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(54) **Title**: TARGETED TRANSDUCTION OF AAV VECTORS

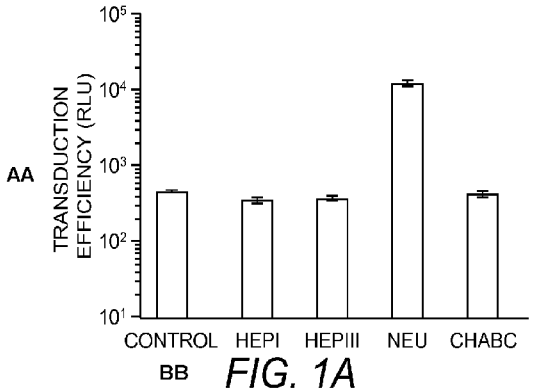


FIG. 1A

(57) **Abstract**: This invention relates to methods of enhancing transduction of cells with adeno-associated virus (AAV) vectors that bind asialoglycans on the cell surface and methods of targeting cells with these vectors by desialylating cells. The invention further relates to methods, compositions and kits for delivering nucleic acid s to cells using the AAV vectors.

Targeted Transduction of AAV Vectors

Statement of Priority

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 61/440,140, filed February 7, 2011, the entire contents of which is incorporated by reference herein.

Statement of Federal Support

[0002] This invention was made with government support under HL089221 awarded by the National Institutes of Health. The government has certain rights in the invention.

Field of the Invention

[0003] This invention relates to methods of enhancing transduction of cells with adeno-associated virus (AAV) vectors that bind asialoglycans on the cell surface and methods of targeting cells with these vectors by desialylating cells. The invention further relates to methods, compositions and kits for delivering nucleic acids to cells using the AAV vectors.

Background of the Invention

[0004] Cell surface glycans have been shown to play a critical role in the infectious pathways of viruses (Olofsson *et al.*, *Ann. Med. 37*:154 (2005)). Detailed studies of virus-glycan interactions have yielded significant insight into mechanisms underlying emergence and transmission of viral pathogens in different hosts. Amongst various glycolipids, glycoproteins, or proteoglycans anchored to the plasma membrane, sialylated glycans and heparan sulfate proteoglycans appear to serve as predominant substrates for viral attachment to the cell surface. For instance, heparan sulfate serves as a primary receptor for *Herpesviridae* (Akhtar *et al.*, *FEBS J.* 276:7228 (2009)) as well as certain adenoviruses (Dechecchi *et al.*, *J. Virol. 75*:8772 (2001)) and parvoviruses (Summerford *et al.*, *J. Virol. 72*:1438 (1998); Schmidt *et al.*, *J. Virol. 82*:8911 (2008)). Interactions between sialylated glycans and members of the *Orthomyxoviridae* (Viswanathan *et al.*, *Glycoconj. J. 27*:561 (2010)), *Reoviridae* (Guglielmi *et al.*, *Curr. Top. Microbiol. Immunol. 309*:1 (2006)), *Polyomaviridae* (Neu *et al.*, *Virology 384*:389 (2009)) families and certain parvoviruses are also well known (Nam *et al.*, *J. Biol. Chem.*

281:25670 (2006); Walters *et al.*, *J. Biol. Chem. 276*:20610 (2001); Wu *et al.*, *J. Virol. 80*:9093 (2006)).

[0005] AAV are small, single-stranded DNA viruses that belong to the genus Dependovirus of the *Parvoviridae* family (Bowles *et al.*, (2006) The genus *dependovirus*. In *Parvoviruses* pp. 15-24. Edited by J. R. Kerr, S. F. Cotmore, M. E. Bloom *et al.*, New York, Edward Arnold Ltd.). Recombinant AAV vectors, by virtue of their lack of pathogenicity and low immunogenicity, are currently being evaluated as lead candidates in clinical gene therapy trials (Mueller *et al.*, *Gene Ther. 15*:858 (2008)). The discovery of a large number of AAV isolates over the past decade has accelerated efforts to exploit tissue tropisms displayed by different strains for therapeutic gene transfer applications (Gao *et al.*, *Curr. Gene Ther. 5*:285 (2005); Mitchell *et al.*, *Curr. Gene Ther. 10*:319 (2010)). As with other viruses, attachment to cell surface glycans constitutes the first step in the AAV infectious pathway. For instance, several AAV serotypes have been shown to bind heparan sulfate proteoglycans (AAV2 (Summerford *et al.*, *J. Virol. 72*:1438 (1998)); AAV6 (Wu *et al.*, *J. Virol. 80*:9093 (2006)), while others utilize sialic acid for cell surface binding and entry (AAV4 (Kaludov *et al.*, *J. Virol. 75*:6884 (2001)); AAV5 (Walters *et al.*, *J. Biol. Chem. 276*:20610 (2001)); AAV1/6 (Wu *et al.*, *J. Virol. 80*:9093 (2006)); Bovine AAV (Schmidt *et al.*, *J. Virol. 80*:5516 (2006))).

[0006] Sialylated glycans that serve as primary receptors for the latter AAV strains vary at the level of *N*-acetylneuraminic acid (Neu5Ac) linkage to underlying sugars, i.e., α2-3 or α2-6 linked to galactose residues (Wu *et al.*, *J. Virol. 80*:9093 (2006); Kaludov *et al.*, *J. Virol. 75*:6884 (2001)). Further receptor specificity has been demonstrated at the level of *N*-linked or *O*-linked glycans displayed on the cell surface (Wu *et al.*, *J. Virol. 80*:9093 (2006); Kaludov *et al.*, *J. Virol. 75*:6884 (2001)). Selective recognition of such linkages and underlying core glycan types (Cohen *et al.*, *OMICS 14*:455 (2010)) is likely enabled by differences in the capsid surface topology of AAV serotypes (Govindasamy *et al.*, *J. Virol. 80*:11556 (2006); Ng *et al.*, *J. Virol. 84*:12945 (2010)). In general, dependence of AAV infectivity on sialic acid has been demonstrated using a battery of chemical, biochemical and genetic tools to desialylate cell surface glycans.

[0007] Recombinant AAV9 vectors display widespread and robust transduction following systemic administration in animal models, but fail to infect cells in culture (Gao *et al.*, *J. Virol. 78*:6381 (2004); Zincarelli *et al.*, *Mol. Ther. 16*:1073 (2008)).

[0008] The present invention provides a solution to the inability of AAV9 and related vectors to infect cells in culture. Additionally, the present invention provides methods for targeting vectors to cells and tissues in live subjects.

Summary of the Invention

[0009] The present invention relates to the discovery that certain AAV vectors preferentially bind to and infect cells carrying asialoglycans on their surface and that removal of sialyl groups from cell surface glycans increases binding of these vectors to the cells. This discovery can be utilized to increase the transduction of cells in culture and tissues in live subjects. This discovery can further be used advantageously to target this class of AAV vectors to specific cells by desialylating the cells. This discovery can be utilized to restrict gene transfer to a specific site in a subject without spread into peripheral non-target organs.

[0010] Thus, one aspect of the invention relates to a method for increasing transduction of a cell with an AAV vector that binds asialoglycans, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a cell that has not been desialylated.

[0011] Another aspect of the invention relates to a method of targeting an AAV vector that binds asialoglycans to a cell, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a cell that has not been desialylated.

[0012] A further aspect of the invention relates to a method of restricting AAV vectors that bind asialoglycans to the site of delivery in a subject, comprising:

(a) delivering a desialylating agent to a site in a subject; and

(b) delivering the AAV vector to the same site;

wherein the AAV vector is targeted to desialylated cells and systemic dissemination of the AAV vector is restricted.

[0013] An additional aspect of the invention relates to a method of delivering a nucleic acid to a cell, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with an AAV vector that binds asialoglycans;

wherein the AAV vector comprises the nucleic acid.

[0014] Another aspect of the invention relates to a method of delivering a nucleic acid to a mammalian subject, comprising delivering to the mammalian subject a cell that has been desialylated and contacted with an AAV vector that binds asialoglycans and comprises the nucleic acid under conditions sufficient for the AAV vector genome to enter the cell.

[0015] A further aspect of the invention relates to a method of delivering a nucleic acid to a mammalian subject, comprising delivering to the mammalian subject a desialylating agent and an AAV vector that binds asialoglycans and comprises the nucleic acid.

[0016] An additional aspect of the invention relates to a composition comprising an AAV vector that binds asialoglycans and a desialylating agent.

[0017] Another aspect of the invention relates to a kit comprising an AAV vector that binds asialoglycans and a desialylating agent.

[0018] These and other aspects of the invention are set forth in more detail in the description of the invention below.

Brief Description of the Drawings

[0019] Figures 1A-1C show the effect of enzymatic desialylation on AAV9 transduction.

[0020] Figure 2 shows the effect of enzymatic desialylation on AAV9 binding to different human cell lines.

[0021] Figure 3 shows the effect of desialylation on the internalization of AAV1 and AAV9 in U87 cells.

[0022] Figures 4A-4C show the effect of glycan chain composition on cell surface binding and transduction of AAV9.

[0023] Figures 5A-5C show the effect of glycosylation inhibitors and enzymatic resialylation on AAV9 transduction.

[0024] Figures 6A-6C show the effect of lectin competition on AAV9 transduction.

[0025] Figures 7A-7B show the effect of endo-β-galactosidase and α-fucosidase on AAV9 transduction efficiency.

[0026] Figure 8 shows the effect of desialylation on cell surface binding of AAV9 particles.

[0027] Figures 9A-9D show the effect of enzymatic desialylation on AAV9 transduction efficiency in HAE cultures *in vitro* and murine airways *in vivo*.

[0028] Figures 10A-10D show the effect of enzymatic desialylation on AAV9 transduction in murine airways *in vivo*.

[0029] Figure 11 shows the inhibition of AAV9 infectivity by different glycans.

[0030] Figures 12A-12B show localized sialidase pretreatment increases AAV9 transgene expression at a low dose of AAV9-CBA-luciferase in joints.

[0031] Figures 13A-13C show localized sialidase pretreatment prevents AAV9 from leakage into the systemic circulation after intraarticular injection.

[0032] Figures 14A-14B show intravitreous sialidase pretreatment increases AAV9 transgene expression in retina.

[0033] Figure 15 shows galactose levels and viral particle levels in endothelial cells after intravenous administration of sialidase and AAV9.

[0034] Figures 16A-16E show increased liver transduction efficiency after intravenous injection of sialidase and AAV9.

Detailed Description of the Invention

[0035] The present invention will now be described with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0036] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[0037] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. For example, features described in relation to one embodiment may also be applicable to and combinable with other embodiments and aspects of the invention.

[0038] Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted.

[0039] Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 CFR §1.822 and established usage. *See, e.g., PatentIn User Manual*, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office).

[0040] Except as otherwise indicated, standard methods known to those skilled in the art may be used for the construction of recombinant parvovirus and rAAV constructs, packaging vectors expressing the parvovirus Rep and/or Cap sequences, and transiently and stably transfected packaging cells. Such techniques are known to those skilled in the art. *See, e.g.*, SAMBROOK *et al.* MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

Definitions

[0041] The following terms are used in the description herein and the appended claims:

[0042] The singular forms "a" and "an" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0043] Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of the length of a polynucleotide or polypeptide sequence, dose, time, temperature, and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount.

[0044] Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0045] The terms "comprise," "comprises," and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[0046] As used herein, the transitional phrase "consisting essentially of" is to be interpreted as encompassing the recited materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention (*e.g.*, rAAV replication). *See*, *In re Herz*, 537 F.2d 549, 551-52, 190 U.S.P.Q. 461, 463 (CCPA 1976) (emphasis in the original); *see also* MPEP § 2111.03. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

[0047] The term "parvovirus" as used herein encompasses the family *Parvoviridae*, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, snake parvovirus, and B19 virus (*See*, *e.g.*, Figs. 20-23). Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.*, FIELDS *et al.* VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

[0048] The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV. *See, e.g.*, Figs. 8-19; FIELDS *et al.* VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers); and **Table 1**.

[0049] As used herein, the term "adeno-associated virus" (AAV), includes but is not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, AAV type 12, AAV type 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, and any other AAV now known or later discovered. *See, e.g.*, FIELDS *et al.* VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of relatively new AAV serotypes and clades have been identified (*See, e.g.*, Gao *et al.* (2004) *J. Virol.* 78:6381; Moris *et al.* (2004) *Virol.* 33-:375; and **Table 1**).

Table 1

Complete Genomes	GenBank Accession Number		GenBank Accession Number		GenBank Accession Number
Adeno-associated virus 1	NC_002077,AF063497	**Hu T88**	AY695375	**Clade E**	
Adeno-associated virus 2	NC_001401	**Hu T71**	AY695374	Rh38	AY530558
Adeno-associated virus 3	NC_001729	**Hu T70**	AY695373	Hu66	AY530626
Adeno-associated virus 3B	NC_001863	**Hu T40**	AY695372	Hu42	AY530605
Adeno-associated virus 4	NC_001829	**Hu T32**	AY695371	Hu67	AY530627
Adeno-associated virus 5	Y18065, AF085716	Hu T17	AY695370	Hu40	AY530603
Adeno-associated virus 6	NC_001862	Hu LG15	AY695377	Hu41	AY530604
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828			Hu37	AY530600
Avian AAV strain DA-1	NC_006263, AY629583	**Clade C**		Rh40	AY530559
Bovine AAV	NC_005889, AY388617	Hu9	AY530629	Rh2	AY243007
		Hu10	AY530576	Bb1	AY243023
Clade A		Hu11	AY530577	Bb2	AY243022
AAV1	NC_002077,AF063497	Hu53	AY530615	Rh10	AY243015
AAV6	NC_001862	Hu55	AY530617	Hu17	AY530582
Hu.48	AY530611	Hu54	AY530616	Hu6	AY530621
Hu 43	AY530606	Hu7	AY530628	Rh25	AY530557
Hu 44	AY530607	Hu18	AY530583	Pi2	AY530554
Hu 46	AY530609	Hu15	AY530580	Pi1	AY530553
		Hu16	AY530581	Pi3	AY530555
Clade B		Hu25	AY530591	Rh57	AY530569
Hu. 19	AY530584	Hu60	AY530622	Rh50	AY530563
Hu. 20	AY530586	Ch5	AY243021	Rh49	AY530562
Hu 23	AY530589	Hu3	AY530595	Hu39	AY530601
Hu22	AY530588	Hu1	AY530575	Rh58	AY530570
Hu24	AY530590	Hu4	AY530602	Rh61	AY530572
Hu21	AY530587	Hu2	AY530585	Rh52	AY530565
Hu27	AY530592	Hu61	AY530623	Rh53	AY530566
Hu28	AY530593			Rh51	AY530564
Hu 29	AY530594	**Clade D**		Rh64	AY530574
Hu63	AY530624	Rh62	AY530573	Rh43	AY530560
Hu64	AY530625	Rh48	AY530561	**AAV8**	AF513852
Hu13	AY530578	Rh54	AY530567	Rh8	AY242997
Hu56	AY530618	Rh55	AY530568	Rh1	AY530556
Hu57	AY530619	Cy2	AY243020		
Hu49	AY530612	**AAV7**	AF513851	**Clade F**	
Hu58	AY530620	Rh35	AY243000	Hu14 **(AAV9)**	AY530579
Hu34	AY530598	Rh37	AY242998	Hu31	AY530596
Hu35	AY530599	Rh36	AY242999	Hu32	AY530597
AAV2	NC_001401	Cy6	AY243016		
Hu45	AY530608	Cy4	AY243018	**Clonal Isolate**	
Hu47	AY530610	Cy3	AY243019	**AAV5**	Y18065, AF085716
Hu51	AY530613	Cy5	AY243017	**AAV 3**	NC_001729
Hu52	AY530614	Rh13	AY243013	**AAV 3B**	NC_001863
Hu T41	AY695378			**AAV4**	NC_001829
Hu S17	AY695376			Rh34	AY243001
				Rh33	AY243002
				Rh32	AY243003

[0050] The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native ITRs, Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. *See, e.g.*, GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, AY631966, AX753250, EU285562, NC_001358, NC_001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also, e.g.*, Bantel-Schaal *et al.* (1999) *J. Virol.* 73: 939; Chiorini *et al.* (1997) *J. Virol.* 71:6823; Chiorini *et al.* (1999) *J. Virol.* 73:1309; Gao *et al.* (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris *et al.* (2004) *Virol.* 33-:375-383; Mori *et al.* (2004) *Virol.* 330:375; Muramatsu *et al.* (1996) *Virol.* 221:208; Ruffing *et al.* (1994) *J. Gen. Virol.* 75:3385; Rutledge *et al.* (1998) *J. Virol.* 72:309; Schmidt *et al.* (2008) *J. Virol.* 82:8911; Shade *et al.*, (1986) *J. Virol.* 58:921; Srivastava *et al.* (1983) *J. Virol.* 45:555; Xiao *et al.* (1999) *J. Virol.* 73:3994; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also* **Table 1**. An early description of the AAV1, AAV2 and AAV3 ITR sequences is provided by Xiao, X., (1996), "Characterization of Adeno-associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein it its entirety).

[0051] The term "tropism" as used herein refers to entry of the virus into the cell, optionally followed by expression (*e.g.*, transcription and, optionally, translation) of sequences carried by the viral genome in the cell, *e.g.*, for a recombinant virus, expression of the heterologous nucleotide sequences(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.*, for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of AAV, gene expression from the viral genome may be from a stably integrated provirus, from a non-integrated episome, as well as any other form in which the virus may take within the cell.

[0052] As used herein, "transduction" or "infection" of a cell by a parvovirus or AAV means that the parvovirus/AAV enters the cell to establish an active (*i.e.*, lytic) infection. As used herein, "transduction" of a cell by AAV means that the AAV enters

the cell to establish a latent infection. *See, e.g.*, FIELDS *et al.* VIROLOGY, volume 2, chapter 69 (3d ed., Lippincott-Raven Publishers).

[0053] The terms "5' portion" and "3' portion" are relative terms to define a spatial relationship between two or more elements. Thus, for example, a "3' portion" of a polynucleotide indicates a segment of the polynucleotide that is downstream of another segment. The term "3' portion" is not intended to indicate that the segment is necessarily at the 3' end of the polynucleotide, or even that it is necessarily in the 3' half of the polynucleotide, although it may be. Likewise, a "5' portion" of a polynucleotide indicates a segment of the polynucleotide that is upstream of another segment. The term "5' portion" is not intended to indicate that the segment is necessarily at the 5' end of the polynucleotide, or even that it is necessarily in the 5' half of the polynucleotide, although it may be.

[0054] As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

[0055] A "polynucleotide" is a sequence of nucleotide bases, and may be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide), and can be either single or double stranded.

[0056] The terms "sequence identity" and "sequence similarity," as used herein, has the standard meaning in the art. As is known in the art, a number of different programs can be used to identify whether a polynucleotide or polypeptide has sequence identity or similarity to a known sequence. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math. 2*:482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol. 48*:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res. 12*:387 (1984), preferably using the default settings, or by inspection.

[0057] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng &

Doolittle, *J. Mol. Evol. 35*:351 (1987); the method is similar to that described by Higgins & Sharp, *CABIOS 5*:151 (1989).

[0058] Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol. 215*:403 (1990) and Karlin *et al., Proc. Natl. Acad. Sci. USA 90*:5873 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, *Meth. Enzymol., 266*:460 (1996); blast.wustl/edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[0059] An additional useful algorithm is gapped BLAST as reported by Altschul *et al., Nucleic Acids Res. 25*:3389 (1997).

[0060] In certain embodiments, a percentage amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

[0061] In a similar manner, in certain embodiments percent nucleic acid sequence identity with respect to the coding sequence of the polypeptides disclosed herein is defined as the percentage of nucleotide residues in the candidate sequence that are identical with the nucleotides in the polynucleotide specifically disclosed herein.

[0062] The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the polypeptides specifically disclosed herein, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical amino acids in relation to the total number of amino acids. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of amino acids in the shorter sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as insertions, deletions, substitutions, *etc.*

[0063] In one embodiment, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0," which obviates the need for a weighted scale or parameters as described below for sequence similarity

calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the "shorter" sequence in the aligned region and multiplying by 100. The "longer" sequence is the one having the most actual residues in the aligned region.

[0064] As used herein, an "isolated" polynucleotide (*e.g.*, an "isolated DNA" or an "isolated RNA") means a polynucleotide separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide.

[0065] Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide. In some embodiments, an isolated polypeptide is one that is at least about 50, 60, 70, 80, 90, or 95% pure or higher.

[0066] A "therapeutic polypeptide" is a polypeptide that may alleviate or reduce symptoms that result from an absence or defect in a protein in a cell or subject. Alternatively, a "therapeutic polypeptide" is one that otherwise confers a benefit to a subject, *e.g.*, anti-cancer effects or improvement in transplant survivability.

[0067] As used herein, the term "modified," as applied to a polynucleotide or polypeptide sequence, refers to a sequence that differs from a wild-type sequence due to one or more deletions, additions, substitutions, or any combination thereof.

[0068] As used herein, by "isolate" or "purify" (or grammatical equivalents) a virus vector, it is meant that the virus vector is at least partially separated from at least some of the other components in the starting material.

[0069] By the terms "treat," "treating" or "treatment of" (and grammatical variations thereof) it is meant that the severity of the subject's condition is reduced, at least partially improved or stabilized and/or that some alleviation, mitigation, decrease or stabilization in at least one clinical symptom is achieved and/or there is a delay in the progression of the disease or disorder.

[0070] The terms "prevent," "preventing" and "prevention" (and grammatical variations thereof) refer to prevention and/or delay of the onset of a disease, disorder and/or a clinical symptom(s) in a subject and/or a reduction in the severity of the onset of the disease, disorder and/or clinical symptom(s) relative to what would occur in the absence of the methods of the invention. The prevention can be complete, *e.g.*, the total

absence of the disease, disorder and/or clinical symptom(s). The prevention can also be partial, such that the occurrence of the disease, disorder and/or clinical symptom(s) in the subject and/or the severity of onset is less than what would occur in the absence of the present invention.

[0071] A "treatment effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a "treatment effective" amount is an amount that will provide some alleviation, mitigation, decrease or stabilization in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[0072] A "prevention effective" amount as used herein is an amount that is sufficient to prevent and/or delay the onset of a disease, disorder and/or clinical symptoms in a subject and/or to reduce and/or delay the severity of the onset of a disease, disorder and/or clinical symptoms in a subject relative to what would occur in the absence of the methods of the invention. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

[0073] The terms "increase" and "decrease," as used herein, *e.g.*, with reference to binding of an AAV vector to a cell, refers to a positive or negative change compared to a control of at least about 10%, *e.g.*, at least about 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, or 500% or more.

[0074] The terms "heterologous nucleotide sequence" and "heterologous nucleic acid" are used interchangeably herein and refer to a sequence that is not naturally occurring in the virus. Generally, the heterologous nucleic acid comprises an open reading frame that encodes a polypeptide or nontranslated RNA of interest (*e.g.*, for delivery to a cell or subject).

[0075] As used herein, the terms "virus vector," "vector" or "gene delivery vector" refer to a virus (*e.g.*, AAV) particle that functions as a nucleic acid delivery vehicle, and which comprises the vector genome (*e.g.*, viral DNA [vDNA]) packaged within a virion. Alternatively, in some contexts, the term "vector" may be used to refer to the vector genome/vDNA alone.

[0076] The virus vectors of the invention can further be duplexed parvovirus particles as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Thus, in some embodiments, double stranded (duplex) genomes can be packaged into the virus capsids of the invention.

[0077] A "rAAV vector genome" or "rAAV genome" is an AAV genome (*i.e.*, vDNA) that comprises one or more heterologous nucleic acid sequences. rAAV vectors generally require only the 145 base ITR in *cis* to generate virus. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka (1992) *Curr. Topics Microbiol. Immunol.* 158:97). Typically, the rAAV vector genome will only retain the one or more ITR sequence so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences may be provided in *trans* (*e.g.*, from a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). In embodiments of the invention the rAAV vector genome comprises at least one ITR sequence (*e.g.*, AAV ITR sequence), optionally two ITRs (*e.g.*, two AAV ITRs), which typically will be at the 5' and 3' ends of the vector genome and flank the heterologous nucleic acid, but need not be contiguous thereto. The ITRs can be the same or different from each other.

[0078] The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (*i.e.*, mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The ITR can be an AAV ITR or a non-AAV ITR. For example, a non-AAV ITR sequence such as those of other parvoviruses (*e.g.*, canine parvovirus, bovine parvovirus, mouse parvovirus, porcine parvovirus, human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as an ITR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the ITR can be partially or completely synthetic, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.*

[0079] Parvovirus genomes have palindromic sequences at both their 5' and 3' ends. The palindromic nature of the sequences leads to the formation of a hairpin structure that is stabilized by the formation of hydrogen bonds between the complementary base pairs. This hairpin structure is believed to adopt a "Y" or a "T" shape. *See*, *e.g.*, FIELDS *et al.* VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0080] An "AAV inverted terminal repeat" or "AAV ITR" may be from any AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, snake AAV, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, goat AAV, shrimp AAV, or any other AAV now known or later discovered (*see, e.g.*, Table 1). In certain embodiments the AAV ITR is from a clade F AAV. An AAV ITR need not

have the native terminal repeat sequence (*e.g.*, a native AAV ITR sequence may be altered by insertion, deletion, truncation and/or missense mutations), as long as the terminal repeat mediates the desired functions, *e.g.*, replication, virus packaging, integration, and/or provirus rescue, and the like.

[0081] The virus vectors of the invention can further be "targeted" virus vectors (*e.g.*, having a directed tropism) and/or a "hybrid" parvovirus (*i.e.*, in which the viral ITRs and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao *et al.*, (2000) *Mol. Therapy* 2:619.

[0082] Further, the viral capsid or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

[0083] As used herein, the term "amino acid" encompasses any naturally occurring amino acids, modified forms thereof, and synthetic amino acids.

[0084] Naturally occurring, levorotatory (L-) amino acids are shown in **Table 2.**

[0085] Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in **Table 3**) or can be an amino acid that is modified by post-translation modification (*e.g.*, acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation).

Table 2

Amino Acid Residue	Abbreviation	
	Three-Letter Code	One-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Table 3

Amino Acid Residue Derivatives	
Modified Amino Acid Residue	Abbreviation
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminoproprionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-Butylalanine	t-BuA
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2'-Diaminopimelic acid	Dpm
2,3-Diaminoproprionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	aIle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-Cl)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg
Beta-2-thienylalanine	Thi

[0086] Further, the non-naturally occurring amino acid can be an "unnatural" amino acid as described by Wang *et al.* (2006) *Annu. Rev. Biophys. Biomol. Struct.* 35:225-49. These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein.

[0087] The term "template" or "substrate" is used herein to refer to a polynucleotide sequence that may be replicated to produce the parvovirus viral DNA. For the purpose of vector production, the template will typically be embedded within a larger nucleotide sequence or construct, including but not limited to a plasmid, naked DNA vector, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) or a viral vector (*e.g.*, adenovirus, herpesvirus, Epstein-Barr Virus, AAV, baculoviral, retroviral vectors, and the like). Alternatively, the template may be stably incorporated into the chromosome of a packaging cell.

[0088] As used herein, parvovirus or AAV "Rep coding sequences" indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, *e.g.*, FIELDS *et al.* VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0089] The "Rep coding sequences" need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the Rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced Rep68 and Rep40 proteins. In representative embodiments, the Rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The Rep coding sequences will generally encode at least one large Rep protein (*i.e.*, Rep78/68) and one small Rep protein (*i.e.*, Rep52/40). In particular embodiments, the Rep coding sequences encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the Rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the Rep coding sequences encode the Rep68 and Rep52 proteins, Rep68 and Rep40 proteins, Rep78 and Rep52 proteins, or Rep78 and Rep40 proteins.

[0090] As used herein, the term "large Rep protein" refers to Rep68 and/or Rep78. Large Rep proteins of the claimed invention may be either wild-type or synthetic. A wild-type large Rep protein may be from any AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see, e.g.*, Table 1). In certain embodiments, the large Rep protein may be from a clade F AAV. A synthetic large Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

[0091] As used herein, the term "small Rep protein" refers to Rep52 and/or Rep40. Small Rep proteins of the claimed invention may be either wild-type or synthetic.

A wild-type small Rep protein may be from any AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see, e.g.*, Table 1). In certain embodiments, the small Rep protein may be from a clade F AAV. A synthetic small Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

[0092] Those skilled in the art will further appreciate that it is not necessary that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (*e.g.*, AAV p19 promoter) may not be recognized by the cell, and it is therefore convenient to express the large and small Rep proteins from separate expression cassettes. In other instances, it may be desirable to express the large Rep and small Rep proteins separately, *i.e.*, under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.*, Rep78/68) to avoid toxicity to the cells (*see, e.g.*, Urabe *et al.*, (2002) *Human Gene Therapy* 13:1935).

[0093] As used herein, the parvovirus or AAV "cap coding sequences" encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.*, can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule. Cap proteins of the claimed invention may be either wild-type or synthetic.

[0094] The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

[0095] The term "AAV vector that binds asialoglycans," as used herein, refers to AAV vectors that bind to asialoglycans on the cell surface as compared to sialoglycans. In certain embodiments, the AAV vector preferentially binds asialoglycans. The term includes naturally occurring AAV particles that bind to asialoglycans as well as AAV

vectors that have been engineered to bind to asialoglycans. The ability of an AAV vector to bind to asialoglycans can be quantitated by any method known in the art and as described in the examples below.

[0096] The term "asialoglycans," as used herein, refers to glycans that do not have a terminal sialic acid group.

[0097] The terms "desialylation," "desialylate(s)," or "desialylating," as used herein, refer to the removal of a terminal sialic acid group from sialoglycans present on the surface of a cell.

[0098] The term "desialylating a cell," as used herein, refers to the removal of one or more sialyl groups from the surface of a cell. In certain embodiments, the term encompasses the removal of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% or more of the sialyl groups present on the cell surface.

[0099] The term "desialylating agent," as used herein, refers to any compound, molecule, or environmental condition that removes sialyl groups from sialoglycans on the surface of a cell, prevents the synthesis of sialoglycans, and/or prevents the transportation of sialoglycans to the cell surface.

[0100] The term "cell comprising sialyl groups," as used herein, refers to any cell that contains at least one cell surface glycan comprising a terminal sialyl group.

[0101] The term "site of delivery," as used herein, refers to the location of delivery of AAV vector to a subject. The site of delivery can be a specific cell, tissue, organ, or region in a subject.

Targeted Transduction of Vectors

[0102] The present invention is based on the discovery that Clade F AAV vectors target cells having asialoglycans on the surface and that the presence of sialoglycans on cells inhibits the binding of Clade F AAV vectors to the cells. Thus, the present invention provides methods for targeting AAV vectors that bind asialoglycans to cells by desialylating the cells to remove some or substantially all of the sialyl groups on the cell surface.

[0103] One aspect of the invention relates to a method for increasing transduction of a cell with an adeno-associated virus (AAV) vector that binds asialoglycans, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a suitable control cell, *e.g.*, a cell that has not been desialylated. The control cell can be a cell of the same type and/or location as the desialylated cell but has not undergone a desialylation step.

[0104] In one embodiment, the AAV vector is from clade F, *e.g.*, AAV9 (also known as Hu.14), Hu.31 or Hu.32. In another embodiment, the AAV vector is a chimeric or hybrid vector comprising a viral genome from any serotype of AAV and a capsid from a clade F AAV. In other embodiments, the chimeric or hybrid AAV comprises a capsid made up of at least a portion of a capsid protein from a clade F AAV, *e.g.*, a portion of the capsid protein that provides binding specificity to asialoglycans.

[0105] In some embodiments, the AAV vector is an engineered vector capable of binding asialoglycans. For example, the vector may comprise a modified capsid that has been identified as capable of binding asialoglycans, *e.g.*, by screening capsid protein mutant libraries for binding ability. In other embodiments, the engineered vector comprises all or a portion of a naturally-occurring capsid protein that has been identified as capable of binding asialoglycans.

[0106] In some embodiments of the invention, the desialylating step comprises removing a portion of the sialyl groups (*e.g.*, one or more sialyl groups but not all of the sialyl groups) from the termini of the cell surface glycans. In other embodiments, substantially all of the sialyl groups are removed. As used here, the term "substantially all" refers to the removal of at least about 80% of the sialyl groups on the cell surface, *e.g.*, at least about 85, 90, 95, 96, 97, 98, or 99% of the sialyl groups.

[0107] The cell to be desialylated can be any cell that is to be targeted for AAV transduction. In some embodiments, the cell is one that is known in the art to contain or is discovered to contain sialyl groups on the termini of one or more of the cell surface glycans. In some embodiments, the cell is an *in vitro* cell, *e.g.*, a cell in culture. The *in vitro* cell may be from an established cell line (*e.g.*, CHO, COS, HEK293, U87, Huh-7, or Neuro2a cells) or a primary cell that has been isolated from a subject and cultured.

[0108] In other embodiments, the cell is an *ex vivo* cell. The *ex vivo* cell may be one that has been isolated from a subject with the objective of returning the cell back to the subject from which it was isolated or to a different subject, *e.g.*, after the cell has been modified, such as by transduction with an AAV vector.

[0109] In other embodiments, the cell is an *in vivo* cell, *i.e.*, one that is present in a subject. The subject may be, for example, a patient in need of treatment or prevention of a disorder or an animal model for use in research.

[0110] The *in vitro*, *ex vivo*, or *in vivo* cell may be any type of cell for which transduction is desired. For example, the cell may be selected from the group consisting of a dendritic cell, T cell, B cell, neural cell, muscle cell, pancreatic cell, hepatic cell, lung cell, retinal cell, epithelial cell, smooth muscle cell, skeletal muscle cell, diaphragm muscle cell, cardiac muscle cell, kidney cell, myocardial cell, bone cell, spleen cell, keratinocyte, fibroblast, endothelial cell, prostate cell, germ cell, progenitor cell, and stem cell.

[0111] In different embodiments of the invention, the desialylating step can be carried out before, during, and/or after the step of contacting the cell with the AAV vector. For example, the cell can be contacted with a desialylating agent or genetically modified to prevent the production of sialoglycans prior to the cell being contacted with the AAV vector, *e.g.*, about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, or 24 hours or more prior to the cell being contacted with the AAV vector. In other embodiments, the cell is contacted with the desialylating agent and the AAV vector concurrently. As used herein, the word "concurrently" means sufficiently close in time to produce a combined effect (that is, concurrently can be simultaneously, or it can be two or more events occurring within a short time period before or after each other). In one embodiment, the desialylating agent and the AAV vector are present in the same composition that contacts the cell. In other embodiments, the desialylating agent and the AAV vector are in separate compositions that contact the cell concurrently. In a further embodiment, the cell is contacted with the AAV vector and then contacted with a desialylating agent, *e.g.*, about 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, or 24 hours or more after the cell is contacted with the AAV vector.

[0112] The desialylation of a cell comprising sialyl groups can be carried out by any means known in the art. In some embodiments, the desialylation is carried out by removing sialyl groups that are present on the cell surface. In one embodiment, the desialylation is carried out enzymatically using an enzyme known in the art to remove sialyl groups from sialoglycans. In one embodiment, the enzyme is a neuraminidase, *e.g.*, neuraminidase type III from *Vibrio cholerae* or 2,3,6,8-neuraminidase from *Arthrobacter ureafaciens*. In another embodiment the enzyme is a sialidase, *e.g.*, a bacterial sialidase such as those from *Clostridium perfringens*, *Actinomyces viscosus*, *Arthrobacter ureafaciens*, or *Micromonospora viridifaciens*, or a mammalian sialidase such as those encoded by the genes NEU2 and NEU4.

[0113] In other embodiments, the desialylation is carried out chemically. For example, the cell can be contacted with an inhibitor of a sialic acid transporter or

sialyltransferase, *e.g.*, swainsonine, soyasaponin, and α-benzyl-*O*-GalNAc, to inhibit the synthesis of sialoglycans. The sialyltransferase can be, for example, α2,3-(N)-sialyltransferase, α2,6-(N)-sialyltransferase, or α2,3-(O)-sialyltransferase. In another embodiment, existing sialoglycans can be desialylated under appropriate environmental conditions, such as mildly acidic conditions.

[0114] In other embodiments, the desialylation is carried out genetically. In one embodiment, the cell is genetically modified to disrupt the synthesis and/or transport of sialoglycans, *e.g.*, by inactivation of a gene encoding a sialic acid transporter or sialyltransferase, *e.g.*, a CMP-sialic acid transporter. In other embodiments, the expression of a sialic acid transporter or sialyltransferase can be inhibited, *e.g.*, using antisense, siRNA, microRNA, or ribozyme molecules.

[0115] The coding and noncoding nucleotide sequences for sialic acid transporters and sialyltransferases are known to those of skill in the art and are readily available in sequence databases such as GenBank. An antisense, siRNA, microRNA, or ribozyme nucleotide sequence or nucleic acid encoding an antisense, siRNA, microRNA, or ribozyme nucleotide sequence can be generated to any portion thereof in accordance with known techniques.

[0116] The term "antisense nucleotide sequence" or "antisense oligonucleotide" as used herein, refers to a nucleotide sequence that is complementary to a specified DNA or RNA sequence. Antisense oligonucleotides and nucleic acids that express the same can be made in accordance with conventional techniques. *See, e.g.*, U.S. Patent No. 5,023,243 to Tullis; U.S. Patent No. 5,149,797 to Pederson *et al.* The antisense nucleotide sequence can be complementary to the entire nucleotide sequence encoding the polypeptide or a portion thereof of at least 10, 20, 40, 50, 75, 100, 150, 200, 300, or 500 contiguous bases and will reduce the level of polypeptide production.

[0117] Those skilled in the art will appreciate that it is not necessary that the antisense nucleotide sequence be fully complementary to the target sequence as long as the degree of sequence similarity is sufficient for the antisense nucleotide sequence to hybridize to its target and reduce production of the polypeptide. As is known in the art, a higher degree of sequence similarity is generally required for short antisense nucleotide sequences, whereas a greater degree of mismatched bases will be tolerated by longer antisense nucleotide sequences.

[0118] For example, hybridization of such nucleotide sequences can be carried out under conditions of reduced stringency, medium stringency or even stringent conditions (*e.g.*, conditions represented by a wash stringency of 35-40% formamide with

5x Denhardt's solution, 0.5% SDS and 1x SSPE at 37°C; conditions represented by a wash stringency of 40-45% formamide with 5x Denhardt's solution, 0.5% SDS, and 1x SSPE at 42°C; and/or conditions represented by a wash stringency of 50% formamide with 5x Denhardt's solution, 0.5% SDS and 1x SSPE at 42°C, respectively) to the nucleotide sequences specifically disclosed herein. *See, e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 2nd Ed. (Cold Spring Harbor, NY, 1989).

[0119] In other embodiments, antisense nucleotide sequences of the invention have at least about 70%, 80%, 90%, 95%, 97%, 98% or higher sequence similarity with the complement of the coding sequences and will reduce the level of polypeptide production.

[0120] The length of the antisense nucleotide sequence (*i.e.*, the number of nucleotides therein) is not critical as long as it binds selectively to the intended location and reduces transcription and/or translation of the target sequence, and can be determined in accordance with routine procedures. In general, the antisense nucleotide sequence will be from about eight, ten or twelve nucleotides in length up to about 20, 30, 50, 75 or 100 nucleotides, or longer, in length.

[0121] An antisense nucleotide sequence can be constructed using chemical synthesis and enzymatic ligation reactions by procedures known in the art. For example, an antisense nucleotide sequence can be chemically synthesized using naturally occurring nucleotides or various modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleotide sequences, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleotide sequence include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomet- hyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopenten- yladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively,

the antisense nucleotide sequence can be produced using an expression vector into which a nucleic acid has been cloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

[0122] The antisense nucleotide sequences further include nucleotide sequences wherein at least one, or all, of the internucleotide bridging phosphate residues are modified phosphates, such as methyl phosphonates, methyl phosphonothioates, phosphoromorpholidates, phosphoropiperazidates and phosphoramidates. For example, every other one of the internucleotide bridging phosphate residues can be modified as described. In another non-limiting example, the antisense nucleotide sequence is a nucleotide sequence in which one, or all, of the nucleotides contain a 2' lower alkyl moiety (*e.g.*, C_1-C_4, linear or branched, saturated or unsaturated alkyl, such as methyl, ethyl, ethenyl, propyl, 1-propenyl, 2-propenyl, and isopropyl). For example, every other one of the nucleotides can be modified as described. *See also*, Furdon *et al.*, *Nucleic Acids Res. 17*:9193 (1989); Agrawal *et al.*, *Proc. Natl. Acad. Sci. USA 87*:1401 (1990); Baker *et al.*, *Nucleic Acids Res.* 18:3537 (1990); Sproat *et al.*, *Nucleic Acids Res. 17*:3373 (1989); Walder and Walder, *Proc. Natl. Acad. Sci. USA 85*:5011 (1988); incorporated by reference herein in their entireties for their teaching of methods of making antisense molecules, including those containing modified nucleotide bases).

[0123] Triple helix base-pairing methods can also be employed to inhibit production of polypeptides. Triple helix pairing is believed to work by inhibiting the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, (1994) In: Huber *et al.*, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY).

[0124] Small Interference (si) RNA, also known as RNA interference (RNAi) molecules, provides another approach for modulating the expression of polypeptides. siRNA is a mechanism of post-transcriptional gene silencing in which double-stranded RNA (dsRNA) corresponding to a coding sequence of interest is introduced into a cell or an organism, resulting in degradation of the corresponding mRNA. The mechanism by which siRNA achieves gene silencing has been reviewed in Sharp *et al.*, *Genes Dev. 15*:485 (2001); and Hammond *et al.*, *Nature Rev. Gen. 2*:110 (2001)). The siRNA effect persists for multiple cell divisions before gene expression is regained. siRNA is therefore a powerful method for making targeted knockouts or "knockdowns" at the RNA level. siRNA has proven successful in human cells, including human embryonic kidney and

HeLa cells (*see, e.g.*, Elbashir *et al.*, *Nature 411*:494 (2001)). In one embodiment, silencing can be induced in mammalian cells by enforcing endogenous expression of RNA hairpins (*see* Paddison *et al.*, *Proc. Natl. Acad. Sci. USA 99*:1443 (2002)). In another embodiment, transfection of small (21-23 nt) dsRNA specifically inhibits nucleic acid expression (reviewed in Caplen, *Trends Biotechnol. 20*:49 (2002)).

[0125] siRNA technology utilizes standard molecular biology methods. dsRNA corresponding to all or a part of a target coding sequence to be inactivated can be produced by standard methods, *e.g.*, by simultaneous transcription of both strands of a template DNA (corresponding to the target sequence) with T7 RNA polymerase. Kits for production of dsRNA for use in siRNA are available commercially, *e.g.*, from New England Biolabs, Inc. Methods of transfection of dsRNA or plasmids engineered to make dsRNA are routine in the art.

[0126] MicroRNA (miRNA), single stranded RNA molecules of about 21-23 nucleotides in length, can be used in a similar fashion to siRNA to modulate gene expression (see U.S. Patent No. 7,217,807).

[0127] Silencing effects similar to those produced by siRNA have been reported in mammalian cells with transfection of a mRNA-cDNA hybrid construct (Lin *et al.*, *Biochem. Biophys. Res. Commun. 281*:639 (2001)), providing yet another strategy for silencing a coding sequence of interest.

[0128] The expression of polypeptides can also be inhibited using ribozymes. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim *et al.*, *Proc. Natl. Acad. Sci. USA 84*:8788 (1987); Gerlach *et al.*, *Nature 328*:802 (1987); Forster and Symons, *Cell 49*:211 (1987)). For example, a large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Michel and Westhof, *J. Mol. Biol. 216*:585 (1990); Reinhold-Hurek and Shub, *Nature 357*:173 (1992)). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence ("IGS") of the ribozyme prior to chemical reaction.

[0129] Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, *Nature 338*:217 (1989)). For example, U.S. Patent No. 5,354,855 reports that certain ribozymes can act as endonucleases with a sequence specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-

mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, *Proc. Natl. Acad. Sci. USA 88*:10591 (1991); Sarver *et al.*, *Science 247*:1222 (1990); Sioud *et al.*, *J. Mol. Biol. 223*:831 (1992)).

[0130] In one aspect, the methods of the present invention can be used to target an AAV vector to a specific cell, tissue, or region *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, by desialylating the specific cell, tissue, or region and then contacting the desialylated cell, tissue, or region with an AAV vector. Thus, one aspect of the invention relates to a method of targeting an AAV vector that binds asialoglycans to a cell, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a suitable control cell, *e.g.*, a cell that has not been desialylated.

[0131] In one embodiment, the cell is a cultured cell. In another embodiment, the cell is present in a subject in a specific tissue or region, *e.g.*, selected from the group consisting of eye, ear, nose, joints, thymus, spleen, kidney, lungs, liver, heart, spinal cord, brain, subarachnoid cisternae, ventricles, skeletal muscle, diaphragm, gastrointestinal tract, and pancreas.

[0132] In certain embodiments, the method comprises delivering a desialylating agent and the AAV vector to the specific tissue or region. The desialylating agent can be any of the enzymatic, chemical, or genetic agents described above. In one embodiment, the desialylating agent is delivered prior to the AAV vector. In another embodiment, the desialylating agent is delivered concurrently with the AAV vector. In a further embodiment, the desialylating agent is delivered after the AAV vector. In certain embodiments, the desialylating agent and the AAV vector are delivered in one composition. In other embodiments, the desialylating agent and the AAV vector are delivered in separate compositions.

[0133] The methods of the present invention can be used advantageously in the delivery of AAV vectors to subjects, *e.g.*, for the expression of therapeutic, prophylactic, or diagnostic proteins or polynucleotides. By desialylating cells, tissues, or regions in a subject, the AAV vectors of the invention may be preferentially targeted to the desialylated cells, tissues, or regions, thereby limiting the spread of the vectors from the site of delivery and minimizing systemic dissemination of the vectors.

[0134] Thus, one aspect of the invention relates to a method of restricting AAV vectors that bind asialoglycans to the site of delivery in a subject, comprising:

(a) delivering a desialylating agent to a site in a subject; and

(b) delivering the AAV vector to the same site;

wherein the AAV vector is targeted to desialylated cells and systemic dissemination of the AAV vector is restricted.

[0135] In one embodiment, the site of delivery is selected from the group consisting of spinal column, lung, eye, ear, joints, nose, cranium, subarachnoid cisternae, ventricles, myocardium, pancreatic duct, and intraportal vein.

[0136] The phrase "systemic dissemination of the AAV vector is restricted" is defined as the presence of less than about 25% (*e.g.*, less that about 20%, 15%, 10%, or 5%) of the delivered AAV vectors (or a nucleic acid delivered by the vector) in the plasma or in a tissue remote from the site of delivery at any time after the AAV vector is delivered to the subject. For example, when AAV vector is delivered intrathecally to the central nervous system, less than about 25% of the delivered AAV vectors can be detected circulating in the blood.

Methods of Producing Virus Vectors

[0137] AAV vectors that binds asialoglycans can be produced by methods well known in the art and described below. For example, the vectors can be produced by a method comprising providing to a cell permissive for AAV replication: (a) a recombinant AAV template comprising (i) a heterologous nucleotide sequence, and (ii) AAV ITRs; and (b) a polynucleotide encoding a Rep and Cap proteins; under conditions sufficient for the replication and packaging of the recombinant AAV template; whereby recombinant AAV vectors are produced in the cell. Conditions sufficient for the replication and packaging of the recombinant AAV template can be, *e.g.*, the presence of AAV sequences sufficient for replication of the AAV template and encapsidation into AAV capsids (*e.g.*, AAV *rep* sequences and AAV *cap* sequences) and helper sequences from adenovirus and/or herpesvirus. In particular embodiments, the AAV template comprises two AAV ITR sequences, which are located 5' and 3' to the heterologous nucleic acid sequence, although they need not be directly contiguous thereto.

[0138] In some embodiments, the recombinant AAV template comprises an ITR that not resolved by Rep to make duplexed AAV vectors as described in international patent publication WO 01/92551.

[0139] The AAV template and AAV *rep* and *cap* sequences are provided under conditions such that virus vector comprising the AAV template packaged within the AAV capsid is produced in the cell. The method can further comprise the step of collecting the

virus vector from the cell. The virus vector can be collected from the medium and/or by lysing the cells.

[0140] The cell can be a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed. In particular embodiments, the cell is a mammalian cell (*e.g.*, a primate or human cell). As another option, the cell can be a trans-complementing packaging cell line that provide functions deleted from a replication-defective helper virus, *e.g.*, 293 cells or other E1a trans-complementing cells.

[0141] The AAV replication and capsid sequences may be provided by any method known in the art. Current protocols typically express the AAV *rep/cap* genes on a single plasmid. The AAV replication and packaging sequences need not be provided together, although it may be convenient to do so. The AAV *rep* and/or *cap* sequences may be provided by any viral or non-viral vector. For example, the *rep/cap* sequences may be provided by a hybrid adenovirus or herpesvirus vector (*e.g.*, inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV *cap* and *rep* genes. One advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (*i.e.*, are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," *see* Margolski, (1992) *Curr. Top. Microbiol. Immun.* 158:67).

[0142] As a further alternative, the *rep/cap* sequences may be stably incorporated into a cell.

[0143] Typically the AAV *rep/cap* sequences will not be flanked by the TRs, to prevent rescue and/or packaging of these sequences.

[0144] The AAV template can be provided to the cell using any method known in the art. For example, the template can be supplied by a non-viral (*e.g.*, plasmid) or viral vector. In particular embodiments, the AAV template is supplied by a herpesvirus or adenovirus vector (*e.g.*, inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo *et al.*, (1998) *J. Virology* 72:5025, describes a baculovirus vector carrying a reporter gene flanked by the AAV TRs. EBV vectors may also be employed to deliver the template, as described above with respect to the *rep/cap* genes.

[0145] In another representative embodiment, the AAV template is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus comprising the AAV template is stably integrated into the chromosome of the cell.

[0146] To enhance virus titers, helper virus functions (*e.g.*, adenovirus or herpesvirus) that promote a productive AAV infection can be provided to the cell. Helper virus sequences necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, *e.g.*, as a non-infectious adenovirus miniplasmid that carries all of the helper genes that promote efficient parvovirus production as described by Ferrari *et al.*, (1997) *Nature Med.* 3:1295, and U.S. Patent Nos. 6,040,183 and 6,093,570.

[0147] Further, the helper virus functions may be provided by a packaging cell with the helper sequences embedded in the chromosome or maintained as a stable extrachromosomal element. Generally, the helper virus sequences cannot be packaged into parvovirus virions, *e.g.*, are not flanked by TRs.

[0148] Those skilled in the art will appreciate that it may be advantageous to provide the AAV replication and capsid sequences and the helper virus sequences (*e.g.*, adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct. As one nonlimiting illustration, the helper construct can be a hybrid adenovirus or hybrid herpesvirus comprising the AAV *rep/cap* genes.

[0149] In one particular embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector can further comprise the AAV template. The AAV *rep/cap* sequences and/or the AAV template can be inserted into a deleted region (*e.g.*, the E1a or E3 regions) of the adenovirus.

[0150] In a further embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. According to this embodiment, the AAV template can be provided as a plasmid template.

[0151] In another illustrative embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the AAV template is integrated into the cell as a provirus. Alternatively, the AAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (*e.g.*, as an EBV based nuclear episome).

[0152] In a further exemplary embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper. The AAV template can be provided as a separate replicating viral vector. For example, the AAV template can be provided by an AAV particle or a second recombinant adenovirus particle.

[0153] According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' *cis* sequences sufficient for adenovirus replication and packaging (*i.e.*, the adenovirus terminal repeats and PAC sequence). The AAV *rep/cap* sequences and, if present, the AAV template are embedded in the adenovirus backbone and are flanked by the 5' and 3' *cis* sequences, so that these sequences may be packaged into adenovirus capsids. As described above, the adenovirus helper sequences and the AAV *rep/cap* sequences are generally not flanked by TRs so that these sequences are not packaged into the AAV virions.

[0154] Zhang *et al.*, ((2001) *Gene Ther.* 18:704-12) describe a chimeric helper comprising both adenovirus and the AAV *rep* and *cap* genes.

[0155] Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway *et al.*, (1999) *Gene Ther.* 6:986 and WO 00/17377.

[0156] As a further alternative, the virus vectors of the invention can be produced in insect cells using baculovirus vectors to deliver the *rep/cap* genes and AAV template as described, for example, by Urabe *et al.*, (2002) *Human Gene Ther.* 13:1935-43.

[0157] AAV vector stocks free of contaminating helper virus may be obtained by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin *et al.* (1999) *Gene Therapy* 6:973). Deleted replication-defective helper viruses can be used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV. Adenovirus mutants defective for late gene expression are known in the art (*e.g.*, ts100K and ts149 adenovirus mutants).

Recombinant Virus Vectors

[0158] AAV vectors that binds asialoglycans are useful for the delivery of nucleic acids to cells *in vitro*, *ex vivo*, and *in vivo*. In particular, the virus vectors can be advantageously employed to deliver or transfer nucleic acids to animal, including mammalian, cells.

[0159] Any heterologous nucleic acid sequence(s) of interest may be delivered in the virus vectors of the present invention. Nucleic acids of interest include nucleic acids encoding polypeptides, including therapeutic (*e.g.*, for medical or veterinary uses) or immunogenic (*e.g.*, for vaccines) polypeptides.

[0160] Therapeutic polypeptides include, but are not limited to, cystic fibrosis transmembrane regulator protein (CFTR), dystrophin (including mini- and micro-dystrophins (*see, e.g.*, Vincent *et al.*, (1993) *Nature Genetics* 5:130; U.S. Patent Publication No. 2003/017131; International publication WO/2008/088895, Wang *et al.*, *Proc. Natl. Acad. Sci. USA* 97:13714-13719 (2000); and Gregorevic *et al.*, *Mol. Ther.* 16:657-64 (2008)), myostatin propeptide, follistatin, activin type II soluble receptor, IGF-1, anti-inflammatory polypeptides such as the I-kappa B dominant mutant, sarcospan, utrophin (Tinsley *et al.*, (1996) *Nature* 384:349), mini-utrophin, clotting factors (*e.g.*, Factor VIII, Factor IX, Factor X, *etc.*), erythropoietin, angiostatin, endostatin, catalase, tyrosine hydroxylase, superoxide dismutase, leptin, the LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, α_1-antitrypsin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase, β-glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase A, branched-chain keto acid dehydrogenase, RP65 protein, cytokines (*e.g.*, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, granulocyte-macrophage colony stimulating factor, lymphotoxin, and the like), peptide growth factors, neurotrophic factors and hormones (*e.g.*, somatotropin, insulin, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, nerve growth factor, neurotrophic factor –3 and –4, brain-derived neurotrophic factor, bone morphogenic proteins [including RANKL and VEGF], glial derived growth factor, transforming growth factor –α and –β, and the like), lysosomal acid α-glucosidase, α-galactosidase A, receptors (*e.g.*, the tumor necrosis growth factorα soluble receptor), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, anti-inflammatory factors such as IRAP, anti-myostatin proteins, aspartoacylase, and monoclonal antibodies (including single chain monoclonal antibodies; an exemplary Mab is the Herceptin® Mab). Other illustrative heterologous nucleic acid sequences encode suicide gene products (*e.g.*, thymidine kinase, cytosine deaminase, diphtheria toxin, and tumor necrosis factor), proteins conferring resistance to a drug used in cancer therapy, tumor suppressor gene products (*e.g.*, p53, Rb, Wt-1), TRAIL, FAS-ligand, and any other polypeptide that has a therapeutic effect in a subject in need thereof. AAV vectors can

also be used to deliver monoclonal antibodies and antibody fragments, for example, an antibody or antibody fragment directed against myostatin (*see, e.g.*, Fang *et al.*, *Nature Biotechnol.* 23:584-590 (2005)).

[0161] Heterologous nucleic acid sequences encoding polypeptides include those encoding reporter polypeptides (*e.g.*, an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, Green Fluorescent Protein, β-galactosidase, alkaline phosphatase, luciferase, and chloramphenicol acetyltransferase gene.

[0162] Alternatively, in particular embodiments of this invention, the heterologous nucleic acid may encode an antisense nucleic acid, a ribozyme (*e.g.*, as described in U.S. Patent No. 5,877,022), RNAs that effect spliceosome-mediated *trans*-splicing (*see*, Puttaraju *et al.*, (1999) *Nature Biotech.* 17:246; U.S. Patent No. 6,013,487; U.S. Patent No. 6,083,702), interfering RNAs (RNAi) including siRNA, shRNA or miRNA that mediate gene silencing (*see*, Sharp *et al.*, (2000) *Science* 287:2431), and other non-translated RNAs, such as "guide" RNAs (Gorman *et al.*, (1998) *Proc. Nat. Acad. Sci. USA* 95:4929; U.S. Patent No. 5,869,248 to Yuan *et al.*), and the like. Exemplary untranslated RNAs include RNAi against a multiple drug resistance (MDR) gene product (*e.g.*, to treat and/or prevent tumors and/or for administration to the heart to prevent damage by chemotherapy), RNAi against myostatin (*e.g.*, for Duchenne muscular dystrophy), RNAi against VEGF (*e.g.*, to treat and/or prevent tumors), RNAi against phospholamban (*e.g.*, to treat cardiovascular disease, *see, e.g.*, Andino *et al.*, *J. Gene Med.* 10:132-142 (2008) and Li *et al.*, *Acta Pharmacol. Sin.* 26:51-55 (2005)); phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E (*e.g.*, to treat cardiovascular disease, *see, e.g.*, Hoshijima *et al. Nat. Med.* 8:864-871 (2002)), RNAi to adenosine kinase (*e.g.*, for epilepsy), RNAi to a sarcoglycan [*e.g.*, α, β, γ], RNAi against myostatin, myostatin propeptide, follistatin, or activin type II soluble receptor, RNAi against anti-inflammatory polypeptides such as the Ikappa B dominant mutant, and RNAi directed against pathogenic organisms and viruses (*e.g.*, hepatitis B virus, human immunodeficiency virus, CMV, herpes simplex virus, human papilloma virus, *etc.*).

[0163] Alternatively, in particular embodiments of this invention, the heterologous nucleic acid may encode protein phosphatase inhibitor I (I-1), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, β2-adrenergic receptor, β2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban

inhibitory or dominant-negative molecules such as phospholamban S16E, enos, inos, or bone morphogenic proteins (including BNP 2, 7, *etc.*, RANKL and/or VEGF).

[0164] The virus vector may also comprise a heterologous nucleic acid that shares homology with and recombines with a locus on a host chromosome. This approach can be utilized, for example, to correct a genetic defect in the host cell.

[0165] The present invention also provides virus vectors that express an immunogenic polypeptide, *e.g.*, for vaccination. The nucleic acid may encode any immunogen of interest known in the art including, but not limited to, immunogens from human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), influenza virus, HIV or SIV gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

[0166] The use of AAV as vaccine vectors is known in the art (*see, e.g.*, Miyamura *et al.*, (1994) *Proc. Nat. Acad. Sci USA* 91:8507; U.S. Patent No. 5,916,563 to Young *et al.*, U.S. Patent No. 5,905,040 to Mazzara *et al.*, U.S. Patent No. 5,882,652, U.S. Patent No. 5,863,541 to Samulski *et al.*). The antigen may be presented in the AAV capsid. Alternatively, the antigen may be expressed from a heterologous nucleic acid introduced into a recombinant vector genome. Any immunogen of interest as described herein and/or as is known in the art can be provided by the virus vector of the present invention.

[0167] An immunogenic polypeptide can be any polypeptide suitable for eliciting an immune response and/or protecting the subject against an infection and/or disease, including, but not limited to, microbial, bacterial, protozoal, parasitic, fungal and/or viral infections and diseases. For example, the immunogenic polypeptide can be an orthomyxovirus immunogen (*e.g.*, an influenza virus immunogen, such as the influenza virus hemagglutinin (HA) surface protein or the influenza virus nucleoprotein, or an equine influenza virus immunogen) or a lentivirus immunogen (*e.g.*, an equine infectious anemia virus immunogen, a Simian Immunodeficiency Virus (SIV) immunogen, or a Human Immunodeficiency Virus (HIV) immunogen, such as the HIV or SIV envelope GP160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV *gag*, *pol* and *env* genes products). The immunogenic polypeptide can also be an arenavirus immunogen (*e.g.*, Lassa fever virus immunogen, such as the Lassa fever virus nucleocapsid protein and the Lassa fever envelope glycoprotein), a poxvirus immunogen (*e.g.*, a vaccinia virus immunogen, such as the vaccinia L1 or L8 gene products), a flavivirus immunogen (*e.g.*, a yellow fever virus immunogen or a Japanese encephalitis virus immunogen), a filovirus immunogen (*e.g.*, an Ebola virus immunogen, or a Marburg

virus immunogen, such as NP and GP gene products), a bunyavirus immunogen (*e.g.*, RVFV, CCHF, and/or SFS virus immunogens), or a coronavirus immunogen (*e.g.*, an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein, or a porcine transmissible gastroenteritis virus immunogen, or an avian infectious bronchitis virus immunogen). The immunogenic polypeptide can further be a polio immunogen, a herpes immunogen (*e.g.*, CMV, EBV, HSV immunogens) a mumps immunogen, a measles immunogen, a rubella immunogen, a diphtheria toxin or other diphtheria immunogen, a pertussis antigen, a hepatitis (*e.g.*, hepatitis A, hepatitis B, hepatitis C, etc.) immunogen, and/or any other vaccine immunogen now known in the art or later identified as an immunogen.

[0168] Alternatively, the immunogenic polypeptide can be any tumor or cancer cell antigen. Optionally, the tumor or cancer antigen is expressed on the surface of the cancer cell. Exemplary cancer and tumor cell antigens are described in S.A. Rosenberg (*Immunity* 10:281 (1991)). Other illustrative cancer and tumor antigens include, but are not limited to: BRCA1 gene product, BRCA2 gene product, gp100, tyrosinase, GAGE-1/2, BAGE, RAGE, LAGE, NY-ESO-1, CDK-4, β-catenin, MUM-1, Caspase-8, KIAA0205, HPVE, SART-1, PRAME, p15, melanoma tumor antigens (Kawakami *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:3515; Kawakami *et al.*, (1994) *J. Exp. Med.*, 180:347; Kawakami *et al.*, (1994) *Cancer Res.* 54:3124), MART-1, gp100 MAGE-1, MAGE-2, MAGE-3, CEA, TRP-1, TRP-2, P-15, tyrosinase (Brichard *et al.*, (1993) *J. Exp. Med.* 178:489); HER-2/neu gene product (U.S. Patent No. 4,968,603), CA 125, LK26, FB5 (endosialin), TAG 72, AFP, CA19-9, NSE, DU-PAN-2, CA50, SPan-1, CA72-4, HCG, STN (sialyl Tn antigen), c-erbB-2 proteins, PSA, L-CanAg, estrogen receptor, milk fat globulin, p53 tumor suppressor protein (Levine, (1993) *Ann. Rev. Biochem.* 62:623); mucin antigens (International Patent Publication No. WO 90/05142); telomerases; nuclear matrix proteins; prostatic acid phosphatase; papilloma virus antigens; and/or antigens now known or later discovered to be associated with the following cancers: melanoma, adenocarcinoma, thymoma, lymphoma (*e.g.*, non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition now known or later identified (*see, e.g.*, Rosenberg, (1996) *Ann. Rev. Med.* 47:481-91).

[0169] As a further alternative, the heterologous nucleic acid can encode any polypeptide that is desirably produced in a cell *in vitro*, *ex vivo*, or *in vivo*. For example,

the virus vectors may be introduced into cultured cells and the expressed gene product isolated therefrom. In another example, the virus vectors may be introduced into animals to create models of disease.

[0170] It will be understood by those skilled in the art that the heterologous nucleic acid(s) of interest can be operably associated with appropriate control sequences. For example, the heterologous nucleic acid can be operably associated with expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), promoters, and/or enhancers, and the like.

[0171] Those skilled in the art will appreciate that a variety of promoter/enhancer elements can be used depending on the level and tissue-specific expression desired. The promoter/enhancer can be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer can be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

[0172] In particular embodiments, the promoter/enhancer elements can be native to the target cell or subject to be treated. In representative embodiments, the promoter/enhancer element can be native to the heterologous nucleic acid sequence. The promoter/enhancer element is generally chosen so that it functions in the target cell(s) of interest. Further, in particular embodiments the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhancer element may be constitutive or inducible.

[0173] Inducible expression control elements are typically advantageous in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery can be tissue-specific or –preferred promoter/enhancer elements, and include muscle specific or preferred (including cardiac, skeletal and/or smooth muscle specific or preferred), neural tissue specific or preferred (including brain-specific or preferred), eye specific or preferred (including retina-specific and cornea-specific), liver specific or preferred, bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited

to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

[0174] In embodiments wherein the heterologous nucleic acid sequence(s) is transcribed and then translated in the target cells, specific initiation signals are generally included for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

[0175] The virus vectors according to the present invention provide a means for delivering heterologous nucleic acids into a broad range of cells, including dividing and non-dividing cells. The virus vectors can be employed to deliver a nucleic acid of interest to a cell *in vitro*, *e.g.*, to produce a polypeptide *in vitro* or for *ex vivo* gene therapy. The virus vectors are additionally useful in a method of delivering a nucleic acid to a subject in need thereof, *e.g.*, to express an immunogenic or therapeutic polypeptide or a functional RNA. In this manner, the polypeptide or functional RNA can be produced *in vivo* in the subject. The subject can be in need of the polypeptide because the subject has a deficiency of the polypeptide. Further, the method can be practiced because the production of the polypeptide or functional RNA in the subject may impart some beneficial effect.

[0176] The virus vectors can also be used to produce a polypeptide of interest or functional RNA in cultured cells or in a subject (*e.g.*, using the subject as a bioreactor to produce the polypeptide or to observe the effects of the functional RNA on the subject, for example, in connection with screening methods).

[0177] In general, the virus vectors of the present invention can be employed to deliver a heterologous nucleic acid encoding a polypeptide or functional RNA to treat and/or prevent any disease state for which it is beneficial to deliver a therapeutic polypeptide or functional RNA. Illustrative disease states include, but are not limited to: cystic fibrosis (cystic fibrosis transmembrane regulator protein) and other diseases of the lung, hemophilia A (Factor VIII), hemophilia B (Factor IX), thalassemia (ß-globin), anemia (erythropoietin) and other blood disorders, Alzheimer's disease (GDF; neprilysin), multiple sclerosis (ß-interferon), Parkinson's disease (glial-cell line derived neurotrophic factor [GDNF]), Huntington's disease (RNAi to remove repeats), amyotrophic lateral sclerosis, epilepsy (galanin, neurotrophic factors), and other neurological disorders, cancer (endostatin, angiostatin, TRAIL, FAS-ligand, cytokines including interferons; RNAi including RNAi against VEGF or the multiple drug resistance gene product), diabetes mellitus (insulin), muscular dystrophies including

Duchenne (dystrophin, mini-dystrophin, insulin-like growth factor I, a sarcoglycan [*e.g.*, α, β, γ], RNAi against myostatin, myostatin propeptide, follistatin, activin type II soluble receptor, anti-inflammatory polypeptides such as the Ikappa B dominant mutant, sarcospan, utrophin, mini-utrophin, RNAi against splice junctions in the dystrophin gene to induce exon skipping [*see, e.g.*, WO/2003/095647], antisense against U7 snRNAs to induce exon skipping [*see, e.g.*, WO/2006/021724], and antibodies or antibody fragments against myostatin or myostatin propeptide) and Becker, Gaucher disease (glucocerebrosidase), Hurler's disease (α-L-iduronidase), adenosine deaminase deficiency (adenosine deaminase), glycogen storage diseases (*e.g.*, Fabry disease [α-galactosidase] and Pompe disease [lysosomal acid α-glucosidase]) and other metabolic defects, congenital emphysema (α1-antitrypsin), Lesch-Nyhan Syndrome (hypoxanthine guanine phosphoribosyl transferase), Niemann-Pick disease (sphingomyelinase), Tays Sachs disease (lysosomal hexosaminidase A), Maple Syrup Urine Disease (branched-chain keto acid dehydrogenase), retinal degenerative diseases (and other diseases of the eye and retina; *e.g.*, PDGF for macular degeneration), diseases of solid organs such as brain (including Parkinson's Disease [GDNF], astrocytomas [endostatin, angiostatin and/or RNAi against VEGF], glioblastomas [endostatin, angiostatin and/or RNAi against VEGF]), liver, kidney, heart including congestive heart failure or peripheral artery disease (PAD) (*e.g.*, by delivering protein phosphatase inhibitor I (I-1), serca2a, zinc finger proteins that regulate the phospholamban gene, Barkct, β2-adrenergic receptor, β2-adrenergic receptor kinase (BARK), phosphoinositide-3 kinase (PI3 kinase), S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct; calsarcin, RNAi against phospholamban; phospholamban inhibitory or dominant-negative molecules such as phospholamban S16E, *etc.*), arthritis (insulin-like growth factors), joint disorders (insulin-like growth factor 1 and/or 2), intimal hyperplasia (*e.g.*, by delivering enos, inos), improve survival of heart transplants (superoxide dismutase), AIDS (soluble CD4), muscle wasting (insulin-like growth factor I), kidney deficiency (erythropoietin), anemia (erythropoietin), arthritis (anti-inflammatory factors such as IRAP and TNFα soluble receptor), hepatitis (α-interferon), LDL receptor deficiency (LDL receptor), hyperammonemia (ornithine transcarbamylase), Krabbe's disease (galactocerebrosidase), Batten's disease, spinal cerebral ataxias including SCA1, SCA2 and SCA3, phenylketonuria (phenylalanine hydroxylase), autoimmune diseases, and the like. The invention can further be used following organ transplantation to increase the success of the transplant and/or to reduce the negative side effects of organ transplantation or adjunct

therapies (*e.g.*, by administering immunosuppressant agents or inhibitory nucleic acids to block cytokine production). As another example, bone morphogenic proteins (including BNP 2, 7, *etc.*, RANKL and/or VEGF) can be administered with a bone allograft, for example, following a break or surgical removal in a cancer patient.

[0178] Gene transfer has substantial potential use for understanding and providing therapy for disease states. There are a number of inherited diseases in which defective genes are known and have been cloned. In general, the above disease states fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and unbalanced states, which may involve regulatory or structural proteins, and which are typically inherited in a dominant manner. For deficiency state diseases, gene transfer can be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced disease states, gene transfer can be used to create a disease state in a model system, which can then be used in efforts to counteract the disease state. Thus, virus vectors according to the present invention permit the treatment and/or prevention of genetic diseases.

[0179] The virus vectors according to the present invention may also be employed to provide a functional RNA to a cell *in vitro* or *in vivo*. Expression of the functional RNA in the cell, for example, can diminish expression of a particular target protein by the cell. Accordingly, functional RNA can be administered to decrease expression of a particular protein in a subject in need thereof. Functional RNA can also be administered to cells *in vitro* to regulate gene expression and/or cell physiology, *e.g.*, to optimize cell or tissue culture systems or in screening methods.

[0180] Virus vectors according to the instant invention find use in diagnostic and screening methods, whereby a nucleic acid of interest is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

[0181] The virus vectors of the present invention can also be used for various non-therapeutic purposes, including but not limited to use in protocols to assess gene targeting, clearance, transcription, translation, *etc.*, as would be apparent to one skilled in the art. The virus vectors can also be used for the purpose of evaluating safety (spread, toxicity, immunogenicity, *etc.*). Such data, for example, are considered by the United States Food and Drug Administration as part of the regulatory approval process prior to evaluation of clinical efficacy.

[0182] As a further aspect, the virus vectors of the present invention may be used to produce an immune response in a subject. According to this embodiment, a virus

vector comprising a heterologous nucleic acid sequence encoding an immunogenic polypeptide can be administered to a subject, and an active immune response is mounted by the subject against the immunogenic polypeptide. Immunogenic polypeptides are as described hereinabove. In some embodiments, a protective immune response is elicited.

[0183] Alternatively, the virus vector may be administered to a cell *ex vivo* and the altered cell is administered to the subject. The virus vector comprising the heterologous nucleic acid is introduced into the cell, and the cell is administered to the subject, where the heterologous nucleic acid encoding the immunogen can be expressed and induce an immune response in the subject against the immunogen. In particular embodiments, the cell is an antigen-presenting cell (*e.g.*, a dendritic cell).

[0184] An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscowitz, *Immunophysiology: Cell Function and Cellular Interactions in Antibody Formation, in* IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to an immunogen by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

[0185] A "protective" immune response or "protective" immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence of disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment and/or prevention of disease, in particular cancer or tumors (*e.g.*, by preventing cancer or tumor formation, by causing regression of a cancer or tumor and/or by preventing metastasis and/or by preventing growth of metastatic nodules). The protective effects may be complete or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

[0186] In particular embodiments, the virus vector or cell comprising the heterologous nucleic acid can be administered in an immunogenically effective amount, as described below.

[0187] The virus vectors of the present invention can also be administered for cancer immunotherapy by administration of a virus vector expressing one or more cancer cell antigens (or an immunologically similar molecule) or any other immunogen that

produces an immune response against a cancer cell. To illustrate, an immune response can be produced against a cancer cell antigen in a subject by administering a virus vector comprising a heterologous nucleic acid encoding the cancer cell antigen, for example to treat a patient with cancer and/or to prevent cancer from developing in the subject. The virus vector may be administered to a subject *in vivo* or by using *ex vivo* methods, as described herein. Alternatively, the cancer antigen can be expressed as part of the virus capsid or be otherwise associated with the virus capsid as described above.

[0188] As another alternative, any other therapeutic nucleic acid (*e.g.*, RNAi) or polypeptide (*e.g.*, cytokine) known in the art can be administered to treat and/or prevent cancer.

[0189] As used herein, the term "cancer" encompasses tumor-forming cancers. Likewise, the term "cancerous tissue" encompasses tumors. A "cancer cell antigen" encompasses tumor antigens.

[0190] The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to melanoma, adenocarcinoma, thymoma, lymphoma (*e.g.*, non-Hodgkin's lymphoma, Hodgkin's lymphoma), sarcoma, lung cancer, liver cancer, colon cancer, leukemia, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical cancer, bladder cancer, kidney cancer, pancreatic cancer, brain cancer and any other cancer or malignant condition now known or later identified. In representative embodiments, the invention provides a method of treating and/or preventing tumor-forming cancers.

[0191] The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. In representative embodiments, the methods disclosed herein are used to prevent and treat malignant tumors.

[0192] By the terms "treating cancer," "treatment of cancer" and equivalent terms it is intended that the severity of the cancer is reduced or at least partially eliminated and/or the progression of the disease is slowed and/or controlled and/or the disease is stabilized. In particular embodiments, these terms indicate that metastasis of the cancer is prevented or reduced or at least partially eliminated and/or that growth of metastatic nodules is prevented or reduced or at least partially eliminated.

[0193] By the terms "prevention of cancer" or "preventing cancer" and equivalent terms it is intended that the methods at least partially eliminate or reduce

and/or delay the incidence and/or severity of the onset of cancer. Alternatively stated, the onset of cancer in the subject may be reduced in likelihood or probability and/or delayed.

[0194] In particular embodiments, cells may be removed from a subject with cancer and contacted with a virus vector according to the instant invention. The modified cell is then administered to the subject, whereby an immune response against the cancer cell antigen is elicited. This method can be advantageously employed with immunocompromised subjects that cannot mount a sufficient immune response *in vivo* (*i.e.*, cannot produce enhancing antibodies in sufficient quantities).

[0195] It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (*e.g.*, α-interferon, β-interferon, γ-interferon, ω-interferon, τ-interferon, interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin 12, interleukin-13, interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor-α, tumor necrosis factor-β, monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin). Accordingly, immunomodulatory cytokines (preferably, CTL inductive cytokines) may be administered to a subject in conjunction with the virus vector.

[0196] Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleic acid encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

Compositions and Kits

[0197] One aspect of the invention relates to compositions comprising an AAV vector that binds asialoglycans and a desialylating agent. In one embodiment, the composition comprises a sufficient amount of AAV vector to infect cells. In an additional embodiment, the composition comprises a therapeutically effective amount of the AAV vector. The desialylating agent can be any agent described above. In another embodiment, the composition further comprises a physiologically acceptable carrier. In further embodiments, the composition further comprises one or more excipients, such as buffers, stabilizers, etc. The composition can be in the form of a liquid, semi-solid, or solid.

[0198] Another aspect of the invention relates to a kit comprising a desialylating agent and an AAV vector that binds asialoglycans. In one embodiment, the desialylating agent and the AAV vector are present in one container. In another embodiment, the

desialylating agent and the AAV vector are present in separate containers. The kits are useful for carrying out the methods of the invention. The kits can comprise other reagents for delivery of viral vectors and/or detection of expression polypeptides or functional RNAs encoded by the vectors. The reagents may be nucleic acids (*e.g.*, an oligonucleotide that specifically hybridizes to a portion of the vector and can be used as a hybridization probe or an amplification primer), antibodies (*e.g.*, one the specifically binds to a polypeptide encoded by the vector), or other agents that specifically recognize the polynucleotides or polypeptides of the invention.

[0199] The reagents can be conjugated to a detectable tag or detectable label. Such a tag can be any suitable tag which allows for detection of the reagents and includes, but is not limited to, any composition or label detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein, Texas red, rhodamine, green fluorescent protein, and the like), radiolabels (*e.g.*, ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, *etc.*) beads.

[0200] In addition, the reagents can be immobilized on a substrate. Such a substrate can include any suitable substrate for immobilization of a detection reagent such as would be used in any of the previously described methods of detection. Briefly, a substrate suitable for immobilization of a detection reagent includes any solid support, such as any solid organic, biopolymer or inorganic support that can form a bond with the detection reagent without significantly effecting the activity and/or ability of the detection reagent to detect the desired target molecule. Exemplary organic solid supports include polymers such as polystyrene, nylon, phenol-formaldehyde resins, acrylic copolymers (*e.g.*, polyacrylamide), stabilized intact whole cells, and stabilized crude whole cell/membrane homogenates. Exemplary biopolymer supports include cellulose, polydextrans (*e.g.*, Sephadex®), agarose, collagen and chitin. Exemplary inorganic supports include glass beads (porous and nonporous), stainless steel, metal oxides (*e.g.*, porous ceramics such as ZrO_2, TiO_2, Al_2O_3, and NiO) and sand.

[0201] The kits may further comprise other components useful for delivery of vectors and/or detecting expression or activity, *e.g.*, buffers, cells, culture medium, enzymes, labeling reagents, containers, *etc.*

Subjects, Pharmaceutical Formulations, and Modes of Administration

[0202] Virus vectors according to the present invention find use in both veterinary and medical applications. Suitable subjects include both avians and mammals. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys, pheasant, parrots, parakeets, and the like. The term "mammal" as used herein includes, but is not limited to, humans, non-human primates, bovines, ovines, caprines, equines, felines, canines, lagomorphs, *etc.* Human subjects include neonates, infants, juveniles and adults.

[0203] In particular embodiments, the present invention provides a pharmaceutical composition comprising a virus vector of the invention in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, *etc.* In certain embodiments, the pharmaceutical composition will further comprise a desialylating agent. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and optionally can be in solid or liquid particulate form.

[0204] By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects.

[0205] One aspect of the present invention is a method of transferring a nucleic acid to a cell *in vitro*. The virus vector may be introduced into the cells at the appropriate multiplicity of infection according to standard transduction methods suitable for the particular target cells. Titers of virus vector to administer can vary, depending upon the target cell type and number, and the particular virus vector, and can be determined by those of skill in the art without undue experimentation. In representative embodiments, at least about 10^3 infectious units, more preferably at least about 10^5 infectious units are introduced to the cell. The virus vector may be introduced into the cells before, during and/or after desialylating the cells.

[0206] The cell(s) into which the virus vector is introduced can be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons and oligodendrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), blood vessel cells (*e.g.*, endothelial cells, intimal cells), epithelial cells (*e.g.*, gut and respiratory epithelial cells), muscle cells (*e.g.*, skeletal muscle cells, cardiac muscle cells, smooth muscle cells and/or diaphragm muscle cells), dendritic cells, pancreatic cells

(including islet cells), hepatic cells, kidney cells, myocardial cells, bone cells (*e.g.*, bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. In representative embodiments, the cell can be any progenitor cell. As a further possibility, the cell can be a stem cell (*e.g.*, neural stem cell, liver stem cell). As still a further alternative, the cell can be a cancer or tumor cell. Moreover, the cell can be from any species of origin, as indicated above.

[0207] The virus vector can be introduced into cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, the virus vector is introduced therein, and the cells are then administered back into the subject. The desialylating step can occur before and/or after the cells are removed from the subject. The virus vector may be introduced into the cells before, during and/or after desialylating the cells. Methods of removing cells from subject for manipulation *ex vivo*, followed by introduction back into the subject are known in the art (*see, e.g.*, U.S. Patent No. 5,399,346). Alternatively, the recombinant virus vector can be introduced into cells from a donor subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof (*i.e.*, a "recipient" subject).

[0208] Suitable cells for *ex vivo* gene delivery are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 cells or at least about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In particular embodiments, the cells transduced with the virus vector are administered to the subject in a treatment effective or prevention effective amount in combination with a pharmaceutical carrier.

[0209] In some embodiments, the virus vector is introduced into a cell and the cell can be administered to a subject to elicit an immunogenic response against the delivered polypeptide (*e.g.*, expressed as a transgene or in the capsid). Typically, a quantity of cells expressing an immunogenically effective amount of the polypeptide in combination with a pharmaceutically acceptable carrier is administered. An "immunogenically effective amount" is an amount of the expressed polypeptide that is sufficient to evoke an active immune response against the polypeptide in the subject to which the pharmaceutical formulation is administered. In particular embodiments, the dosage is sufficient to produce a protective immune response (as defined above). The

degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof.

[0210] A further aspect of the invention is a method of administering the virus vector to subjects. Administration of the virus vectors according to the present invention to a human subject or an animal in need thereof can be by any means known in the art. Optionally, the virus vector is delivered in a treatment effective or prevention effective dose in a pharmaceutically acceptable carrier. The virus vector may be delivered to the subject before, during and/or after delivering a desialylating agent to the subject.

[0211] The virus vectors of the invention can further be administered to elicit an immunogenic response (*e.g.*, as a vaccine). Typically, immunogenic compositions of the present invention comprise an immunogenically effective amount of virus vector in combination with a pharmaceutically acceptable carrier. Optionally, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof. Subjects and immunogens are as described above.

[0212] Dosages of the virus vector to be administered to a subject depend upon the mode of administration, the disease or condition to be treated and/or prevented, the individual subject's condition, the particular virus vector, and the nucleic acid to be delivered, and the like, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are titers of at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} transducing units, optionally about $10^8 - 10^{13}$ transducing units.

[0213] In particular embodiments, more than one administration (*e.g.*, two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, *e.g.*, daily, weekly, monthly, yearly, *etc.*

[0214] Exemplary modes of administration include oral, rectal, transmucosal, intranasal, inhalation (*e.g.*, via an aerosol), buccal (*e.g.*, sublingual), vaginal, intrathecal, intraocular, transdermal, intraendothelial, *in utero* (or *in ovo*), parenteral (*e.g.*, intravenous, intraarterial, intraportal, subcutaneous, intradermal, intracranial, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, intracisternal, and intraarticular), topical (*e.g.*, to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.*, to liver, eye, skeletal muscle, cardiac muscle, diaphragm muscle or brain).

[0215] Administration can be to any site in a subject, including, without limitation, a site selected from the group consisting of the brain, a joint, a skeletal muscle, a smooth muscle, the heart, the diaphragm, the airway epithelium, the liver, the kidney, the spleen, the pancreas, the skin, and the eye.

[0216] Administration can also be to a tumor (*e.g.*, in or near a tumor or a lymph node). The most suitable route in any given case will depend on the nature and severity of the condition being treated and/or prevented and on the nature of the particular vector that is being used.

[0217] Administration to skeletal muscle according to the present invention includes but is not limited to administration to skeletal muscle in the limbs (*e.g.*, upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (*e.g.*, tongue), thorax, abdomen, pelvis/perineum, and/or digits. Suitable skeletal muscles include but are not limited to abductor digiti minimi (in the hand), abductor digiti minimi (in the foot), abductor hallucis, abductor ossis metatarsi quinti, abductor pollicis brevis, abductor pollicis longus, adductor brevis, adductor hallucis, adductor longus, adductor magnus, adductor pollicis, anconeus, anterior scalene, articularis genus, biceps brachii, biceps femoris, brachialis, brachioradialis, buccinator, coracobrachialis, corrugator supercilii, deltoid, depressor anguli oris, depressor labii inferioris, digastric, dorsal interossei (in the hand), dorsal interossei (in the foot), extensor carpi radialis brevis, extensor carpi radialis longus, extensor carpi ulnaris, extensor digiti minimi, extensor digitorum, extensor digitorum brevis, extensor digitorum longus, extensor hallucis brevis, extensor hallucis longus, extensor indicis, extensor pollicis brevis, extensor pollicis longus, flexor carpi radialis, flexor carpi ulnaris, flexor digiti minimi brevis (in the hand), flexor digiti minimi brevis (in the foot), flexor digitorum brevis, flexor digitorum longus, flexor digitorum profundus, flexor digitorum superficialis, flexor hallucis brevis, flexor hallucis longus, flexor pollicis brevis, flexor pollicis longus, frontalis, gastrocnemius, geniohyoid, gluteus maximus, gluteus medius, gluteus minimus, gracilis, iliocostalis cervicis, iliocostalis lumborum, iliocostalis thoracis, illiacus, inferior gemellus, inferior oblique, inferior rectus, infraspinatus, interspinalis, intertransversi, lateral pterygoid, lateral rectus, latissimus dorsi, levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, levator palpebrae superioris, levator scapulae, long rotators, longissimus capitis, longissimus cervicis, longissimus thoracis, longus capitis, longus colli, lumbricals (in the hand), lumbricals (in the foot), masseter, medial pterygoid, medial rectus, middle scalene, multifidus, mylohyoid, obliquus capitis inferior, obliquus capitis superior, obturator externus, obturator internus, occipitalis, omohyoid, opponens digiti minimi,

opponens pollicis, orbicularis oculi, orbicularis oris, palmar interossei, palmaris brevis, palmaris longus, pectineus, pectoralis major, pectoralis minor, peroneus brevis, peroneus longus, peroneus tertius, piriformis, plantar interossei, plantaris, platysma, popliteus, posterior scalene, pronator quadratus, pronator teres, psoas major, quadratus femoris, quadratus plantae, rectus capitis anterior, rectus capitis lateralis, rectus capitis posterior major, rectus capitis posterior minor, rectus femoris, rhomboid major, rhomboid minor, risorius, sartorius, scalenus minimus, semimembranosus, semispinalis capitis, semispinalis cervicis, semispinalis thoracis, semitendinosus, serratus anterior, short rotators, soleus, spinalis capitis, spinalis cervicis, spinalis thoracis, splenius capitis, splenius cervicis, sternocleidomastoid, sternohyoid, sternothyroid, stylohyoid, subclavius, subscapularis, superior gemellus, superior oblique, superior rectus, supinator, supraspinatus, temporalis, tensor fascia lata, teres major, teres minor, thoracis, thyrohyoid, tibialis anterior, tibialis posterior, trapezius, triceps brachii, vastus intermedius, vastus lateralis, vastus medialis, zygomaticus major, and zygomaticus minor, and any other suitable skeletal muscle as known in the art.

[0218] The virus vector can be delivered to skeletal muscle by intravenous administration, intra-arterial administration, intraperitoneal administration, limb perfusion, (optionally, isolated limb perfusion of a leg and/or arm; *see, e.g.* Arruda *et al.*, (2005) *Blood* 105: 3458-3464), and/or direct intramuscular injection. In particular embodiments, the virus vector is administered to a limb (arm and/or leg) of a subject (*e.g.*, a subject with muscular dystrophy such as DMD) by limb perfusion, optionally isolated limb perfusion (*e.g.*, by intravenous or intra-articular administration. In embodiments of the invention, the virus vectors of the invention can advantageously be administered without employing "hydrodynamic" techniques. Tissue delivery (*e.g.*, to muscle) of prior art vectors is often enhanced by hydrodynamic techniques (*e.g.*, intravenous/intravenous administration in a large volume), which increase pressure in the vasculature and facilitate the ability of the vector to cross the endothelial cell barrier. In particular embodiments, the viral vectors of the invention can be administered in the absence of hydrodynamic techniques such as high volume infusions and/or elevated intravascular pressure (*e.g.*, greater than normal systolic pressure, for example, less than or equal to a 5%, 10%, 15%, 20%, 25% increase in intravascular pressure over normal systolic pressure). Such methods may reduce or avoid the side effects associated with hydrodynamic techniques such as edema, nerve damage and/or compartment syndrome.

[0219] Administration to cardiac muscle includes administration to the left atrium, right atrium, left ventricle, right ventricle and/or septum. The virus vector can be

delivered to cardiac muscle by intravenous administration, intra-arterial administration such as intra-aortic administration, direct cardiac injection (*e.g.*, into left atrium, right atrium, left ventricle, right ventricle), and/or coronary artery perfusion.

[0220] Administration to diaphragm muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration.

[0221] Administration to smooth muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration. In one embodiment, administration can be to endothelial cells present in, near, and/or on smooth muscle.

[0222] Delivery to a target tissue can also be achieved by delivering a depot comprising the virus vector. In representative embodiments, a depot comprising the virus vector is implanted into skeletal, smooth, cardiac and/or diaphragm muscle tissue or the tissue can be contacted with a film or other matrix comprising the virus vector. Such implantable matrices or substrates are described in U.S. Patent No. 7,201,898.

[0223] In particular embodiments, a virus vector according to the present invention is administered to skeletal muscle, diaphragm muscle and/or cardiac muscle (*e.g.*, to treat and/or prevent muscular dystrophy or heart disease [for example, PAD or congestive heart failure]).

[0224] In representative embodiments, the invention is used to treat and/or prevent disorders of skeletal, cardiac and/or diaphragm muscle.

[0225] In a representative embodiment, the invention provides a method of treating and/or preventing muscular dystrophy in a subject in need thereof, the method comprising: administering a treatment or prevention effective amount of a virus vector of the invention to a mammalian subject, wherein the virus vector comprises a heterologous nucleic acid encoding dystrophin, a mini-dystrophin, a micro-dystrophin, myostatin propeptide, follistatin, activin type II soluble receptor, IGF-1, anti-inflammatory polypeptides such as the I-kappa B dominant mutant, sarcospan, utrophin, a micro-dystrophin, laminin-α2, α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, δ-sarcoglycan, IGF-1, an antibody or antibody fragment against myostatin or myostatin propeptide, and/or RNAi against myostatin. In particular embodiments, the virus vector can be administered to skeletal, diaphragm and/or cardiac muscle as described elsewhere herein.

[0226] Alternatively, the invention can be practiced to deliver a nucleic acid to skeletal, cardiac or diaphragm muscle, which is used as a platform for production of a polypeptide (*e.g.*, an enzyme) or functional RNA (*e.g.*, RNAi, microRNA, antisense

RNA) that normally circulates in the blood or for systemic delivery to other tissues to treat and/or prevent a disorder (*e.g.*, a metabolic disorder, such as diabetes (*e.g.*, insulin), hemophilia (*e.g.*, Factor IX or Factor VIII), a mucopolysaccharide disorder (*e.g.*, Sly syndrome, Hurler Syndrome, Scheie Syndrome, Hurler-Scheie Syndrome, Hunter's Syndrome, Sanfilippo Syndrome A, B, C, D, Morquio Syndrome, Maroteaux-Lamy Syndrome, *etc.*) or a lysosomal storage disorder (such as Gaucher's disease [glucocerebrosidase], Pompe disease [lysosomal acid α-glucosidase] or Fabry disease [α-galactosidase A]) or a glycogen storage disorder (such as Pompe disease [lysosomal acid α glucosidase]). Other suitable proteins for treating and/or preventing metabolic disorders are described above. The use of muscle as a platform to express a nucleic acid of interest is described in U.S. Patent Publication No. 2002/0192189.

[0227] Thus, as one aspect, the invention further encompasses a method of treating and/or preventing a metabolic disorder in a subject in need thereof, the method comprising: administering a treatment or prevention effective amount of a virus vector of the invention to a subject (*e.g.*, to skeletal muscle of a subject), wherein the virus vector comprises a heterologous nucleic acid encoding a polypeptide, wherein the metabolic disorder is a result of a deficiency and/or defect in the polypeptide. Illustrative metabolic disorders and heterologous nucleic acids encoding polypeptides are described herein. Optionally, the polypeptide is secreted (*e.g.*, a polypeptide that is a secreted polypeptide in its native state or that has been engineered to be secreted, for example, by operable association with a secretory signal sequence as is known in the art). Without being limited by any particular theory of the invention, according to this embodiment, administration to the skeletal muscle can result in secretion of the polypeptide into the systemic circulation and delivery to target tissue(s). Methods of delivering virus vectors to skeletal muscle are described in more detail herein.

[0228] The invention can also be practiced to produce antisense RNA, RNAi or other functional RNA (*e.g.*, a ribozyme) for systemic delivery.

[0229] The invention also provides a method of treating and/or preventing congenital heart failure or PAD in a subject in need thereof, the method comprising administering a treatment or prevention effective amount of a virus vector of the invention to a mammalian subject, wherein the virus vector comprises a heterologous nucleic acid encoding, for example, a sarcoplasmic endoreticulum Ca^{2+}-ATPase (SERCA2a), an angiogenic factor, phosphatase inhibitor I (I-1), RNAi against phospholamban; a phospholamban inhibitory or dominant-negative molecule such as phospholamban S16E, a zinc finger protein that regulates the phospholamban gene, β2-

adrenergic receptor, β2-adrenergic receptor kinase (BARK), PI3 kinase, calsarcan, a β-adrenergic receptor kinase inhibitor (βARKct), inhibitor 1 of protein phosphatase 1, S100A1, parvalbumin, adenylyl cyclase type 6, a molecule that effects G-protein coupled receptor kinase type 2 knockdown such as a truncated constitutively active bARKct, Pim-1, PGC-1α, SOD-1, SOD-2, EC-SOD, kallikrein, HIF, thymosin-β4, mir-1, mir-133, mir-206 and/or mir-208.

[0230] The virus vectors disclosed herein can be administered to the lungs of a subject by any suitable means, optionally by administering an aerosol suspension of respirable particles comprised of the virus vectors, which the subject inhales. The respirable particles can be liquid or solid. Aerosols of liquid particles comprising the virus vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the virus vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0231] The virus vectors can be administered to tissues of the CNS (*e.g.*, brain, eye) and may advantageously result in a more restricted distribution of the virus vector than would be observed in the absence of the present invention.

[0232] In particular embodiments, the delivery vectors of the invention may be administered to treat diseases of the CNS, including genetic disorders, neurodegenerative disorders, psychiatric disorders and tumors. Illustrative diseases of the CNS include, but are not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, Canavan disease, Leigh's disease, Refsum disease, Tourette syndrome, primary lateral sclerosis, amyotrophic lateral sclerosis, progressive muscular atrophy, Pick's disease, muscular dystrophy, multiple sclerosis, myasthenia gravis, Binswanger's disease, trauma due to spinal cord or head injury, Tay Sachs disease, Lesch-Nyan disease, epilepsy, cerebral infarcts, psychiatric disorders including mood disorders (*e.g.*, depression, bipolar affective disorder, persistent affective disorder, secondary mood disorder), schizophrenia, drug dependency (*e.g.*, alcoholism and other substance dependencies), neuroses (*e.g.*, anxiety, obsessional disorder, somatoform disorder, dissociative disorder, grief, post-partum depression), psychosis (*e.g.*, hallucinations and delusions), dementia, paranoia, attention deficit disorder, psychosexual disorders, sleeping disorders, pain disorders, eating or weight disorders (*e.g.*, obesity, cachexia, anorexia nervosa, and bulemia) and cancers and tumors (*e.g.*, pituitary tumors) of the CNS.

[0233] Disorders of the CNS include ophthalmic disorders involving the retina, posterior tract, and optic nerve (*e.g.*, retinitis pigmentosa, diabetic retinopathy and other retinal degenerative diseases, uveitis, age-related macular degeneration, glaucoma).

[0234] Most, if not all, ophthalmic diseases and disorders are associated with one or more of three types of indications: (1) angiogenesis, (2) inflammation, and (3) degeneration. The delivery vectors of the present invention can be employed to deliver anti-angiogenic factors; anti-inflammatory factors; factors that retard cell degeneration, promote cell sparing, or promote cell growth and combinations of the foregoing.

[0235] Diabetic retinopathy, for example, is characterized by angiogenesis. Diabetic retinopathy can be treated by delivering one or more anti-angiogenic factors either intraocularly (*e.g.*, in the vitreous) or periocularly(*e.g.*, in the sub-Tenon's region). One or more neurotrophic factors may also be co-delivered, either intraocularly (*e.g.*, intravitreally) or periocularly.

[0236] Uveitis involves inflammation. One or more anti-inflammatory factors can be administered by intraocular (*e.g.*, vitreous or anterior chamber) administration of a delivery vector of the invention.

[0237] Retinitis pigmentosa, by comparison, is characterized by retinal degeneration. In representative embodiments, retinitis pigmentosa can be treated by intraocular (*e.g.*, vitreal administration) of a delivery vector encoding one or more neurotrophic factors.

[0238] Age-related macular degeneration involves both angiogenesis and retinal degeneration. This disorder can be treated by administering the inventive delivery vectors encoding one or more neurotrophic factors intraocularly (*e.g.*, vitreous) and/or one or more anti-angiogenic factors intraocularly or periocularly (*e.g.*, in the sub-Tenon's region).

[0239] Glaucoma is characterized by increased ocular pressure and loss of retinal ganglion cells. Treatments for glaucoma include administration of one or more neuroprotective agents that protect cells from excitotoxic damage using the inventive delivery vectors. Such agents include N-methyl-D-aspartate (NMDA) antagonists, cytokines, and neurotrophic factors, delivered intraocularly, optionally intravitreally.

[0240] In other embodiments, the present invention may be used to treat seizures, *e.g.*, to reduce the onset, incidence or severity of seizures. The efficacy of a therapeutic treatment for seizures can be assessed by behavioral (*e.g.*, shaking, ticks of the eye or mouth) and/or electrographic means (most seizures have signature electrographic

abnormalities). Thus, the invention can also be used to treat epilepsy, which is marked by multiple seizures over time.

[0241] In one representative embodiment, somatostatin (or an active fragment thereof) is administered to the brain using a delivery vector of the invention to treat a pituitary tumor. According to this embodiment, the delivery vector encoding somatostatin (or an active fragment thereof) is administered by microinfusion into the pituitary. Likewise, such treatment can be used to treat acromegaly (abnormal growth hormone secretion from the pituitary). The nucleic acid (*e.g.*, GenBank Accession No. J00306) and amino acid (*e.g.*, GenBank Accession No. P01166; contains processed active peptides somatostatin-28 and somatostatin-14) sequences of somatostatins as are known in the art.

[0242] In particular embodiments, the vector can comprise a secretory signal as described in U.S. Patent No. 7,071,172.

[0243] In representative embodiments of the invention, the virus vector is administered to the CNS (*e.g.*, to the brain or to the eye). The virus vector may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes. cortex, basal ganglia, hippocampus and portaamygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The virus vector may also be administered to different regions of the eye such as the retina, cornea and/or optic nerve.

[0244] The virus vector may be delivered into the cerebrospinal fluid (*e.g.*, by lumbar puncture) for more disperse administration of the delivery vector. The virus vector may further be administered intravascularly to the CNS in situations in which the blood-brain barrier has been perturbed (*e.g.*, brain tumor or cerebral infarct).

[0245] The virus vector can be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intra-ocular, intracerebral, intraventricular, intravenous (*e.g.*, in the presence of a sugar such as mannitol), intranasal, intra-aural, intra-ocular (*e.g.*, intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.*, sub-Tenon's region) delivery as well as intramuscular delivery with retrograde delivery to motor neurons.

[0246] In particular embodiments, the virus vector is administered in a liquid formulation by direct injection (*e.g.*, stereotactic injection) to the desired region or compartment in the CNS. In other embodiments, the virus vector may be provided by

topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye, may be by topical application of liquid droplets. As a further alternative, the virus vector may be administered as a solid, slow-release formulation (*see, e.g.,* U.S. Patent No. 7,201,898).

[0247] In yet additional embodiments, the virus vector can used for retrograde transport to treat and/or prevent diseases and disorders involving motor neurons (*e.g.*, amyotrophic lateral sclerosis (ALS); spinal muscular atrophy (SMA), etc.). For example, the virus vector can be delivered to muscle tissue from which it can migrate into neurons.

[0248] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus vector of the invention in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the virus vector can be delivered adhered to or impregnated within a surgically implantable matrix such as a sheet or mesh (*e.g.*, as described in U.S. Patent Publication No. 2004-0013645).

[0249] Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

Example 1

Materials and Methods

[0250] Plasmids and viruses. All plasmids were obtained from the UNC vector core. The triple plasmid transfection protocol (Grieger *et al., Nat. Protoc. 1*:1412 (2006)) utilized for production of AAV9 vectors, includes (i) the AAV helper plasmid, pXR9, containing AAV2 *Rep* and AAV9 *Cap* genes, (ii) the Adenoviral helper plasmid, pXX6-80 and (iii) the vector genome cassette, pTR-CBA-Luc, containing the firefly luciferase gene driven by the chicken beta-actin (CBA) promoter and flanked by inverted terminal repeats (ITRs) required for packaging. The ITRs are the only elements within the vector genome cassette derived from the wild-type AAV genome, thereby eliminating 96% of viral elements. Recombinant AAV9 vectors generated thus allow quantitation of viral infectivity (transduction efficiency) through luciferase transgene expression assays. HEK293 cells utilized for production of recombinant AAV9 vectors were obtained from the UNC vector core. Sonicated cell lysates and PEG8000 precipitates from supernatant were pooled and subjected to cesium chloride ultracentrifugation as described earlier (23). Dialyzed peak fractions were subjected to quantitative PCR using a Roche Light Cycler

instrument with luc transgene-specific primers to determine viral vector titers (forward 5'-AAA AGC ACT CTG ATT GAC AAA TAC-3' (SEQ ID NO:1); reverse 5'-CCT TCG CTT CAA AAA ATG GAA C-3' (SEQ ID NO:2)).

[0251] Cell lines. All cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin, streptomycin, amphotericin B (Sigma) and maintained in 5% CO_2 at 37°C unless mentioned otherwise. COS-1 (monkey kidney), Neuro2a (mouse neuroblastoma), U87 (human glioma) and Huh7 (human hepatocarcinoma) were obtained from the UNC tissue culture facility and utilized in viral infectivity assays. Chinese hamster ovary (CHO) Pro5 and mutant Lec1, Lec2 cell lines were a gift from Dr. Jude Samulski (UNC-Chapel Hill) and the CHO Lec8 cell line purchased from ATCC. All CHO cells, utilized for viral binding and infectivity assays, were cultured in α-MEM (GIBCO) supplemented with 10% FBS and penicillin, streptomycin, amphotericin B as outlined above. Well-differentiated human airway epithelial (HAE) cultures (4-6 weeks) grown on permeable membrane supports (Millipore, Corning, NY) at the air-liquid interface were provided by the Cell Culture Models Core and the UNC Cystic Fibrosis/Pulmonary Research Center.

[0252] Transduction assays. Different cell lines were seeded at 10^5 cells/well in 24-well plates and allowed to adhere overnight at 37°C. Plates were then pre-chilled at 4°C for 30 min and incubated with AAV9 vectors at a multiplicity of infection (MOI) of 1000 vector genomes per cell (vg/cell) to allow binding to the cell surface for 1.5 hrs at 4°C. Unbound virus was then removed by washing three times with ice cold 1x phosphate-buffered saline (1xPBS) and 0.5 mL of DMEM added to each well. Luciferase transgene expression levels were quantitated after incubation for 24 hrs from cell lysates using a Victor 2 luminometer (Perkin Elmer). For studies with HAE, well-differentiated cultures were pretreated with 1.25 U/ml of sialidase A (Prozyme #GK80040) at 37°C for 3 hrs followed by three washes with ice-cold 1xPBS. Cultures were then incubated with scAAV9-CMV-GFP vectors (MOI = 10^5 vg/cell) as outlined above. Fluorescence micrographs of green fluorescent protein (GFP) expression in HAE cultures at 2 weeks post-transduction were obtained using an Olympus epifluorescence microscope equipped with a Hamamatsu camera.

[0253] Enzymatic desialylation assays. Different cell lines were treated with Heparinase I and III (from *Flavobacterium heparinum*; Sigma #H2519 and #H8891), Chondroitinase ABC (from *Proteus vulgaris* Sigma #C2905) and Neuraminidase Type III (from *Vibrio cholerae* Sigma #N7885) to determine the role of cell surface glycans in AAV9 infection. Briefly, Cos1 cells were seeded at 10^5 cells/well in 24-well plates and

pretreated with 50 mU/ml neuraminidase, 3 U/ml heparinase I, 1.5 U/ml heparinase III and 1.5 U/ml chondroitinase ABC in serum-free DMEM at 37°C for 2 hrs. Neuro2a, U87, HEK293 and Huh7 cells were treated with neuraminidase alone. Cells were then washed three times with 1xPBS and subjected to AAV9 infection at an MOI of 1000 vg/cell. Luciferase transgene expression assays were carried out as described above at 24 hrs post-infection.

[0254] Chemical inhibition assays. CHO Lec2 cells were seeded at 10^5 cells/well in 24-well plates and pretreated for 24 hrs with small molecule inhibitors of glycosylation, Swainsonine (10 μM; Sigma, #S8195) and α-benzyl-GalNAc (1 μg/mL; Sigma, #B4894) to determine the role of *N*- and *O*-glycans in AAV9 infection. Cells pretreated with chemicals were subjected to AAV9 infection at an MOI of 1000 vg/cell and luciferase transgene expression assays carried out as described above.

[0255] Enzymatic resialylation assays. The sialic acid-deficient cell line, CHO Lec2 was treated with 50 mU/mL each of α2,3-(N)-sialyltransferase (Calbiochem #566218), α2,6-(N)-sialyltransferase (Calbiochem #566222) or α2,3-(O)-sialyltransferase (Calbiochem, #566227) and 1 mM CMP-sialic acid (Sigma) in serum-free media for 3 hrs at 37°C. Untreated CHO Pro5 cells with endogenous sialic acid were included as control. Cells were then rinsed three times with 1xPBS and subjected to AAV9 infection at an MOI of 1000 vg/cell and luciferase transgene expression assays carried out as described earlier.

[0256] Lectin inhibition and staining assays. Competitive inhibition of AAV9 infection was carried out using a panel of lectins, Concanavalin A (Con A), Wheat germ agglutinin (WGA), *Maackia amurensis* lectin (MAL I), *Sambucus nigra* lectin (SNA) and *Erythrina cristagalli* lectin (ECL) obtained from Vector Labs. Briefly, prechilled CHO Pro5 or mutant Lec2 cells were incubated with 100 mg/mL FITC-conjugated lectin (fluorescent labeling assay) in serum-free α-MEM media or along with AAV9 particles (infectivity assay at MOI = 10,000 vg/cell) for a period 1.5 hrs at 4°C. After three washes with ice cold 1xPBS to remove unbound virus and/or lectins, cells were subjected to epifluorescence microscopy using an Olympus 100 microscope (Olympus) or incubated for 24 hrs at 37°C prior to luciferase transgene expression analysis.

[0257] Cell surface binding assays. CHO Pro5 cells were seeded at a density of 10^4 cells/well in 96-well plates prior to treatment with 50 mU/mL neuraminidase type III from *Vibrio cholerae* for 2 hrs at 37°C. Untreated Pro5 and Lec2 cells were included as negative and positive controls respectively. Cells were then pre-chilled at 4°C for 30 minutes, followed by incubation with AAV9 particles at MOI of 10^2, $5x10^2$, 10^3, $5x10^3$,

10^4, $5x10^4$, 10^5, $5x10^5$ vg/cell for 1.5 hrs at 4°C. Cells were then subjected to three washes with ice cold 1xPBS to remove unbound virions. Cell surface-bound virions were collected along with cell lysates following three freeze-thaw cycles and vector genome copy numbers per cell determined using Q-PCR as outlined above. Binding curves were generated using GraphPad Prism 5 software by applying the single site binding model ($\mathbf{Y = B_{max}*X/(K_d + X)}$), where **Y** represents number of bound virions/cell determined by Q-PCR; **X** represents MOI; $\mathbf{B_{max}}$ is the maximum binding capacity and $\mathbf{K_d}$', the observed disassociation constant.

[0258] Animal studies. All experiments were carried out with 6-8 week old female BALB/c mice (Jackson Labs, Bar Harbor, ME) maintained and treated in accordance with National Institutes of Health guidelines and as approved by IACUC at UNC-Chapel Hill. Mice were administered via intranasal instillation with either 100 µl PBS (50 µl/nostril) or 100 µl Neuraminidase Type III from *Vibrio cholerae* (200 mU, Sigma, St. Louis, MO). At 2 hrs post-treatment, a dose of $5x10^{10}$ AAV9 particles in 1xPBS (50 µl/nostril) was administered. Luciferase transgene expression in live animals was obtained using a Xenogen IVIS Lumina® imaging system (Caliper Lifesciences, CA) after intranasal instillation of luciferin substrate (120 mg/kg; Nanolight). Image analysis was carried out using the Living Image software® (Caliper Lifesciences) and luciferase expression reported in relative light units (photons/sec/cm^2/sr).

Example 2

Neuraminidase treatment selectively increases infectivity of AAV9 in different cell types

[0259] Enzymatic removal of different cell surface glycans was achieved by treating cells with different glycosidases. First, different enzymes (heparinase I; heparinase III, neuraminidase from *Vibrio Cholerae* and chondroitinase ABC) were utilized to deglycosylate prominently expressed cell surface glycans on COS-1 cells. Transduction assays with AAV9 vectors (MOI = 1000 vg/cell) were carried out 2 hrs post-enzymatic treatment. Next, neuraminidase treatment of COS-1 cells prior to infection with AAV1, AAV2 or AAV9 vectors (MOI = 1000 vg/cell) was carried out to demonstrate serotype selective effects of enzymatic desialylation on transduction efficiency. Finally, different cell lines (HEK293, U87, Huh-7 and Neuro2a) were untreated (white bars) or pretreated with neuraminidase from *Vibrio Cholerae* (grey bars) followed by subsequent infection with AAV9 vectors (MOI = 1000 vg/cell). Luciferase transgene expression (Relative Light Units, RLU) was quantified for both studies at 24

hrs post-infection. All experiments were carried out in triplicate. Error bars represent standard error mean.

[0260] Removal of terminal sialic acid residues using neuraminidase (from *V. Cholerae*) enhanced infectivity of AAV9 by > 1 log unit when compared to virus alone on COS1 cells (**FIG. 1A**). In contrast, hydrolysis of cell surface heparan sulfate or chondroitin sulfate proteoglycans using heparinase I/III or chondroitinase ABC, respectively, had no effect on viral infectivity compared to control. Neuraminidase treatment abrogated AAV1 transduction, while AAV2 remained unaffected under these conditions (**Fig. 1B**). In addition, treatment of cell lines derived from different tissues with neuraminidase (grey bars) prior to AAV9 infection enhanced transduction efficiency by nearly 2 log units in contrast to infection with virus alone (white bars) (**FIG. 1C**).

[0261] These results were corroborated by increased binding and internalization of AAV9, but not AAV1 vectors upon sialidase treatment in these cell lines. U87 and Huh-7 cells were seeded at a density of $1x10^5$ cells/well on a 24-well plate. Cells were untreated (white bars) or pretreated with 50 mU/ml of Neuraminidase Type III from *Vibrio Cholerae* (grey bars) (**FIG. 2**). The cells were prechilled at 4°C for 30 min followed by incubation with AAV9-CBA-Luciferase vectors (MOI = 1000 vg/cell) at 4°C for 1.5 hrs. Unbound virions were removed by three washes with ice-cold lx PBS. The amount of cell surface-bound AAV9 virion was quantified using Q-PCR. The amount of bound virions was expressed as vector genome copy numbers (vg) per cell. All experiments were carried out in quadruplicate. Error bars represent standard error mean.

[0262] The effect of desialylation on cell surface binding and infectivity of AAV1 and AAV9 was examined. Human glioma U87 cells were untreated (white bars) or pretreated with Neuraminidase Type III from *Vibrio Cholerae* (grey bars) followed by incubation with AAV1 or AAV9 at MOI = 10^5 vg/cell. At 60 min post incubation on ice, unbound virions were removed with three lx PBS washes. Cells were then incubated at 37°C for 30 min to allow uptake of AAV9 particles. Cells were then treated with 100 µl/well of 0.05% Trypsin EDTA (Cellgro #25-052-CI) for 5 min at 37°C to remove cell surface bound virions. Cells were then pelleted by centrifugation and washed thrice with lx PBS. Cell lysates were then analyzed using Q-PCR to quantitate cell-associated (internalized virions). Internalized virions are expressed as vector genome copy numbers (vg) per cell. All experiments were carried out at n=5 and error bars represent standard error mean.

[0263] Thus, sialic acid appears to mask cell surface glycans that selectively facilitate AAV9 infection *in vitro*. Further, enzymatic desialylation might serve as a

facile biochemical strategy to enhance transduction efficiency of AAV9 vectors and might enable detailed analysis of the intracellular trafficking pathways of AAV9 *in vitro*.

Example 3

Mutant CHO Lec2 cells lacking terminal sialic acid are highly permissive to AAV9 infection

[0264] Analysis of cell surface binding and infectivity of AAV9 on parental (Pro5) and mutant CHO cell lines was carried out to further understand the role of core glycans under sialic acid in AAV9 infection. **FIG. 4A** shows a schematic representation of *N*-glycan compositions of the parental CHO Pro5 cell line and mutants Lec2, Lec8 and Lec1 (North *et al.*, *J. Biol. Chem. 285*:5759 (2010)) using nomenclature proposed by the Consortium for Functional Glycomics nomenclature committee (● Mannose, ■ GlcNac, ○ Galactose, ♦ Sialic acid). Binding of AAV1 (white bars) and AAV9 (grey bars) particles to wild type and parental CHO cell lines was analyzed using Q-PCR. Number of bound virions is expressed as vector genome copy number (vg) per cell (**FIG. 4B**). Transduction efficiency (Relative Light Units, RLU) of AAV1 (white bars) and AAV9 (grey bars) particles (MOI = 1000 vg/cell) on parental and mutant CHO cell lines were analyzed by quantifying luciferase transgene expression at 24 hrs post-infection (**FIG. 4C**). All binding experiments were carried out in quadruplicate and infectivity assays in triplicate. Error bars represent standard error mean.

[0265] The CHO Lec2 cell line lacks terminal sialic acid due to a defect in CMP-sialic acid transport (Deutscher *et al.*, *Cell 39*:295 (1984)), while Lec8 and Lec1 cell lines are defective in translocation of UDP-galactose and N-acetylglucosaminyltransferase activity (Deutscher *et al.*, *J. Biol. Chem. 261*:96 (1986); Stanley *et al.*, (1985) *Mol. Cell. Biol. 5*:1204 (1985)), respectively. Correspondingly, cell surface glycans on CHO Lec2 cells contain terminal galactosyl residues, while Lec8 and Lec1 cell lines predominantly display terminal N-acetylglucosamine and mannosylated glycans, respectively (**FIG. 4A**). As seen in **FIGS. 4B and 4C**, cell surface binding and infectivity of AAV9 particles on Lec2 cells is significantly increased (> 1 log unit) when compared to the parental Pro5 cell line. In contrast, no major changes in binding and infectivity are observed in the case of Lec8 and Lec1 cells. These results suggest that galactosylated glycans immediately underlying sialic acid can facilitate AAV9 cell surface binding and entry. The results also support the notion that AAV9 particles might exploit an inefficient and non-specific uptake mechanism in the parental Pro5, mutant Lec8 and Lec1 cell lines.

Example 4

Sialylation of N-linked glycans blocks AAV9 infection

[0266] In order to further elucidate the nature of glycans required for AAV9 infection, small molecule inhibitors of glycosylation and sialyltransferases were utilized to modify terminal galactosyl residues on the sialic acid-deficient Lec2 cell surface. Prior to infection with AAV9 vectors (MOI = 1000 vg/cell), CHO Lec2 cells were treated with α-Benzyl-GalNAc (1 μg/mL) or swainsonine (10 μM), inhibitors of *O*- and *N*-glycosylation, respectively (**FIG. 5A**). Next, CHO Lec2 cells were treated with 50 mU/mL each of α2,3-(N)-sialyltransferase (α2,3NST), α2,6-(N)-sialyltransferase (α2,6NST) or α2,3-(O)-sialyltransferase (α2,3OST) and 1 mM CMP-sialic acid (Sigma) to resialylate cell surface asialoglycans. Luciferase transgene expression was quantified at 24 hrs post-infection and expressed as % infectivity with respect to untreated or wild type (CHO Pro5) control (**FIG. 5B**). Finally, CHO Lec2 cells treated with CMP-Sialic acid alone or with CMP-Sialic acid and different sialyltransferases were subjected to lectin staining using FITC-labeled ECL, which exclusively recognizes Gal(β1,4)GlcNAc, or FITC-labeled MAL, which recognizes α2,3-Sialylated Gal(β1,4)GlcNAc (**FIG. 5C**). Untreated wild type CHO Pro5 cells, which show high levels of FITC-MAL I staining and untreated Lec2 cells, which show high levels of FITC-ECL staining were included as controls. All experiments were carried out in triplicate. Error bars indicate standard error mean.

[0267] Swainsonine (Elbein *et al.*, *Proc. Natl. Acad. Sci. U. S. A. 78*:7393 (1981)) and α-benzyl-*O*-GalNAc (Kuan *et al.*, *J. Biol. Chem. 264*:19271 (1989)) are chemical inhibitors of *N*-linked and *O*-linked glycosylation, respectively. Treatment with these reagents results in a corresponding decrease in cell surface expression of *N*-linked glycans and *O*-linked glycans. As seen in **FIG. 5A**, AAV9 infection is significantly blocked by swainsonine (~ 75%), while α-benzyl-*O*-GalNAc has a modest inhibitory effect (~ 25%). These results suggest that AAV9 prefers *N*-linked cell surface glycans for infection. In addition, resialylation of the sialic acid-deficient Lec2 cell surface was carried out to understand the nature of the sialic acid linkage that blocks AAV9 infection (**FIG. 5B**). The abilities of different sialyltransferases to partially block AAV9 infection was observed to follow the order, α2,3-*N*-sialyltransferase (3'NST) > α2,6-*N*-sialyltransferase (6'NST) > α2,3-*O*-sialyltransferase (3'OST). Alteration of cell surface glycans upon resialylation was confirmed by staining with lectins recognizing different glycan residues and linkages (**FIG. 3C**). As expected, Pro5 expressing sialylated glycans and Lec2 cells expressing asialoglycans demonstrated preferential staining by FITC-MAL

I and FITC-ECL, respectively. Resialylation of Gal(β1,4)GlcNAc residues with 3'NST, but not 3'OST partially restored FITC-MAL I staining concurrent with a decrease in AAV9 infectivity. The lack of MAL I staining in 6'NST-treated Lec2 cells is expected due to lack of recognition of α2,6-sialylated glycans by MAL I. Taken together, these results not only corroborate the important role played by core *N*-linked glycans in AAV9 infection, but also the potential for α2,3 and α2,6 sialic acid linkages to block infection by masking underlying glycoconjugates on the cell surface.

Example 5

Terminal galactosyl residues are critical for AAV9 infection

[0268] To better understand the nature of AAV9-glycan interactions that mediate infection, competitive inhibition studies were carried out with lectins that recognize different glycan linkages on the cell surface (**FIGS. 6A and 6B**). Specifically, we utilized (i) *Maackia amurensis* lectin (MAL I), which recognizes native, α2,3-sialylated or sulfated glycoconjugates having Gal-(β1,4)-*N*-GlcNAc structures (Wang *et al.*, *J. Biol. Chem. 263*:4576 (1988); Bai *et al.*, *Glycobiology 11*:621 (2001)); (ii) *Sambucus nigra* lectin (SNA), which binds preferentially to α2,6-sialylated galactose residues (Shibuya *et al.*, *J. Biol. Chem. 262*:1596 (1987)); (iii) *Erythrina cristagalli* lectin (ECL), which demonstrates specificity towards galactose residues, in particular, galactosyl-(β1,4)-*N*-acetylglucosamine (Gal-(β1,4)-*N*-GlcNAc) (Wu *et al.*, *Glycoconj. J. 24*:591 (2007)); (iv) Wheat germ agglutinin (WGA), which binds *N*-acetylglucosamine (*N*-GlcNAc) and also tolerates glycoconjugates containing sialic acid with different linkages (Yamamoto *et al.*, *Biochemistry 20*:5894 (1981)) and (v) Concanavalin A (Con A), which recognizes mannose residues (Ohyama *et al.*, *J. Biol. Chem. 260*:6882 (1985)). Wild type CHO Pro5 (**FIG. 6A**) and sialic acid-deficient Lec2 (**FIG. 6B**) cell lines were co-incubated with lectins recognizing different glycan linkages (100 mg/mL) and AAV9 vectors (MOI = 10,000 vg/cell). Briefly, ConA lectin recognizes mannose residues, while WGA lectin recognizes GlcNAc residues as well as sialic acid residues. MAL and SNA lectins recognize α2,3- and α2,6-linked sialic acid residues, respectively, while ECL exclusively recognizes Gal(β1,4)GlcNAc residues. Luciferase transgene expression (% Infectivity) was quantified at 24 hrs post-infection. All experiments were carried out in triplicate. Error bars represent standard error mean. Lectin staining of Pro5 and Lec2 cell lines were carried out using FITC-labeled lectins and fluorescent images obtained (**FIG. 6C**).

[0269] The SNA lectin had no effect on AAV9 infection and can be explained by low levels of α2,6-sialylated glycans in both cell lines of rodent (hamster) origin (Stults *et*

al., J. Biol. Chem. 264:19956 (1989)). On the other hand, the MAL I lectin demonstrated 5 to 10-fold inhibition of AAV9 infection in both the Pro5 and Lec2 cell lines. More importantly, a striking difference in AAV9 infectivity was observed in the case of ECL-treated cells with 5-fold inhibition in parental Pro5 cells and nearly 200-fold inhibitory activity in the Lec2 cell line demonstrating the importance of core Gal β1-linked residues. The Con A and WGA lectins demonstrated broad inhibitory activity in both parental Pro5 cells and the sialic acid-deficient Lec2 cell line suggesting that underlying *N*-GlcNAc and core mannose residues might contribute to AAV9-glycan interactions. These results were corroborated by fluorescent staining of Pro5 and Lec2 cell lines with the aforementioned lectins (**FIG. 6C**). As expected, MAL I and WGA lectins, which bind to α2,3-sialylated glycans, preferentially label Pro5 cells. In contrast, preferential ECL staining is noted in Lec2 cells. The SNA lectin, which recognizes α2,6-sialic acid does not stain either Pro5 or Lec2 cells confirming the lack (or modest expression) of α2,6-sialylated glycans on these hamster-derived cell lines.

[0270] Further evidence supporting the important role of galactose in mediating AAV9 infection was obtained by treatment of CHO Pro5 cells with endo-β-galactosidase. Cos-1 cells were untreated (-) or pretreated (+) with Neuraminidase Type III from *Vibrio Cholerae* followed by no treatment (-) or treatment (+) with (A) endo-β-galactosidase (80 mU/ml, from *Pseudomonas* sp., Sigma #G6920) and (B) α-fucosidase (50 mU/ml, from bovine kidney, Prozyme #GKX-5006). Transduction of AAV9 were carried out with MOI = 1000 vg/cell. Transduction efficiency (RLU) was quantified at 24 hrs post-infection. All experiments were carried out in triplicate. Error bars represent standard error mean. Removal of galactose residues, but not fucose abrogated AAV9 transduction. Taken together, these results suggest that terminal galactosyl residues serve as the primary receptor for AAV9.

Example 6

Desialylation increases the cell surface binding potential of AAV9 particles

[0271] Cell surface binding curves were generated to establish a quantitative biochemical rationale for the observed increase in AAV9 infectivity following desialylation. Binding of AAV9 particles to the surface of parental CHO Pro5 cells, sialidase-treated Pro5 cells, or sialic acid-deficient Lec2 cells was carried out over a range of multiplicities of infection (genome-containing viral particles (VG) per cell). Sialic acid-deficient Lec2 cells (■); untreated wild type Pro5 cells (●) and sialidase-treated Pro5

cells (○) were prechilled and incubated with AAV9 particles at different MOI ranging from 100 to 500,000 (across 4.5 orders of magnitude) at 4°C to allow binding, without cellular uptake (**FIG. 8**). Quantitative analysis of dose-dependent AAV9 binding to cell surface asialo *N*-glycans was carried out by generating binding curves using a single-site binding model. The inset shows linear range of the binding curve from X-axis values ranging from 100 to 10,000 vg/cell. Calculated binding parameters are listed in **Table 4**. All experiments were carried out in triplicate. Error bars represent standard error mean.

Table 4

	Pro5	Pro5 + Sialidase	Lec2
B_{max} (vg/cell)	$3.75x10^2 \pm 8.60x10^1$	$1.03x10^3 \pm 1.88x10^2$	$1.97x10^3 \pm 2.52x10^2$
K_d' (vg/cell)	$3.58x10^5 \pm 1.64x10^5$	$3.34x10^5 \pm 1.26x10^5$	$1.22x10^5 \pm 4.12x10^4$
B_{max}/K_d'	$1.05x10^{-3}$	$3.07x10^{-3}$	$1.62x10^{-2}$
R^2	0.97	0.97	0.96

[0272] As seen in **FIG. 8**, enzymatic removal of sialic acid partially recapitulates the effect of genetic desialylation by increasing the number of cell surface-bound AAV9 particles. Non-linear regression analysis of binding data was carried out using the single site binding model ($\mathbf{Y = B_{max}*X/(K_d' + X)}$), where **X** and **Y** represent multiplicity of infection and number of bound AAV9 particles, respectively; $\mathbf{B_{max}}$ is the maximum binding capacity and $\mathbf{K_d'}$ is the relative (observed) binding affinity. Calculation of the aforementioned parameters reveals $\mathbf{B_{max}}$ values averaging 375 vg/cell on wild type Pro5 cells, a ~3-fold increase to 1075 vg/cell on sialidase-treated Pro5 cells and a ~5-fold increase to 1971 vg/cell on the sialic acid-deficient Lec2 cell line. These results support the observation that AAV9 prefers asialo *N*-glycans with terminal Galβ1-linked residues. In addition, an apparent decrease in $\mathbf{K_d'}$ (~3-fold) is observed in Lec2 cells as well as a corresponding 10-fold increase in relative binding potential ($\mathbf{B_{max}/K_d'}$; **Table 4**). These results support the notion that enhanced avidity plays a role in mediating AAV9 binding to asialo *N*-glycans.

Example 7

Sialidase pretreatment increases AAV9 gene transfer efficiency in HAE and murine airways

[0273] To evaluate the potential of enzymatic desialylation as a strategy to enhance gene transfer by AAV9 vectors, the effect of sialidase pretreatment on AAV9

transduction efficiency was evaluated in well differentiated HAE cultures and murine airways. Well differentiated human airway epithelial (HAE) cells were untreated (A) or pretreated with neuraminidase from *Arthrobacter Ureafaciens* (B) followed by subsequent infection with self-complementary (sc) AAV9-CMV-GFP vectors (MOI = 10^5 vg/cell). Representative fluorescence micrographs of GFP transgene expression were obtained using an Olympus microscope equipped with a Hamamatsu camera. (C) Representative live animal bioluminescent images of luciferase expression in Balb/c mice pretreated with intranasally administered PBS or neuraminidase (200 mU/50µl/nostril). Intranasal instillation of AAV9-CBA-Luciferase vectors ($5x10^{10}$ vg/50µl/nostril) was carried out 2 hrs post-sialidase treatment and bioluminescent images obtained at 4 weeks post-administration. (D) Bioluminescence intensity was quantified using Living Image ® software and expressed as relative light units (RLU). Error bars represent standard error mean.

[0274] A marked increase in GFP positive HAE was observed upon treatment with neuraminidase from *Arthrobacter Ureafaciens* (Sialidase A) prior to infection with scAAV9/CMV-GFP vectors (**FIGS. 9A and 9B**). Further, quantitation of live animal bioluminescent images of Balb/c mice following intranasal instillation of sialidase showed ~ 10-fold increase in luciferase transgene expression in murine airways mediated by AAV9/CBA-Luc vectors (**FIGS. 9C and 9D).**

[0275] In another experiment, luciferase transgene expression in the nasal cavity of Balb/C mice pretreated with PBS (control) or Neuraminidase from *Arthrobacter Ureafaciens* (200 mU/50µL/nostril) was monitored at 4weeks post-administration with AAV9-CBA-Luciferase vectors ($5x10^{10}$ vg/mouse) via intranasal instillation. Bioluminescence images of mice (dorsal or ventral views) were obtained using a Xenogen IVIS Lumina® system (Caliper Lifesciences) after intranasal instillation of luciferin substrate (120 mg/kg; Nanolight). Image analysis was carried out using Living Image software (**FIGS. 10A-10D**).

Example 8

Inhibition of AAV9 infectivity by different glycans

[0276] AAV9 was preincubated with 0.5 mg/ml of LacNAc (LN), α2,3-sialylated LN (3'SLN) or 3'S-Di-LN at room temperature for 1.5 hrs prior to incubation with prechilled CHO-Lec2 cells (MOI = 10^4 vg/cell) at 4°C. After 90 min co-incubation with AAV9 on cell cultures, unbound virions were removed by three ice-cold 1xPBS washes. Transduction efficiency was determined by quantifying luciferase activity (RLU) at 24hrs

post-infection, and normalized to that of control. All experiments were carried out in triplicate. Error bars represent standard error mean. **FIG. 11** show that a modest, yet statistically significant inhibitory effect on transduction efficiency was seen in the case of AAV9 vectors pretreated with LN ($p = 0.017$ (LN vs. Control); $p = 0.012$ (LN vs. 3'SLN); $p = 0.065$ (LN vs. 3'S-Di-LN) as determined by student t-test).

[0277] Glycomic profiles of major *N*-and *O*-glycans expressed in the parental CHO Pro5 and the CHO Lec2 mutant (Stults *et al.*, *J. Biol. Chem. 264*:19956 (1989); North *et al.*, *J. Biol. Chem. 285*:5759 (2010)) were instrumental towards delineating the nature of glycans that play a role in cell surface binding and infection by AAV9 vectors. The *N*-glycan profile of CHO Pro5 cells has been shown to possess complex bi-, tri-, and tetra-antennary structures bearing multiple *N*-acetyllactosamine (LacNAc) extensions, capped with sialic acid (NeuAc) residues. In addition, the *O*-glycan profile contains Gal-(β1,3)-GalNAc core structures that are mono- or di-sialylated. In contrast, the most abundant glycans produced by the sialic acid-deficient Lec2 cell line are asialo *N*-glycans, possessing between 2 and 7 LacNAc units. The *O*-glycan profile is known to be similarly affected by altered sialylation (North *et al.*, *J. Biol. Chem. 285*:5759 (2010)). These observations suggest that LacNAc units might serve as cell surface attachment factors for AAV9. Treatment of the Lec2 cell line with chemical inhibitors and sialyltransferase enzymes confirmed that Galβ1-linked *N*-glycans rather than *O*-glycans were the preferred substrate for attachment. These results were further corroborated by competitive inhibition studies with the LacNAc-specific *Erythrina cristagalli* lectin (ECL). In addition, ConA and WGA lectins revealed a potential contributing role for mannose and GlcNAc residues underlying terminal galactose in mediating AAV9 infection. Further evidence supporting the aforementioned results was also obtained from competitive inhibition studies of AAV9 particles co-incubated with different glycans residues. Although only a modest inhibitory effect was observed, LacNAc (LN), but not α2,3-sialylated LN (3'-SLN) or α2,3-sialylated di-LN (3'-S-Di-LN) glycans appear to selectively block AAV9 infection **(FIG. 11)**.

[0278] Cell surface binding studies constitute a direct approach to determine the relative binding potential ($\mathbf{B_{max}/K_d}$') of AAV9 particles under physiological conditions. Upon desialylation, the observed increase in number of binding sites ($\mathbf{B_{max}}$) is likely due to increased availability of asialo *N*-glycans on the cell surface. The increase in relative binding affinity ($\mathbf{K_d}$') is potentially a consequence of the increased number of binding sites and can be explained by enhanced avidity arising from multivalent interactions. The aforementioned results suggest that the relatively low abundance of asialo *N*-glycans

provides a molecular basis for the low infectivity displayed by AAV9 in cell culture. In contrast to *in vitro* assays, studies in rodent, canine and primate models have demonstrated robust and widespread systemic gene transfer using AAV9 vectors following intravenous administration (Zincarelli *et al.*, *Mol. Ther. 16*:1073 (2008); Kornegay *et al.*, *Mol. Ther. 18*:1501 (2010); Pacak *et al.*, *Circ. Res. 99*:e3 (2006)). As such, the nature of AAV9-glycan interactions does not provide sufficient rationale for the observed lack of *in vitro-in vivo* correlation outlined above. As seen in the case of other viruses, potential interactions with blood components (Baker *et al.*, *Mol. Ther. 15*:1410 (2007)) or co-receptors such as integrins (Nemerow *et al.*, *Virology 384*:380 (2009)) might play an important role in dictating the transduction profile observed in animal models. Nevertheless, it is tempting to speculate that the relative abundance of asialo *N*-glycans in various animal tissues likely contributes to the broad biodistribution pattern and tissue tropism displayed by AAV9 vectors *in vivo*.

[0279] Carbohydrate receptors utilized by members of different AAV clades appear to fall under two classes, namely, heparan sulfate proteoglycans and sialylated glycans (Wu *et al.*, *Mol. Ther. 14*:316 (2006)). For instance, AAV2, a Clade B member utilizes heparan sulfate proteoglycan as a primary receptor (Summerford *et al.*, *J. Virol. 72*:1438 (1998)). The closely related strains, AAV1 and AAV6 of Clade A appear to equally prefer α2,3- and α2,6-*N*-linked sialic acid for infection (Wu *et al.*, *J. Virol. 80*:9093 (2006)). Further, AAV6 has been shown to bind heparin implying a potential dual mechanism of interaction with cell surface glycans (Wu *et al.*, *J. Virol. 80*:11393 (2006)). Serotypes AAV4 and AAV5, which have not been assigned to any clade thus far, require α2,3-*O*- and α2,3-*N*-linked sialic acid for cell surface binding, respectively (Kaludov *et al.*, *J. Virol. 75*:6884 (2001)). The current study identifies a third class of glycan receptors lacking terminal sialic acid utilized by the AAV strain Hu.14/AAV9 for infection. The latter serotype has been classified under Clade F within the AAV phylogenetic tree (Gao *et al.*, *J. Virol. 78*:6381 (2004)). The major capsid protein (VP3, viral protein subunit 3) of other AAV isolates within Clade F is largely similar to Hu.14/AAV9 (GenBank# AY530579.1). Specifically, isolate Hu.31 (GenBank# AY530596.1) differs from Hu.14/AAV9 by 2 amino acid residues (S386G, N716S), while the VP3 subunit of isolate Hu.32 (GenBank# AY530597.1) is identical to Hu.14/AAV9. These observations suggest that at least one other member of Clade F (Hu.32) might utilize non-sialylated glycans for cell surface binding and entry. Lastly, Akache *et al.* (Akache *et al.*, *Mol. Ther.* 15:330 (2007)) have previously reported the role of laminin receptor (LamR) in mediating transduction by AAV9. Whether the 2.5-fold

increase in AAV9 infectivity mediated by LamR in transfected NIH 3T3 cells is due to enhanced binding or internalization is unknown. Nevertheless, low levels of LamR expressed in CHO cells (Hundt *et al.*, *EMBO J 20*:5876 (2001)) might account for residual infectivity of AAV9 seen in this cell type.

[0280] In the current study, enzymatic desialylation was found to markedly enhance binding and transduction of AAV9 across different cell types regardless of host or tissue type. In addition, intranasal instillation of sialidase markedly enhanced gene transfer efficiency of AAV9 in murine airways supporting the potential application of recombinant sialidase as an adjuvant in therapeutic gene transfer applications. Specifically, co-administration or pretreatment of different tissue types in animal models such as the lung, CNS or eye with recombinant sialidase might serve as (i) a strategy to expose high avidity glycan binding sites and consequently restrict AAV9 transduction to these specific tissue types; (ii) a facile biochemical strategy to increase gene transfer efficiency of AAV9 vectors and (iii) evaluate AAV9 vectors in desialylated preclinical animal models eliminating cross-species variation in sialic acid linkage patterns.

Example 9

Intraarticular administration of sialidase and AAV9

[0281] The effect of localized pretreatment of joints with sialidase was tested. BALB/c mice were pretreated with intraarticularly administered PBS or sialidase from *Vibrio cholera*. Intraarticular injections of recombinant sialidase (200 milliunits/10 μl per joint) were followed by injections of AAV9-CBA-luciferase vectors (1 x 10^9 vg/10 μl per joint) through the same route 2 h later. Live bioluminescent images were obtained at 6 days post administration. Bioluminescence intensity was quantified using Living Image® software and is expressed in terms of relative light units (RLU). **FIG. 12A** shows representative live animal bioluminescent images of luciferase expression. **FIG. 12B** shows quantification of luciferase transgene expression levels at 6 days post injection in liver, leg muscle (gastrocnemius), and joint. Luciferase expression levels are represented as RLU. All experiments were carried out in triplicate. Error bars represent standard error.

[0282] The effect of intraarticular injection of sialidase on leakage of AAV9 into the systemic circulation was tested. BALB/c mice were pretreated with intraarticularly administered PBS or sialidase from *Vibrio cholera*. Intraarticular injections of recombinant sialidase (200 milliunits/10 μl per joint) were followed by injections of AAV9-CBA-luciferase vectors (1 x 10^{10} vg/10 μl per joint) through the same route 2 h

later. Live bioluminescent images were obtained at 6 days post administration. Bioluminescence intensity was quantified using Living Image® software and is expressed in terms of relative light units (RLU). **FIG. 13A** shows representative live animal bioluminescent images of luciferase expression. **FIG. 13B** shows quantification of luciferase transgene expression levels at 7 days post injection in liver, leg muscle (gastrocnemius), and joint. Luciferase expression levels are represented as RLU. **FIG. 13C** shows vector genome copy numbers of AAV9-CBA-luciferase at 7 days post injection in liver, leg muscle, and joint. Vector genome copy numbers are normalized per μg of genomic DNA. All experiments were carried out in triplicate. Error bars represent standard error.

Example 10

Intravitreous administration of sialidase and AAV9

[0283] The effect of localized pretreatment of eyes with sialidase was tested. BALB/c mice were co-administered AAV9 vectors with PBS or sialidase from *Vibrio cholera*. AAV9-CBA-luciferase vectors (1×10^9 vg) were premixed with PBS or 4 mU of sialidase to reach a total volume of 1 μl for each injection. The left eye of each mouse was injected with AAV9/sialidase while the right eye was injected with AAV9/PBS as a control. Experiments were carried out in duplicate as two separate groups. Bioluminescent images were obtained at 16 days and 157 days post injection. Bioluminescence intensity was quantified using Living Image® software and is expressed as counts of photons. **FIG. 14A** shows representative live animal bioluminescent images of luciferase expression. **FIG. 14B** shows quantification of luciferase transgene expression levels at 4 weeks post injection in retina and sclera tissues. Four weeks post intravitreous injection of AAV9/PBS and AAV9/sialidase mixture into mice eyeballs, the animals were sacrificed for tissue harvesting. Retina and sclera tissues were separated, minced and ground before measuring their luciferase activity. Luciferase expression levels are represented as RLU. Error bars represent standard error.

Example 11

Intravenous administration of sialidase and AAV9

[0284] The effect of intravenous administration of sialidase on AAV9 accumulation was tested. BALB/c mice were injected intravenously with sialidase 2 h prior to AAV9 administration. The results are shown in **FIG. 15**. The pretreatment with sialidase resulted in an increase in galactose levels as demonstrated by green lectin

staining (ECL), which results in accumulation of viral particles (ADK9) in endothelial cells as demonstrated by co-staining with CD16/CD32 antibody (a marker for liver endothelium).

[0285] The intravenous pretreatment resulted in an increase in liver transduction efficiency as demonstrated by live animal bioluminescence imaging (**FIG. 16A**) and quantitation of luciferase activity in the liver and the heart (**FIGS. 16C and 16E**). These data were corroborated by quantitative PCR results which demonstrate higher viral particle numbers in liver compared to heart (**FIGS. 16B and 16D**).

[0286] The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

1. A method for increasing transduction of a cell with an adeno-associated virus (AAV) vector that binds asialoglycans, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a cell that has not been desialylated.

2. The method of claim 1, wherein the AAV vector is from clade F.

3. The method of claim 2, wherein the clade F AAV vector is AAV9 (Hu.14).

4. The method of claim 2, wherein the clade F AAV is Hu.31 or Hu.32.

5. The method of claim 1, wherein the AAV vector comprises at least a portion of a capsid protein from a clade F AAV.

6. The method of claim 1, wherein the AAV vector is an engineered vector capable of binding asialoglycans.

7. The method of claim 1, wherein the desialylating comprises removing a portion of the sialyl groups on the cell.

8. The method of claim 1, wherein the desialylating comprises removing substantially all of the sialyl groups on the cell.

9. The method of claim 1, wherein the cell is *in vitro*.

10. The method of claim 9, wherein the cell is a cultured cell.

11. The method of claim 10, wherein the cell is selected from the group consisting of CHO, COS, HEK293, U87, Huh-7, and Neuro2a cells.

12. The method of claim 1, wherein the cell is *in vivo*.

13. The method of claim 12, wherein the cell is selected from the group consisting of a dendritic cell, T cell, B cell, neural cell, muscle cell, pancreatic cell, hepatic cell, lung cell, retinal cell, epithelial cell, smooth muscle cell, skeletal muscle cell, diaphragm muscle cell, cardiac muscle cell, kidney cell, myocardial cell, bone cell, spleen cell, keratinocyte, fibroblast, endothelial cell, prostate cell, germ cell, progenitor cell, and stem cell.

14. The method of claim 1, wherein the cell is *ex vivo*.

15. The method of claim 1, wherein the desialylating occurs prior to contacting the cell with the AAV vector.

16. The method of claim 1, wherein the desialylating occurs concurrently with contacting the cell with the AAV vector.

17. The method of claim 1, wherein the desialylating occurs enzymatically.

18. The method of claim 17, wherein the cell is contacted with a neuraminidase.

19. The method of claim 1, wherein the desialylating occurs chemically.

20. The method of claim 19, wherein the cell is contacted with an inhibitor of sialyltransferase.

21. The method of claim 19, wherein the cell is contacted with swainsonine or α-benzyl-*O*-GalNAc.

22. The method of claim 1, wherein the desialylating occurs genetically.

23. The method of claim 22, wherein expression of a sialic acid transporter and/or sialyltransferase is inhibited.

24. The method of claim 22, wherein the cell is modified to disrupt CMP-sialic acid transport.

25. A method of targeting an AAV vector that binds asialoglycans to a cell, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with the AAV vector;

wherein binding of the AAV vector to the cell is increased relative to binding to a cell that has not been desialylated.

26. The method of claim 25, wherein the cell is present in a subject in a specific tissue or region.

27. The method of claim 26, wherein the tissue or region is selected from the group consisting of eye, ear, nose, joints, thymus, spleen, kidney, lungs, liver, heart, spinal cord, brain, subarachnoid cisternae, ventricles, skeletal muscle, diaphragm, gastrointestinal tract, and pancreas.

28. The method of claim 25, comprising delivering a desialylating agent and the AAV vector to the specific tissue or region.

29. The method of claim 28, wherein the desialylating agent is delivered prior to the AAV vector.

30. The method of claim 28, wherein the desialylating agent is delivered concurrently with the AAV vector.

31. The method of claim 30, wherein the desialylating agent and the AAV vector are delivered in one composition.

32. The method of claim 30, wherein the desialylating agent and the AAV vector are delivered in separate compositions.

33. A method of restricting AAV vectors that bind asialoglycans to the site of delivery in a subject, comprising:

(a) delivering a desialylating agent to a site in a subject; and

(b) delivering the AAV vector to the same site;

wherein the AAV vector is targeted to desialylated cells and systemic dissemination of the AAV vector is restricted.

34. The method of claim 33, wherein the site of delivery is selected from the group consisting of spinal column, lung, nose, cranium, myocardium, pancreatic duct, and intraportal vein.

35. A method of delivering a nucleic acid to a cell, comprising:

(a) desialylating a cell comprising sialyl groups; and

(b) contacting the cell with an AAV vector that binds asialoglycans;

wherein the AAV vector comprises the nucleic acid.

36. The method of claim 35, wherein the cell is selected from the group consisting of a neural cell, lung cell, retinal cell, epithelial cell, smooth muscle cell, skeletal muscle cell, diaphragm cell, cardiac muscle cell, pancreatic cell, hepatic cell, kidney cell, myocardial cell, bone cell, spleen cell, keratinocyte, fibroblast, endothelial cell, prostate cell, germ cell, progenitor cell, and stem cell.

37. A method of delivering a nucleic acid to a mammalian subject comprising delivering to the mammalian subject a cell that has been desialylated and contacted with an AAV vector that binds asialoglycans and comprises the nucleic acid under conditions sufficient for the AAV vector genome to enter the cell.

38. A method of delivering a nucleic acid to a mammalian subject comprising delivering to the mammalian subject a desialylating agent and an AAV vector that binds asialoglycans and comprises the nucleic acid.

39. The method of claim 37 or 38, wherein the subject is a human subject.

40. The method of claim 38, wherein the AAV vector and desialylating agent are administered by a route selected from the group consisting of oral, rectal, transmucosal, transdermal, inhalation, intravenous, subcutaneous, intradermal, intracranial, intramuscular, intraendothelial, and intraarticular administration.

41. The method of claim 38, wherein the AAV vector and desialylating agent are administered to a site selected from the group consisting of a tumor, the brain, a skeletal muscle, a smooth muscle, the heart, the diaphragm, the airway epithelium, the liver, the kidney, the spleen, the pancreas, the skin, and the eye.

42. A composition comprising an AAV vector that binds asialoglycans and a desialylating agent.

43. A kit comprising an AAV vector that binds asialoglycans and a desialylating agent.

44. The kit of claim 43, wherein the AAV vector and the desialylating agent are present in one container.

45. The kit of claim 43, wherein the AAV vector and the desialylating agent are present in separate containers.

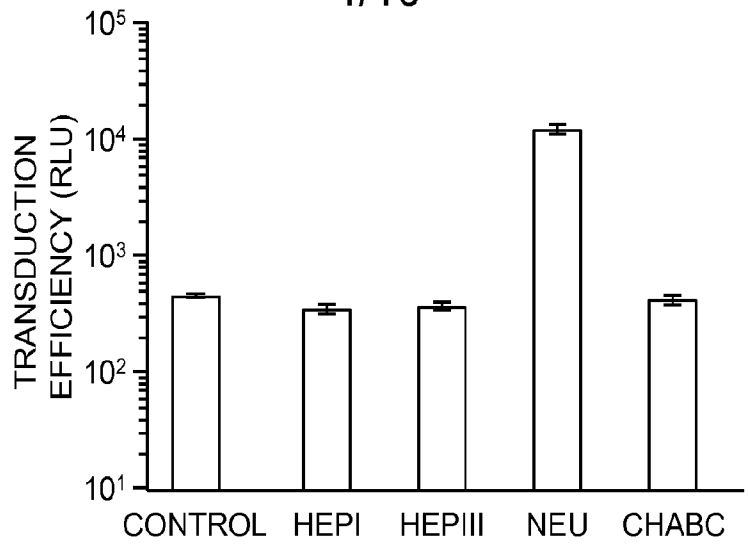


FIG. 1A

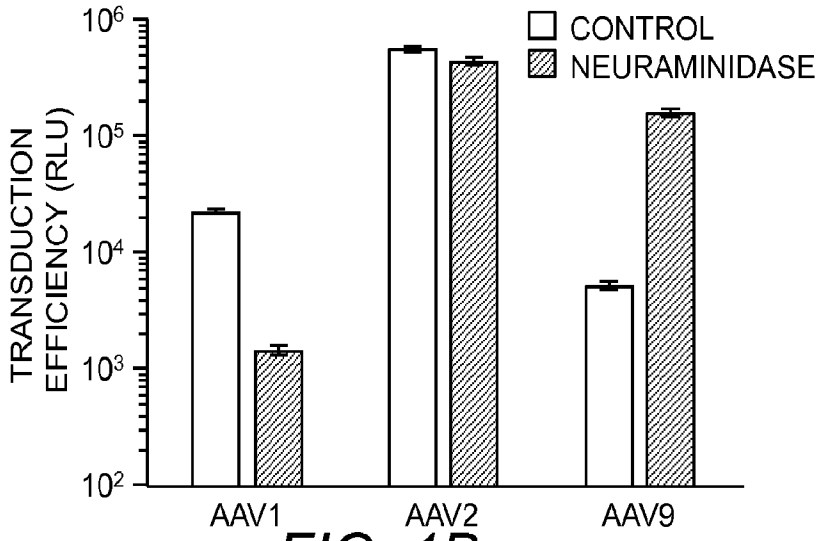


FIG. 1B

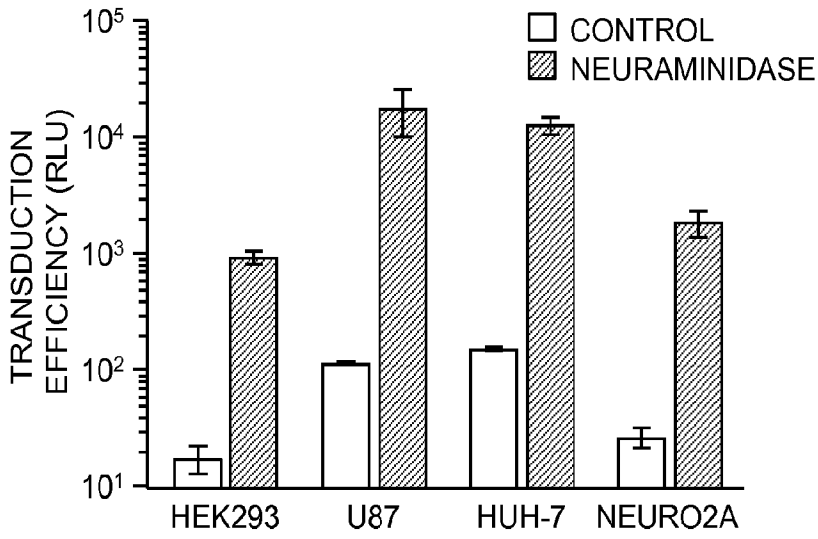


FIG. 1C

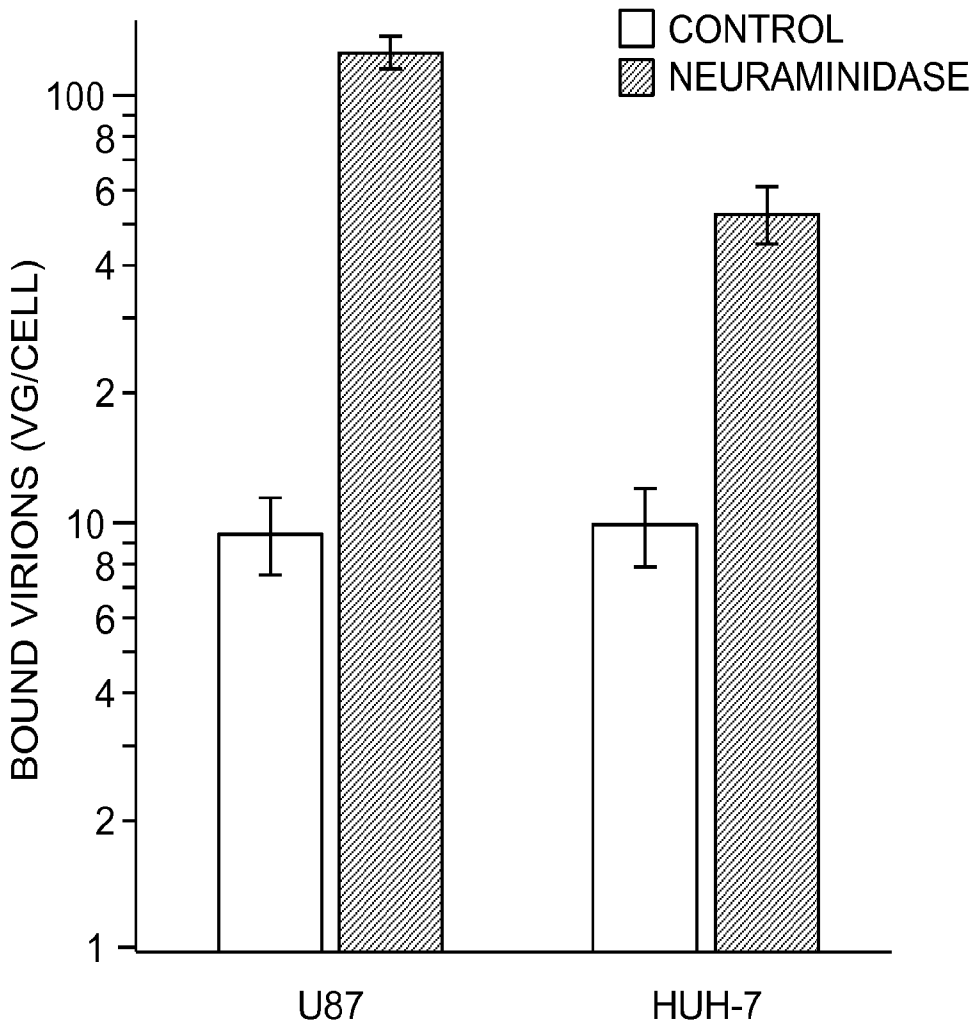

CONTROL
NEURAMINIDASE
BOUND VIRIONS (VG/CELL)
100
8
6
4
2
10
8
6
4
2
1
U87
HUH-7

FIG. 2

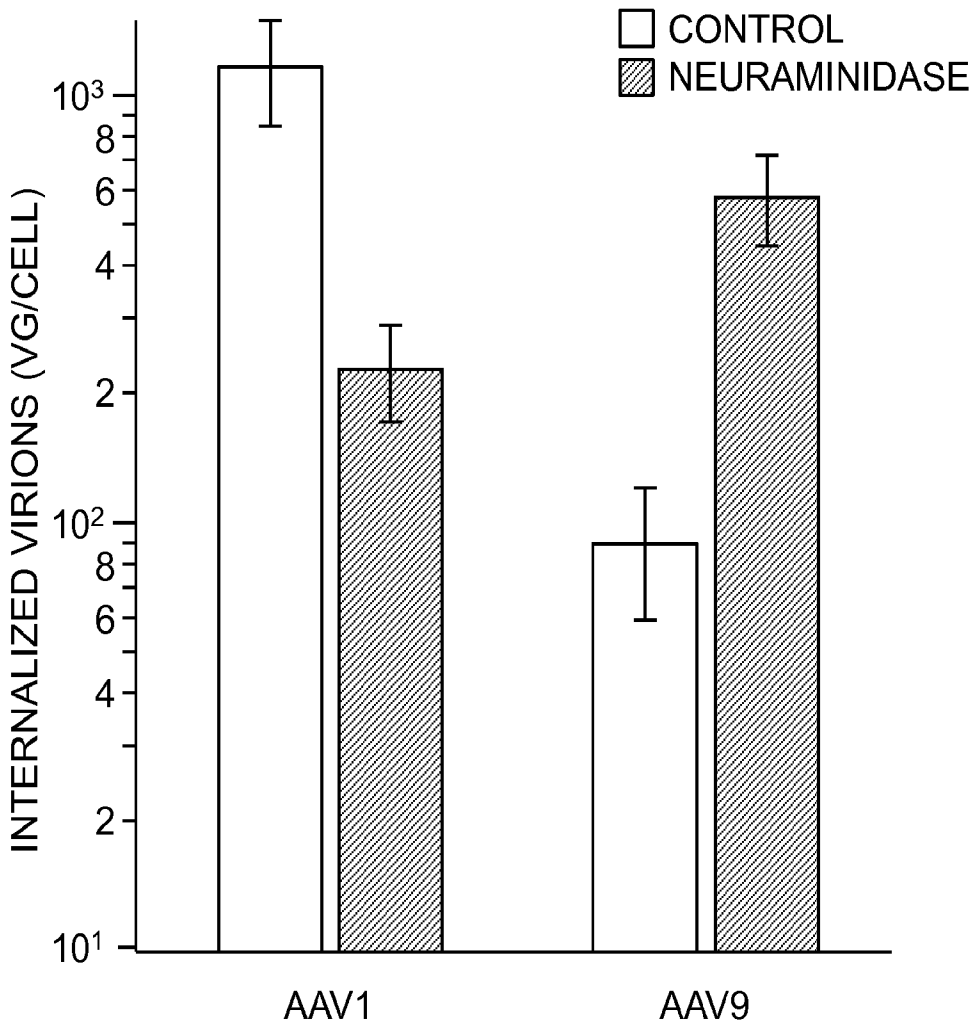

CONTROL
NEURAMINIDASE
INTERNALIZED VIRIONS (VG/CELL)
10³
8
6
4
2
10²
8
6
4
2
10¹
AAV1
AAV9

FIG. 3

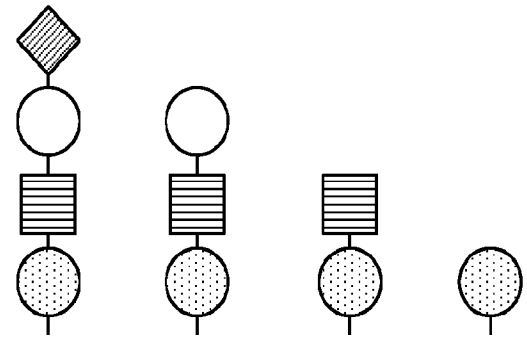


FIG. 4A

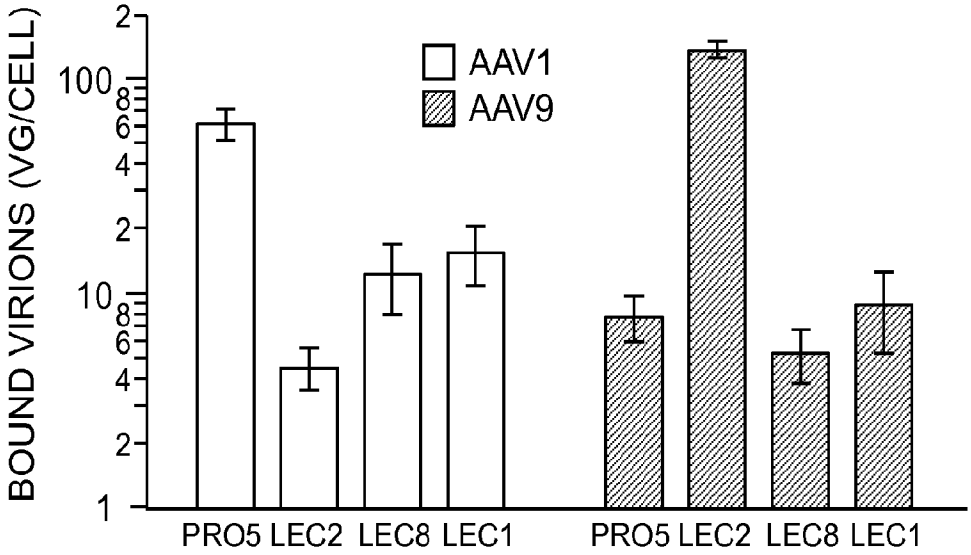


FIG. 4B

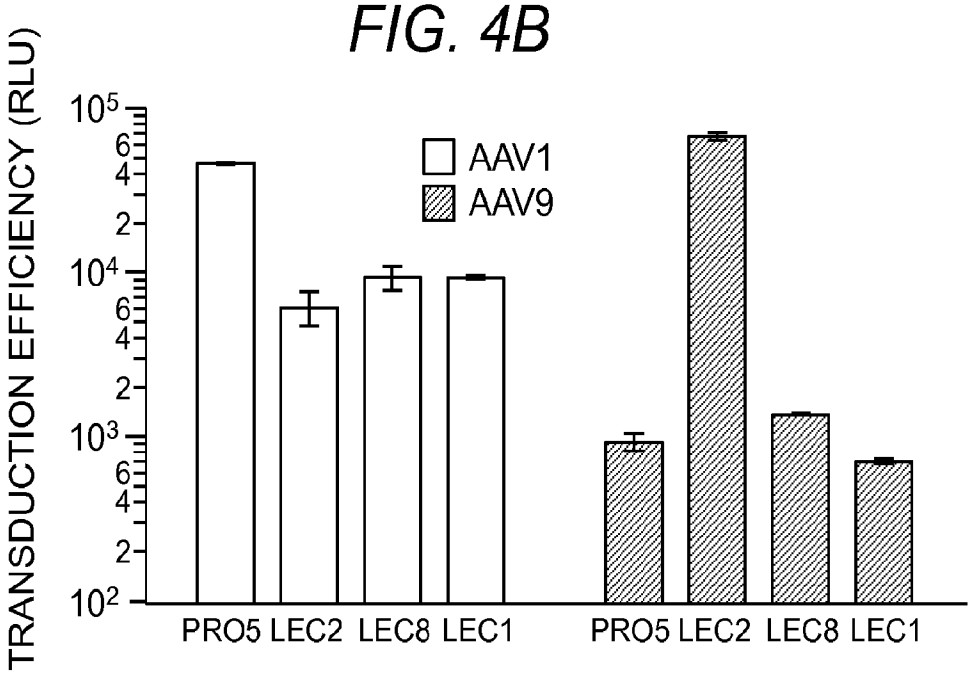


FIG. 4C

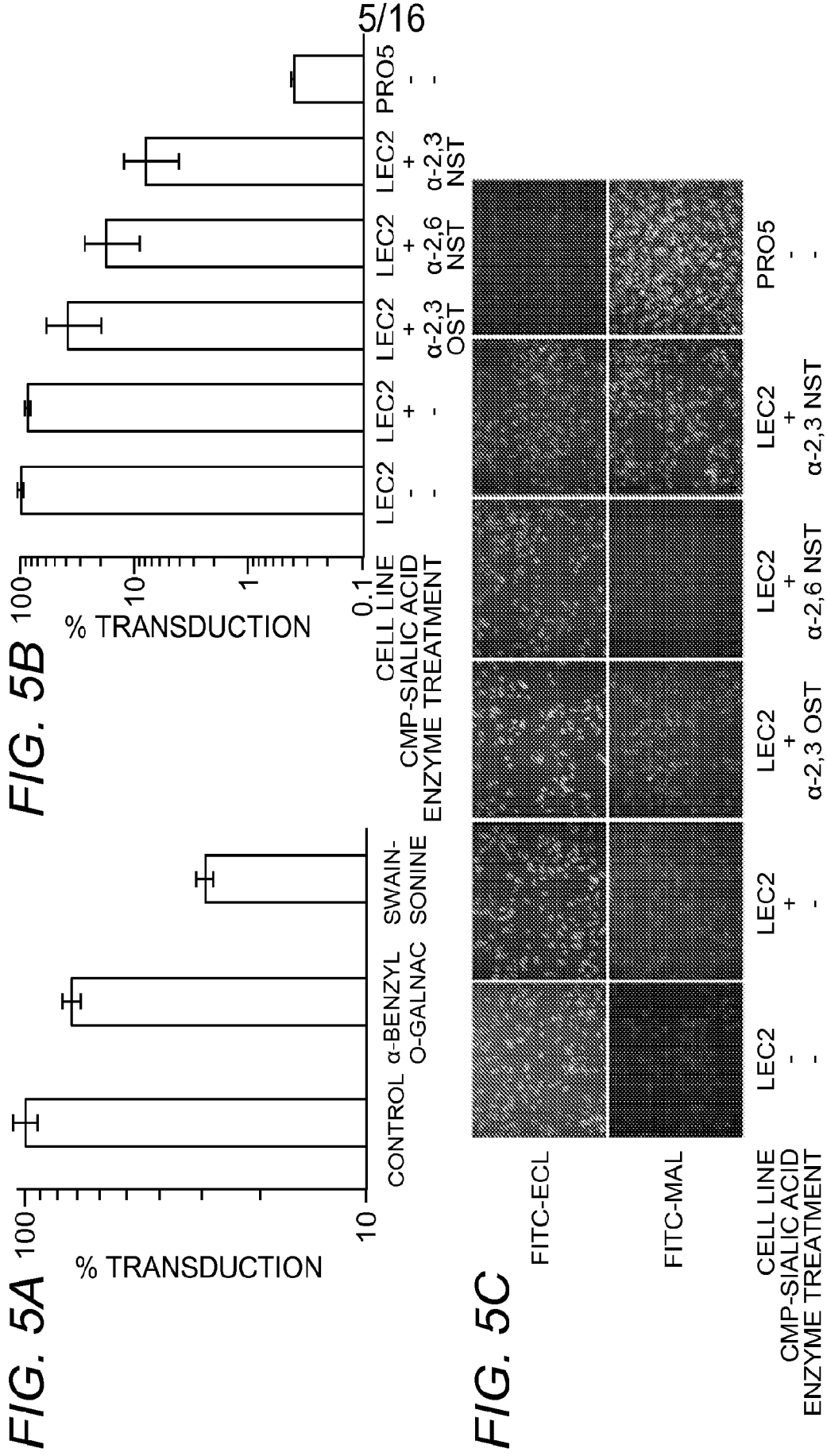


FIG. 5A
100
10
% TRANSDUCTION
CONTROL
α-BENZYL O-GALNAC
SWAIN-SONINE
FIG. 5B
100
10
1
0.1
% TRANSDUCTION
CELL LINE
CMP-SIALIC ACID
ENZYME TREATMENT
LEC2 - -
LEC2 + -
LEC2 + α-2,3 OST
LEC2 + α-2,6 NST
LEC2 + α-2,3 NST
PRO5 - -
FIG. 5C
FITC-ECL
FITC-MAL
CELL LINE
CMP-SIALIC ACID
ENZYME TREATMENT
LEC2 - -
LEC2 + -
LEC2 + α-2,3 OST
LEC2 + α-2,6 NST
LEC2 + α-2,3 NST
PRO5 - -

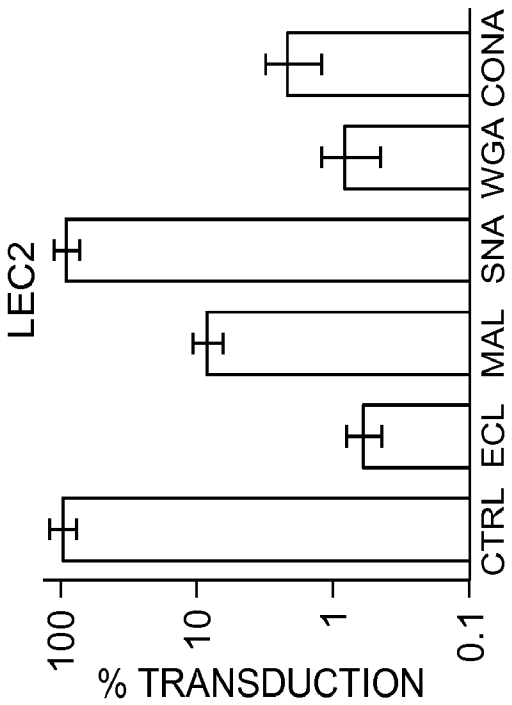


FIG. 6A

FIG. 6B

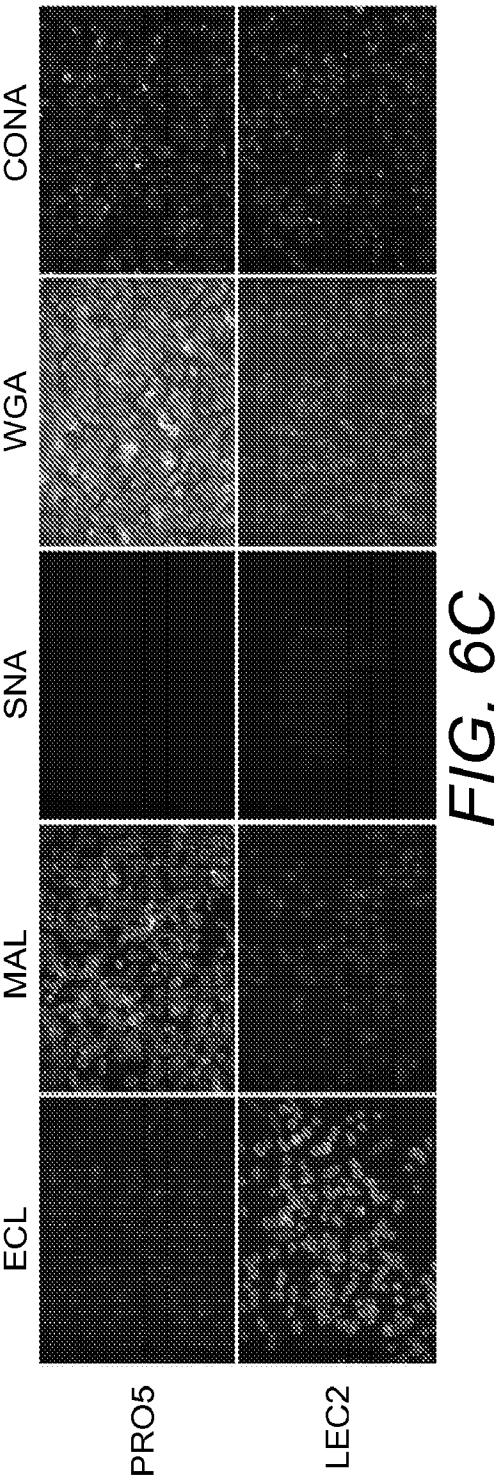


FIG. 6C

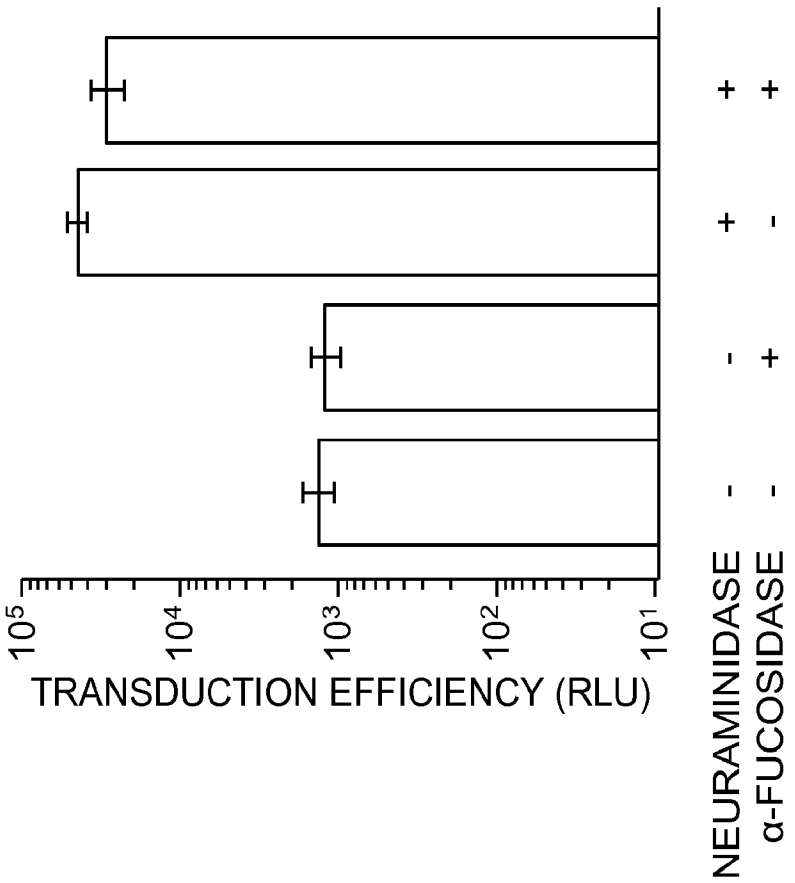

TRANSDUCTION EFFICIENCY (RLU)
10^5
10^4
10^3
10^2
10^1
NEURAMINIDASE - - + +
α-FUCOSIDASE - + - +

FIG. 7B

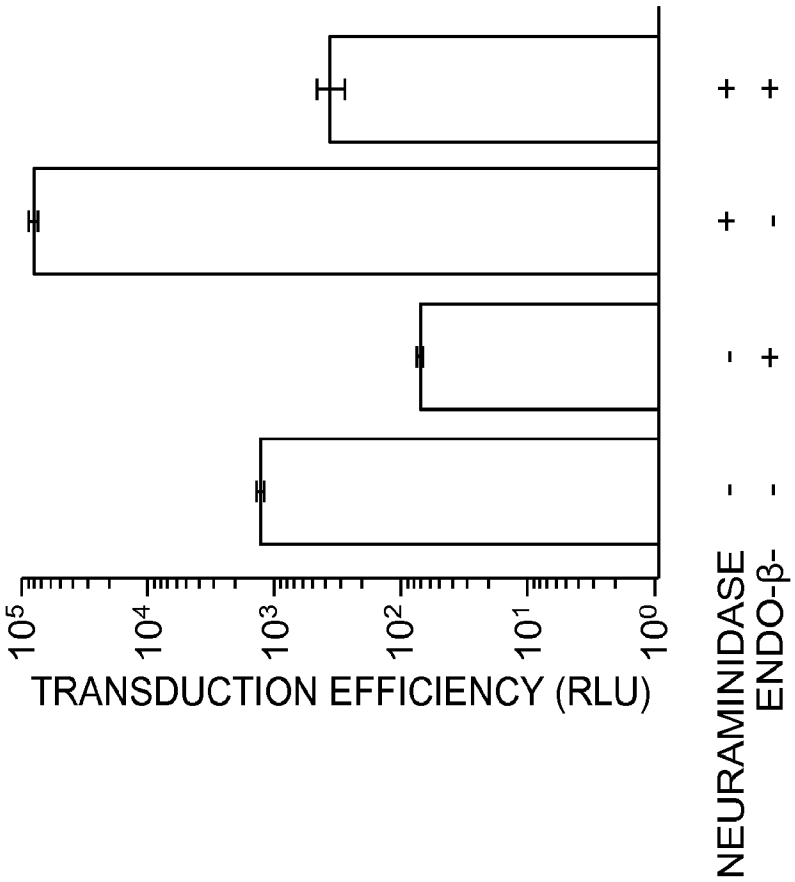

TRANSDUCTION EFFICIENCY (RLU)
10^5
10^4
10^3
10^2
10^1
10^0
NEURAMINIDASE - - + +
ENDO-β-GALACTOSIDASE - + - +

FIG. 7A

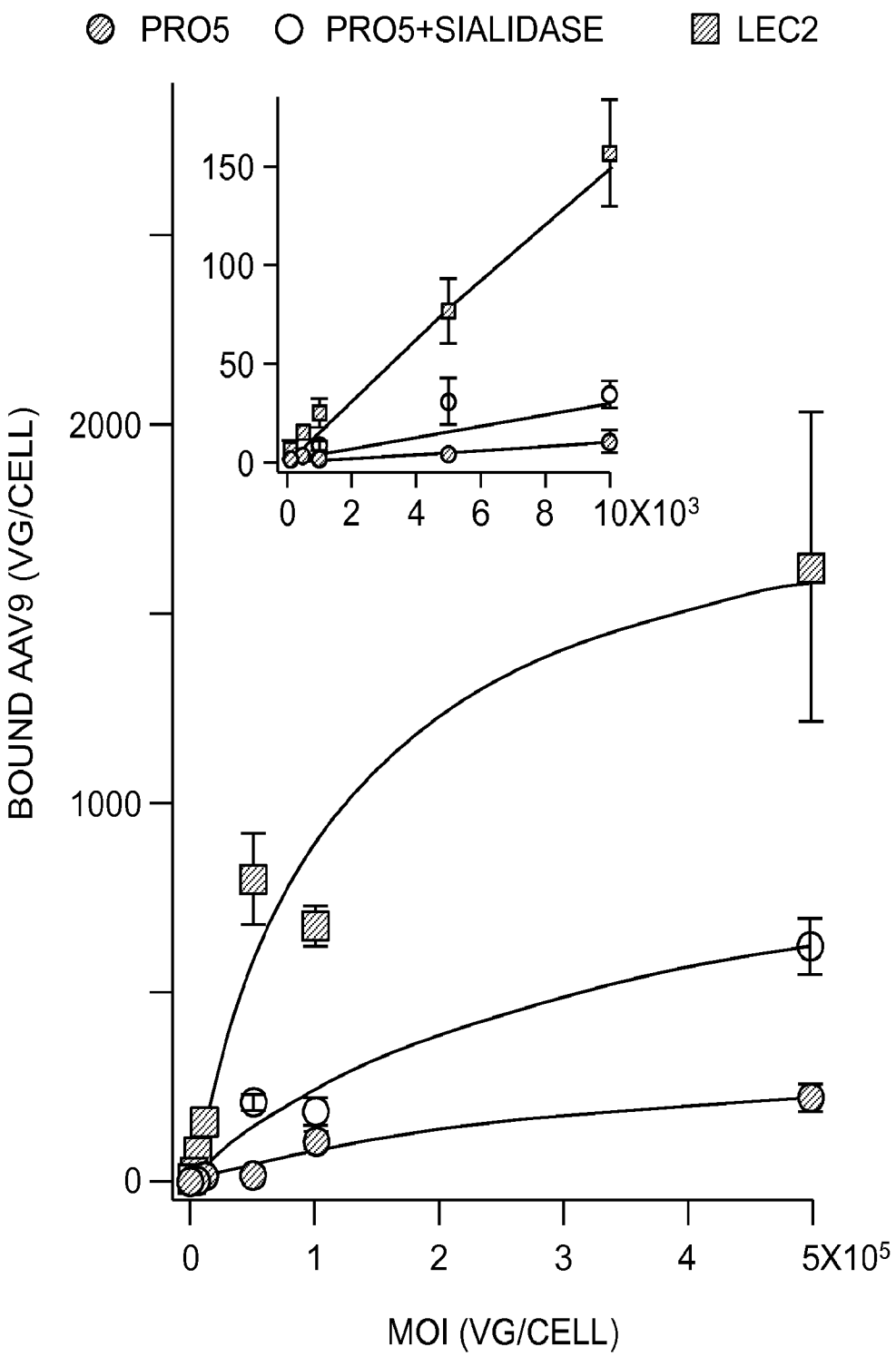

PRO5
PRO5+SIALIDASE
LEC2
BOUND AAV9 (VG/CELL)
2000
1000
0
150
100
50
0
0
2
4
6
8
10X10³
0
1
2
3
4
5X10⁵
MOI (VG/CELL)

FIG. 8

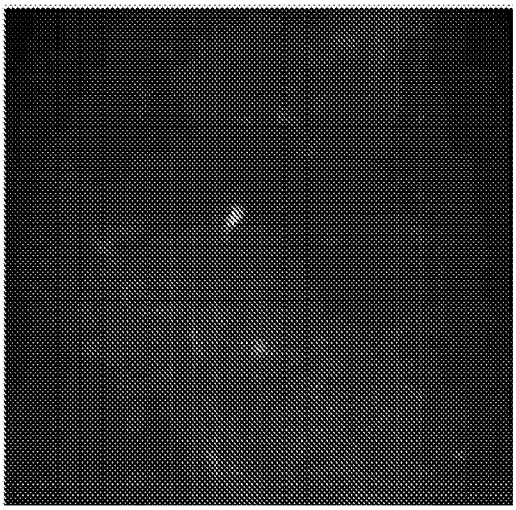


FIG. 9A

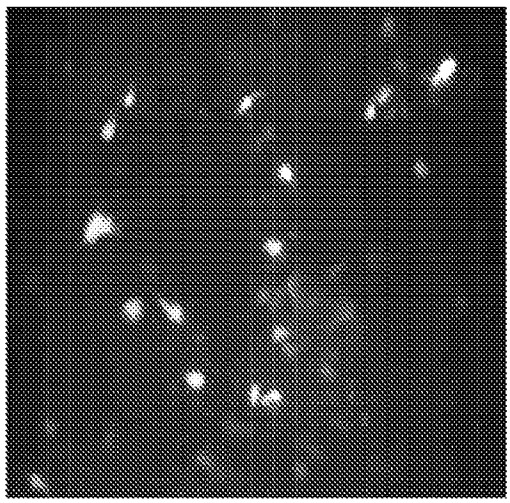


FIG. 9B

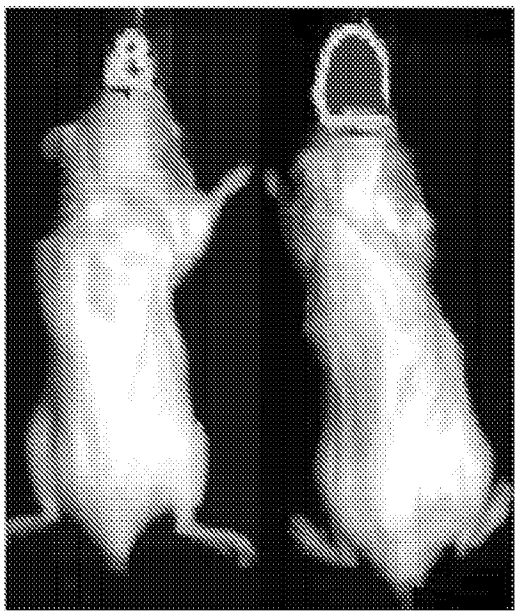


FIG. 9C

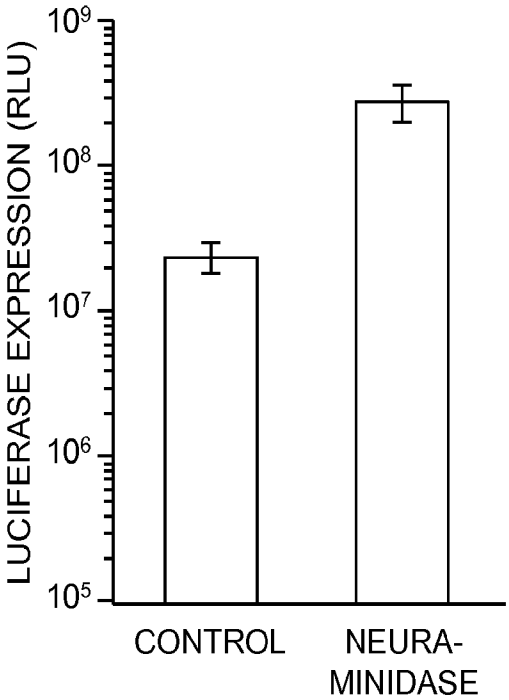


FIG. 9D

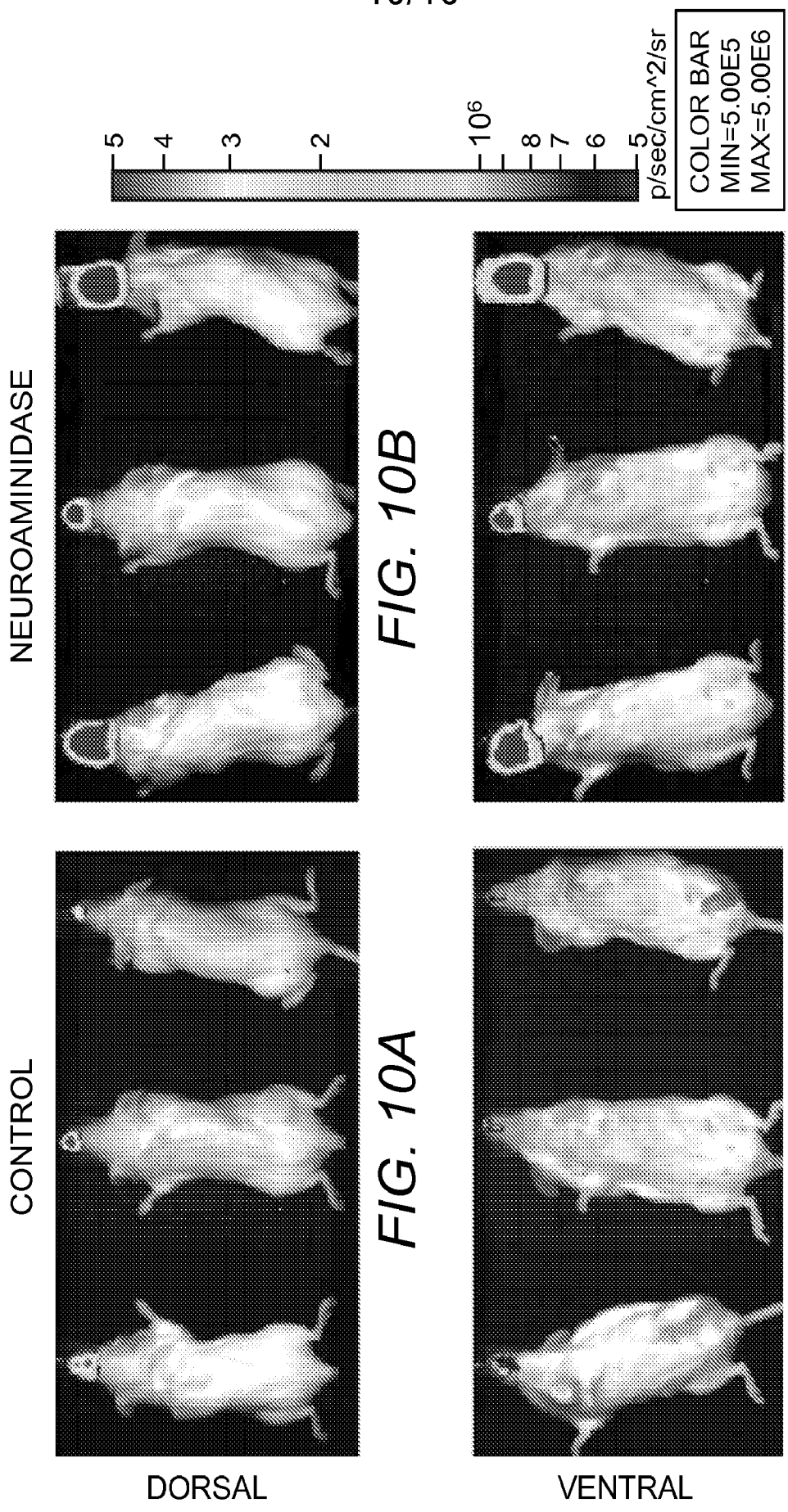


FIG. 10B

FIG. 10D

FIG. 10A

FIG. 10C

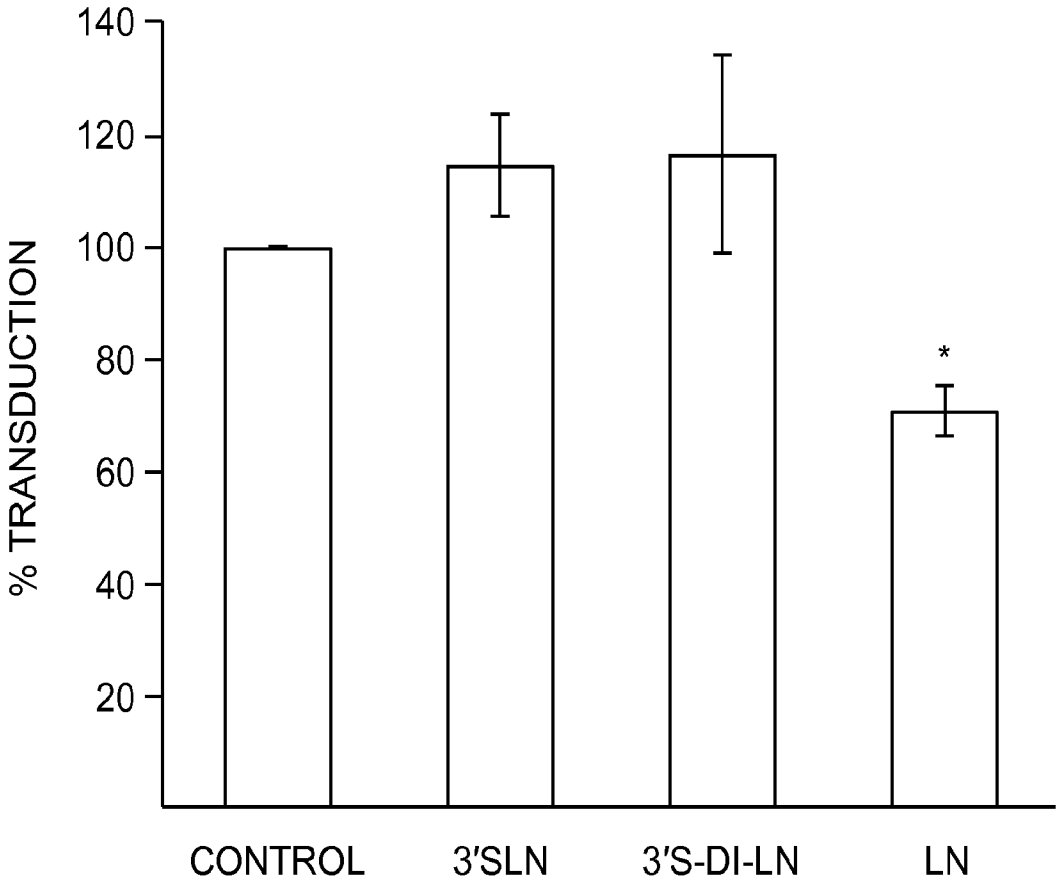
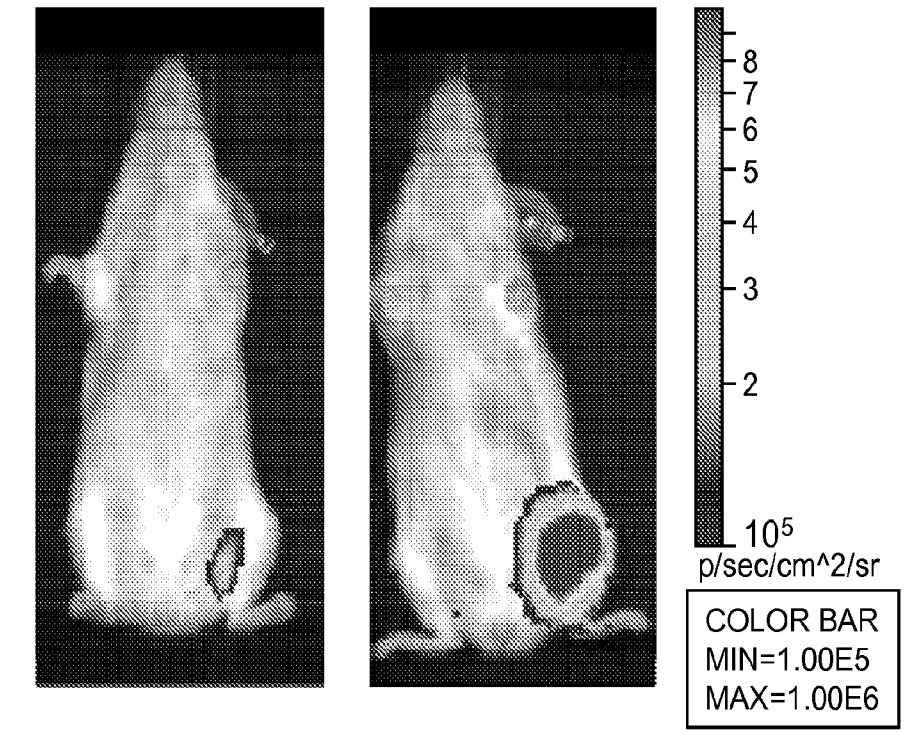


FIG. 11


8
7
6
5
4
3
2
10^5
p/sec/cm^2/sr
COLOR BAR
MIN=1.00E5
MAX=1.00E6

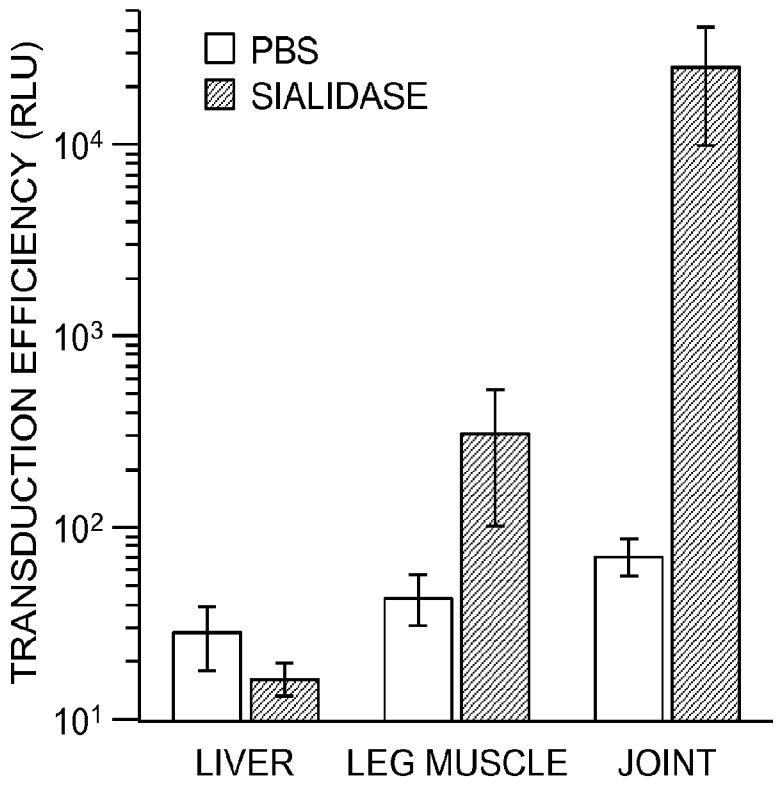

PBS
SIALIDASE
TRANSDUCTION EFFICIENCY (RLU)
10^4
10^3
10^2
10^1
LIVER
LEG MUSCLE
JOINT

FIG. 12

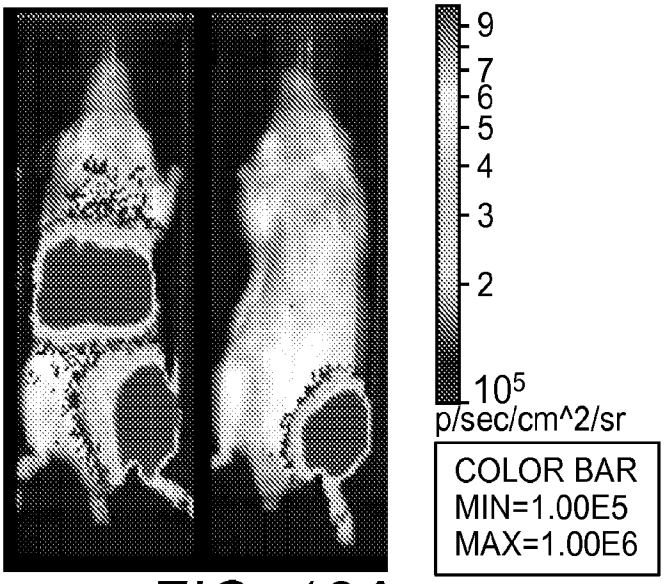


FIG. 13A

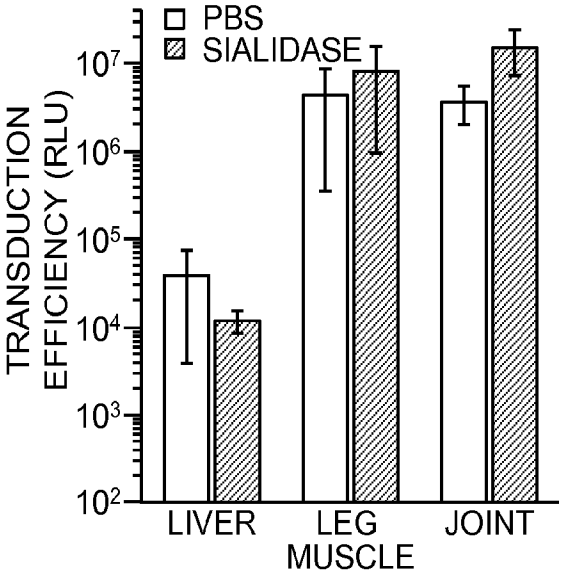


FIG. 13B

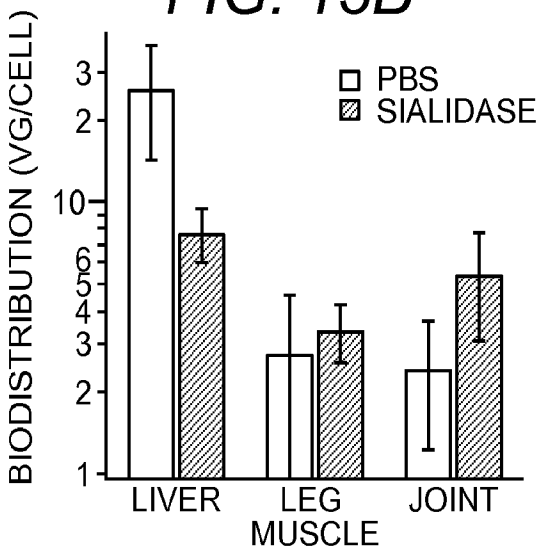


FIG. 13C

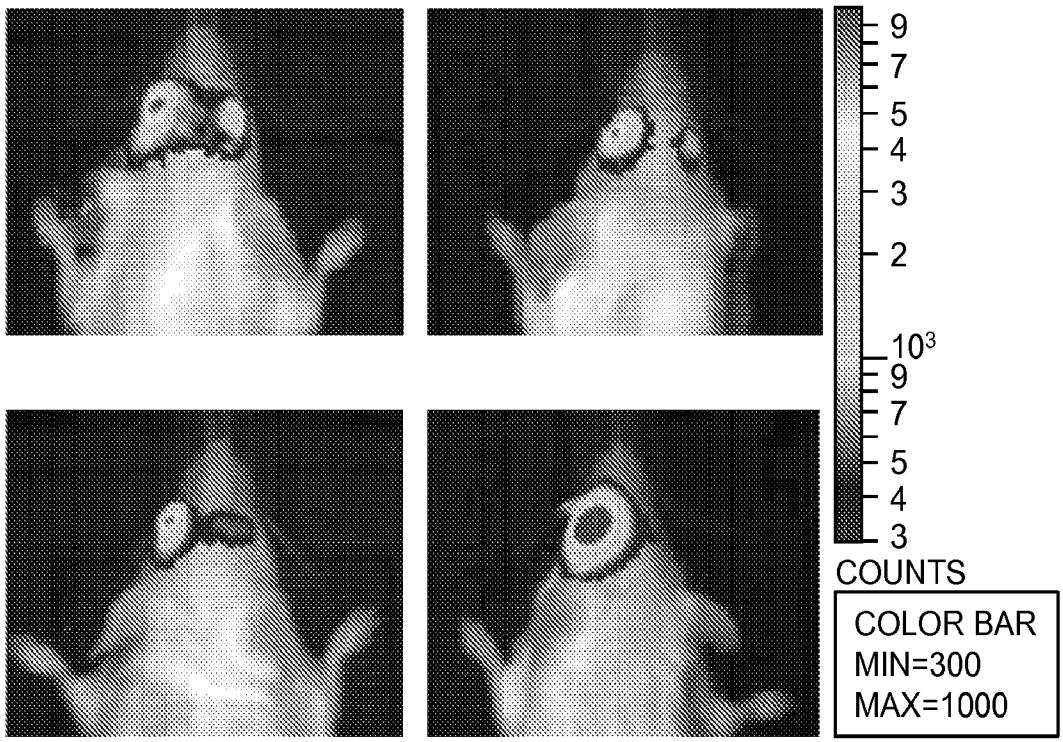


FIG. 14A

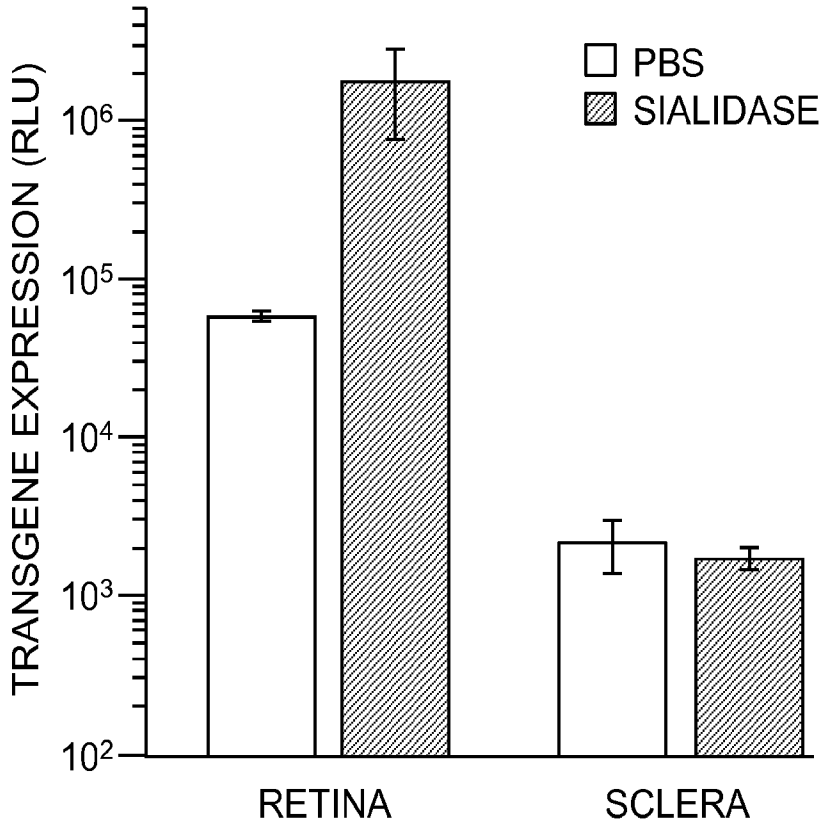


FIG. 14B

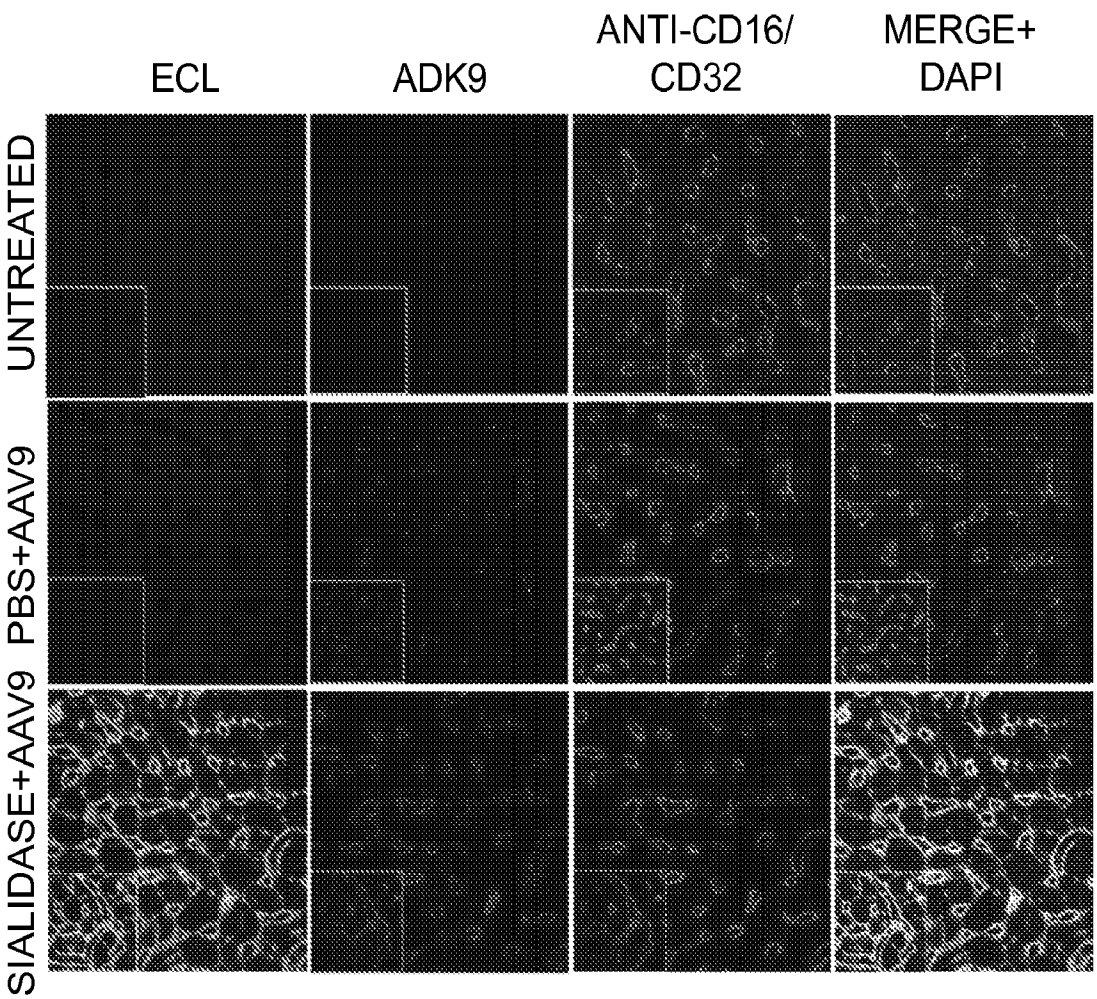

ECL
ADK9
ANTI-CD16/
CD32
MERGE+
DAPI
UNTREATED
PBS+AAV9
SIALIDASE+AAV9

FIG. 15

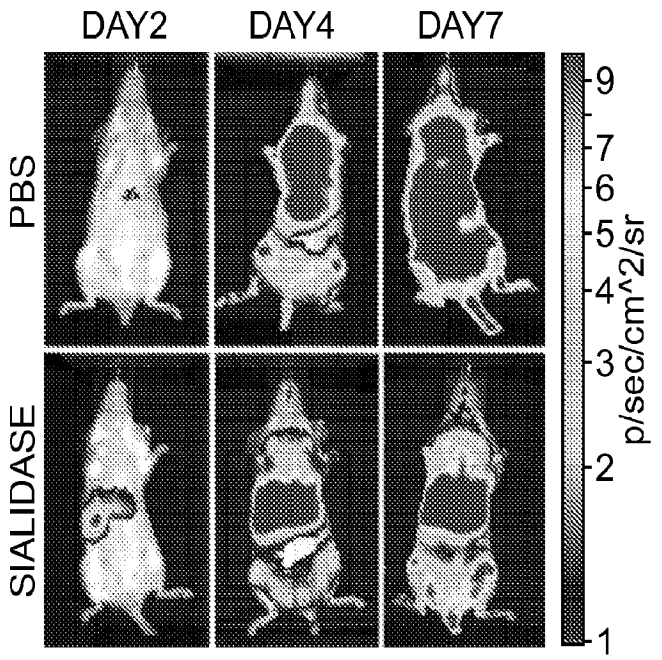


FIG. 16A

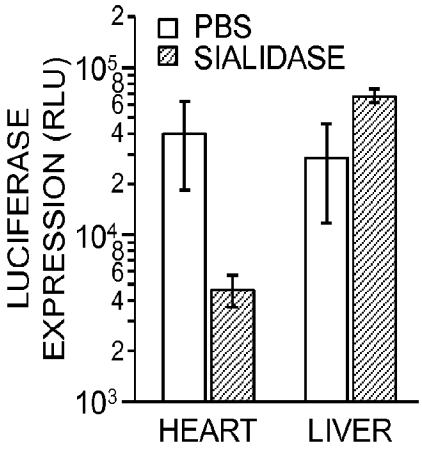


FIG. 16B

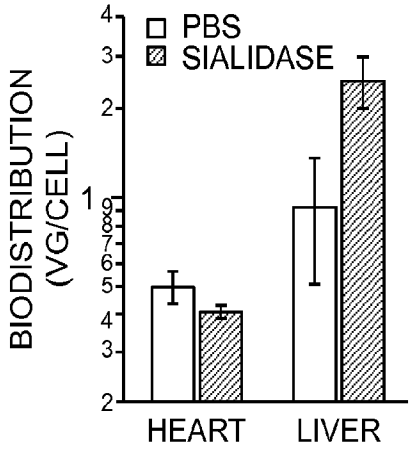


FIG. 16C

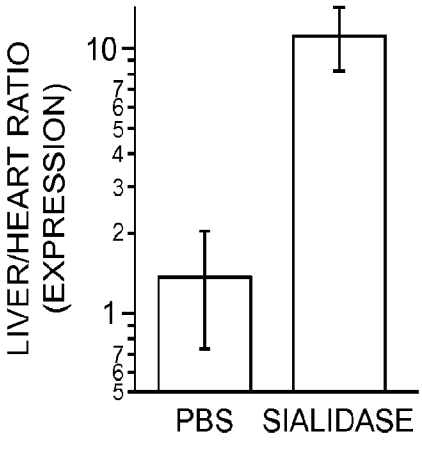


FIG. 16D

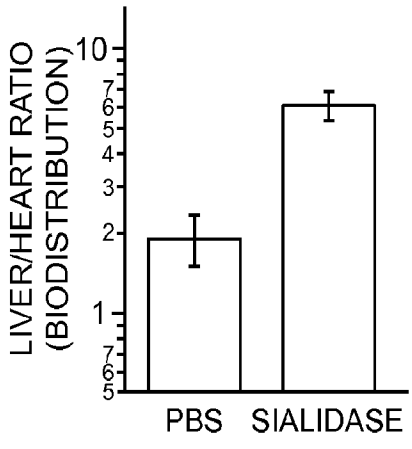


FIG. 16E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2012/024108

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/861 *(2006.01)*
C07K 14/005 *(2006.01)*
A61K 48/00 *(2006.01)*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 15/861, C07K 14/005, A61K 48/00

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

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

CIPO, DEPATISnet, DWPI, EAPATIS, EMBL, EPO-Internal, Esp@ce, Esp@cenet, Google, KIPRIS, PAJ, PabMed, RUPTO, SCIENCEDIRECT, SIPO, USPTO, WIPO, VINITI, NCBI, Patsearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0287122 A1 (CHILDREN'S HOSPITAL INC.) 29.12.2005	1-45
A	WO 2005/005610 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 20.01.2005	1-45

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2012 (17.04.2012)	24 May 2012 (24.05.2012)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer O. Kolontaevskaya Telephone No. (495)531-65-15