AADC POLYNUCLEOTIDES FOR THE TREATMENT OF PARKINSON'S DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/075,298, entitled AADC Polynucleotides for the Treatment of Parkinson's Disease, filed November 5, 2014, U.S. Provisional Patent Application No. 62/155,692, entitled AADC Polynucleotides for the Treatment of Parkinson's Disease, filed May 1, 2015, U.S. Provisional Patent Application No. 62/199,578, entitled AADC Polynucleotides for the Treatment of Parkinson's Disease, filed July 31, 2015 and U.S. Provisional Patent Application No. 62/243,537, entitled AADC Polynucleotides for the Treatment of Parkinson's Disease, filed October 19, 2015; the contents of each of which is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled 1010PCTSEQLST.txt, was created on November 5, 2015 and is 178,144 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The invention relates to compositions, particularly nucleic acid molecules, e.g., polynucleotides encoding AADC, for use in the treatment of Parkinson's disease. In some embodiments such AADC polynucleotides may be encoded by or within recombinant adeno-associated viruses (AAVs).

BACKGROUND OF THE INVENTION

[0004] Aromatic L-amino acid decarboxylase (AADC) is a homodimeric pyridoxal phosphate-dependent enzyme responsible for the synthesis of dopamine and serotonin. The encoded protein catalyzes the decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA or levodopa) to dopamine; L-5-hydroxytryptophan to serotonin; and L-tryptophan to tryptamine. Defects in this gene are the cause of aromatic L-amino-acid decarboxylase deficiency (AADCD), which is an inborn error in neurotransmitter metabolism leading to combined serotonin and catecholamine deficiency that results in severe motor and autonomic dysfunctions.

[0005] Parkinson's Disease (PD) is a progressive neurodegenerative disease of the central nervous system (CNS) producing sensory and motor symptoms. Dopamine replacement (i.e., levodopa) has been the standard pharmacotherapy for motor impairment in PD. However, the benefit of dopamine therapy becomes less marked over time, due, in part, to the progressive

death of dopamine-generating cells and corresponding loss of AADC activity. Furthermore, systemic administration of high-dose dopamine is complicated by side effects, such as fluctuations in motor performance, dyskinesias, and hallucinations, resulting from dopaminergic stimulation of the mesolimbic system. One strategy to restore dopaminergic function and minimize side effects is the use of gene therapy to deliver AADC directly to a targeted region of the CNS.

[0006] The adeno-associated virus (AAV) has emerged as an attractive vector for gene therapy due to its long-term gene expression, the inability to autonomously replicate without a helper virus, the ability to transduce dividing and non-diving cells, and the lack of pathogenicity from wild-type infections (See e.g., Hadaczek et al. Mol. Ther. 18(8), 1458-1461, Aug. 2010). AAV is a helper-dependent DNA parvovirus which belongs to the genus *Dependovirus*.

[0007] The present invention provides such improved nucleic acid constructs, e.g., polynucleotides, for use with AAV-derived vectors comprising dopa carboxylase ("DDC") gene sequence which encodes a full-length AADC protein for the purpose of gene therapy in the treatment of Parkinson's Disease.

[0008] The nucleic acid constructs described herein comprise at least a 5'-ITR and a 3'-ITR, each or both of which may be derived from an AAV, positioned about a *DDC gene* sequence, as well as additional components required for gene expression and clone selection.

SUMMARY OF THE INVENTION

[0009] Described herein are compositions, methods, processes, kits and devices for the design, preparation, manufacture and/or formulation of AADC polynucleotides.

[00010] In some embodiments such AADC polynucleotides may be encoded by or contained within plasmids or vectors or recombinant adeno-associated viruses (AAV).

[00011] The details of various embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

Compositions of the Invention

[00012] According to the present invention, AADC polynucleotides are provided which function alone or in combination with additional nucleic acid sequence(s) to encode the AADC protein. As used herein an "AADC polynucleotide" is any nucleic acid polymer which encodes an AADC protein and when present in a vector, plasmid or translatable construct, expresses such AADC protein in a cell, tissue, organ or organism.

[00013] AADC polynucleotides include precursor molecules which are processed inside the cell. AADC polynucleotides or the processed forms thereof may be encoded in a plasmid, vector, genome or other nucleic acid expression vector for delivery to a cell.

[00014] In some embodiments AADC polynucleotides are designed as components of AAV viral genomes and packaged in AAV viral particles which are processed within the cell to produce the wild type AADC protein.

[00015] As used herein, the wild type AADC protein may be any of the naturally occurring isoforms or variants from the *DDC gene*. Multiple alternatively spliced transcript variants encoding different isoforms of AADC have been identified. Specifically, the *DDC gene* produces seven transcript variants that encode six distinct isoforms. DDC transcript variants 1 and 2 both encode AADC isoform 1. In some embodiments, the AADC polynucleotides encode DDC transcript variant 2, thereby encoding a native AADC isoform 1 (NCBI Reference Sequence: NP_000781.1). This sequence is given here:

MNASEFRRRGKEMVDYVANYMEGIEGRQVYPDVEPGYLRPLIPAAAPQEPDTFEDIIND
VEKIIMPGVTHWHSPYFFAYFPTASSYPAMLADMLCGAIGCIGFSWAASPACTELETVM
MDWLGKMLELPKAFLNEKAGEGGGVIQGSASEATLVALLAARTKVIHRLQAASPELTQ
AAIMEKLVAYSSDQAHSSVERAGLIGGVKLKAIPSDGNFAMRASALQEALERDKAAGLI
PFFMVATLGTTTCCSFDNLLEVGPICNKEDIWLHVDAAYAGSAFICPEFRHLLNGVEFAD
SFNFNPHKWLLVNFDCSAMWVKKRTDLTGAFRLDPTYLKHSHQDSGLITDYRHWQIPL
GRRFRSLKMWFVFRMYGVKGLQAYIRKHVQLSHEFESLVRQDPRFEICVEVILGLVCFR
LKGSNKVNEALLQRINSAKKIHLVPCHLRDKFVLRFAICSRTVESAHVQRAWEHIKELA
ADVLRAERE (SEQ ID NO:1)

[00016] The AADC polynucleotides of the invention, may be engineered to contain modular elements and/or sequence motifs assembled to create AADC polynucleotide constructs.

AADC polynucleotide constructs

[00017] According to the present invention, AADC polynucleotides are provided. Such polynucleotides comprise nucleic acid polymers which comprise a region of linked nucleosides encoding one or more isoforms or variants of the AADC protein.

[00018] In some embodiments, the AADC polynucleotide comprises a codon optimized transcript encoding an AADC protein.

[00019] In some embodiments, the AADC polynucleotide comprises a sequence region encoding one or more wild type isoforms or variants of an AADC protein. Such polynucleotides may also comprise a sequence region encoding any one or more of the following: a 5' ITR, a cytomegalovirus (CMV) Enhancer, a CMV Promoter, an ie1 exon 1, an ie1 intron1, an

hbBglobin intron2, an hBglobin exon 3, a 5' UTR, a 3' UTR, an hGH poly(A) signal, and/or a 3' ITR. Such sequence regions are taught herein or may be any of those known in the art.

[00020] In some embodiments, the AADC polynucleotide comprises a sequence of any of SEQ ID NOs. 2-23.

[00021] In one embodiment, the AADC polynucleotide comprises a sequence which has a percent identity to any of SEQ ID NOs: 2-23. The AADC polynucleotide may have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% identity to any of SEQ ID NOs: 2-23. The AADC polynucleotide may have 1-10%, 10-20%, 30-40%, 50-60%, 50-70%, 50-80%, 50-90%, 50-99%, 50-100%, 60-70%, 60-80%, 60-90%, 60-99%, 60-100%, 70-80%, 70-90%, 70-99%, 70-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-100%, 90-95%, 90-99%, or 90-100% to any of SEQ ID NOs: 2-23. As a non-limiting example, the AADC polynucleotide comprises a sequence which as 80% identity to any of SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23. As another non-limiting example, the AADC polynucleotide comprises a sequence which as 85% identity to any of SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23. As another non-limiting example, the AADC polynucleotide comprises a sequence which as 90% identity to any of SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23. As another non-limiting example, the AADC polynucleotide comprises a sequence which as 95% identity to any of SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23. As another non-limiting example, the AADC polynucleotide comprises a sequence which as 99% identity to any of SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23.

[00022] In some embodiments the AADC coding region is 1440 nucleotides in length. Such an AADC polynucleotide may be codon optimized over all or a portion of the polynucleotide.

[00023] In some embodiments the AADC coding region is 1443 nucleotides in length. In such case, an additional codon may be present at the 3' end of the polynucleotide.

[00024] In some embodiments the AADC coding region is 1449 nucleotides in length. In such case, additional codons may be present at the 3' end of the polynucleotide.

[00025] In some embodiments, the AADC polynucleotide comprises any of SEQ ID NOs 6-9, 17-23 but lacking the 5' and/or 3' ITRs. Such a polynucleotide may be incorporated into a plasmid or vector and utilized to express the encoded AADC protein.

[00026] In one embodiment, the AADC polynucleotides may be produced in insect cells (e.g., Sf9 cells).

[00027] In one embodiment, the AADC polynucleotides may be produced using triple transfection.

[00028] In one embodiment, the AADC polynucleotide may comprise a codon optimized open reading frame of an AADC mRNA, at least one 5'ITR and at least one 3'UTR where the one or more of the 5'ITRs may be located at the 5'end of the promoter region and one or more 3' ITRs may be located at the 3' end of the poly(A) signal. The AADC mRNA may comprise a promoter region, a 5'untranslated region (UTR), a 3'UTR and a poly(A) signal. The promoter region may inclue, but is not limited to, enhancer element, a promoter element, a first exon region, a first intron region, a second intron region and a second exon region. As a non-limiting example, the enhancer element and the promoter element are derived from CMV. As another non-limiting example, the first exon region is ie1 exon 1 or fragments thereof, the first intron region is ie1 intron 1 or fragments thereof, the second intron region is hbBglobin intron 2 or fragments thereof and the second exon region is hbBglobin exon 3 or fragments thereof. As yet another non-limiting example, the poly(A) signal is derived from human growth hormone.

[00029] In one embodiment, the AADC polynucleotide is encoded in a plasmid or vector, which may be derived from an adeno-associated virus (AAV). The AAV may be a recombinant AAV virus and may comprise a capsid serotype such as, but not limited to, of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ, and AAV-DJ8. As a non-limiting example, the capsid of the recombinant AAV virus is AAV2. As a non-limiting example, the capsid of the recombinant AAV virus is AAVrh10. As a non-limiting example, the capsid of the recombinant AAV virus is AAV9(hu14). As a non-limiting example, the capsid of the recombinant AAV virus is AAV-DJ. As a non-limiting example, the capsid of the recombinant AAV virus is AAV9.47. As a non-limiting example, the capsid of the recombinant AAV virus is AAV-DJ8.

Promoters

[00030] A person skilled in the art may recognize that a target cell may require a specific promoter including but not limited to a promoter that is species specific, inducible, tissue-specific, or cell cycle-specific Parr et al., *Nat. Med.*3:1145-9 (1997); the contents of which are herein incorporated by reference in its entirety).

[00031] In one embodiment, the promoter is a promoter deemed to be efficient for the AADC polynucleotide.

[00032] In one embodiment, the promoter is a promoter deemed to be efficient for the cell being targeted.

[00033] In one embodiment, the promoter is a weak promoter which provides expression of a payload for a period of time in targeted tissues such as, but not limited to, nervous system

tissues. Expression may be for a period of 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 3 weeks, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more than 10 years. Expression may be for 1-5 hours, 1-12 hours, 1-2 days, 1-5 days, 1-2 weeks, 1-3 weeks, 1-4 weeks, 1-2 months, 1-4 months, 1-6 months, 2-6 months, 3-6 months, 3-9 months, 4-8 months, 6-12 months, 1-2 years, 1-5 years, 2-5 years, 3-6 years, 3-8 years, 4-8 years or 5-10 years. As a non-limiting example, the promoter is a weak promoter for sustained expression of a payload in nervous tissues. As another non-limiting example, the promoter is a weak promoter for sustained frataxin expression in nervous system tissue such as, but not limited to, neuronal tissue and glial tissue.

[00034] In one embodiment, the FRDA promoter is used with the AADC polynucleotides described herein.

[00035] In one embodiment, there is a region located approximately ~5 kb upstream of the first exon of the payload. As a non-limiting example, there is a 17 bp region located approximately 4.9 kb upstream of the first exon of the frataxin gene in order to allow for expression with the FRDA promoter (See e.g., Puspasari et al. *Long Range Regulation of Human FXN Gene Expression*, PLOS ONE, 2011; the contents of which is herein incorporated by reference in its entirety).

[00036] In one embodiment, the promoter may be a promoter which is less than 1 kb. The promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. The promoter may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800.

[00037] In one embodiment, the promoter may be a combination of two or more components such as, but not limited to, CMV and CBA. Each component may have a length of 200, 210,

220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. Each component may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800. As a non-limiting example, the promoter is a combination of a 382 nucleotide CMV-enhancer sequence and a 260 nucleotide CBA-promoter sequence.

[00038] In one embodiment, at least one element may be used with the AADC polynucleotides described herein to enhance the transgene target specificity and expression (See e.g., Powell et al. *Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy*, 2015; the contents of which are herein incorporated by reference in its entirety). Non-limiting examples of elements to enhance the transgene target specificity and expression include promoters, endogenous miRNAs, post-transcriptional regulatory elements (PREs), polyadenylation (PolyA) signal sequences and upstream enhancers (USEs), CMV enhancers and introns.

[00039] In one embodiment, at least one element may be used with the AADC polynucleotides described herein to enhance the transgene target specificity and expression (See e.g., Powell et al. *Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy*, 2015; the contents of which are herein incorporated by reference in its entirety) such as promoters. Promoters for which promote expression in most tissues include, but are not limited to, human elongation factor 1α-subunit (EF1α), immediate-early cytomegalovirus (CMV), chicken β-actin (CBA) and its derivative CAG, the β glucuronidase (GUSB), or ubiquitin C (UBC). Tissue-specific expression elements can be used to restrict expression to certain cell types such as, but not limited to, nervous system promoters which can be used to restrict expression to neurons, astrocytes, or oligodendrocytes. Non-limiting example of tissue-specific expression elements for neurons include neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF-β), the synapsin (Syn), the methyl-CpG binding protein 2 (MeCP2), CaMKII, mGluR2, NFL, NFH, nβ2, PPE, Enk and EAAT2 promoters. A non-limiting example of a tissue-specific expression elements for astrocytes include the glial fibrillary acidic protein (GFAP) and EAAT2 promoters. A non-limiting example of a tissue-specific expression element for oligodendrocytes include the myelin basic protein (MBP) promoter.

[00040] In one embodiment, a ubiquitous promoter may be used with the AADC polynucleotides described herein. Non-limiting examples of ubiquitous promoters include CMV, CBA (including derivatives CAG, CBh, etc.), EF-1α, PGK, UBC, GUSB (hGBp), and UCOE (promoter of HNRPA2B1-CBX3). Yu et al. (Molecular Pain 2011, 7:63; the contents of which are herein incorporated by reference in its entirety) evaluated the expression of eGFP under the CAG, EFIα, PGK and UBC promoters in rat DRG cells and primary DRG cells using lentiviral vectors and found that UBC showed weaker expression than the other 3 promoters and there was only 10-12% glia expression seen for all promoters. Soderblom et al. (E. Neuro 2015; the contents of which are herein incorporated by reference in its entirety) the expression of eGFP in AAV8 with CMV and UBC promoters and AAV2 with the CMV promoter after injection in the motor cortex. Intranasal administration of a plasmid containing a UBC or EFIα promoter showed a sustained airway expression greater than the expression with the CMV promoter (See e.g., Gill et al., Gene Therapy 2001, Vol. 8, 1539-1546; the contents of which are herein incorporated by reference in its entirety). Husain et al. (Gene Therapy 2009; the contents of which are herein incorporated by reference in its entirety) evaluated a HβH construct with a hGUSB promoter, a HSV-1LAT promoter and a NSE promoter and found that the HβH construct showed weaker expression than NSE in mice brain. Passini and Wolfe (J. Virol. 2001, 12382-12392, the contents of which are herein incorporated by reference in its entirety) evaluated the long term effects of the HβH vector following an intraventricular injection in neonatal mice and found that there was sustained expression for at least 1 year. Low expression in all brain regions was found by Xu et al. (Gene Therapy 2001, 8, 1323-1332; the contents of which are herein incorporated by reference in its entirety) when NF-L and NF-H promoters were used as compared to the CMV-lacZ, CMV-luc, EF, GFAP, hENK, nAChR, PPE, PPE + wpre, NSE (0.3 kb), NSE (1.8 kb) and NSE (1.8 kb + wpre). Xu et al. found that the promoter activity in descending order was NSE (1.8 kb), EF, NSE (0.3 kb), GFAP, CMV, hENK, PPE, NFL and NFH. NFL is a 650 nucleotide promoter and NFH is a 920 nucleotide promoter which are both absent in the liver but NFH is abundant in the sensory proprioceptive neurons, brain and spinal cord and NFH is present in the heart. Scn8a is a 470 nucleotide promoter which expresses throughout the DRG, spinal cord and brain with particularly high expression seen in the hippocampal neurons and cerebellar Purkinje cells, cortex, thalamus and hypothalamus (See e.g., Drews et al. 2007 and Raymond et al. 2004; the contents of each of which are herein incorporated by reference in their entireties).

[00041] In one embodiment, an UBC promoter may be used with the AADC polynucleotides described herein. The UBC promoter may have a size of 300-350 nucleotides. As a non-limiting example, the UBC promoter is 332 nucleotides.

[00042] In one embodiment, a GUSB promoter may be used with the AADC polynucleotides described herein. The GUSB promoter may have a size of 350-400 nucleotides. As a non-limiting example, the GUSB promoter is 378 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-hFXN-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00043] In one embodiment, a NFL promoter may be used with the AADC polynucleotides described herein. The NFL promoter may have a size of 600-700 nucleotides. As a non-limiting example, the NFL promoter is 650 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-hFXN-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00044] In one embodiment, a NFH promoter may be used with the AADC polynucleotides described herein. The NFH promoter may have a size of 900-950 nucleotides. As a non-limiting example, the NFH promoter is 920 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-hFXN-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00045] In one embodiment, a scn8a promoter may be used with the AADC polynucleotides described herein. The scn8a promoter may have a size of 450-500 nucleotides. As a non-limiting example, the scn8a promoter is 470 nucleotides. As a non-limiting example, the construct may be AAV-promoter-CMV/globin intron-hFXN-RBG, where the AAV may be self-complementary and the AAV may be the DJ serotype.

[00046] In one embodiment, a FXN promoter may be used with the AADC polynucleotides described herein.

[00047] In one embodiment, a PGK promoter may be used with the AADC polynucleotides described herein.

[00048] In one embodiment, a CBA promote may be used with the AADC polynucleotides described herein.

[00049] In one embodiment, a CMV promoter may be used with the AADC polynucleotides described herein.

[00050] In one embodiment, a liver or a skeletal muscle promoter may be used with the AADC polynucleotides described herein. Non-limiting examples of liver promoters include

hAAT and TBG. Non-limiting examples of skeletal muscle promoters include Desmin, MCK and C5-12.

[00051] In one embodiment, an enhancer element, a promoter and/or a 5'UTR intron may be used with the AADC polynucleotides described herein. The enhancer may be, but is not limited to, a CMV enhancer, the promoter may be, but is not limited to, a CMV, CBA, UBC, GUSB, NSE, Sunapsin, MeCP2, and GFAP promoter and the 5'UTR/intron may be, but is not limited to, SV40, and CBA-MVM. As a non-limiting example, the enhancer, promoter and/or intron used in combination may be: (1) CMV enhancer, CMV promoter, SV40 5'UTR intron; (2) CMV enhancer, CBA promoter, SV 40 5'UTR intron; (3) CMV enhancer, CBA promoter, CBA-MVM 5'UTR intron; (4) UBC promoter; (5) GUSB promoter; (6) NSE promoter; (7) Synapsin promoter; (8) MeCP2 promoter and (9) GFAP promoter.

[00052] In one embodiment, an engineered promoter may be used with the AADC polynucleotides described herein.

Introns

[00053] In one embodiment, at least one element may be used with the AADC polynucleotides described herein to enhance the transgene target specificity and expression (See e.g., Powell et al. *Viral Expression Cassette Elements to Enhance Transgene Target Specificity and Expression in Gene Therapy*, 2015; the contents of which are herein incorporated by reference in its entirety) such as an intron. Non-limiting examples of introns include, MVM (67-97 bps), F.IX truncated intron 1 (300 bps), β-globin SD/immunoglobulin heavy chain splice acceptor (250 bps), adenovirus splice donor/immunoglobin splice acceptor (500 bps), SV40 late splice donor/splice acceptor (19S/16S) (180 bps) and hybrid adenovirus splice donor/IgG splice acceptor (230 bps).

[00054] In one embodiment, the intron may be 100-500 nucleotides in length. The intron may have a length of 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490 or 500. The intron may have a length between 80-100, 80-120, 80-140, 80-160, 80-180, 80-200, 80-250, 80-300, 80-350, 80-400, 80-450, 80-500, 200-300, 200-400, 200-500, 300-400, 300-500, or 400-500.

Introduction into cells

[00055] The AADC polynucleotides of the invention can be introduced into host cells using any of a variety of approaches. Infection with a viral vector comprising the AADC

polynucleotide can be effected. Examples of suitable viral vectors include replication defective retroviral vectors, adenoviral vectors, adeno-associated vectors and lentiviral vectors.

[00056] According to the present invention, viral vectors for use in therapeutics and/or diagnostics comprise a virus that has been distilled or reduced to the minimum components necessary for transduction of a nucleic acid payload or cargo of interest.

[00057] In this manner, viral vectors are engineered as vehicles for specific delivery while lacking the deleterious replication and/or integration features found in wild-type virus.

[00058] As used herein, a "vector" is any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule such as the polynucleotides of the invention. A "viral vector" is a vector which comprises one or more polynucleotide regions encoding or comprising payload molecule of interest, e.g., a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide. Viral vectors of the present invention may be produced recombinantly and may be based on adeno-associated virus (AAV) parent or reference sequence. Serotypes which may be useful in the present invention include any of those arising from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ, and AAV-DJ8.

[00059] In one embodiment, the serotype which may be useful in the present invention may be AAV-DJ8. The amino acid sequence of AAV-DJ8 may comprise two or more mutations in order to remove the heparin binding domain (HBD). As a non-limiting example, the AAV-DJ sequence described as SEQ ID NO: 1 in US Patent No. 7,588,772, the contents of which are herein incorporated by reference in its entirety, may comprise two mutations: (1) R587Q where arginine (R; arg) at amino acid 587 is changed to glutamine (Q; gln) and (2) R590T where arginine (R; arg) at amino acid 590 is changed to threonine (T; thr). As another non-limiting example, may comprise three mutations: (1) K406R where lysine (K; lys) at amino acid 406 is changed to arginine (R; arg), (2) R587Q where arginine (R; arg) at amino acid 587 is changed to glutamine (Q; gln) and (3) R590T where arginine (R; arg) at amino acid 590 is changed to threonine (T; thr).

[00060] AAV vectors may also comprise self-complementary AAV vectors (scAAVs). scAAV vectors contain both DNA strands which anneal together to form double stranded DNA. By skipping second strand synthesis, scAAVs allow for rapid expression in the cell.

Pharmaceutical compositions

[00061] Although the descriptions of pharmaceutical compositions, e.g., those polynucleotides (including the encoding plasmids or expression vectors, such as viruses, e.g., AAV) comprising a payload, e.g., AADC encoding sequences, to be delivered, provided herein

are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other animal, *e.g.,* to non-human animals, e.g. non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00062] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase "active ingredient" generally refers either to the viral vector carrying the payload or to the polynucleotide payload molecule delivered by a viral vector as described herein.

[00063] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00064] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered.

[00065] In one embodiment, the pharmaceutical composition comprises a recombinant adeno-associated virus (AAV) vector comprising an AAV capsid and an AAV vector genome. The AAV vector genome may comprise at least one AADC polynucleotide described herein, such as, but not limited to, SEQ ID NOs 6, 7, 8, 9, 17, 18, 19, 20, 21, 22, and 23 or variants having at least 95% identity thereto. The recombinant AAV vectors in the pharmaceutical composition may have at least 70% which contain an AAV vector genome.

Formulation

[00066] The AADC polynucleotides or viral vectors encoding them can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection or transduction; (3)

permit the sustained or delayed release; or (4) alter the biodistribution (e.g., target the viral vector to specific tissues or cell types).

[00067] Formulations of the present invention can include, without limitation, saline, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with viral vectors (e.g., for transplantation into a subject), nanoparticle mimics and combinations thereof. Further, the viral vectors of the present invention may be formulated using self-assembled nucleic acid nanoparticles.

[00068] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00069] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" refers to a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00070] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, e.g., between .5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

[00071] In some embodiments, the formulations described herein may contain at least one payload molecule, e.g., an AADC polynucleotide. As a non-limiting example, the formulations may contain 1, 2, 3, 4 or 5 AADC polynucleotide payload molecules.

[00072] In some embodiments, a pharmaceutically acceptable excipient may be at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use for humans and for veterinary use. In some embodiments, an excipient may be approved by United States Food and Drug Administration. In some embodiments, an excipient may be of pharmaceutical grade. In some embodiments, an excipient

may meet the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00073] Excipients, which, as used herein, includes, but is not limited to, any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, and the like, as suited to the particular dosage form desired. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference in its entirety). The use of a conventional excipient medium may be contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00074] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00075] In one embodiment, the AADC polynucleotides may be formulated in a hydrogel prior to administration. Hydrogels have a degree of flexibility which is similar to natural tissue as a result of their significant water content.

[00076] In another embodiment, a hydrogel may be administered to a subject prior to the administration of an AADC polynucleotide formulation. As a non-limiting example, the site of administration of the hydrogel may be within 3 inches (e.g., within 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2., 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1 or less than 0.1 inches) of the site of administration of the AADC polynucleotide formulation.

[00077] In one embodiment, the AADC polynucleotides may be administered in saline. As a non-limiting example, the formulation may be phosphate buffered saline (PBS) with 0.001% Pluronic acid (F-68). Additionally the formulation may be sterilized.

Inactive Ingredients

[00078] In some embodiments, AADC polynucleotide formulations may comprise at least one excipient which is an inactive ingredient. As used herein, the term "inactive ingredient" refers to one or more inactive agents included in formulations. In some embodiments, all, none or some of

the inactive ingredients which may be used in the formulations of the present invention may be approved by the US Food and Drug Administration (FDA).

[00079] Formulations of viral vectors carrying AADC polynucleotides disclosed herein may include cations or anions. In one embodiment, the formulations include metal cations such as, but not limited to, Zn2+, Ca2+, Cu2+, Mg+ and combinations thereof. As a non-limiting example, formulations may include polymers and AADC polynucleotides complexed with a metal cation (See e.g., U.S. Pat. Nos. 6,265,389 and 6,555,525, each of which is herein incorporated by reference in its entirety).

Administration

[00080] The viral vectors comprising AADC polynucleotides of the present invention may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to epidural (into the dura matter), oral (by way of the mouth), transdermal, peridural, intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intravenous bolus, intravenous drip, intraarterial (into an artery), intrathecal (into the spinal canal), endocervical, intracaudal (within the cauda equine), intracisternal (within the cisterna magna cerebellomedularis), intradiscal (within a disc), intradural (within or beneath the dura), intrameningeal (within the meninges), intrapleural (within the pleura), intraspinal (within the vertebral column), intrathecal (within the cerebrospinal fluid at any level of the cerebrospinal axis), intrathoracic (within the thorax), intrastriatal (within the striatum, caudate nucleus and/or putamen), caudal block, nerve block, or spinal. In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier. In one embodiment, a formulation for a route of administration may include at least one inactive ingredient.

[00081] In one embodiment, the viral vectors comprising AADC polynucleotides of the present invention may be administered to the right putamen and/or the left putamen. The administration may be at one or more sites in the putamen such as, but not limited to, 2 sites, 3 sites, 4 sites or more than 4 sites. As a non-limiting example, the viral vectors comprising AADC polynucleotides of the present invention are delivered to 2 sites in the left putamen and 2 sites in the right putamen.

[00082] In one embodiment, the administration of the formulation of the viral vectors comprising the AADC polynucleotides of the present invention to a subject provides coverage of the putamen of a subject (e.g., the left and/or right putamen). In one aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide at least 8%, 9%, 10%,

13%, 14%, 15%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more than 95% to the left and/or right putamen of a subject. As a non-limiting example, the coverage is at least 20%. As a non-limiting example, the coverage is at least 40%. In another aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide at least 8%, 9%, 10%, 13%, 14%, 15%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more than 95% coverage of the surface area of the left and/or right putamen of a subject. As a non-limiting example, the total coverage is at least 20%. As a non-limiting example, the total coverage is at least 40%. In yet another aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide 10-40%, 20-40%, 20-30%, 20-35%, 20-50%, 30-40%, 35-40%, 30-60%, 40-70%, 50-80% or 60-90% coverage to the left and/or right putamen of a subject or to the total surface area of the left and/or right putamen of a subject.

[00083] In one embodiment, the administration of the formulation of the viral vectors comprising the AADC polynucleotides of the present invention to a subject provides coverage of the posterior putamen of a subject (e.g., the left and/or right posterior putamen). In one aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide at least 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more than 95% to the left and/or right posterior putamen of a subject. As a non-limiting example, the coverage is at least 20%. As a non-limiting example, the coverage is at least 40%. In another aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide at least 10%, 15%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more than 95% coverage of the surface area of the left and/or right posterior putamen of a subject. As a non-limiting example, the total coverage is at least 20%. As a non-limiting example, the total coverage is at least 40%. In yet another aspect, the administration of the viral vectors comprising the AADC polynucleotides may provide 10-40%, 20-50%, 30-60%, 40-70%,

50-80% or 60-90% coverage to the left and/or right posterior putamen of a subject or to the total surface area of the left and/or right putamen of a subject.

[00084] In one embodiment, a subject may be administered the viral-vectors comprising AADC polynucleotides of the present invention safely delivered to substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) via bilateral infusions, or alternatively, intrastriatally (into the caudate nucleus and putamen), or into the subthalamic nucleus (STN).

[00085] In one embodiment, the AADC polynucleotides described herein may be administered using acute bilateral placement of catheters into each putamen. The placement may use magnetic resonance image (MRI)-guided stereotactic neurosurgical techniques known in the art or described herein. Additionally, a contrast agent such as, but not limited to a gadolinium based contrast agent (e.g., PROHANCE®) may be used in the formulation to monitor and confirm the distribution of the formulation.

[00086] In one embodiment, a subject may be administered the viral vectors comprising AADC polynucleotides of the present invention in a bilateral stereotactic CED-assisted step infusion into the putamen (e.g., the post commissural putamen).

[00087] In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) comprises a rate of delivery defined by [VG/hour = mL/hour * VG/mL] wherein VG is viral genomes, VG/mL is composition concentration, and mL/hour is rate of prolonged infusion.

[00088] In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) comprises infusion of up to 1 mL. In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) may comprise infusion of 0.001, 0.002, 0.003, 0.004, 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.040, 0.050, 0.060, 0.070, 0.080, 0.090, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 mL.

[00089] In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) comprises infusion of between about 1 mL to about 120 mL. In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) may comprise infusion of 0.1, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77,

78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120 mL. In one embodiment, delivery of AAV particles to cells of the central nervous system (e.g., parenchyma) comprises infusion of at least 3 mL. In one embodiment, delivery of AAV particles to cells of the central nervous system (e.g., parenchyma) consists of infusion of 3 mL. In one embodiment, delivery of AAV particles to cells of the central nervous system (e.g., parenchyma) comprises infusion of at least 10 mL. In one embodiment, delivery of AAV particles to cells of the central nervous system (e.g., parenchyma) consists of infusion of 10 mL.

[00090] In one embodiment, the volume of the viral vector pharmaceutical composition delivered to the cells of the central nervous system (e.g., parenchyma) of a subject is 50 ul, 100 ul, 200 ul, 300 ul, 400 ul, 500 ul, 600 ul, 700 ul, 800 ul, 900 ul, 1000 ul, 1100 ul, 1200 ul, 1300 ul, 1400 ul, 1500 ul, 1600 ul, 1700 ul, 1800 ul, 1900 ul, 2000 ul or more than 2000 ul.

[00091] In one embodiment, the volume of the viral vector pharmaceutical composition delivered to a region in both hemispheres of a subject brain is 50 ul, 100 ul, 200 ul, 300 ul, 400 ul, 500 ul, 600 ul, 700 ul, 800 ul, 900 ul, 1000 ul, 1100 ul, 1200 ul, 1300 ul, 1400 ul, 1500 ul, 1600 ul, 1700 ul, 1800 ul, 1900 ul, 2000 ul or more than 2000 ul. As a non-limiting example, the volume delivered to a region in both hemispheres is 200 ul. As another non-limiting example, the volume delivered to a region in both hemispheres is 900 ul. As yet another non-limiting example, the volume delivered to a region in both hemispheres is 1800 ul.

[00092] In one embodiment, the volume of the viral vector pharmaceutical composition delivered to the putamen in both hemispheres of a subject brain is 50 ul, 100 ul, 200 ul, 300 ul, 400 ul, 450 ul, 500 ul, 600 ul, 700 ul, 800 ul, 900 ul, 1000 ul, 1100 ul, 1200 ul, 1300 ul, 1400 ul, 1500 ul, 1600 ul, 1700 ul, 1800 ul, 1900 ul, 2000 ul or more than 2000 ul. As a non-limiting example, the volume delivered to the putamen in both hemispheres is 100 ul. As another non-limiting example, the volume delivered to the putamen in both hemispheres is 200 ul. As a non-limiting example, the volume delivered to the putamen in both hemispheres is 300 ul. As another non-limiting example, the volume delivered to the putamen in both hemispheres is 450 ul. As another non-limiting example, the volume delivered to the putamen in both hemispheres is 900 ul. As yet another non-limiting example, the volume delivered to the putamen both hemispheres is 1800 ul.

[00093] In one embodiment, the total volume delivered to a subject may be split between one or more administration sites e.g., 1, 2, 3, 4, 5 or more than 5 sites. As a non-limiting example, the total volume is split between administration to the left and right putamen. As another non-

limiting example, the total volume is split between two sites of administration to each of the left and right putamen.

[00094] In one embodiment, the viral vector pharmaceutical composition is administered using a fenestrated needle. Non-limiting examples of fenestrated needles are described in US Patent Nos. 8,333,734, 7,135,010, 7,575,572, 7,699,852, 4,411,657, 6,890,319, 6,613,026, 6,726,659, 6,565,572, 6,520,949, 6,382,212, 5,848,996, 5,759,179, 5 674,267, 5,588,960, 5,484,401, 5,199,441, 5,012,818, 4,474,569, 3,766,907, 3,552,394, the contents of each of which are herein incorporated by reference in its entirety.

[00095] In one embodiment, a composition comprises AADC polynucleotides described herein and the AADC polynucleotides are components of an AAV viral genome packaged in an AAV viral particle. The percent (%) ratio of AAV viral particles comprising the AADC polynucleotide (also referred to herein and AADC particles) to the AAV viral particles without the AADC polynucleotide (also referred to herein as empty capsids) in the composition may be 0:100, 1:99, 0: 90, 15:85, 25:75, 30:70, 50:50, 70:30, 85:15, 90:10, 99:1 or 100:0. As a non-limiting example, the percent ratio of AADC particles to empty capsids is 50:50. As another non-limiting example, the percent ratio of AADC particles to empty capsids is 70:30. As another non-limiting example, the percent ratio of AADC particles to empty capsids is 85:15. As another non-limiting example, the percent ratio of AADC particles to empty capsids is 100:0.

[00096] In one embodiment, the composition described herein comprises at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, 52, 53, 54, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or greater than 99% AADC particles. As a non-limiting example, the composition comprises at least 50% AADC particles. As another non-limiting example, the composition comprises at least 52% AADC particles. As another non-limiting example, the composition comprises at least 58% AADC particles. As another non-limiting example, the composition comprises at least 70% AADC particles. As another non-limiting example, the composition comprises at least 83% AADC particles. As another non-limiting example, the composition comprises at least 85% AADC particles. As another non-limiting example, the composition comprises at least 99% AADC particles. As another non-limiting example, the composition comprises 100% AADC particles.

[00097] In one embodiment, the composition described herein comprises 1-10%, 10-20%, 30-40%, 50-60%, 50-70%, 50-80%, 50-90%, 50-99%, 50-100%, 60-70%, 60-80%, 60-90%, 60-99%, 60-100%, 70-80%, 70-90%, 70-99%, 70-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-100%, 90-95%, 90-99%, or 90-100% AADC particles. As a non-limiting example, the composition described herein comprises 50-100% AADC particles. As another non-limiting

example, the composition described herein comprises 50-60% AADC particles. As another non-limiting example, the composition described herein comprises 80-99% AADC particles. As another non-limiting example, the composition described herein comprises 80-90% AADC particles. As a non-limiting example, the composition described herein comprises 80-95% AADC particles. As a non-limiting example, the composition described herein comprises 80-85% AADC particles.

[00098] In one embodiment, the composition described herein comprises less than 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, 52, 53, 54, 55, 60, 65, 70, 75, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% empty particles. As a non-limiting example, the composition comprises less than 50% empty particles. As a non-limiting example, the composition comprises less than 45% empty particles. As a non-limiting example, the composition comprises less than 40% empty particles. As a non-limiting example, the composition comprises less than 35% empty particles. As a non-limiting example, the composition comprises less than 30% empty particles. As a non-limiting example, the composition comprises less than 25% empty particles. As a non-limiting example, the composition comprises less than 20% empty particles. As a non-limiting example, the composition comprises less than 15% empty particles. As a non-limiting example, the composition comprises less than 10% empty particles. As a non-limiting example, the composition comprises less than 5% empty particles. As a non-limiting example, the composition comprises less than 1% empty particles.

[00099] In the composition described herein comprises 1-10%, 10-20%, 30-40%, 50-60%, 50-70%, 50-80%, 50-90%, 50-99%, 50-100%, 60-70%, 60-80%, 60-90%, 60-99%, 60-100%, 70-80%, 70-90%, 70-99%, 70-100%, 80-85%, 80-90%, 80-95%, 80-99%, 80-100%, 90-95%, 90-99%, or 90-100% empty particles. As a non-limiting example, the composition described herein comprises 30-40% empty particles. As another non-limiting example, the composition described herein comprises 30-50% empty particles. As another non-limiting example, the composition described herein comprises 30-60% empty particles. As another non-limiting example, the composition described herein comprises 30-70% empty particles. As a non-limiting example, the composition described herein comprises 30-80% empty particles. As a non-limiting example, the composition described herein comprises 30-90% empty particles.

[000100] In one embodiment, the AADC polynucleotides described herein may be administered to a subject who is also undergoing levodopa therapy. As a non-limiting example, the subject may have a positive response to levodopa therapy and at least one symptom of PD is reduced. As another non-limiting example, the subject may have a response to levodopa therapy

where the symptoms of PD experienced by the subject are stable. As yet another non-limiting example, the subject may have a negative response to levodopa therapy where the symptoms of PD experienced by the subject are increasing.

[000101] In one embodiment, the dose of levodopa administered to the subject prior to the AADC polynucleotides is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more than 25 mg/kg. As a non-limiting example, the dose is 3 mg/kg. As another non-limiting example, the dose is 10 mg/kg. As yet another non-limiting example, the dose is 20 mg/kg. The subject's response (e.g., behavioral response) to levodopa may be assessed prior to administration of the AADC polynucleotides. Additionally, the subject may be administered levodopa again after the administration of the AADC polynucleotides (e.g., 1 week, 2, weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year or more than 1 year after the administration of AADC polynucleotides). The behavioral response can be re-assessed and compared to the initial response to determine the effects of the AADC polynucleotides. The subject may have 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100% behavioral improvement.

[000102] In one embodiment, Levodopa may be administered multiple times after the administration of the AADC polynucleotides. Levodopa may be administered on a repeating schedule (e.g., every 5 days, weekly, every 10 days, every 15 days, every 30 days, monthly, bimonthly, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months or yearly) or as symptoms arise. As a non-limiting example, 3 years post administration of AADC polynucleotides a subject may have 1-10%, 5-15%, 10-20%, 15-30%, 20-40%, 25-50%, 30-50%, 40-50%, 40-60%, 50-70%, 50-80%, 60-70%, 60-75%, 60-80%, 60-90%, 70-80%, 70-90%, 75-90%, 80-90%, 90-100% of the striatal neurons within the infused region of the putamen to be AADC-immunoreactive. As a non-limiting example, 6 years post administration of AADC polynucleotides a subject may have 1-10%, 5-15%, 10-20%, 15-30%, 20-40%, 25-50%, 30-50%, 40-50%, 40-60%, 50-70%, 50-80%, 60-70%, 60-75%, 60-80%, 60-90%, 70-80%, 70-90%, 75-90%, 80-90%, 90-100% of the striatal neurons within the infused region of the putamen to be AADC-immunoreactive. As a non-limiting example, 9 years post administration of AADC polynucleotides a subject may have 1-10%, 5-15%, 10-20%, 15-30%, 20-40%, 25-50%, 30-50%, 40-50%, 40-60%, 50-70%, 50-80%, 60-70%, 60-75%, 60-80%, 60-90%, 70-80%, 70-90%, 75-90%, 80-90%, 90-100% of the striatal neurons within the infused region of the putamen to be AADC-immunoreactive.

[000103] In one embodiment, a subject who may be administered the AADC polynucleotides described herein have a documented response to levodopa therapy but have medically refractory fluctuations and are considered good surgical candidates. The determination if a subject is a good surgical candidate may be made by the physician treating the subject for PD or the physician administering the AADC polynucleotides who takes into consideration the overall risk to benefit ratio for the patient for the surgical intervention required for delivery of the AADC polynucleotides.

[000104] In one embodiment, the ratio of distribution volume in the parenchyma of an area of a subject to the infusion volume of an area of a subject may be 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0 or more than 6.0. As a non-limiting example, the ratio of distribution volume in the parenchyma to infusion volume was 1.6 in the caudate nucleus. As a non-limiting example, the ratio of distribution volume in the parenchyma to infusion volume was 3.1 in the putamen. As a non-limiting example, the distribution of the AADC polynucleotides in the putamen may be 2-3 times the volume infused.

Dosing

[000105] The present invention provides methods comprising administering viral vectors and their AADC polynucleotide payload or complexes in accordance with the invention to a subject in need thereof. Viral vector pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition (*e.g.*, a disease, disorder, and/or condition relating to working memory deficits). The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in unit dosage form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the

specific polynucleotide payload employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[000106] In certain embodiments, viral vector pharmaceutical compositions in accordance with the present invention may be administered at AADC polynucleotide dosage levels sufficient to deliver from about 0.0001 mg/kg to about 100 mg/kg, from about 0.001 mg/kg to about 0.05 mg/kg, from about 0.005 mg/kg to about 0.05 mg/kg, from about 0.001 mg/kg to about 0.005 mg/kg, from about 0.05 mg/kg to about 0.5 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, or from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. The desired AADC polynucleotide dosage may be delivered three times in a single day, two times in a single day, once in a single day or in a period of 24 hours the dosage may be delivered once, twice, three times or more than three times. In certain embodiments, the desired AADC polynucleotide dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. As used herein, a "split dose" is the division of single unit dose or total dose into two or more doses, e.g., two or more administrations of the single unit dose. As used herein, a "single unit dose" is a dose of any polynucleotide therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose. In one embodiment, the viral vectors comprising the AADC polynucleotides of the present invention are administered to a subject in split doses. They may be formulated in buffer only or in a formulation described herein.

[000107] In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) may comprise a total concentration between about $1x10^6$ VG/mL and about $1x10^{16}$ VG/mL. In some embodiments, delivery may comprise a composition concentration of about $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$, $8x10^6$, $9x10^6$, $1x10^7$, $2x10^7$, $3x10^7$, $4x10^7$, $5x10^7$, $6x10^7$, $7x10^7$, $8x10^7$, $9x10^7$, $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, $9x10^{10}$, $1x10^{11}$, $1.8x10^{11}$, $2x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $5.5x10^{11}$,

$6x10^{11}$, $7x10^{11}$, $8x10^{11}$, $9x10^{11}$, $0.8x10^{12}$, $0.83x10^{12}$, $1x10^{12}$, $1.1x10^{12}$, $1.2x10^{12}$, $1.3x10^{12}$, $1.4x10^{12}$, $1.5x10^{12}$, $1.6x10^{12}$, $1.7x10^{12}$, $1.8x10^{12}$, $1.9x10^{12}$, $2x10^{12}$, $2.1x10^{12}$, $2.2x10^{12}$, $2.3x10^{12}$, $2.4x10^{12}$, $2.5x10^{12}$, $2.6x10^{12}$, $2.7x10^{12}$, $2.8x10^{12}$, $2.9x10^{12}$, $3x10^{12}$, $3.1x10^{12}$, $3.2x10^{12}$, $3.3x10^{12}$, $3.4x10^{12}$, $3.5x10^{12}$, $3.6x10^{12}$, $3.7x10^{12}$, $3.8x10^{12}$, $3.9x10^{12}$, $4x10^{12}$, $4.1x10^{12}$, $4.2x10^{12}$, $4.3x10^{12}$, $4.4x10^{12}$, $4.5x10^{12}$, $4.6x10^{12}$, $4.7x10^{12}$, $4.8x10^{12}$, $4.9x10^{12}$, $5x10^{12}$, $6x10^{12}$, $7x10^{12}$, $8x10^{12}$, $9x10^{12}$, $1x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $7x10^{13}$, $8x10^{13}$, $9x10^{13}$, $1x10^{14}$, $2x10^{14}$, $3x10^{14}$, $4x10^{14}$, $5x10^{14}$, $6x10^{14}$, $7x10^{14}$, $8x10^{14}$, $9x10^{14}$, $1x10^{15}$, $2x10^{15}$, $3x10^{15}$, $4x10^{15}$, $5x10^{15}$, $6x10^{15}$, $7x10^{15}$, $8x10^{15}$, $9x10^{15}$, or $1x10^{16}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $1x10^{13}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $3x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $1.1x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $3.7x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $8x10^{11}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $2.6x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $4.9x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $0.8x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is $0.83x10^{12}$ VG/mL. In one embodiment, the concentration of the viral vector in the composition is the maximum final dose which can be contained in a vial.

[000108] In one embodiment, delivery of viral vector pharmaceutical compositions in accordance with the present invention to cells of the central nervous system (e.g., parenchyma) may comprise a total concentration per subject between about $1x10^6$ VG and about $1x10^{16}$ VG. In some embodiments, delivery may comprise a composition concentration of about $1x10^6$, $2x10^6$, $3x10^6$, $4x10^6$, $5x10^6$, $6x10^6$, $7x10^6$, $8x10^6$, $9x10^6$, $1x10^7$, $2x10^7$, $3x10^7$, $4x10^7$, $5x10^7$, $6x10^7$, $7x10^7$, $8x10^7$, $9x10^7$, $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $1.5x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, $5x10^{10}$, $6x10^{10}$, $7x10^{10}$, $8x10^{10}$, $9x10^{10}$, $1x10^{11}$, $1.3x10^{11}$, $2x10^{11}$, $2.1x10^{11}$, $2.2x10^{11}$, $2.3x10^{11}$, $2.4x10^{11}$, $2.5x10^{11}$, $2.6x10^{11}$, $2.7x10^{11}$, $2.8x10^{11}$, $2.9x10^{11}$, $3x10^{11}$, $4x10^{11}$, $5x10^{11}$, $5.4x10^{11}$, $6x10^{11}$, $7x10^{11}$, $7.1x10^{11}$, $7.2x10^{11}$, $7.3x10^{11}$, $7.4x10^{11}$, $7.5x10^{11}$, $7.6x10^{11}$, $7.7x10^{11}$, $7.8x10^{11}$, $7.9x10^{11}$, $8x10^{11}$, $9x10^{11}$, $9.4x10^{11}$, $1x10^{12}$, $1.1\ x10^{12}$, $1.2x10^{12}$, $1.3x10^{12}$, $1.4x10^{12}$, $1.5x10^{12}$, $1.6x10^{12}$, $1.7x10^{12}$, $1.8x10^{12}$, $1.9x10^{12}$, $2x10^{12}$, $2.3x10^{12}$, $2.4x10^{12}$, $3x10^{12}$, $4x10^{12}$, $4.1x10^{12}$, $4.2x10^{12}$, $4.3x10^{12}$, $4.4x10^{12}$, $4.5x10^{12}$,$4.6x10^{12}$, $4.7x10^{12}$, $4.8x10^{12}$, $4.9x10^{12}$, $5x10^{12}$, $6x10^{12}$, $7x10^{12}$, $8x10^{12}$, $8.1x10^{12}$, $8.2x10^{12}$, $8.3x10^{12}$, $8.4x10^{12}$, $8.5x10^{12}$, $8.6x10^{12}$, $8.7x10^{12}$, $8.8\ x10^{12}$, $8.9x10^{12}$, $9x10^{12}$, $1x10^{13}$, $2x10^{13}$, $3x10^{13}$, $4x10^{13}$, $5x10^{13}$, $6x10^{13}$, $7x10^{13}$, $8x10^{13}$, $9x10^{13}$, $1x10^{14}$, $2x10^{14}$, $3x10^{14}$, $4x10^{14}$, $5x10^{14}$, $6x10^{14}$, $7x10^{14}$, $8x10^{14}$, $9x10^{14}$, $1x10^{15}$, $2x10^{15}$, $3x10^{15}$,

$4x10^{15}$, $5x10^{15}$, $6x10^{15}$, $7x10^{15}$, $8x10^{15}$, $9x10^{15}$, or $1x10^{16}$ VG/subject. In one embodiment, the concentration of the viral vector in the composition is $1x10^{13}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $3x10^{12}$ VG/ subject. As a non-limiting example, the composition administered to the subject has a concentration of about $3x10^{11}$ VG/subject. As a non-limiting example, the composition administered to the subject has a concentration of about $9x10^{11}$ VG/subject. In one embodiment, the concentration of the viral vector in the composition is $2.3x10^{11}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $7.2x10^{11}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $7.5x10^{11}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $1.4x10^{12}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $4.8x10^{12}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $8.8x10^{12}$ VG/ subject. In one embodiment, the concentration of the viral vector in the composition is $2.3x10^{12}$ VG/ subject.

[000109] In one embodiment, the effectiveness of the dose, route of administration and/or volume of administration may be evaluated using various methods described herein such as, but not limited to, PET imaging, L-DOPA challenge test (e.g., see Forsayeth et al. 2006, Mol. Ther. 14(4): 571-577), UPDRS scores and patient diaries. As a non-limiting example, a subject may have decreased dyskinesia or periods of decreased dyskinesia after administration of the AADC polynucleotide composition. As another non-limiting example, a subject may have a decrease in Parkinson's Disease related symptoms including limited mobility and dyskinesia. As yet another non-limiting example, a subject may show improvement in off time and motor fluctuations. The improvement may be at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or greater than 90%. The improvement may last for minutes (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 , 15, 20, 25, 30, 35, 40, 45, 50, 55 or more than 55), hours (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or more than 24), days (e.g., 1, 2, 3, 4, 5, 6 or more than 7), weeks (1, 2, 3, 4, 5, 6, 7 or more than 7), months (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or more than 11) or years (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or more than 9).

[000110] In one embodiment, the selection of subjects for administration of the viral vectors described herein and/or the effectiveness of the dose, route of administration and/or volume of administration may be evaluated using imaging of the perivascular spaces (PVS) which are also known as Virchow-Robin spaces. PVS surround the arterioles and venules as they perforate brain parenchyma and are filled with cerebrospinal fluid (CSF)/interstitial fluid. PVS are

common in the midbrain, BG, and centrum semiovale. While not wishing to be bound by theory, PVS may play a role in the normal clearance of metabolites and have been associated with worse cognition and several disease states including Parkinson's disease. PVS are usually normal in size but they can increase in size in a number of disease states. Potter et al. (Cerebrovasc Dis. 2015 Jan; 39(4): 224–231; the contents of which are herein incorporated by reference in its entirety) developed a grading method where they studied a full range of PVS and rated basal ganglia, centrum semiovale and midbrain PVS. They used the frequency and range of PVS used by Mac and Lullich et al. (J Neurol Neurosurg Psychiatry. 2004 Nov;75(11):1519-23; the contents of which are herein incorporated by reference in its entirety) and Potter et al. gave 5 ratings to basal ganglia and centrum semiovale PVS: 0 (none), 1 (1-10), 2 (11-20), 3 (21-40) and 4 (>40) and 2 ratings to midbrain PVS: 0 (non visible) or 1 (visible). The user guide for the rating system by Potter et al. can be found at: www.sbirc.ed.ac.uk/documents/epvs-rating-scale-user-guide.pdf.

[000111] In one embodiment, the selection of subjects for administration of the viral vectors described herein and/or the effectiveness of the dose, route of administration and/or volume of administration may be evaluated using positron emission tomography (PET) measurements of neuroimaging biomarkers such as, but not limited to [^{18}F]FDOPA. Neuroimaging biomarkers such as [^{18}F]FDOPA may be used to identify affected individuals and/or may be used to detect a nigrostriatal defect prior to the onset of clinical manifestations. Further, PET-based criteria may be used to categorize subjects based on their nigrostriatal neuronal integrity (e.g., abnormal, normal or uncertain nigrostriatal neuronal integrity) (Rachette et al. Am J Med Genet B Neuropsychiatr Genet. 2006 Apr 5; 141B(3): 245–249; the contents of which are herein incorporated by reference in its entirety).

[000112] In one embodiment, a subject who may be administered a dose of the AADC polynucleotides described herein may have advanced PD and still respond to levodopa therapy but the subject also experiences medically refractory motor complications (e.g., sever motor fluctuations and/or dyskinesias that occur during levodopa and other dopaminergic therapies despite adjustments in and optimization of medication). The subject may be healthy enough to undergo a neurosurgical procedure which may be determined by methods known in the art. As a non-limiting example, the subject may meet the selection criteria for deep brain stimulation (DBS). The subject may have idiopathic PD, younger than 69 years of age, have pronounced responses to levodopa, have medication-refractory symptoms (e.g., motor fluctuation and/or dyskinesia) and/or have little or no cognitive dysfunction.

[000113] In one embodiment, a subject who may be administered a dose of the AADC polynucleotides described herein may also suffer from dementia or cognitive impairment.

[000114] In one embodiment, a subject who may be administered a dose of the AADC polynucleotides described herein may have been previously treated with the same or similar therapeutic. In another embodiment, a subject may have been treated with a therapeutic which has been shown to reduce the symptoms of Parkinson's Disease.

[000115] In one embodiment, a subject who may be administered a dose of the AADC polynucleotides described herein may have failed to derive adequate benefit from standard medical therapy. As a non-limiting example, the subject may not have responded to treatment. As another non-limiting example, a subject may have residual disability despite treatment.

[000116] In one embodiment, a subject who may be administered a dose of the AADC polynucleotides described herein may undergo testing to evaluate the levels of neurotransmitter analytes to determine the effectiveness of the dose. As a non-limiting example, CSF neurotransmitters, plasma AADC activity and/or urine VLA may be analyzed.

[000117] In one embodiment, a subject who may be administered a dose of the AADC polynucleotide described herein may be videotaped or recorded in order to monitor the progress of the subject during the course of treatment.

Combinations

[000118] The viral vectors comprising the AADC polynucleotide may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

Delivery

[000119] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for the delivery of AAV virions described in European Patent Application No. EP1857552, the contents of which are herein incorporated by reference in its entirety.

[000120] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering proteins using AAV vectors described in European Patent Application No. EP2678433, the contents of which are herein incorporated by reference in its entirety.

[000121] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering DNA molecules using AAV vectors described in US Patent No. US 5858351, the contents of which are herein incorporated by reference in its entirety.

[000122] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering DNA to the bloodstream described in US Patent No. US 6211163, the contents of which are herein incorporated by reference in its entirety.

[000123] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering AAV virions described in US Patent No. US 6325998, the contents of which are herein incorporated by reference in its entirety.

[000124] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering a payload to the central nervous system described in US Patent No. US 7588757, the contents of which are herein incorporated by reference in its entirety.

[000125] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering a payload described in US Patent No. US 8283151, the contents of which are herein incorporated by reference in its entirety.

[000126] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering a payload using a glutamic acid decarboxylase (GAD) delivery vector described in International Patent Publication No. WO2001089583, the contents of which are herein incorporated by reference in its entirety.

[000127] In one embodiment, the viral vector comprising an AADC polynucleotide may be administered or delivered using the methods for delivering a payload to neural cells described in International Patent Publication No. WO2012057363, the contents of which are herein incorporated by reference in its entirety.

[000128] The pharmaceutical compositions of viral vectors described herein may be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

Bioavailability

[000129] Viral vectors comprising an AADC polynucleotide of the present invention, when formulated into compositions with delivery/formulation agents or vehicles as described herein, may exhibit increased bioavailability as compared to compositions lacking delivery agents as described herein. As used herein, the term "bioavailability" refers to the systemic availability of a given amount of a particular agent administered to a subject. Bioavailability may be assessed by measuring the area under the curve (AUC) or the maximum serum or plasma concentration (C_{max}) of the unchanged form of a compound following administration of the compound to a mammal. AUC is a determination of the area under the curve plotting the serum or plasma concentration of a compound along the ordinate (Y-axis) against time along the abscissa (X-axis). Generally, the AUC for a particular compound may be calculated using methods known to those of ordinary skill in the art and as described in G. S. Banker, Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences, v. 72, Marcel Dekker, New York, Inc., 1996, the contents of which are herein incorporated by reference in their entirety.

[000130] C_{max} values are maximum concentrations of compounds achieved in serum or plasma of a subject following administration of compounds to the subject. C_{max} values of particular compounds may be measured using methods known to those of ordinary skill in the art. As used herein, the phrases "increasing bioavailability" or "improving the pharmacokinetics," refer to actions that may increase the systemic availability of a viral vector of the present invention (as measured by AUC, C_{max}, or C_{min}) in a subject. In some embodiments, such actions may comprise co-administration with one or more delivery agents as described herein. In some embodiments, the bioavailability of viral vectors may increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or about 100%.

Therapeutic window

[000131] Viral vectors comprising an AADC polynucleotide of the present invention, when formulated with one or more delivery agents as described herein, may exhibit increases in the therapeutic window of compound and/or composition administration as compared to the therapeutic window of viral vectors administered without one or more delivery agents as described herein. As used herein, the term "therapeutic window" refers to the range of plasma concentrations, or the range of levels of therapeutically active substance at the site of action, with a high probability of eliciting a therapeutic effect. In some embodiments, therapeutic windows of viral vectors when administered in a formulation may increase by at least about 2%, at least

about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or about 100%.

Volume of distribution

[000132] Viral vectors comprising an AADC polynucleotide of the present invention, when formulated with one or more delivery agents as described herein, may exhibit an improved volume of distribution (V_{dist}), e.g., reduced or targeted, relative to formulations lacking one or more delivery agents as described herein. V_{dist} relates the amount of an agent in the body to the concentration of the same agent in the blood or plasma. As used herein, the term "volume of distribution" refers to the fluid volume that would be required to contain the total amount of an agent in the body at the same concentration as in the blood or plasma: V_{dist} equals the amount of an agent in the body/concentration of the agent in blood or plasma. For example, for a 10 mg dose of a given agent and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which an agent is present in the extravascular tissue. Large volumes of distribution reflect the tendency of agents to bind to the tissue components as compared with plasma proteins. In clinical settings, V_{dist} may be used to determine loading doses to achieve steady state concentrations. In some embodiments, volumes of distribution of viral vector compositions of the present invention when co-administered with one or more delivery agents as described herein may decrease at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%.

Kits and devices

[000133] The invention provides a variety of kits for conveniently and/or effectively carrying out methods of the present invention. Typically kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[000134] Any of the AADC vectors, AADC constructs, AADC polynucleotides, or AADC polypeptides of the present invention may be comprised in a kit. In some embodiments, kits may further include reagents and/or instructions for creating and/or synthesizing compounds and/or compositions of the present invention. In some embodiments, kits may also include one or more buffers. In some embodiments, kits of the invention may include components for making protein or nucleic acid arrays or libraries and thus, may include, for example, solid supports.

[000135] In some embodiments, kit components may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one kit component, (labeling reagent and label may be packaged together), kits may also generally contain second, third or other additional containers into which additional components may be separately placed. In some embodiments, kits may also comprise second container means for containing sterile, pharmaceutically acceptable buffers and/or other diluents. In some embodiments, various combinations of components may be comprised in one or more vial. Kits of the present invention may also typically include means for containing compounds and/or compositions of the present invention, e.g., proteins, nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which desired vials are retained.

[000136] In some embodiments, kit components are provided in one and/or more liquid solutions. In some embodiments, liquid solutions are aqueous solutions, with sterile aqueous solutions being particularly preferred. In some embodiments, kit components may be provided as dried powder(s). When reagents and/or components are provided as dry powders, such powders may be reconstituted by the addition of suitable volumes of solvent. In some embodiments, it is envisioned that solvents may also be provided in another container means. In some embodiments, labeling dyes are provided as dried powders. In some embodiments, it is contemplated that 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000 micrograms or at least or at most those amounts of dried dye are provided in kits of the invention. In such embodiments, dye may then be resuspended in any suitable solvent, such as DMSO.

[000137] In some embodiments, kits may include instructions for employing kit components as well the use of any other reagent not included in the kit. Instructions may include variations that may be implemented.

Devices

[000138] In some embodiments, AADC compounds and/or AADC compositions of the present invention may be combined with, coated onto or embedded in a device. Devices may include, but are not limited to stents, pumps, and/or other implantable therapeutic device. Additionally AADC compounds and/or AADC compositions may be delivered to a subject while the subject is using a compression device such as, but not limited to, a compression device to reduce the chances of deep vein thrombosis (DVT) in a subject.

[000139] The present invention provides for devices which may incorporate viral vectors that encode one or more AADC polynucleotide payload molecules. These devices contain in a stable formulation the viral vectors which may be immediately delivered to a subject in need thereof, such as a human patient.

[000140] Devices for administration may be employed to deliver the viral vectors comprising an AADC polynucleotide of the present invention according to single, multi- or split-dosing regimens taught herein.

[000141] Method and devices known in the art for multi-administration to cells, organs and tissues are contemplated for use in conjunction with the methods and compositions disclosed herein as embodiments of the present invention. These include, for example, those methods and devices having multiple needles, hybrid devices employing for example lumens or catheters as well as devices utilizing heat, electric current or radiation driven mechanisms.

[000142] In some embodiments, AADC compounds and/or AADC compositions of the present invention may be delivered using a device such as, but not limited to, a stent, a tube, a catheter, a pipe, a straw, needle and/or a duct. Methods of using these devices are described herein and are known in the art.

[000143] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using delivery systems which integrate image guided therapy and integrate imaging such as, but not limited to, laser, MRgFUS, endoscopic and robotic surgery devices.

[000144] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the CLEARPOINT® neuro intervention system by MRI Interventions, Inc. The CLEARPOINT® neuro intervention system may be used alone or in combination with any of the other administration methods and devices described herein. The CLEARPOINT® neuro intervention system helps to provide stereotactic guidance in the placement and operation of instruments or devices during the planning and operation of neurological procedures.

[000145] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the NEUROMATE® stereotactic robot system by Renishaw PLC. The NEUROMATE® system may be used alone or in combination with any of the other administration methods and devices described herein. As a non-limiting example, the NEUROMATE® system may be used with head holders, CT image localizers, frame attachments, remote controls and software.

[000146] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the Elekta MICRODRIVE™ device by Elekta AB. The MICRODRIVE™ device may be used alone or in combination with any of the other administration methods and devices described herein. As a non-limiting example, the MICRODRIVE™ device may be used to position electrodes (e.g., for micro electrode recording (MER), macro stimulation and deep brain stimulation (DBS) electrode implantation), implantation of catheters, tubes or DBS electrodes using cross-hair and A-P holders to verify position, biopsies, injections and aspirations, brain lesioning, endoscope guidance and GAMMA KNIFE® radiosurgery.

[000147] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the AXIIIS® stereotactic miniframe by MONTERIS® Medical, Inc. The AXIIIS® stereotactic miniframe may be used alone or in combination with any of the other administration methods and devices described herein. The AXIIIS® stereotactic miniframe is a trajectory alignment device which may be used for laser coagulation, biopsies, catheter placement, electrode implant, endoscopy, and clot evacuation. The miniframe allows for 360 degree interface and provides access to multiple intracranial targets with a simple adjustment. Further, the miniframe is compatible with MRI.

[000148] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the INTEGRA™ CRW® system by Integra LifeSciences Corporation. The INTEGRA™ CRW® system may be used alone or in combination with any of the other administration methods and devices described herein. The CRW® system may be used for various applications such as, but not limited to, stereotactic surgery, microsurgery, catheterization and biopsy. The CRW® system is designed to provide accuracy to those who use the system (e.g., thumb lock screws, Vernier scaling, double bolt fixation, and a solid frame).

[000149] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using the EPOCH® solution system by Stereotaxis, Inc. which may include the NIOBE® ES magnetic navigation system, the VDRIVE® robotic navigation system and/or the ODYSSEY® information solution (all by Stereotaxis, Inc.). The EPOCH® solution system may be used alone or in combination with any of the other administration methods and devices described herein. As a non-limiting example, the NIOBE® ES magnetic navigation system may be used to accurately contact a subject. As another non-limiting example the NIOBE® ES magnetic system may be used with the VDRIVE® robotic navigation system to provide precise movement and stability.

[000150] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using a NeuroStation workstation which uses frameless stereotactic methods to provide image-guidance for applications such as, but not limited to, surgical planning, biopsies, craniotomies, endoscopy, intra-operative ultrasound and radiation therapy.

[000151] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using a robotic stereotaxis system such as, but not limited to the device described in US Patent No. 5,078,140, the contents of which are herein incorporated by reference in its entirety. The robotic arm of the device may be used to precisely orient the surgical tools or other implements used to conduct a procedure.

[000152] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject using an automatic delivery system such as, but not limited to the device described in US Patent No. 5,865,744, the contents of which are herein incorporated by reference in its entirety. Based on the images gathered by the delivery system, the computer adjusts the administration of the needle to be the appropriate depth for the particular subject.

[000153] In one embodiment, the AADC polynucleotides of the present invention may be administered to a subject who is simultaneously using during administration, and/or uses for a period of time before and/or after administration a compression device such as, but not limited to, a compression device which reduces the chances of deep vein thrombosis (DVT) in a subject. The compression device may be used for at least 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, or more than 8 hours before a subject is administered the AADC polynucleotides. The compression device may be used for at least 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks or a month after the AADC polynucleotides are administered. As a non-limiting example, the compression device is used simultaneously during the procedure of the delivery of the AADC polynucleotides. As another non-limiting example, the compression device is used before the administration of the AADC polynucleotides. As another non-limiting example, the compression device is used after administration of the AADC polynucleotides. As another non-limiting example, the compression device is used before, during and after administration of the AADC polynucleotides.

[000154] Non-limiting examples, of compression devices include ActiveCare +S.F.T. intermittent compression device, ActiveCare +S.F.T pneumatic compression device, DVTlite's

Venowave, KCI system compression pump, Aircast VenaFlow system, SCD Express Compression System or Bio Compression Systems, Inc. pneumatic compression therapy equipment (e.g., the pump may be selected from Model SC-2004, Model SC-2004-FC, Model SC-3004, Model SC-3004-FC, Model SC-2008, Model SC-2008-DL, Model SC-3008-T, the BioCryo system, Model IC-BAP-DL or multi-flo DVT combo IC_1545-DL and the garment used with the pump may be a 4 chamber, 8 chamber, BioCryo, Multi-Flo or BioArterial garment).

CNS diseases

[000155] The polynucleotides of the present invention may be used in the treatment, prophylaxis or amelioration of any disease or disorder characterized by aberrant or undesired target expression. In one embodiment, the invention relates to compositions, particularly nucleic acid molecules, e.g., polynucleotides encoding AADC, for use in the treatment of Parkinson's disease.

[000156] In some embodiments, the polynucleotides of the invention may be used in the treatment, prophylaxis or amelioration of any disease or disorder characterized by aberrant or undesired target expression wherein the payload, i.e. AADC, is swapped for an alternate payload.

[000157] The present disclosure provides a method for treating a disease, disorder and/or condition in a mammalian subject, including a human subject, comprising administering to the subject any of the viral particles e.g., AAV, AAV polynucleotides or AAV genomes described herein (i.e., viral genomes or "VG") or administering to the subject a particle comprising said AAV polynucleotide or AAV genome, or administering to the subject any of the described compositions, including pharmaceutical compositions.

[000158] In one embodiment, the disease, disorder and/or condition is a neurological disease, disorder and/or condition. The CNS diseases may be diseases that affect any component of the brain (including the cerebral hemispheres, diencephalon, brain stem, and cerebellum) or the spinal cord.

[000159] In some embodiments, viral particles of the present invention, through delivery of a functional payload that is a therapeutic product that can modulate the level or function of a gene product in the CNS, may be used to treat a neurodegenerative diseases and/or diseases or disorders that are characteristic with neurodegeneration, neuromuscular diseases, lysosomal diseases, trauma, bone marrow injuries, pain (including neuropathic pain), cancers of the nervous system, demyelinating diseases, autoimmune diseases of the nervous system, neurotoxic syndromes, sleeping disorders, genetic brain disorders and developmental CNS disorders. A

functional payload may alleviate or reduce symptoms that result from abnormal level and/or function of a gene product (e.g., an absence or defect in a protein) in a subject in need thereof or that otherwise confers a benefit to a CNS disorder in a subject in need thereof.

[000160] As non-limiting examples, therapeutic products delivered by viral particles of the present invention may include, but are not limited to, growth and trophic factors, cytokines, hormones, neurotransmitters, enzymes, anti-apoptotic factors, angiogenic factors, and any protein known to be mutated in pathological disorders such as the "survival of motor neuron " protein (SMN); antisense RNA or RNAi targeting messenger RNAs coding for proteins having a therapeutic interest in any of CNS diseases discussed herein; or microRNAs that function in gene silencing and post-transcriptionally regulation of gene expression in the CNS (e.g., brain specific Mir-128a, See Adlakha and Saini, Molecular cancer, 2014, 13:33) . For example, an RNAi targeting the superoxide dismutase enzyme may be packaged by viral particles of the present invention, for the treatment of ALS.

[000161] The growth and trophic factors may include, but are not limited to brain-derived growth factor (BDNF), epidermal growth factor (EGF), basic Fibroblast growth factor (bFGF), Ciliary neurotrophic factor (CNTF), corticotropin-releasing factor (CRF), Glial cell line derived growth factor (GDNF), Insulin-like growth factor-1 (IGF-1), nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and vascular endothelial growth factor (VEGF). Cytokines may include interleukin-10 (IL-10), interleukin-6, Interleukin-8, chemokine CXCL12 (SDF-1), TGF-beta, and Growth and differentiation factor (GDF-1/10).

[000162] In some embodiments, the neurological disorders may be neurodegenerative disorders including, but not limited to, Alzheimer's Diseases (AD); Amyotrophic lateral sclerosis (ALS); Creutzfeldt-Jakob Disease (CJD); Huntingtin's disease (HD); Friedreich's ataxia (FA); Parkinson Disease (PD); Multiple System Atrophy (MSA); Spinal Muscular Atrophy (SMA), Multiple Sclerosis (MS); Primary progressive aphasia; Progressive supranuclear palsy (PSP); Dementia; Brain Cancer, Degenerative Nerve Diseases, Encephalitis, Epilepsy, Genetic Brain Disorders that cause neurodegeneration, Retinitis pigmentosa (RP), Head and Brain Malformations, Hydrocephalus, Stroke, Prion disease, Infantile neuronal ceroid lipofuscinosis (INCL) (a neurodegenerative disease of children caused by a deficiency in the lysosomal enzyme palmitoyl protein thioesterase-1 (PPT1)), and others.

[000163] In some embodiments, viral particles of the present invention may be used to treat diseases that are associated with impairments of the growth and development of the CNS, i.e., neurodevelopmental disorders. In some aspects, such neurodevelopmental disorders may be caused by genetic mutations, including but not limited to, Fragile X syndrome (caused by

mutations in FMR1 gene), Down syndrome (caused by trisomy of chromosome 21), Rett syndrome, Williams syndrome, Angelman syndrome, Smith-Magenis syndrome, ATR-X syndrome, Barth syndrome, Immune dysfunction and/or infectious diseases during infancy such as Sydenham's chorea, Schizophrenia Congenital toxoplasmosis, Congenital rubella syndrome, Metabolic disorders such as diabetes mellitus and phenylketonuria; nutritional defects and/or brain trauma, Autism and autism spectrum.

[000164] In some embodiments, viral particles of the present invention, may be used to treat a tumor in the CNS, including but not limited to, acoustic neuroma, Astrocytoma (Grades I, II, III and IV), Chordoma, CNS Lymphoma, Craniopharyngioma, Gliomas (e.g., brain stem glioma, ependymoma, optical nerve glioma, subependymoma), Medulloblastoma, Meningioma, Metastatic brain tumors, Oligodendroglioma, Pituitary Tumors, Primitive neuroectodermal (PNET), and Schwannoma.

[000165] In some embodiments, the neurological disorders may be functional neurological disorders with motor and/or sensory symptoms which have neurological origin in the CNS. As non-limiting examples, functional neurological disorders may be chronic pain, seizures, speech problems, involuntary movements, and sleep disturbances.

[000166] In some embodiments, the neurological disorders may be white matter disorders (a group of diseases that affects nerve fibers in the CNS) including but not limited to, Pelizaeus-Merzbacher disease, Hypomyelination with atrophy of basal ganglia and cerebellum, Aicardi-Goutières syndrome, Megalencephalic leukoencephalopathy with subcortical cysts, Congenital muscular dystrophies, Myotonic dystrophy, Wilson disease, Lowe syndrome, Sjögren-Larsson syndrome, PIBD or Tay syndrome, Cockayne's disease, erebrotendinous xanthomatosis, Zellweger syndrome, Neonatal adrenoleukodystrophy, Infantile Refsum disease, Zellweger-like syndrome, Pseudo-Zellweger syndrome, Pseudo-neonatal adrenoleukodystrophy, Bifunctional protein deficiency, X-linked adrenoleukodystrophy and adrenomyeloneuropathy and Refsum disease.

[000167] In some embodiments, the neurological disorders may be lysosomal storage disorders (LSDs) caused by the inability of cells in the CNS to break down metabolic end products, including but not limited to Niemann-Pick disease (a LSD resulting from inherited deficiency in acid sphingomyelinase (ASM); Metachromatic leukodystrophy (MLD) (a LSD characterized by accumulation of sulfatides in glial cells and neurons, the result of an inherited deficiency of arylsulfatase A (ARSA)); Globoid-cell leukodystrophy (GLD) (a LSD caused by mutations in galactosylceramidase); Fabry disease (a LSD caused by mutations in the alpha-galactosidase A (GLA) gene); Gaucher disease (caused by mutations in the beta-glucocerebrosidase (GBA)

gene); GM1/GM2 gangliosidosis; Mucopolysaccharidoses disorder; Pompe disease; and Neuronal ceroid lipofuscinosis.

[000168] In one embodiment, the neurological disease, disorder and/or condition is Parkinson's disease. In one embodiment the polynucleotide used to treat Parkinson's disease comprises any one of SEQ ID NOs 2-23, such as, but not limited to SEQ ID NOs: 6-9 and 17-23, wherein the payload is replaced by AADC or any other payload known in the art for treating Parkinson's disease. As a non-limiting example, the condition is early stage Parkinson's disease. As another non-limiting example, the condition is late stage Parkinson's disease.

[000169] In one embodiment, the subject is a human patient who has a minimum motor score of about 30 to a maximum score of about 100, about 10 to a maximum score of about 100, about 20 to a maximum score of about 100 in the Unified Parkinson's Disease Rating Scale.

[000170] In one embodiment, the subject has been diagnosed with Parkinson's disease within the past 5 years prior to treatment with the compositions described herein. As a non-limiting example, the subject may have been diagnosed with Parkinson's disease within a week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 1 year, 2 years, 3 years, 4 years or less than 5 years prior to treatment with the compositions described herein.

[000171] In one embodiment, the subject has been diagnosed with Parkinson's disease between 5 and 10 years prior to treatment with the compositions described herein. As a non-limiting example, the subject may have been diagnosed with Parkinson's disease 5, 5.5., 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 years prior to treatment with the compositions described herein.

[000172] In one embodiment, the subject has been diagnosed with Parkinson's disease more than 10 years prior to treatment with the compositions described herein. As a non-limiting example, the subject may have been diagnosed with Parkinson's disease 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24 or more than 24 years prior to treatment with the compositions described herein.

[000173] In one embodiment, a subject has seen a change in motor symptoms such as tremors and movements prior to administration of the composition described herein. Non-limiting examples of tremors include, unilateral or bilateral mild tremors, bilateral or midline moderate tremors or intractable tremors. Non-limiting examples of movements include mild bradykinesia, moderate bradykinesia, severe bradykinesia and morning akinesia.

[000174] In one embodiment, a subject may have changes in balance such as, but not limited to, impaired balance, impaired righting reflexes, significant balance disorder or falling.

[000175] In one embodiment, a subject may have a reduced quality of life. As a non-limiting example, the subject may have a moderate impact on their quality of life such as experiencing some limitations to activities of daily living. As another non-limiting example, the subject may have a quality of life which has been diminished by illness.

[000176] In one embodiment, a subject has seen a change in non-motor symptoms prior to administration of the composition described herein. As a non-limiting example, the subject may have mild to moderate cognitive impairment prior to administration to the composition described herein. As another non-limiting example, the subject may have significant cognitive impairment such as dementia which may also include behavioral disturbances such as hallucinations.

[000177] In one embodiment, a subject may have a satisfactory response with limited fluctuations on one or more dopaminergic medications prior to administration of the compositions described herein.

[000178] In one embodiment, a subject may have motor fluctuations causing mild to moderate disability on one or more dopaminergic medications prior to administration of the compositions described herein.

[000179] In one embodiment, a subject may have medically refractory motor fluctuations consisting of "wearing off" and/or levodopa-induced dyskinesias causing significant disability prior to administration of the compositions described herein.

[000180] In one embodiment, a subject may have mild symptoms associated with Parkinson's disease such as, but not limited to, no cognitive impairment, diagnosed within the past 5 years, satisfactory response with limited fluctuations on one or more dopaminergic medications, unilateral or bilateral mild tremors, little to no impact on the quality of life, and/or no balance impairment.

[000181] In one embodiment, a subject may have moderate symptoms associated with Parkinson's disease such as, but not limited to, mild to moderate cognitive impairment, first signs of impaired balance and righting reflexes, motor fluctuations causing mild-moderate disability on one or more dopaminergic medications, diagnosed within the past 5 to 10 years, bilateral or midline moderate tremors, moderate bradykinesia and/or subject experiencing some limitations to activities of daily living.

[000182] In one embodiment, a subject may have advanced symptoms associated with Parkinson's disease such as, but not limited to, being diagnosed with Parkinson's more than 10 years, medium refractory motor fluctuations wearing off and/or levodopa-induced dyskinesia causing significant disability, intractable tremors, significant balance disorder and/or falling,

significant cognitive impairment (such as dementia with or without behavioral disturbances), sever bradykinesia, quality of life markedly diminished by illness and/or morning akinesia.

[000183] In one embodiment, a subject has been referred to a movement disorder specialist (MDS) but has not undergone deep brain stimulation.

[000184] In one embodiment, a subject is using DUOPA™ in combination with the compositions described herein. As a non-limiting example, the subject may have success with using DUOPA™ alone. As a non-limiting example, the subject may not have any success or limited success using DUOPA™ alone.

[000185] In another embodiment, the neurological disease, disorder and/or condition is Friedreich's Ataxia. In one embodiment the polynucleotide used to treat Friedreich's Ataxia comprises any one of SEQ ID NOs 2-23, such as, but not limited to SEQ ID NOs: 6-9 and 17-23, wherein the payload is replaced by Frataxin or any other payload known in the art for treating Friedreich's Ataxia.

[000186] In another embodiment, the neurological disease, disorder and/or condition is Amyotrophic lateral sclerosis (ALS). In one embodiment the polynucleotide used to treat ALS comprises any one of SEQ ID NOs 2-23, such as, but not limited to SEQ ID NOs: 6-9 and 17-23, wherein the payload is replaced by an shRNA, miRNA, siRNA, RNAi for SOD1 or any other payload known in the art for treating ALS.

[000187] In another embodiment, the neurological disease, disorder and/or condition is Huntington's disease. In one embodiment the polynucleotide used to treat Huntington's disease comprises any one of SEQ ID NOs 2-23, such as, but not limited to SEQ ID NOs: 6-9 and 17-23, wherein the payload is replaced by an shRNA, miRNA, siRNA, RNAi for Htt or any other payload known in the art for treating Huntington's disease.

[000188] In another embodiment, the neurological disease, disorder or condition is spinal muscular atrophy (SMA). In one embodiment the polynucleotide used to treat SMA comprises any one of SEQ ID NOs 2-23, such as, but not limited to SEQ ID NOs: 6-9 and 17-23, wherein the payload is replaced by SMN or any other payload known in the art for treating SMA.

Circadian Rhythm and Sleep-Wake Cycles

[000189] Circadian rhythms are physical, mental and behavioral changes that tend to follow a 24 hour cycle. Circadian rhythms can influence sleep-wake cycles, hormone release, body temperature and other bodily functions. Changes in the circadian rhythm can cause conditions and/or disorder such as, but not limited to sleep disorders (e.g., insomnia), depression, bipolar disorder, seasonal affective disorder, obesity and diabetes.

[000190] In one embodiment, the AADC polynucleotides described herein may be used to treat insomnia.

[000191] The sleep-wake cycle comprises periods of sleep and periods of wake. Generally, in a 24 hour period the total hours of sleep are less than the total hours of wakefulness. As a non-limiting example, the sleep-wake cycle comprises 7-9 hours of sleep and 15-17 hours of wakefulness. As a non-limiting example, the sleep-wake cycle comprises 8 hours of sleep and 16 hours of wakefulness. As a non-limiting example, the sleep-wake cycle comprises 8-10 hours of sleep and 14-16 hours of wakefulness.

[000192] In one embodiment, the sleep-wake cycle of a subject is improved by administered to the subject the AADC polynucleotides described herein.

[000193] In one embodiment, the sleep-wake cycle of a subject is regulated by administering to the subject the AADC polynucleotides described herein. As a non-limiting example, the regulation may be the correction of more periods of sleep occurring at night and less periods of sleep occurring

[000194] In one embodiment, the sleep-wake cycle of a subject administered the AADC polynucleotides described herein improves as compared to the sleep-wake cycle of the subject prior to administration of the AADC polynucleotides. As a non-limiting example, the subject has an increased period of sleep and a decreased period of wakefulness. As another non-limiting example, the subject has a decreased period of sleep and an increased period of wakefulness.

[000195] In one embodiment, the sleep-wake cycle of a subject administered the AADC polynucleotides described herein is regulated as compared to the sleep-wake cycle of the subject prior to administration of the AADC polynucleotides. As a non-limiting example, the length of the periods of sleep and the periods of wakefulness may be about the same (e.g., +/- 1 hour) for at least 2 days. As another non-limiting example, the length of the periods of sleep and the periods of wakefulness if a 24 hours period may be within 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.5 hours, or 2 hours of the previous 24 hour period.

[000196] In one embodiment, the amount of rapid eye movement (REM) sleep a subject experiences in a 24 hour period is altered after the subject is administered the AADC polynucleotides described herein. REM sleep is generally considered an active period of sleep marked by intense brain activity where brain waves are fast and desynchronized. An adult, on average, spends about 20-25% of their total daily sleep period in REM sleep. As a non-limiting example, the amount of REM sleep is decreased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or

more than 65%. As a non-limiting example, the amount of REM sleep is decreased by 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%. As a non-limiting example, the amount of REM sleep is increased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of REM sleep is increased by 1-5%, 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%.

[000197] In one embodiment, the amount of non-REM (NREM) sleep a subject experiences in a 24 hour period is altered after the subject is administered the AADC polynucleotides described herein. NREM sleep is generally characterized by a reduction in physiological activity since as the brain waves, measured by EEG, get slower and have greater amplitude. NREM has four stages: Stage 1 is the time of drowsiness or transition from being awake to falling asleep where the brain waves and muscle activity begin to slow; Stage 2 is a period of light sleep during which eye movements stop and brain waves become slower with occasional bursts of rapid waves (sometimes called sleep spindles); Stage 3 and Stage 4 (collectively referred to as slow wave sleep) are characterized by the presence of slow brain waves (delta waves) interspersed with smaller faster waves where there are no eye movements. An adult, on average, spends about 75-80% of their total daily sleep period in NREM sleep with about half of their total daily sleep time in NREM stage 2 sleep.

[000198] In one embodiment, the amount of NREM sleep a subject experiences is altered after the subject is administered the AADC polynucleotides described herein. As a non-limiting example, the amount of NREM sleep is decreased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM sleep is decreased by 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%. As a non-limiting example, the amount of NREM sleep is increased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM sleep is increased by 1-5%, 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%.

[000199] In one embodiment, the amount of NREM Stage 1 sleep a subject experiences is altered after the subject is administered the AADC polynucleotides described herein. As a non-

limiting example, the amount of NREM Stage 1 sleep is decreased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 1 sleep is decreased by 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%. As a non-limiting example, the amount of NREM Stage 1 sleep is increased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 1 sleep is increased by 1-5%, 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%.

[000200] In one embodiment, the amount of NREM Stage 2 sleep a subject experiences is altered after the subject is administered the AADC polynucleotides described herein. As a non-limiting example, the amount of NREM Stage 2 sleep is decreased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 2 sleep is decreased by 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%. As a non-limiting example, the amount of NREM Stage 2 sleep is increased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 2 sleep is increased by 1-5%, 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%.

[000201] In one embodiment, the amount of NREM Stage 3 and 4 sleep a subject experiences is altered after the subject is administered the AADC polynucleotides described herein. As a non-limiting example, the amount of NREM Stage 3 and 4 sleep is decreased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 3 and 4 sleep is decreased by 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%. As a non-limiting example, the amount of NREM Stage 3 and 4 sleep is increased by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65% or more than 65%. As a non-limiting example, the amount of NREM Stage 3 and 4 sleep is increased by 1-5%, 1-10%, 5-10%, 5-15%, 10-15%, 15-20%, 15-25%, 20-25%, 20-30%, 25-30%, 25-35%, 30-35%, 30-40%, 35-40%, 40-50% or 40-60%.

[000202] In one embodiment, periods of NREM and REM cycles are more consistent in a subject after the subject is administered the AADC polynucleotides described herein. Generally NREM and REM cycles alternate every 90 to 110 minutes four to six times per night.

DEFINITIONS

[000203] At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges.

[000204] *About:* As used herein, the term "about" means +/- 10% of the recited value.

[000205] *Activity*: As used herein, the term "activity" refers to the condition in which things are happening or being done. Compositions described herein may have activity and this activity may involve one or more biological events.

[000206] *Adeno-associated virus*: The term "adeno-associated virus" or "AAV" as used herein refers to members of the dependovirus genus comprising any particle, sequence, gene, protein, or component derived therefrom. The term "AAV particle" as used herein comprises a capsid and a polynucleotide. The AAV particle may be derived from any serotype, described herein or known in the art, including combinations of serotypes (i.e., "pseudotyped" AAV) or from various genomes (e.g., single stranded or self-complementary). In addition, the AAV particle may be replication defective and/or targeted.

[000207] *Administered in combination:* As used herein, the term "administered in combination" or "combined administration" means that two or more agents (e.g., AAV) are administered to a subject at the same time or within an interval such that there may be an overlap of an effect of each agent on the patient and/or the subject is at some point in time simultaneously exposed to both. In some embodiments, they are administered within about 60, 30, 15, 10, 5, or 1 minutes of one another or within about 24 hours, 12 hours, 6 hours, 3 hours of at least one dose of one or more other agents. In some embodiments, administration occurs in overlapping dosage regimens. As used herein, the term "dosage regimen" refers to a plurality of doses spaced apart in time. Such doses may occur at regular intervals or may include one or more hiatus in administration. In some embodiments, the administrations of the agents are spaced sufficiently closely together such that a combinatorial (*e.g.*, a synergistic) effect is achieved.

[000208] *Amelioration*: As used herein, the term "amelioration" or "ameliorating" refers to a lessening of severity of at least one indicator of a condition or disease. For example, in the context of neurodegeneration disorder, amelioration includes the reduction of neuron loss.

[000209] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans at any stage of development. In some embodiments, "animal" refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[000210] *Antisense strand*: As used herein, the term "the antisense strand" or "the first strand" or "the guide strand" of a siRNA molecule refers to a strand that is substantially complementary to a section of about 10-50 nucleotides, e.g., about 15-30, 16-25, 18-23 or 19-22 nucleotides of the mRNA of the gene targeted for silencing. The antisense strand or first strand has sequence sufficiently complementary to the desired target mRNA sequence to direct target-specific silencing, e.g., complementarity sufficient to trigger the destruction of the desired target mRNA by the RNAi machinery or process.

[000211] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[000212] *Associated with:* As used herein, the terms "associated with," "conjugated," "linked," "attached," and "tethered," when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An "association" need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the "associated" entities remain physically associated.

[000213] *Bifunctional:* As used herein, the term "bifunctional" refers to any substance, molecule or moiety which is capable of or maintains at least two functions. The functions may affect the same outcome or a different outcome. The structure that produces the function may be the same or different.

[000214] *Biologically active*: As used herein, the phrase "biologically active" refers to a characteristic of any substance (e.g., AAV) that has activity in a biological system and/or organism. For instance, a substance that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, a polynucleotide of the present invention may be considered biologically active if even a portion of the polynucleotides is biologically active or mimics an activity considered biologically relevant.

[000215] *Biological system*: As used herein, the term "biological system" refers to a group of organs, tissues, cells, intracellular components, proteins, nucleic acids, molecules (including, but not limited to biomolecules) that function together to perform a certain biological task within cellular membranes, cellular compartments, cells, tissues, organs, organ systems, multicellular organisms, or any biological entity. In some embodiments, biological systems are cell signaling pathways comprising intracellular and/or extracellular cell signaling biomolecules. In some embodiments, biological systems comprise growth factor signaling events within the extracellular/cellular matrix and/or cellular niches.

[000216] *Biomolecule*: As used herein, the term "biomolecule" is any natural molecule which is amino acid-based, nucleic acid-based, carbohydrate-based or lipid-based, and the like.

[000217] *Complementary and substantially complementary*: As used herein, the term "complementary" refers to the ability of polynucleotides to form base pairs with one another. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can form base pairs in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is the base that is considered to be complementary to adenosine. However, when a U is denoted in the context of the present invention, the ability to substitute a T is implied, unless otherwise stated. Perfect complementarity or 100% complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can form a hydrogen bond with a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can form hydrogen bonds with each other. For example, for two 20-mers, if only two base pairs on each strand can form hydrogen bonds with each other, the polynucleotide strands exhibit 10% complementarity. In the same example, if 18 base pairs on each strand can form hydrogen bonds with each other, the polynucleotide strands exhibit 90% complementarity. As used herein, the term "substantially complementary" means that the siRNA has a sequence (e.g., in the antisense

strand) which is sufficient to bind the desired target mRNA, and to trigger the RNA silencing of the target mRNA.

[000218] *Compound*: As used herein, the term "compound," refers to a distinct chemical entity. In some embodiments, a particular compound may exist in one or more isomeric or isotopic forms (including, but not limited to stereoisomers, geometric isomers and isotopes). In some embodiments, a compound is provided or utilized in only a single such form. In some embodiments, a compound is provided or utilized as a mixture of two or more such forms (including, but not limited to a racemic mixture of stereoisomers). Those of skill in the art appreciate that some compounds exist in different such forms, show different properties and/or activities (including, but not limited to biological activities). In such cases it is within the ordinary skill of those in the art to select or avoid particular forms of the compound for use in accordance with the present invention. For example, compounds that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

[000219] Compounds of the present disclosure also include tautomeric forms. Tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. Tautomeric forms include prototropic tautomers which are isomeric protonation states having the same empirical formula and total charge.

[000220] Compounds of the present disclosure also include all of the isotopes of the atoms occurring in the intermediate or final compounds. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium.

[000221] The compounds and salts of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[000222] *Conserved*: As used herein, the term "conserved" refers to nucleotides or amino acid residues of a polynucleotide sequence or polypeptide sequence, respectively, that are those that occur unaltered in the same position of two or more sequences being compared. Nucleotides or amino acids that are relatively conserved are those that are conserved amongst more related sequences than nucleotides or amino acids appearing elsewhere in the sequences.

[000223] In some embodiments, two or more sequences are said to be "completely conserved" if they are 100% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80% identical, about 90% identical, about 95%, about 98%, or about 99% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are at least 30% identical, at least 40% identical, at least 50% identical, at least 60% identical, at least 70% identical, at least 80% identical, at least 90% identical, or at least 95% identical to one another. In some embodiments, two or more sequences are said to be "conserved" if they are about 30% identical, about 40% identical, about 50% identical, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. Conservation of sequence may apply to the entire length of an oligonucleotide, a polynucleotide or polypeptide or may apply to a portion, region or feature thereof.

[000224] In one embodiment, conserved sequences are not contiguous. Those skilled in the art are able to appreciate how to achieve alignment when gaps in contiguous alignment are present between sequences, and to align corresponding residues not withstanding insertions or deletions present.

[000225] In one embodiment, conserved sequences are not contiguous. Those skilled in the art are able to appreciate how to achieve alignment when gaps in contiguous alignment are present between sequences, and to align corresponding residues not withstanding insertions or deletions present.

[000226] *Delivery*: As used herein, "delivery" refers to the act or manner of delivering a compound such as a parvovirus, e.g. an AAV and/or AAV compound, substance, entity, moiety, cargo or payload to a target. Such target may be a cell, tissue, organ, organism, or system (whether biological or production).

[000227] *Delivery Agent*: As used herein, "delivery agent" refers to any agent or substance which facilitates, at least in part, the *in vivo* and/or *in vitro* delivery of a polynucleotide and/or one or more substances (including, but not limited to a compounds and/or compositions of the present invention, e.g., viral particles or expression vectors) to targeted cells.

[000228] *Destabilized*: As used herein, the term "destable," "destabilize," or "destabilizing region" means a region or molecule that is less stable than a starting, reference, wild-type or native form of the same region or molecule.

[000229] *Detectable label:* As used herein, "detectable label" refers to one or more markers, signals, or moieties which are attached, incorporated or associated with another entity that is readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance immunological detection, and the like. Detectable labels may include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels may be located at any position in the entity with which they are attached, incorporated or associated. For example, when attached, incorporated in or associated with a peptide or protein, they may be within the amino acids, the peptides, or proteins, or located at the N- or C- termini.

[000230] *Dosing regimen*: As used herein, a "dosing regimen" is a schedule of administration or physician determined regimen of treatment, prophylaxis, or palliative care.

[000231] *Effective Amount:* As used herein, the term "effective amount" of an agent is that amount sufficient to effect beneficial or desired results, for example, upon single or multiple dose administration to a subject cell, in curing, alleviating, relieving or improving one or more symptoms of a disorder, clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied. For example, in the context of administering an agent that treats Parkinson's Disease, an effective amount of an agent is, for example, an amount sufficient to achieve treatment, as defined herein, of Parkinson's Disease, as compared to the response obtained without administration of the agent.

[000232] *Encapsulate:* As used herein, the term "encapsulate" means to enclose, surround or encase.

[000233] *Engineered*: As used herein, embodiments are "engineered" when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild-type or native molecule. Thus, engineered agents or entities are those whose design and/or production include an act of the hand of man.

[000234] *Epitope*: As used herein, an "epitope" refers to a surface or region on a molecule that is capable of interacting with a biomolecule. For example a protein may contain one or more amino acids, e.g., an epitope, which interacts with an antibody, e.g., a biomolecule. In some embodiments, when referring to a protein or protein module, an epitope may comprise a linear stretch of amino acids or a three dimensional structure formed by folded amino acid chains.

[000235] *Expression*: As used herein, "expression" of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (*e.g.*, by transcription); (2) processing of an RNA transcript (*e.g.*, by splicing, editing, 5′ cap

formation, and/or 3′ end processing); (3) translation of an RNA into a polypeptide or protein; (4) folding of a polypeptide or protein; and (5) post-translational modification of a polypeptide or protein.

[000236] *Feature:* As used herein, a "feature" refers to a characteristic, a property, or a distinctive element.

[000237] *Formulation*: As used herein, a "formulation" includes at least one polynucleotide and/or compound and/or composition of the present disclosure (e.g., a vector, AAV particle, etc.) and a delivery agent.

[000238] *Fragment:* A "fragment," as used herein, refers to a contiguous portion of a whole. For example, fragments of proteins may comprise polypeptides obtained by digesting full-length protein isolated from cultured cells. In some embodiments, a fragment of a protein includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250 or more amino acids. In some embodiments, fragments of an antibody include portions of an antibody subjected to enzymatic digestion or synthesized as such.

[000239] *Functional*: As used herein, a "functional" biological molecule is a biological molecule and/or entity with a structure and in a form in which it exhibits a property and/or activity by which it is characterized.

[000240] *Gene expression*: The term "gene expression" refers to the process by which a nucleic acid sequence undergoes successful transcription and in most instances translation to produce a protein or peptide. For clarity, when reference is made to measurement of "gene expression", this should be understood to mean that measurements may be of the nucleic acid product of transcription, e.g., RNA or mRNA or of the amino acid product of translation, e.g., polypeptides or peptides. Methods of measuring the amount or levels of RNA, mRNA, polypeptides and peptides are well known in the art.

[000241] *Homology*: As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the invention, two polynucleotide sequences are considered to be homologous if the polypeptides they encode are at least about 50%, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least

about 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in length, homology is typically determined by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. In accordance with the invention, two protein sequences are considered to be homologous if the proteins are at least about 50%, 60%, 70%, 80%, or 90% identical for at least one stretch of at least about 20 amino acids. In many embodiments, homologous protein may show a large overall degree of homology and a high degree of homology over at least one short stretch of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 or more amino acids. In many embodiments, homologous proteins share one or more characteristic sequence elements. As used herein, the term "characteristic sequence element" refers to a motif present in related proteins. In some embodiments, the presence of such motifs correlates with a particular activity (such as biological activity).

[000242] *Identity*: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, *e.g.*, between oligonucleotide and/or polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of the percent identity of two polynucleotide sequences, for example, may be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M.,

and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference in its entirety. For example, the percent identity between two nucleotide sequences can be determined, for example using the algorithm of Meyers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference in its entirety. Techniques for determining identity are codified in publicly available computer programs. Exemplary computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al.*, *J. Molec. Biol.*, 215, 403 (1990)).

[000243] *Inhibit expression of a gene*: As used herein, the phrase "inhibit expression of a gene" means to cause a reduction in the amount of an expression product of the gene. The expression product may be RNA transcribed from the gene (e.g. mRNA) or a polypeptide translated from mRNA transcribed from the gene. Typically a reduction in the level of mRNA results in a reduction in the level of a polypeptide translated therefrom. The level of expression may be determined using standard techniques for measuring mRNA or protein.

[000244] *In vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, in a Petri dish, *etc.*, rather than within an organism (*e.g.*, animal, plant, or microbe).

[000245] *In vivo*: As used herein, the term "*in vivo*" refers to events that occur within an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof).

[000246] *Isolated*: As used herein, the term "isolated" is synonymous with "separated", but carries with it the inference separation was carried out by the hand of man. In one embodiment, an isolated substance or entity is one that has been separated from at least some of the components with which it was previously associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In

some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components.

[000247] *Substantially isolated*: By "substantially isolated" is meant that the compound is substantially separated from the environment in which it was formed or detected. Partial separation can include, for example, a composition enriched in the compound of the present disclosure. Substantial separation can include compositions containing at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% by weight of the compound of the present disclosure, or salt thereof. Methods for isolating compounds and their salts are routine in the art. In some embodiments, isolation of a substance or entity includes disruption of chemical associations and/or bonds. In some embodiments, isolation includes only the separation from components with which the isolated substance or entity was previously combined and does not include such disruption.

[000248] *Modified:* As used herein, the term "modified" refers to a changed state or structure of a molecule or entity of the invention as compared with a parent or reference molecule or entity. Molecules may be modified in many ways including chemically, structurally, and functionally. In some embodiments, compounds and/or compositions of the present disclosure are modified by the introduction of non-natural amino acids, or non-natural nucleotides.

[000249] *Mutation*: As used herein, the term "mutation" refers to a change and/or alteration. In some embodiments, mutations may be changes and/or alterations to proteins (including peptides and polypeptides) and/or nucleic acids (including polynucleic acids). In some embodiments, mutations comprise changes and/or alterations to a protein and/or nucleic acid sequence. Such changes and/or alterations may comprise the addition, substitution and or deletion of one or more amino acids (in the case of proteins and/or peptides) and/or nucleotides (in the case of nucleic acids and or polynucleic acids). In embodiments wherein mutations comprise the addition and/or substitution of amino acids and/or nucleotides, such additions and/or substitutions may comprise 1 or more amino acid and/or nucleotide residues and may include modified amino acids and/or nucleotides.

[000250] *Naturally occurring:* As used herein, "naturally occurring" means existing in nature without artificial aid or involvement of the hand of man

[000251] *Non-human vertebrate:* As used herein, a "non-human vertebrate" includes all vertebrates except *Homo sapiens*, including wild and domesticated species. Examples of non-

human vertebrates include, but are not limited to, mammals, such as alpaca, banteng, bison, camel, cat, cattle, deer, dog, donkey, gayal, goat, guinea pig, horse, llama, mule, pig, rabbit, reindeer, sheep water buffalo, and yak.

[000252] *Nucleic acid*: As used herein, the term "nucleic acid", "polynucleotide" and 'oligonucleotide" refer to any nucleic acid polymers composed of either polydeoxyribonucleotides (containing 2-deoxy-D-ribose), or polyribonucleotides (containing D-ribose), or any other type of polynucleotide which is an N glycoside of a purine or pyrimidine base, or modified purine or pyrimidine bases. There is no intended distinction in length between the term "nucleic acid", "polynucleotide" and "oligonucleotide", and these terms will be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single stranded RNA.

[000253] *Off-target*: As used herein, "off target" refers to any unintended effect on any one or more target, gene and/or cellular transcript.

[000254] *Open reading frame:* As used herein, "open reading frame" or "ORF" refers to a sequence which does not contain a stop codon in a given reading frame.

[000255] *Operably linked:* As used herein, the phrase "operably linked" refers to a functional connection between two or more molecules, constructs, transcripts, entities, moieties or the like.

[000256] *Particle*: As used herein, a "particle" is a virus comprised of at least two components, a protein capsid and a polynucleotide sequence enclosed within the capsid.

[000257] *Patient:* As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition, such as for example Parkinson's Disease.

[000258] *Payload*: As used herein, "payload" refers to one or more polynucleotides or polynucleotide regions encoded by or within a viral genome or an expression product of such polynucleotide or polynucleotide region, e.g., a transgene, a polynucleotide encoding a polypeptide or multi-polypeptide or a modulatory nucleic acid or regulatory nucleic acid.

[000259] *Payload construct*: As used herein, "payload construct" is one or more polynucleotide regions encoding or comprising a payload that is flanked on one or both sides by an inverted terminal repeat (ITR) sequence. The payload construct is a template that is replicated in a viral production cell to produce a viral genome.

[000260] *Payload construct vector*: As used herein, "payload construct vector" is a vector encoding or comprising a payload construct, and regulatory regions for replication and expression in bacterial cells.

[000261] *Payload construct expression vector*: As used herein, a "payload construct expression vector" is a vector encoding or comprising a payload construct and which further comprises one or more polynucleotide regions encoding or comprising components for viral expression in a viral replication cell.

[000262] *Peptide:* As used herein, "peptide" is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[000263] *Pharmaceutically acceptable*: The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[000264] *Pharmaceutically acceptable excipients:* The phrase "pharmaceutically acceptable excipient," as used herein, refers to any ingredient other than the compounds and/or active agents (e.g. as described herein) present in pharmaceutical compositions and having the properties of being substantially nontoxic and non-inflammatory in a subject such as a patient. In some embodiments, pharmaceutically acceptable excipients are vehicles capable of suspending and/or dissolving active agents. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspension or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[000265] *Pharmaceutically acceptable salts*: Pharmaceutically acceptable salts of the compounds described herein. As used herein, "pharmaceutically acceptable salts" refers to derivatives or forms of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g., as generated by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include,

but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, acetic acid, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzene sulfonic acid, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. In some embodiments a pharmaceutically acceptable salt of the present disclosure can be synthesized salt prepared from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, Pharmaceutical Salts: Properties, Selection, and Use, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., Journal of Pharmaceutical Science, 66, 1-19 (1977), each of which is incorporated herein by reference in its entirety.

[000266] *Pharmaceutically acceptable solvate*: The term "pharmaceutically acceptable solvate," as used herein, refers to a crystalline form of a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), N-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), N,N'-

dimethylformamide (DMF), N,N'-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate." In some embodiments, the solvent incorporated into a solvate is of a type or at a level that is physiologically tolerable to an organism to which the solvate is administered (e.g., in a unit dosage form of a pharmaceutical composition).

[000267] *Pharmacokinetic:* As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism (or Biotransformation) is the irreversible transformation of parent compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[000268] *Physicochemical:* As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[000269] *Preventing*: As used herein, the term "preventing" refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition, such as for example Parkinson's Disease.

[000270] *Prodrug*: The present disclosure also includes prodrugs of the compounds described herein. As used herein, "prodrugs" refer to any substance, molecule or entity which is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may by covalently bonded or sequestered in some way and which release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in *Bioreversible*

Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

[000271] *Proliferate*: As used herein, the term "proliferate" means to grow, expand, replicate or increase or cause to grow, expand, replicate or increase. "Proliferative" means having the ability to proliferate. "Anti-proliferative" means having properties counter to or in opposition to proliferative properties.

[000272] *Prophylactic*: As used herein, "prophylactic" refers to a therapeutic or course of action used to prevent the spread of disease.

[000273] *Prophylaxis:* As used herein, a "prophylaxis" refers to a measure taken to maintain health and prevent the spread of disease.

[000274] *Protein of interest:* As used herein, the terms "proteins of interest" or "desired proteins" include those provided herein and fragments, mutants, variants, and alterations thereof.

[000275] *Purified:* As used herein, "purify," "purified," "purification" means to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection. "Purified" refers to the state of being pure. "Purification" refers to the process of making pure.

[000276] *Region*: As used herein, the term "region" refers to a zone or general area. In some embodiments, when referring to a protein or protein module, a region may comprise a linear sequence of amino acids along the protein or protein module or may comprise a three dimensional area, an epitope and/or a cluster of epitopes. In some embodiments, regions comprise terminal regions. As used herein, the term "terminal region" refers to regions located at the ends or termini of a given agent. When referring to proteins, terminal regions may comprise N- and/or C-termini. N-termini refer to the end of a protein comprising an amino acid with a free amino group. C-termini refer to the end of a protein comprising an amino acid with a free carboxyl group. N- and/or C-terminal regions may therefore comprise the N- and/or C-termini as well as surrounding amino acids. In some embodiments, N- and/or C-terminal regions comprise from about 3 amino acid to about 30 amino acids, from about 5 amino acids to about 40 amino acids, from about 10 amino acids to about 50 amino acids, from about 20 amino acids to about 100 amino acids and/or at least 100 amino acids. In some embodiments, N-terminal regions may comprise any length of amino acids that includes the N-terminus, but does not include the C-terminus. In some embodiments, C-terminal regions may comprise any length of amino acids, which include the C-terminus, but do not comprise the N-terminus.

[000277] In some embodiments, when referring to a polynucleotide, a region may comprise a linear sequence of nucleic acids along the polynucleotide or may comprise a three dimensional

area, secondary structure, or tertiary structure. In some embodiments, regions comprise terminal regions. As used herein, the term "terminal region" refers to regions located at the ends or termini of a given agent. When referring to polynucleotides, terminal regions may comprise 5' and 3' termini. 5' termini refer to the end of a polynucleotide comprising a nucleic acid with a free phosphate group. 3' termini refer to the end of a polynucleotide comprising a nucleic acid with a free hydroxyl group. 5' and 3' regions may therefore comprise the 5' and 3' termini as well as surrounding nucleic acids. In some embodiments, 5' and 3' terminal regions comprise from about 9 nucleic acids to about 90 nucleic acids, from about 15 nucleic acids to about 120 nucleic acids, from about 30 nucleic acids to about 150 nucleic acids, from about 60 nucleic acids to about 300 nucleic acids and/or at least 300 nucleic acids. In some embodiments, 5' regions may comprise any length of nucleic acids that includes the 5' terminus, but does not include the 3' terminus. In some embodiments, 3' regions may comprise any length of nucleic acids, which include the 3' terminus, but does not comprise the 5' terminus.

[000278] *RNA or RNA molecule*: As used herein, the term "RNA" or "RNA molecule" or "ribonucleic acid molecule" refers to a polymer of ribonucleotides; the term "DNA" or "DNA molecule" or "deoxyribonucleic acid molecule" refers to a polymer of deoxyribonucleotides. DNA and RNA can be synthesized naturally, e.g., by DNA replication and transcription of DNA, respectively; or be chemically synthesized. DNA and RNA can be single-stranded (i.e., ssRNA or ssDNA, respectively) or multi-stranded (e.g., double stranded, i.e., dsRNA and dsDNA, respectively). The term "mRNA" or "messenger RNA", as used herein, refers to a single stranded RNA that encodes the amino acid sequence of one or more polypeptide chains.

[000279] *RNA interference*: As used herein, the term "RNA interference" or "RNAi" refers to a sequence specific regulatory mechanism mediated by RNA molecules which results in the inhibition or interference or "silencing" of the expression of a corresponding protein-coding gene.

[000280] *Sample:* As used herein, the term "sample" refers to an aliquot, subset or portion taken from a source and/or provided for analysis or processing. In some embodiments, a sample is from a biological source such as a tissue, cell or component part (e.g. a body fluid, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). In some embodiments, a sample may be or comprise a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, or

organs. In some embodiments, a sample is or comprises a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule. In some embodiments, a "primary" sample is an aliquot of the source. In some embodiments, a primary sample is subjected to one or more processing (e.g., separation, purification, etc.) steps to prepare a sample for analysis or other use.

[000281] *Self-complementary viral particle*: As used herein, a "self-complementary viral particle" is a particle comprised of at least two components, a protein capsid and a polynucleotide sequence encoding a self-complementary genome enclosed within the capsid.

[000282] *Sense strand*: As used herein, the term "the sense strand" or "the second strand" or "the passenger strand" of a siRNA molecule refers to a strand that is complementary to the antisense strand or first strand. The antisense and sense strands of a siRNA molecule are hybridized to form a duplex structure. As used herein, a "siRNA duplex" includes a siRNA strand having sufficient complementarity to a section of about 10-50 nucleotides of the mRNA of the gene targeted for silencing and a siRNA strand having sufficient complementarity to form a duplex with the siRNA strand.

[000283] *Signal Sequences*: As used herein, the phrase "signal sequences" refers to a sequence which can direct the transport or localization.

[000284] *Single unit dose*: As used herein, a "single unit dose" is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. . In some embodiments, a single unit dose is provided as a discrete dosage form (e.g., a tablet, capsule, patch, loaded syringe, vial, etc.).

[000285] *Similarity*: As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.* between polynucleotide molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art.

[000286] *Small/short interfering RNA*: As used herein, the term "small/short interfering RNA" or "siRNA" refers to an RNA molecule (or RNA analog) comprising between about 5-60 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNAi. Preferably, a siRNA molecule comprises between about 15-30 nucleotides or nucleotide analogs, more preferably between about 16-25 nucleotides (or nucleotide analogs), even more preferably between about 18-23 nucleotides (or nucleotide analogs), and even more preferably between about 19-22 nucleotides (or nucleotide analogs) (e.g., 19, 20, 21 or 22 nucleotides or nucleotide

analogs). The term "short" siRNA refers to a siRNA comprising 5-23 nucleotides, preferably 21 nucleotides (or nucleotide analogs), for example, 19, 20, 21 or 22 nucleotides. The term "long" siRNA refers to a siRNA comprising 24-60 nucleotides, preferably about 24-25 nucleotides, for example, 23, 24, 25 or 26 nucleotides. Short siRNAs may, in some instances, include fewer than 19 nucleotides, e.g., 16, 17 or 18 nucleotides, or as few as 5 nucleotides, provided that the shorter siRNA retains the ability to mediate RNAi. Likewise, long siRNAs may, in some instances, include more than 26 nucleotides, e.g., 27, 28, 29, 30, 35, 40, 45, 50, 55, or even 60 nucleotides, provided that the longer siRNA retains the ability to mediate RNAi or translational repression absent further processing, e.g., enzymatic processing, to a short siRNA. siRNAs can be single stranded RNA molecules (ss-siRNAs) or double stranded RNA molecules (ds-siRNAs) comprising a sense strand and an antisense strand which hybridized to form a duplex structure called siRNA duplex.

[000287] *Split dose*: As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[000288] *Stable:* As used herein "stable" refers to a compound or entity that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and preferably capable of formulation into an efficacious therapeutic agent.

[000289] *Stabilized:* As used herein, the term "stabilize", "stabilized," "stabilized region" means to make or become stable. In some embodiments, stability is measured relative to an absolute value. In some embodiments, stability is measured relative to a reference compound or entity.

[000290] *Subject:* As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the invention may be administered, *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants. . In some embodiments, the subject may be an infant, neonate, or a child under the age of 12 years old. In some embodiments, the subject may be in utero.

[000291] *Substantially*: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[000292] *Substantially equal*: As used herein as it relates to time differences between doses, the term means plus/minus 2%.

[000293] *Substantially simultaneously*: As used herein and as it relates to plurality of doses, the term typically means within about 2 seconds.

[000294] *Suffering from*: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition such as for example Parkinson's Disease.

[000295] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[000296] *Sustained release:* As used herein, the term "sustained release" refers to a pharmaceutical composition or compound release profile that conforms to a release rate over a specific period of time.

[000297] *Synthetic*: The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[000298] *Targeting*: As used herein, "targeting" means the process of design and selection of nucleic acid sequence that will hybridize to a target nucleic acid and induce a desired effect.

[000299] *Targeted Cells:* As used herein, "targeted cells" refers to any one or more cells of interest. The cells may be found *in vitro*, *in vivo*, *in situ* or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[000300] *Therapeutic Agent:* The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[000301] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition such as for example Parkinson's Disease. In some embodiments, a therapeutically effective amount is provided in a single dose. In some embodiments, a therapeutically effective amount is administered in a dosage regimen comprising a plurality of doses. Those skilled in the art will appreciate that in some embodiments, a unit dosage form may be considered to comprise a therapeutically effective amount of a particular agent or entity if it comprises an amount that is effective when administered as part of such a dosage regimen.

[000302] *Therapeutically effective outcome*: As used herein, the term "therapeutically effective outcome" means an outcome that is sufficient in a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[000303] *Total daily dose:* As used herein, a "total daily dose" is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose.

[000304] *Transfection*: As used herein, the term "transfection" refers to methods to introduce exogenous nucleic acids into a cell. Methods of transfection include, but are not limited to, chemical methods, physical treatments and cationic lipids or mixtures.

[000305] *Treating*: As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition such as for example Parkinson's Disease.

[000306] *Unmodified*: As used herein, "unmodified" refers to any substance, compound or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the

wild type or native form of a biomolecule or entity. Molecules or entities may undergo a series of modifications whereby each modified substance, compound, molecule or entity may serve as the "unmodified" starting molecule for a subsequent modification.

[000307] *Vector*: As used herein, a "vector" is any molecule or moiety which transports, transduces or otherwise acts as a carrier of a heterologous molecule. Vectors of the present disclosure may be produced recombinantly and may be based on and/or may comprise adeno-associated virus (AAV) parent or reference sequence. Such parent or reference AAV sequences may serve as an original, second, third or subsequent sequence for engineering vectors. In non-limiting examples, such parent or reference AAV sequences may comprise any one or more of the following sequences: a polynucleotide sequence encoding a polypeptide or multi-polypeptide, which sequence may be wild-type or modified from wild-type and which sequence may encode full-length or partial sequence of a protein, protein domain, or one or more subunits of a protein; a polynucleotide comprising a modulatory or regulatory nucleic acid which sequence may be wild-type or modified from wild-type; and a transgene that may or may not be modified from wild-type sequence. These AAV sequences may serve as either the "donor" sequence of one or more codons (at the nucleic acid level) or amino acids (at the polypeptide level) or "acceptor" sequences of one or more codons (at the nucleic acid level) or amino acids (at the polypeptide level).

[000308] *Viral construct vector*: As used herein, a "viral construct vector" is a vector which comprises one or more polynucleotide regions encoding or comprising Rep and or Cap protein.

[000309] *Viral construct expression vector*: As used herein, a "viral construct expression vector" is a vector which comprises one or more polynucleotide regions encoding or comprising Rep and or Cap that further comprises one or more polynucleotide regions encoding or comprising components for viral expression in a viral replication cell.

[000310] *Viral genome*: As used herein, a "viral genome" is a polynucleotide encoding at least one inverted terminal repeat (ITR), at least one regulatory sequence, and at least one payload. The viral genome is derived by replication of a payload construct from the payload construct expression vector. A viral genome encodes at least one copy of the payload construct.

EQUIVALENTS AND SCOPE

[000311] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[000312] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[000313] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

[000314] 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. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used.

[000315] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[000316] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (*e.g.*, any nucleic acid or protein encoded thereby; any method of production; any method of use; *etc.*) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[000317] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[000318] Section and table headings are not intended to be limiting.

EXAMPLES

Example 1. Design of AADC polynucleotides

[000319] AADC polynucleotides are designed to comprise at a minimum a nucleic acid sequence encoding an AADC protein.

[000320] Once designed, the sequence is engineered or synthesized or inserted in a plasmid or vector and administered to a cell or organism. Suitable plasmids or vectors are any which transduce or transfect the target cell.

[000321] Adeno-associated viral vectors (AAV), viral particles or entire viruses may be used.

[000322] Administration results in the processing of the AADC polynucleotide to generate the AADC protein which alters the etiology of the disease, in this case Parkinson's Disease.

[000323] In one non-limiting example, plasmids containing an AADC polynucleotide of the invention are given in Table 1. These AADC polynucleotides in the table are contained in a Fastback plasmid and have a CMV promoter and encode AADC. In some embodiments the open reading frame of the AADC protein mRNA is codon optimized (e.g., codop).

Table 1. AADC polynucleotide-containing plasmids/vectors.

Construct	SEQ ID NO
pFB CMV hAADC-1	2
pFB CMV hAADC-2	3
pFB CMV hAADC-3	4
pFB CMV hAADC-4	5

Example 2. AADC polynucleotides: ITR to ITR

[000324] AADC polynucleotides suitable for use in an AAV viral vector include those in Table 2.

[000325] Given in Table 2 are the ITR to ITR sequences from Table 1.

Table 2. ITR to ITR AADC polynucleotides

Construct	SEQ ID NO
pFB CMV hAADC-1 (ITR to ITR)	6
pFB CMV hAADC-2 (ITR to ITR)	7
pFB CMV hAADC-3 (ITR to ITR)	8
pFB CMV hAADC-4 (ITR to ITR)	9

Example 3. Relative to the ITR to ITR parent sequence

[000326] AADC polynucleotides are designed according to Table 3 and Table 4. The start and stop positions given are relative to the ITR to ITR AADC polynucleotides described in Table 2.

Table 3. Component modules or sequence regions of AADC polynucleotides

	pFB CMV hAADC-1 (ITR to ITR)		pFB CMV hAADC-2 (ITR to ITR)		pFB CMV hAADC-3 (ITR to ITR)		pFB CMV hAADC-4 (ITR to ITR)	
	Start	Stop	Start	Stop	Start	Stop	Start	Stop
5' ITR	1	130	1	130	1	130	1	130
CMV Enhancer	263	566	263	566	263	566	296	599
CMV Promoter	567	769	567	769	567	769	600	802
ie1 exon 1	784	917	784	917	784	917	817	950
ie1 intron1	918	949	918	949	918	949	951	982
hbBglobin intron2	950	1296	950	1296	950	1296	983	1329
hBglobin exon 3	1297	1349	1297	1349	1297	1349	1330	1382
5' UTR	--	--	--	--	--	--	1398	1468
hAADC	1374	2822	--	--	1374	2822	1473	2921
hAADC codop	--	--	1374	2816	--	--	--	--
3' UTR	--	--	--	--	2823	3221	2922	3361
hGH poly(A) signal	2841	3317	2835	3311	3240	3716	3380	3856
3' ITR	3417	3535	3416	3534	3822	3940	3961	4079

Table 4. Component modules or sequence regions of AADC polynucleotides

	hAADC_1k		hAADC_2k		hAADC_3k		hAADC_4	
	Start	Stop	Start	Stop	Start	Stop	Start	Stop
5' ITR	1	141	1	141	1	141	1	141
CMV Enhancer	245	548	245	548	245	548	245	548
CMV Promoter	549	751	549	751	549	751	549	751
ie1 exon 1	766	899	766	899	766	899	766	899
ie1 intron1	900	931	900	931	900	931	900	931
hbBglobin intron2	932	1278	932	1278	932	1278	932	1278
hBglobin exon 3	1279	1331	1279	1331	1279	1331	1279	1331
5' UTR	--	--	--	--	--	--	1347	3310
hAADC	1356	2804	1356	2804	--	--	1422	2864
hAADC codop	--	--	--	--	1356	2798	--	--
3' UTR	--	--	--	--	--	--	2865	3310
hGH poly(A) signal	2823	3299	2823	3299	2817	3293	3329	3805
3' ITR	3357	3497	3357	3497	3351	3491	3863	4003

Table 5. Component modules or sequence regions of AADC polynucleotides

	hAADC_5k		hAADC_6k		hAADC_9k	
	Start	Stop	Start	Stop	Start	Stop
5' ITR	1	145	1	141	1	130
CMV Enhancer	249	552	245	548	234	537
CMV Promoter	553	755	549	751	538	740
ie1 exon 1	770	903	766	899	755	888
ie1 intron1	904	935	900	931	889	920
hbBglobin intron2	936	1282	932	1278	921	1267

hBglobin exon 3	1283	1335	1279	1331	1268	1320
5' UTR	--	--	--	--	--	--
hAADC			1356	2798	1345	2793
hAADC codop	1360	2802	--	--	--	--
3' UTR	--	--	2799	3203	--	--
hGH poly(A) signal	2821	3297	3222	3698	2812	3288
3' ITR	3355	3499	3756	3896	3346	3475

Example 4. Design of AADC polynucleotides

[000327] AADC polynucleotides are designed to comprise at a minimum a nucleic acid sequence encoding an AADC protein.

[000328] Once designed, the sequence is engineered or synthesized or inserted in a plasmid or vector and administered to a cell or organism. Suitable plasmids or vectors are any which transduce or transfect the target cell.

[000329] Adeno-associated viral vectors (AAV), viral particles or entire viruses may be used.

[000330] Administration results in the processing of the AADC polynucleotide to generate the AADC protein which alters the etiology of the disease, in this case Parkinson's Disease.

[000331] In one non-limiting example, plasmids containing an AADC polynucleotide of the invention are given in Table 6. These AADC polynucleotides in the table are contained in a Fastback plasmid and have a CMV promoter and encode AADC. In some embodiments the open reading frame of the AADC protein mRNA is codon optimized (e.g., codop).

Table 6. AADC polynucleotide-containing plasmids/vectors.

Construct	SEQ ID NO
phAADC_1k	10
phAADC_2k	11
phAADC_3k	12
phAADC_4	13
phAADC_5k	14
phAADC_6k	15
phAADC_9k	16

[000332] Given in Table 7 are the ITR to ITR sequences from Table 6.

Table 7. ITR to ITR AADC polynucleotides

Construct	SEQ ID NO
phAADC_1k (ITR to ITR)	17
phAADC_2k (ITR to ITR)	18
phAADC_3k (ITR to ITR)	19

phAADC_4 (ITR to ITR)	20
phAADC_5k (ITR to ITR)	21
phAADC_6k (ITR to ITR)	22
phAADC_9k (ITR to ITR)	23

Example 5. Administration of AADC polynucleotide compositions to patients for gene therapy

[000333] AADC polynucleotide-containing recombinant AAV vector compositions are infused into the substantia nigra, and in particular, the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) of patients having Parkinson's Disease and identified as qualified for treatment according to methods known in the art.

[000334] One method of administration contemplated for use in the methods described herein is real-time convection-enhanced delivery (RCD) of AADC polynucleotide-containing AAV vector compositions by co-infusion of gadoteridol (a magnetic resonance (MR) contrast agent) and T1 or T2 magnetic resonance imaging (MRI), which can predict areas of subsequent AADC gene expression. As described in Richardson, *et al.*, 2011, the accuracy of cannula placement and initial infusate distribution may be safely determined by saline infusion without significantly altering the subsequent distribution of the tracer agent (Richardson, *et al.*, 2011, *Neurosurgery*, 69(1):154-163). T2 RCD provides detection of intraparenchymal convection-enhanced delivery in the uninjured brain and may predict subsequent distribution of a transgene after viral vector infusion. Subjects undergo saline infusion/T2 acquisition, immediately followed by gadoteridol infusion/T1 acquisition in the putamen and brainstem. Distribution volumes and spatial patterns are analyzed. Gadoteridol and AAV-encoded AADC are co-infused under alternating T2/T1 acquisition in the thalamus, and hyperintense areas are compared with areas of subsequent transgene expression. Ratios of distribution volume to infusion volume are expected to be similar between saline and gadoteridol RCD. Spatial overlap should correlate well between T2 and T1 images. The second infusate will follow a spatiotemporal pattern similar to that of the first, filling the target area before developing extra-target distribution. Areas of AADC expression should correlate well with areas of both T1 and T2 hyperintensity observed during RCD (Richardson, *et al.*, 2011, *Neurosurgery*, 69(1):154-163).

[000335] Convection-enhanced delivery (CED) of macromolecules directly into the brain parenchyma has been known for over two decades. CED is a term that denotes the use of a pressure gradient to generate bulk flow within the brain parenchyma, *i.e.* convection of macromolecules within the interstitial fluid driven by infusing a solution through a cannula placed directly in the targeted structure. This method allows therapeutic agents to be

homogenously distributed through large volumes of brain tissue by bypassing the blood brain barrier and surpassing simple diffusion (Richardson, *et al.*, 2011, *Stereotact. Funct. Neurosurg.* 89:141–151).

[000336] Salegio, *et al.* recently demonstrated the distribution of nanoparticles of different sizes, including micelles (~15 nm in size), AAV (~20–25 nm) and liposomes (~65 nm), within the CNS of rodents and NHPs (Salegio *et al.*, 2014, *Frontiers in Neuroanatomy*, vol. 8, article 9: pp. 1-8). Simple injections cannot engage the perivascular system, and specialized infusion cannulae are required, enabling constant pressures to be exerted at the tip of the cannula such that the interstitial hydrostatic pressure is exceeded and infusate can flow out into the tissue. Simple needles generate significant reflux; thus, reflux-resistant cannulas have been developed to counter this tendency. The advent of platforms for MRI-guided convection-enhanced infusions further refined understanding of the mechanics of perivascular flow, and it was demonstrated that perivascular distribution of liposomes was linear with respect to time, the slope of the curve was increased in myelinated regions, and cessation of infusion prevented further expansion in the volume of distribution. (Richardson, *et al.*, 2011, *Stereotact. Funct. Neurosurg.* 89:141–151; Salegio *et al.*, 2014, *Frontiers in Neuroanatomy*, vol. 8, article 9: pp. 1-8).

[000337] Intraparenchymal rAAV injections are known to result in robust but relatively local transduction. Such local delivery methods are advantageous when attempting gene therapy for neurological disorders that result from neuropathology that is localized to a specific anatomical region or anatomical circuitry such as in the case of Parkinson's disease. However, in treatments requiring more widespread CNS transduction, intraparenchymal injections are impractical. Treatment of neurological disorders attributable to inborn errors of metabolism and/or single-gene defects, or those that affect motor neurons of the spinal cord can require transduction of large proportions of the brain or spinal cord, respectively. Development of less invasive trans-BBB delivery methods for vectors is an extremely important endeavor. Numerous attempts to use molecules that are known to interact with various active transport mechanisms (probably receptor-mediated) to convey proteins across the BBB have been reported with varying results. Given the large number of AAV serotypes available, one or more serotypes may bind a cell-entry receptor capable of transporting the AAV capsid across the BBB (Manfredsson, *et al.*, 2009, "AAV9: a potential blood-brain barrier buster." Molecular Therapy 17(3):403-405).

Vector and stereotaxic infusion

[000338] A stereotactic approach may be used to surgically deliver the AADC polynucleotides. Although individuals with AADC deficiency lack epinephrine and norepinephrine, these patients

should maintain stable blood pressure and heart rates during the surgery. There should be no notable intracerebral hemorrhages in the postoperative computed tomography (CT) or MRI scans. The needle tracts, as shown on the MRI scans, should show accurate injection into the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA). The patients will be discharged from the hospital about one week after the surgery (Hwu, W.L., *et al.*, 2012. Gene therapy for aromatic L-amino acid decarboxylase deficiency. *Sci. Transl. Med.* Vol. 4, 134ra61).

[000339] Subjects of treatment receive the AAV-vector composition vector, safely delivered to substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) via bilateral infusions, or alternatively, intrastriatally (into the caudate nucleus and putamen), or into the subthalamic nucleus (STN), for example optionally using the FDA-approved SMARTFLOW® neuroventricular cannula (SurgiVision, Inc.) specifically designed for clinical application, with or without the aid of the CLEARPOINT® system to help the treating neurosurgeon(s) target and observe the delivery of the therapeutic agent in the brain (*See*, for example, San Sebastian, *et al.*, 2014, *Mol. Ther. Methods Clin. Dev.* 3: 14049; *See*, for example, Feng and Maguire-Zeiss, 2010, *CNS Drugs* 24(3):177-192).

[000340] For example, during the surgery, two target points are determined in the substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) that are sufficiently separated from each other in dorsolateral directions and identified on a magnetic resonance image. One burr hole is trepanned in each side of the cranial bone, through which the vector is injected into the two target points via the two-track insertion route. The AAV-vector-containing solution is prepared to a concentration of 1.5×10^{12} vector genome/ml, and 50 µl per point of the solution is injected at 1 µl/min; each patient receives 3×10^{11} vector genome of the AAV-vector construct.

[000341] Neutralizing antibody titers against AAV2 are determined by measuring β-galactosidase activities in HEK293 cells transduced with 5×10^{3} vector genome/cell of AAV2 vectors expressing β-galactosidase in various dilutions of sera.

PET

[000342] The AADC expression level in the substantia nigra are assessed on PET imaging with FMT six days before surgery and at one- and six-months after gene transfer. All patients cease taking dopaminergic medications 18 hours before PET and take 2.5mg/kg of carbidopa orally one hour before FMT injection. Subsequently, 0.12mCi/kg of FMT in saline is infused into an antecubital vein, and a 90-minute dynamic acquisition sequence is obtained. The PET and magnetic resonance imaging data are co-registered with a fusion processing program (Syntegra; Philips, Amsterdam, The Netherlands) to produce the fusion images. Radioactivities within volumes of interest drawn in the nigrostriatal pathway are calculated between 80 and 90 minutes

after tracer injection. A change in nigrostriatal pathway FMT uptake from baseline to 24 weeks is assessed using the substantia nigra to striatal ratio of radioactivities.

Statistical analysis

[000343] Values at baseline and 6 months after gene transfer are compared using Student's t-test (paired analyses). A two-sided P value <0.05 is taken to indicate significant differences. Two-way analysis of variance with Bonferroni correction of P values is used for the short-duration response to levodopa. (*See*, for example, Muramatsu, *et al.*, 2010, "A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease." *Mol. Ther.* 18:1731–1735).

[000344] Safety and tolerability of bilateral administration of AAV-vector compositions using real-time image-guided infusion into the brains of Parkinson's Disease subjects may be monitored for up to or after 9 months post-surgery. Broad coverage of targeted areas (substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA)) and widespread AADC protein distribution in the striatum should be achieved without inducing any adverse effects.

[000345] *Changes in growth and motor skills:* The patients should gain weight and exhibit improvement in their motor scores after gene transfer, within a year, post-treatment. Weight will be measured at 3 to 6 months after gene transfer. All patients initially should have raw scores of zero on the Alberta Infant Motor Scale (AIMS) and very low raw scores for the Peabody Developmental Motor Scale, Second Edition (PDMS-II). After the gene transfer, all of the patients should show continuous increases in their raw scores on these two scales, which indicates that their motor functions have improved. The Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT) covers both cognition and motor development. All of the patients should show low raw CDIIT scores before gene transfer, and the subsequent increase in scores demonstrate improvement in both motor and cognitive functions.

Subjective improvements after gene transfer

[000346] To document the symptoms that are more difficult to quantify, spouses, guardians or caretakers of the patients are asked to fill out a questionnaire at the end of the study. The symptoms of the oculogyric crises should lessen, and eye deviations and sleep disruptions, for example, are some mild symptoms of the oculogyric crises that may remain after gene therapy. Subjects may experience increased emotional stability, and/or some improvements in sweating and hyperthermia (a common manifestation of body temperature instability in hot weather). There should be no detectable abnormality in heart rate variability as assessed by 24-hour Holter monitoring either before or after gene transfer. Before gene therapy, patients that were bedridden and showed little spontaneous movement may exhibit less severe ptosis (drooping of the upper

eyelid) one to two weeks after the gene transfer. According to previous studies, dyskinesia may occur one month after gene transfer, but upon observation of a decrease in dyskinesia, motor development should start (Hwu, W.L., *et al.*, 2012. Gene therapy for aromatic L-amino acid decarboxylase deficiency. *Sci. Transl. Med.* Vol. 4, 134ra61). Subjects may exhibit increased head control after three months, sitting with support after six to nine months, sitting up from the prone position after thirteen months, and holding toys and standing with support sixteen months after the gene transfer, for example. Anti-AAV2 antibodies should be negative in the patients before gene therapy, and the titers may increase slightly after gene transfer.

PET scans and CSF analyses

[000347] PET scans and CSF analyses are completed for the treated patients. Six months after gene transfer, PET scans should reveal that uptake of 6-[18F] fluorodopa (FDOPA) increase from baseline in the combined (right and left) treatment sites. The CSF analysis should reveal increases in the levels of homovanillic acid (HVA, a metabolite of dopamine) and 5-hydroxyindoleacetic acid (HIAA, a metabolite of serotonin). However, the levels of L-DOPA and 3-O-methyldopa may remain elevated (Hwu, W.L., *et al.*, 2012. Gene therapy for aromatic L-amino acid decarboxylase deficiency. *Sci. Transl. Med.* Vol. 4, 134ra61).

Example 6. Administration of AADC Polynucleotides

[000348] AADC polynucleotide-containing recombinant AAV vector compositions are infused into the putamen of patients having Parkinson's Disease using the administration methods described in Example 5. The dose, number of patients and volume are outlined in Table 8.

Table 8. Study Design

Study No.	Number of Patients	Dose	Volume
1	6	3×10^{11} vg	100 ul per putamen
2	6	9×10^{11} vg	300 ul per putamen
3	10	2.3×10^{11} vg	100 ul per putamen
4	10	7.5×10^{11} vg	100 ul per putamen
5	5	7.5×10^{11} vg	450 ul per putamen
6	Up to 20	1.4×10^{12} vg	Up to 900 ul per putamen
7	Up to 20	4.8×10^{12} vg	Up to 900 ul per putamen
8	Up to 20	8.8×10^{12} vg	Up to 900 ul per putamen

[000349] During the course of the study the safety and tolerability of the infusion of the AADC polynucleotide-containing recombinant adeno-associated virus (AAV) vector compositions in human patients diagnosed with Parkinson's Disease is evaluated. Patients are evaluated preoperatively and monthly postoperatively for six months, using multiple measures, including the Global Systonia Scale (GDS) (*see* Comella, *et al.*, 2003, *Movement Disorders*, 18(3):303–312), L-DOPA challenge test, UPDRS scores, motor state diaries, and laboratory tests. Using diaries that separate the day into half-hour segments, the caregivers of the patients will record

their mobility during the four days before admission and for another four days at six months after admission to the study site. The patient caregivers are trained to rate subject's condition as sleeping, immobile, mobile without troublesome dyskinesias, or mobile with troublesome dyskinesias. The total number of hours spent in each of these categories is calculated, and the differences between the baseline and the six-month scores are compared between the groups. The short-duration response to levodopa is evaluated at baseline and 6 months after gene transfer; subjects take 100 mg of levodopa orally with 25mg benserazide after 20 hours without dopaminergic medication. Motor symptoms based on GDS and plasma levodopa concentrations are assessed at baseline and 30 minutes, 1, 2, 3, and 4 hours after levodopa intake (*See*, for example, Muramatsu, *et al.*, 2010, "A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease." *Mol. Ther.* 18:1731–1735).

Example 7. *In Vivo* Administration of AADC Polynucleotides

[000350] Two AADC polynucleotide-containing recombinant AAV vector compositions (plasmid SEQ ID NO: 10 and 12; ITR to ITR SEQ ID NO: 17 and 19), a control and a standard were administered to rats (n=5) by bilateral intrastriation (10 ul/side) at a dose level of $2x10^{12}$ vg/ml. The expression of AADC in rat striatum was determined by ELISA after 4 and 8 weeks. Variation was seen between the animals and the hemispheres due to variable delivery between the infusion sites. Both constructs expressed AADC, but the phAADC_3k construct (plasmid SEQ ID NO: 12; ITR to ITR SEQ ID NO: 19) showed up to 200% increase of expression as compared to the standard construct.

Example 8. Dose Response Study of AADC Polynucleotides

[000351] Compositions of AADC polynucleotide-containing recombinant AAV vectors (plasmid SEQ ID NO: 10; ITR to ITR SEQ ID NO: 17) at five different dose levels ranging from $1x10^{11}$ vg/ml to $1x10^{13}$ vg/ml and a control are administered to 6-OHDA lesioned rats. The behavioral response to low-dose levodopa administration is quantified before and after (week 3 and 4) delivery of the composition. 5 weeks after dosing, necropsy is conducted and the AADC enzymatic activity is measured in *ex vivo* striatal tissue assay and the distribution of AADC in the brain is determined by immunohistochemical (IHC) staining.

Example 9. Effect of Empty Particles on Intrastriatal Transduction

[000352] Adult rats (n=6) were administered varying ratios of full:empty vector particles at: 0% full, 50% full, 85% full or 99% full. AADC polynucleotide-containing recombinant AAV vector (plasmid SEQ ID NO: 10; ITR to ITR SEQ ID NO: 17) at a constant dose and volume (5 ul and 1x10 vg) was administered intrastriatally. The rats were evaluated 4 weeks after

administration. The low vector dose resulted in limited AADC vector expression. The volume of distribution for the particles is shown in Table 9 (ELISA) and the striatal levels of AADC expression is shown in Table 10 (Histology).

Table 9. Volume of Distribution

% Ratio of full AAV2-AADC particle to empty capsids	Approximate Volume of Distribution (mm^3)
50:50	2
70:30	2.4
85:15	2.5
100:0	3

Table 10. Striatal Levels

% full of AAV2-AADC particles	AADC pg/ug protein
0	0.4
52	1.1
58	1.7
83	2
>99	2.1

[000353] Distribution was comparable for all groups. Relatively low vector dose resulted in limited AADC expression. There was also a trend to lower AADC expression levels with >30% empty particles.

[000354] While the present invention has been described at some length and with some particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

[000355] All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

Claims

1. An AAV vector genome comprising, in order:
a) a 5’ inverted terminal repeat (ITR), wherein said 5’ ITR is 141 nucleotides in length,
b) a cytomegalovirus (CMV) sequence region, wherein said CMV sequence region is derived from the CMV gene and said CMV sequence region comprises 5’ to 3’ an enhancer region and promoter region, wherein said CMV sequence region is at least 500 nucleotides,
c) an immediate early 1 (IE1) sequence region derived from an IE1 gene,
d) a human beta globin (HB) sequence region derived from a HB gene,
e) an AADC sequence region, said AADC sequence region comprising a nucleotide sequence encoding SEQ ID NO: 1,
f) a poly(A) signal sequence region derived from the human growth hormone (hGH) gene, and
g) a 3’ ITR, wherein said 3’ ITR is 141 nucleotides in length.

2. The AAV vector genome of claim 1, wherein the 5’ ITR and the 3’ ITR are derived from AAV2.

3. The AAV vector genome of claim 2, wherein the CMV sequence region is 507 nucleotides in length.

4. The AAV vector genome of claim 2, wherein the IE1 sequence region comprises a nucleotide sequence from IE1 exon1.

5. The AAV vector genome of claim 4, wherein the IE1 sequence region further comprises a nucleotide sequence from IE1 intron 1 or a fragment thereof.

6. The AAV vector genome of claim 5, wherein the IE1 sequence region is at least 160 nucleotides in length.

7. The AAV vector genome of claim 5, wherein the IE1 sequence region is 166 nucleotides in length.

8. The AAV vector genome of claim 2, wherein the HB sequence region comprises a nucleotide sequence from HB intron 2.

9. The AAV vector genome of claim 8, wherein the HB sequence region further comprises a nucleotide sequence from HB exon 3.

10. The AAV vector genome of claim 9, wherein the HB sequence region is 400 nucleotides in length.

11. The AAV vector genome of claim 2, wherein the poly(A) signal sequence region is at least 450 nucleotides in length.

12. The AAV vector genome of claim 11, wherein the poly(A) signal sequence region is 477 nucleotides in length.

13. The AAV vector genome of claim 2, wherein
i) the IE1 sequence region comprises a nucleotide sequence from IE1 exon1,
ii) the IE1 sequence region comprises a nucleotide sequence from IE1 intron 1 or a fragment thereof,
iii) the HB sequence region comprises a nucleotide sequence from HB intron 2, and
iv) the HB sequence region comprises a nucleotide sequence from HB exon 3.

14. The AAV vector genome of claim 13, wherein the CMV sequence region is 507 nucleotides in length, the IE1 sequence region is 166 nucleotides in length, the HB sequence region is 400 nucleotides in length, the poly(A) signal region is 477 nucleotides in length,

15. The AAV vector genome of claim 1, wherein the AAV vector genome comprises a sequence selected from the group consisting of SEQ ID NO: 1-13, 15, and 16 or variants having at least 95% identity thereto.

16. The AAV vector genome of claim 2, wherein, the AADC region comprises a codon optimized nucleotide sequence encoding SEQ ID NO: 1.

17. The AAV vector genome of claim 16, wherein the AAV vector genome comprises a sequence selected from the group consisting of SEQ ID NO: 7 and 12 or variants having 95% identity thereto.

18. A recombinant AAV virus comprising the AAV vector genome of any one of claims 1-17.

19. The recombinant AAV virus of claim 18, comprising a capsid serotype selected from the group consisting of AAV2, AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV9.47, AAV9(hu14), AAV10, AAV11, AAV12, AAVrh8, AAVrh10, AAV-DJ, and AAV-DJ8.

20. The recombinant AAV virus of claim 19, wherein the capsid serotype is AAV2.

21. A pharmaceutical composition comprising the recombinant AAV virus of any one of claims 18-20.

22. The pharmaceutical composition of claim 21, wherein at least 70% of the recombinant AAV vectors contain an AAV vector genome.

23. The pharmaceutical composition of claim 21 or 22, for use in a method of reducing periods of dyskinesia in a subject in need thereof.

24. The pharmaceutical composition of claim 23, wherein the decrease in periods of dyskinesia is shown by improvement in off time and motor fluctuations by at least 30%.

25. The pharmaceutical composition of claim 24, wherein the improvement lasts for at least 6 hours.

26. The pharmaceutical composition of any one of claims 23-25, wherein the subject has Parkinson's Disease.

27. The pharmaceutical composition of claim 21 or 22, for use in a method of improving the sleep-wake cycle of a subject in need thereof.

28. The pharmaceutical composition of claim 27, where the subject's amount of rapid eye movement (REM) sleep is decreased after administration of the pharmaceutical composition to the subject as compared to the subject's amount of REM sleep prior to the administration of the pharmaceutical composition.

29. The pharmaceutical composition of claim 27, wherein the subject's amount of non-REM (NREM) sleep is increased after administration of the pharmaceutical composition to the subject as compared to the subject's amount of REM sleep prior to the administration of the pharmaceutical composition.

30. The pharmaceutical composition of any one of claims 27-29, wherein the subject has Parkinson's Disease.

31. The pharmaceutical composition of claim 21 or 22, for use in a method of treating a sleep disorder of a subject in need thereof.

32. The pharmaceutical composition of claim 31, wherein the sleep disorder is insomnia.

33. The pharmaceutical composition of any one of claims 31-32, wherein the subject has Parkinson's Disease.

34. The pharmaceutical composition of claim 21 or 22, for use in a method of increasing the level of AADC protein in a subject in need thereof.