeno-associated viral vector (AAV) serotype is AAV9, and wherein the meganuclease is HSV-1m4.

47. The method of any one of claims 44-46, wherein the recurrent infection associated with latent HSV reactivation is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

48. The method of any one of claims 44-46, wherein the recurrent infection associated with latent HSV reactivation is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

49. The method of any one of claims 44-48, wherein the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject.

50. The method of any one of claims 44-49, wherein the latent Herpes simplex virus type I(HSV-1) reactivation is by administering of an effective amount of a latency reversing agent to the subject, wherein the latency reversing agent is a BET protein inhibitor.

51. The method of claim 50, wherein the latency reversing agent is administered prior to, concomitant with, or after administration of the composition comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome.

52. The method of claim 50 or 51, wherein the latency reversing agent is a BET protein inhibitor.

53. The method of any one of claims 44-52, wherein the composition is effective in reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in the subject.

54. A method for reducing or eliminating potential transmission of HSV-1 associated with recurrent latent Herpes simplex virus type 1 (HSV-1) reactivation of HSV-1 in a subject, the method comprising: administering to the subject an effective amount of a composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

55. The method of claim 54, wherein the composition comprises a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

56. The method of claim 54 or claim 55, wherein the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease.

57. The method of claim 55 or claim 56, wherein the first and the second HSV-1-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

58. The method of claim 54-57, wherein the adeno-associated vector further comprises a neuron specific promoter.

59. The method of any one of claims 54-58, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

60. The method of any one of claims 54-58, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

61. The method of any one of claims 54-60, wherein the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in the subject.

62. The method of any one of claims 54-61, wherein the latent Herpes simplex virus type I(HSV-1) reactivation is by administering of an effective amount of a latency reversing agent to the subject.

63. The method of claim 62, wherein the latency reversing agent is administered prior to, concomitant with, or after administration of the composition comprising a plurality of one or more neurotropic adeno-associated viral vector (AAV) serotypes, each

comprising at least one nucleic acid sequence encoding at least one HSV-1-specific meganuclease.

64. The method of claim 62 or 63, wherein the latency reversing agent is a BET protein inhibitor.

65. A composition comprising a plurality of at least three different adeno-associated viral vector (AAV) serotypes, each viral vector serotype comprising a first nucleic acid sequence encoding a first HSV-1-specific meganuclease, and a second nucleic acid sequence encoding a second HSV-1-specific meganuclease, separately.

66. The composition of claim 65, wherein the adeno-associated vector further comprises a neuron specific promoter.

67. The composition of claim 65 or claim 66, wherein the composition comprises: (i) AAV9 comprising a nucleic acid sequence encoding a first HSV-1 specific meganuclease; (ii) AAV9 comprising a nucleic acid sequence encoding a second HSV-1 specific meganuclease; (iii) AAV-DJ/8 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; (iv) AAV-DJ/8 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease; (v) AAV-Rh10 comprising a nucleic acid sequence encoding the first HSV-1 specific meganuclease; and (vi) AAV-Rh10 comprising a nucleic acid sequence encoding the second HSV-1 specific meganuclease.

68. The composition of any one of claims 65-67, wherein the first and the second HSV-l-specific meganucleases are HSV-1m5 and HSV-1m8, respectively.

69. The composition of any one of claims 65-68, wherein the composition is effective for reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation in a subject.

70. The composition of claim 69, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

71. The composition of claim 69, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

72. The composition of any one of claims 65-71, wherein the composition is administered intravenously or transdermally.

73. The composition of claims 65-72, wherein the composition is effective in reducing or eliminating potential transmission to a new host/subject.

74. The composition of any one of claims 69-73, wherein the composition is administered prior to, concomitantly with, or after administration of an effective amount of a latency reversing agent to the subject.

75. The composition of claim 74, wherein the latency reversing agent is a BET protein inhibitor.

76. A composition, comprising a plurality of a single adeno-associated viral vector (AAV) serotype, each of the single AAV serotype viral vector comprising at least one nucleic acid sequence encoding a meganuclease targeting a duplicated region of the HSV-1 genome.

77. The composition of claim 76, wherein the adeno-associated vector serotype further comprises a neuron specific promoter.

78. The composition of claim 76 or claim 77, wherein the composition is effective in treating recurrent Herpes simplex virus (HSV) disease associated with latent Herpes simplex virus type 1(HSV-1) reactivation in a subject.

79. The composition of any one of claims 76-78, wherein the plurality of the single neurotropic adeno-associated viral vector (AAV) serotype consists of AAV9, and wherein the HSV-1-specific meganuclease encoded by the at least one nucleic acid sequence is HSV-1m4.

80. The composition of any one of claims 76-78, wherein the recurrent latent HSV infection is an orofacial infection, wherein the composition is effective in reducing ganglionic HSV load in superior cervical ganglia (SCG) and trigeminal ganglia (TG), and wherein the composition is effective in editing the genome of remaining latent HSV.

81. The composition of any one of claims 76-78, wherein the recurrent latent HSV infection is a genital infection, wherein the composition is effective in reducing ganglionic viral load in dorsal root ganglia (DRG), and wherein the composition is effective in editing the genome of remaining HSV.

82. The composition of any one of claims 76-79, wherein the composition is effective in reducing or eliminating peripheral viral shedding associated with latent Herpes simplex virus type 1 (HSV-1) reactivation.

83. The composition of any one of claims 76-82, wherein the composition is effective in reducing or eliminating potential transmission to a new host/subject.

84. The composition of any one of claims 76-83, wherein the composition is associated with improved tolerability and reduced hepato- and neurotoxicity.