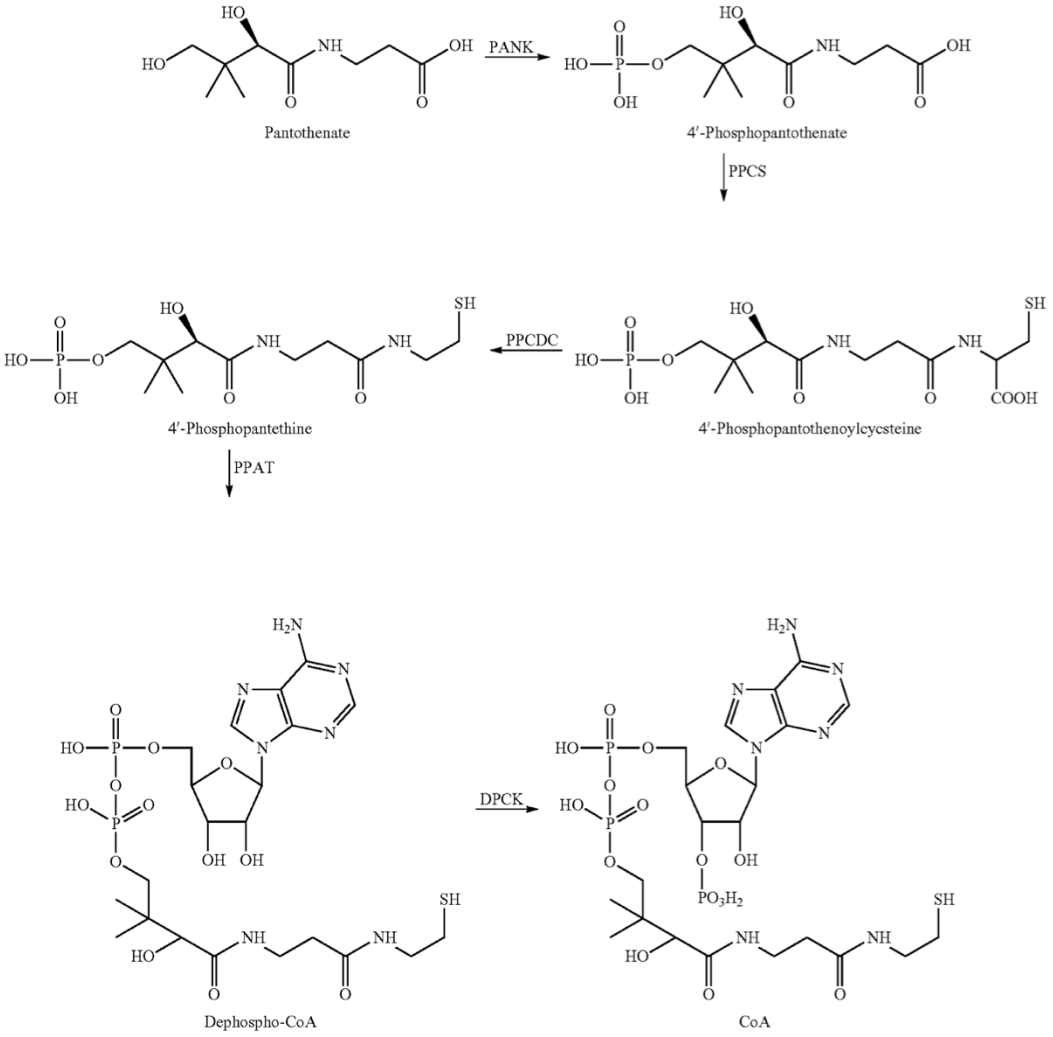
GENE THERAPIES FOR THE TREAMENT OF NEUROLOGICAL DISORDERS

ABSTRACT

The present disclosure relates to compositions and methods for the treatment of neurological disorders (such as pantothenate kinase-associated neurodegeneration and related parkinsonisms). In some embodiments, the disclosure provides expression constructs comprising a transgene encoding of functional Pantothenate Kinase 2*, PKAN2,* or a portion thereof, C19orf12 or PLA2G6, and any combination of the foregoing to a subject in need thereof.

Background

Pantothenate kinase-associated neurodegeneration (PKAN) is a form thought to be responsible for half of neurodegeneration with brain iron accumulation (NBIA) that causes extrapyramidal dysfunction (eg, dystonia rigidity choreoathetosis) (A.M. Gregory and S.J. Hayflic, "Neuro-degeneration With Brain Iron Accumulation", *Orphanet Encyclopedia*, September 2004). PKAN is thought to be a genetic disorder resulting from lack of the enzyme pantothenate kinase, which is responsible for the conversion of pantothenate (Vitamin B-5) to 4'-phosphopanthenate. 4-Phosphopantothenate is subsequently converted into Coenzyme A (CoA) (as shown below) (R. Leonardi, Y.-M. Zhang, C.O. Rock, and S Jackowski, "Coenzyme A: Back in Action", *Progress in Lipid Research,* 2005, 44, 125-153)



In particular, pantothenate is converted to 4’-phosphonate via the enzyme pantothenate kinase(PANK) which is converted to 4’Phosphopantothenoylcsteine via the enzyme 4’phosphopantothenocylsteine synthase (PPCS), and subsequently decarboxylated to 4’phosphopantethine via 4’-phosophopanthenoylcysteine decarboxylase (PPCDC). 4’phsphopantethine is then appended to adenosine by the action of phosphosphpantethine adenlytransferease(PPAT) to afford dephospho CoA, which is finally converted to coenzyme A (CoA) via dephospho-CoA kinase (DPCK). Coenzyme A is critical for the operation of various cellular metabolic processes.

Classic PKAN usually presents in a child’s first ten to fifteen years, though there is also an atypical form that may occur up to age 40. PKAN is a progressively degenerative disease, that leads to loss of musculoskeletal function with a devasting effect on quality of life. Even though

the genetic mutations in the *PANK2* gene might be predicted to affect all cells, the pathology of PKAN appears to be highly focal, centered in neurons of the globus pallidus (GP) and in retinal rods, which have high energy and myelin maintenance demand. (Munshi, Muhammad I et al. "Redesigning therapies for pantothenate kinase-associated neurodegeneration." *The Journal of biological chemistry* vol. 298,3 (2022): 101577.)

One approach to treating PKAN could be gene therapy directed at the *PANK2 gene* which encodes pantothenic acid(vitamin b5) in the biosynthesis of coenzyme A(CoA). This approach has been mentioned in the literature, but it has been noted gene-based therapy for PKAN remains challenging as any vector carrying the gene would have to be delivered across the blood brain barrier. This concern is addressed in the summary below. (Combs, B, Kneynsberg A. Kanaan N.M. "Gene therapy models of Alzheimer's disease and other dementias." *Methods Mol. Biol.* 2016;1382:339-366.)

Summary of Invention

The disclosure is based, in part, on compositions and methods for expression of combinations of gene therapies to treat neurological disorders such as PKAN disease and NBIA. A gene product can be a protein, a fragment(e.g. portion) of a protein an interfering nucleic acid that inhibits a PKAN-associated gene, etc. In some embodiments a gene product is a protein or a protein fragment encoded by a PKAN-associated gene. In some embodiments, a gene product is an interfering nucleic acid(e.g, shRNA, siRNA, miRNA, amiRNA etc,) that inhibits a PKAN-associated gene.

A PKAN-associated gene refers to a gene encoding a gene product that is genetically, biochemically, or functionally associated with PKAN. For example, individuals having mutations in the PANK2 gene (which encodes the protein pantothenate kinase 2 protein consisting of 570 amino acids) has been observed to have an increased risk of developing PKAN disease and other neurological disorders compared to individuals that do not have a mutation in PKAN2. In another example, PKAN is associated with accumulation of iron, NBIA, primarily in the globus pallidus. In some embodiments, an expression cassette described herein encodes a wild-type or isolated nucleic acid and vectors.

An isolated nucleic acid may be DNA or RNA. The disclosure provides, in some aspects, an isolated nucleic acid comprising an expression construct encoding pantothenate Kinase(e.g., the gene product of *PANK2* gene) or a portion thereof. Pantothenate Kinase, refers to a an essential regulatory enzyme in CoA biosynthesis, catalyzing the cytosolic phosphorylation of pantothenate (vitamin B5), N-pantothenoylcysteine, and pantetheine. In humans, PKAN is encoded by the *PANK2* gene located on chromosome 20. In some embodiments, *PANK2* encodes a peptide that is represented by SEQ ID NO: 1 (NCBI Reference Sequence NP_001311120.1).
. In some embodiments, the isolated nucleic acid comprises a pantothenate-encoding sequence that has been codon optimized (e.g. codon optimized for expression in mammalian cells, for

example human cells) such as the sequence set forth in SEQ ID NO: 2 (NCBI Reference Sequence NP_705902.2).

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a Phospholipase A2 (e.g., the gene product of PLA2G6 gene). Phospholipase A2 catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA), a precursor of eicosanoids including prostaglandins (PGs) and leukotrienes(LTs). The same reaction also produces lysophospholipids , which represent another class of lipid mediators. In humans, the PLA2G6 gene is located on chromosome 22. In some embodiments, PLA2G6 gene encodes a peptide that is represented by SEQ ID NO:3 (NCBI Reference Sequence NM_001199562.3). In some embodiments, the isolated nucleic acid comprises Phospholipase A2-encoding sequence that has been codon optimized (e.g codon optimized for expression in mammalian cells, for example human cells), such the sequence set forth in SEQ ID NO:4 (NCBI Reference Sequence NM_001349865.2.

Aspects of the disclosure relate to an isolated nucleic acid comprising an expression construct encoding C19orf12(e.g., the gene product of C19orf12 gene). C19orf12 refers to a small transmembrane protein. Mutations in this gene are a cause of neurodegeneration with brain iron accumulation-4 (NBIA4). Spanning 17 kb and containing 4 exons, expression of C19orf12 increases with differentiation in adipocytes along with genes involved in valine, leucine, and isoleucine degradation and fatty acid metabolism. In some embodiments the C19orf12 gene encodes a peptide that is represented by SEQ ID NO:5 (NCBI Reference Sequence NP_001026896.3). In some embodiments the isolated nucleic acid comprises a C19orf12-encoding sequence that has been codon optimized.

In some aspects, the disclosure provides an isolated nucleic acid comprising an expression construct encoding a first gene product and a second gene product, wherein each gene product independently is selected from the gene products, or portions thereof, set forth in Table 1.

TABLE 1

Examples of Neurodegenerative-associated genes

Name	Gene	Function	NCBI Accession No.
Pantothenate Kinase	PKAN2	regulatory enzyme in CoA biosynthesis	NP_ 001311120.1 WP_0030937 68.1
Phospholipase A2	PLA2G6	catalyzes the hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid (AA).	NM _ 001199562.3 NM_ 001349865.2
C19orf12 proetein	C19orf12	protein plays a role in the maintenance of fat (lipid) molecules, a process known as lipid homeostasis.	NP 0010268963 Isoform 2 NP NP_001269860.1 Isoform 4 NP NP_001242975.1 .1 (Isoform 3)

In some embodiments, a gene product is encoded by a coding portion (e.g., A cDNA) of a naturally occurring gene. In some embodiments, a first gene product is a protein (or a fragment thereof) encoded by the *PKAN2* gene. In some embodiments, a gene product is a protein (or a fragment thereof) encoded by the PLA2G6 gene and or/c19orf12 gene. However, the subject expert recognizes that the order of expression of a first gene product (e.g., Phospholipase A2) and a second gene product (e.g., C19orf12 protein) can generally be reversed (e.g., C19orf12 protein is the first gene product and Phospholipase A2 is the second gene product). In some embodiments, a gene product is a fragment (e.g., portion) of a gene listed in Table 1. A protein fragment may comprise about 50%, about 60%, and 70%, about 80%, or about 99% of a protein encoded by the genes listed in Table 1.

In some embodiments, an expression construct is monocistronic (e.g. the expression construct encodes a single fusion protein comprising a first gene product and a second gene product). In

some embodiments, an expression construct is polycistronic (e.g., the expression construct encodes two distinct gene products, for example two different proteins or protein fragments.)

A polycistronic expression vector may comprise one or more (e.g., 1,2,3,4 or more) promoters. Any suitable promoter can be used, for example, a constitutive promoter, an inducible promoter, an endogenous promoter, a tissue-specific promoter (e.g., a CNS-specific promoter), etc. In some embodiments, a promoter is a chicken beta actin promoter (CBA promoter), a CAG promoter (for example as described by Alexopoulou et al (2008) *BMC Cell Biol*. 9:2, doi:10.1186/1471-2121-9-2), a CD68 promoter, or a JeT promoter (for example as described by Tornøe et al. (2002) *Gene297(1-2):21-32).* In some embodiments, a promoter is operably-linked to a nucleic acid sequence encoding a first gene product, a second gene product, or a first gene product and a second gene product. In some embodiments, an expression cassette comprises one or more additional regulator sequences including but not limited to transcription factor binding sequences, intron splice sites, Poly(A) addition sites, enhancer sequences, repressor binding sites, or any combination of the foregoing.

In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product or a separated by a nucleic acid sequence encoding an internal ribosomal entry site (IRES). Examples of IRES sites are described, for example, by Mokrejs et al. (2006) *Nucleic Acids Res. 34 (*Database issue): D125-30. In some embodiments, a nucleic acid sequence encoding a first gene product and a nucleic acid sequence encoding a second gene product are separated by a nucleic acid sequence encoding a self-cleaving peptide. Examples of self-cleaving peptides include but are not limited to t2A, P2A,E2A,F2A, BmCPV 2A, and BMIFV 2A, and those described by Liu et al. (2017) Sci Rep. 7: 2193. In some embodiments, the self-cleaving peptide is a T2A peptide.

Pathologically, disorders such as PKAN disease and other neurodegenerative disorders are associated with brain iron accumulation (NBIA) contributing to progressive impairment of movement, vision, and cognition. The disease is initially diagnosed on the basis of changes in brain magnetic resonance imaging which indicate an abnormal brain iron accumulation in the basal ganglia. Accordingly, in some embodiments, isolated nucleic acids described herein comprise a catalytic nucleic acid that improves the biosynthesis of coenzyme A (CoA) and achieve balance of cysteine accumulation reducing iron deposits in the basal ganglia and dentate nucleus. A sequence encoding a catalytic nucleic acid may be placed in an untranslated region (e.g., intron, 5'UTR, 3'UTR etc.) of the expression vector.

In some embodiments, an inhibitory nucleic acid is positioned in an intron of an expression construct, for example in an intron upstream of the sequence encoding a first gene product. An inhibitory nucleic acid can be a double stranded RNA (dsRNA), siRNA, micro RNA (miRNA), artificial miRNA(amiRNA), or an RNA aptamer. Generally an inhibitory nucleic acid binds to (e.g., hybridizes with) between about 6 and 30 (e.g., Any integer between 6 and 30, inclusive) contiguous nucleotides of a target RNA (e.g., mRNA). In some embodiments, the inhibitory nucleic acid molecule is an miRNA or amiRNA, for example an miRNA that targets Pantothenate Kinase 2 (the gene encoding PKAN2 protein). In some embodiments, the miRNA does not

comprise any mismatches with the region of Pantothenate Kinase 2 mRNA to which it hybridizes (e.g., the miRNA is "perfected"). In some embodiments, the inhibitory nucleic acid is an shRNA (e.g., an shRNA targeting Pantothenate Kinase 2).
An isolated nucleic acid as described herein may exist on its own or as part of a vector. Generally, a vector can be plasmid, cosmid, phagemid, bacterial artificial chromosome (BAC), or a viral vector (e.g. adenoviral vector, adeno-associated virus AAV, vector retroviral vector, baculoviral vector, etc). In some embodiments, the vector is a plasmid (e.g., a plasmid comprising an isolated nucleic acid as described herein). In some embodiments, the vector is a recombinant AAV (rAAV) vector. In some embodiments, an rAAv vector is single-stranded (e.g., single-stranded DNA). In some embodiments, a vector is a Baculovirus vector (e.g. an *Autographa Californica* nuclear polyhedrosis (AcNPV) vector).

Typically, an rAAV vector (e.g., rAAV genome) comprises a transgene (e.g., an expression construct comprising one or more of each of the following: promoter, intron, enhancer sequence, protein coding sequence, inhibitory RNA coding sequence, Poly(A) tail sequence, etc.) flanked by two AAV inverted terminal repeat (ITR) sequences. In some embodiments, each of the two ITR sequences of an rAAV vector is a full-length ITR(e.g., approximately 145 bp in length, and containing functional REP binding site (RBS) and terminal resolution site (trs)). In some embodiments, one of the ITRs of an rAAv vector is truncated (e.g., shortened or not full-length. In some embodiments, a truncated ITR lacks a functional terminal resolution site (trs) and is used for production of self-complementary AAV vectors (scAAV vectors). In some embodiments, a truncated ITR is a ΔITR, for example as described by McCarty et al. (2003) *Gene Ther.*10(26):2112-8.

Aspects of the disclosure relate to isolated nucleic acids (e.g., rAAV vectors) comprising an ITR having one or more modifications (e.g., nucleic acid additions, deletions, substitutions, etc.) relative to a wild-type AAV ITR, for example relative to wild-type AAV2 ITR(e.g., SEQ ID No: 6). The structure of wild-type AAV2 ITR is shown in **Fig. 19**. Generally, a wild type ITR comprises 125 nucleotide region that self-anneals to form a palindromic double-stranded T-shaped, hairpin structure consisting of two cross arms (formed by sequences referred to as B/B' and C/C', respectively), a longer stem region (formed by sequences A/A, and a single-stranded terminal region referred to as the "D" region. (FIG 19). Generally, the "D" region of an ITR is positioned between the stem region formed by the A/A' sequences and the insert containing the transgene for the rAAV vector (e.g. positioned on the "inside" of the ITR relative to the terminus of the ITR or proximal to the transgene insert or expression construct of the rAAV vector). In some embodiments, a "D" region comprises the sequence set forth in SEQ ID NO: 7. The "D" Region has been observed to play an important role in encapsidation of rAAV vectors by casid proteins, for example as disclosed by Ling et al. (2015) *J Mol Genet Med 9(3).*

The disclosure is based, in part, on the surprising discovery that rAAV vectors comprising a "D" region located on the "outside" of the ITR (e.g., proximal to the terminus of the ITR relative to the transgene insert or expression construct) are efficiently encapsidated by AAV capsid proteins than rAAV vectors having ITRs with unmodified (e.g., wild-type) ITRs. In some embodiments, rAAV vectors having a modified "D" Sequence (e.g. a "D" sequence in the

"outside" position) have reduced toxicity relative to rAAV vectors having wild-type ITR sequences.

In some embodiments, a modified "D" sequence comprises atleast one nucleotide substitution relative to a wild-type "D" sequence (e.g., SEQ ID NO: 8). A modified "D" sequence may have atleast 1, 2,3 4, 5, 6, 7, 8, 9, 10 or more than 10 nucleotide substitutions relative to a wild type "D" sequence (e.g, SEQ ID NO: 9). In some embodiments, a modified "D" sequence comprises at least 10, 11, 12, 13, 14, 15, 16 ,17, 18, or 19 nucleic acid substitution relative to a wild-type "D" sequence (e.g., SEQ ID NO: 10). In some embodiments, a modified "D" sequence is between about 10% and about 99% (e.g., 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,95%, or 99%) identical to wild-type "D" sequence (e.g. SEQ ID NO:11). In some embodiments, a modified "D" sequence comprise the sequence set forth in SEQ ID 12, also referred to as "S" sequence as described in Wang et al. (1995) *J Mol Biol* 250(5):573-80.

An isolated nucleic acid or rAAV vector as described by the disclosure may further comprise a "TRY" sequence, for example as set forth in SEQ ID NO:13 or as described in Francois, et al. The Cellular TATA Binding Protein is required for Rep-Dependent Replication of a Minimal Adeno-Associated Virus Type 2 p5 Elemement. J Virol. 2005. In some embodiments, a TRY sequence is positioned between an ITR (e.g.a 5'ITR) and an expression construct (e.g. a transgene-encoding insert) of an isolated nucleic acid or rAAV vector.

In some aspects, the disclosure relates to Baculovirus vectors comprising an isolated nucleic acid or rAAV vector as described by the disclosure. In some embodiments, the Baculovirus vector is an *Autographa Californica* nuclear polyhedrosis (AcNPV) vector, for example as describe dby Urabe et al. (*2002) Hum Gene Ther* 13(16):1935-43 and Smith et al. (2009) *Mol Ther 17(*11) : 1888-1896.

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or vector as described herein. A host cell can be a prokaryotic cell or eukaryotic cell. For example, a host cell can be a mammalian cell, bacterial cell, insect cell, etc. In some embodiments, a host cell is a mammalian cell, for example a HEK293T cell. In some embodiments, a host cell is a bacterial cell, for example an *E. coli* cell.

In some aspects, the disclosure relates to recombinant AAVs (rAAVs) comprising a transgene that encodes a nucleic acid as described herein (e.g., an rAAV vector as described herein). The term "rAAvs" generally refers to viral particles comprising an RAAV vector encapisadated by one or more AAV capsid proteins. An rAAv described by the disclosure may comprise a capsid protein having a serotype selected from AAV1, AAv2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, and AAV10. In some embodiments, an rAAV comprises a capsid protein from a non-human host, for example a rhesus AAV capsid protein such as AAVrh, 10, AAVrh.39 etc. In some embodiments, an rAAV described by the discloser comprises a capsid protein that is a variant of a wild-type capsid protein such as a capsid protein variant that includes at least

1,2,3,4,5,6,7,8,9,10 or more than 10 (e.g. 15, 20, 25, 50, 100, etc.) amino acid substitutions (e.g. mutations relative to the wild-type AAV capsid protein from which it is derived.

In some embodiments, rAAVs described by the disclosure readily spread through the CNS, particularly when introduced in to CSF space or directly into the brain parenchyma. Accordingly, in some embodiments, rAAVs described by the disclosure comprise a capsid protein that is capable of crossing the blood-brain barrier (BBB). For example, in some embodiments, an rAAv comprises a capsid protein having an AAV9 or AAVrh.10 serotype. Production of rAAVs is described, for example by Samulski et al. (1989) *J Viorl.* 63(9):3822-8 and Wright(2009) *Hum Gene Ther.* 20(7):698-706.

In some embodiments, an rAAV as described by the disclosure (e.g., comprising a recombinant rAAV genome encapsidated by AAV capsid proteins to form an rAAV capsid particle) is produced in a Baculovirus vector expression system (BEVS). Production of rAAVs using BEVS are described, for example by URABE et al. (2002) *Hum Gene Ther 13(16): 1935-43, Smith et al. (2009)* Mol Ther *17 (11) 1888-1896,* U.S. Pat. Nos. 8,945,918, 9,879, 282, and International PCT Publication WO 2017/184879. However, an rAAV can be produced using any suitable method (e.g., using recombinant rep and cap genes).

Pharmaceutical Compositions

In some aspects, the disclosure provides pharmaceutical compositions comprising an isolated nucleic acid or rAAV as described herein and a pharmaceutically acceptable carrier. As use herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or dilutant, which does not abrograte the biological activity or properties of the compound, and is relatively non-toxic, e.g. the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutically accepted carrier means a pharmaceutically acceptable material, composition, or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involving in carrying or transporting a compound useful withing the invention within or to the patient such that it may perform its intended function. Additional ingredients that may be included in the pharmaceutical composition sued in the practice of the invention are known in the art described, for example in Remington’s Pharmaceutical Sciences (Genaro, ED., Mack Publishing CO., 1985, Easton, Pa.), which is incorporated herein by reference.

Compositions (e.g., pharmaceutical compositions) provided herein can be admnistered by an route, including enteral (e.g., oral, parenteral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, transdermal, intradermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops). Mucosal, nasal buccal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation, and/or as an oral spray, nasal spray, and/or aerosol. Specifically contemplated

routes are oral administration, intravenous administration (e.g., systemic intravenous injection), regional administration via blood and/ or lymph supply, and/or direct administration to an affected site. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (e.g., its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (e.g., whether the subject is able to tolerate oral administration). In certain embodiments, the compound or pharmaceutical composition described herein is suitable for topical administration in the eye of a subject.

Methods

The disclosure is based in part, on compositions for expression of combinations of PKAN associated gene products in a subject that act together(synergistically) to treat neurodegenerative disease. As used herein “treat” or “treat-ing” refers to (a) preventing or delaying onset of PKAN disease and other neurological disorders, (b) reducing severity of PKAN and other neurological disease; (c) reducing or preventing development of symptoms characteristic of PKAN and other neurological disease;(d) and/or preventing worsening of symptoms characteristic of PKAN and other neurological disease. Symptoms of PKAN and associated neurological disease include but are not limited to, “ motor dysfunction (e.g., shaking, rigidity, slowness of movement difficulty with walking), cognitive dysfunction (e.g., dementia, depression, anxiety), emotional and behavioral dysfunction.

Accordingly, in some aspects, the disclosure provides a method for treating a subject having or suspected of having PKAN disease, the method comprising administering to the subject a composition (e.g., a composition comprising an isolated nucleic acid or vector or a rAAV) as described by the disclosure.

In some embodiments, a composition administered directly into the CNS of the subject, for example by direct injection into the brain and or/spinal cord of the subject. Examples of CNS-direct administration modalities include but are not limited to intracerebral injection, intraventricular injection, intracisternal injection, intraparenchymal injection, intrathecal injection, and any combination of the foregoing. In some embodiments, direct injection into the CNS of a subject results in transgene expression (e.g., expression of the first gene product, second gene product, and if applicable, third gene product) in the midbrain, striatum and/or cerebral cortex of the subject. In some embodiments, directly injection into the CNS results in transgene expression (e.g., expression of the first gene product, second gene product, and if applicable, third gene product) in the spinal cord and/or CSF of the subject

In some embodiments, direct injection to the CNS of a subject comprises convection enhanced delivery (CED). Convection enhanced delivery is a therapeutic strategy that involves surgical exposure of the brain and placement of a small-diameter catheter directly into a target area of the brain, followed by infusion of a therapeutic agent (e.g. a composition or rAAV as described herein) directly to the brain of the subject. CED is described, for example, by Debinski et al. (2009) *Expert Rev Neurother.* 9(10):1519-27.

In some embodiments, a composition (e.g., a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure is administered both peripherally and directly to the CNS of a subject. For example, in some embodiments, a subject is administered a composition by intra-arterial injection (e.g., injection the carotid artery) and by intraparenchymal injection (e.g., intraparenchymal injection by CED). In some embodiments, the direct injection to the CNS and the peripheral injection are simultaneous (e.g., happen at the same time). In some embodiments, the direct injection occurs prior (e.g., between 1 minute and 1 week or more before) to the peripheral injection. In some embodiments, the direct injection occurs after (e.g., between 1 minute and 1 week, or more after) the peripheral injection.

The amount of composition (e.g., a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure administered to a subject will vary depending on the administration method. For example, in some embodiments, a rAAV as described herein is administered to a subject at a titer between about 10^9 Genome copies (GC)/KG and about 10^14 GC/kg (e.g. about 10^9 GC/kg about 10^10 GC/kg, about 10^11 GC/KG, about 10^12 GC/kg about 10^12 GC/kg about 10^14 GC/kg). In some embodiments, a subject is administered a high titer (e.g., >10^12 Genome Copies GC/kg of an rAAV) by injection to the CSF space, or by intraparenchymal injection.

A composition (e.g., a composition comprising an isolated nucleic acid or a vector or a rAAV) as described by the disclosure can be administered to a subject once or multiple times (e.g., 2,3,4,5,6,7,8,9,10,20 or more) times. In some embodiments, a composition is administered to a subject continuously (e.g., chronically), for example via an infusion pump.

Examples

Example 1: rAAV Vectors

AAV vectors are generated using cells, such as HEK293 cells for triple-plasmid transfection. The ITR sequences flank an expression construct comprising a promoter/enhancer element for each transgene of interest, a 3' poly(A) signal and posttranslational signals such as the WPRE element. Multiple gene products can be expressed simultaneously such as PKAN2 and C19orf12 and or Phospholipase A2, by infusion of the protein sequences; or using a 2A peptide linker, such as T2A or P2A, which leads 2 peptide fragments with added amino acids due to prevention of the creation of a peptide bond; or using an IRES element; or by expression with 2 separate expression cassettes. The presence of a short intronic sequence that is efficiently spliced, upstream of the expressed gene, can improve expression levels. shRNAs and other regulatory RNAs can potentially be included within the sequences. Examples of Plasmids comprising rAAV vectors described by the disclosure are shown in FIGS. 1-6 and in Table 2 below.

TABLE 2

Name	Promoter 1	shRNA	CDS1	PolyA1	Bicistronic element	Promoter 2	CDS2	PolyA2	Length between ITRs
CMVe_CBAp_ PKAN2_WPRE _ bGH	CBA		PKAN2	WPRE-bGH					3741
LT1s_JetLong_ mRNAiaSYn_ C19orf12 -T2A-PKAN2_bGH	JetLong	aSyn	C19orf12	bGH	T2A		PKAN2		4215
LI1_JetLong_ C19orf12 -IRES-PKAN2_bGH	JetLong		C19orf12	bGH	IRES		PKAN2		4399
FP1_JetLong_ PKAN2_bGH_JetLong_ C19orf12 _SV40L	JetLong		PKAN2	bGH		JetLong	C19orf12	SV40L	4464
PrevailVector_ LT2s_JetLong_ mRNAiaSYn_ PLA2G6-T2A-PKAN2_bGH_ 4353nt	JetLong	aSyn	PLA2G6	bGH	T2A	—	PKAN2	—	4353
PrevailVector_ LI2_JetLong_ PLA2G6_IRES_ PKAN2_ SymtheticpolyA_ 4337nt	JetLong	—	PLA2G6	Synthetic pA	IRES	—	PKAN2	—	4337

Using such cell models, genetic mutations resulting in neurodegeneration are quantified in terms of brain iron accumulation-4 (NBIA4), followed by imaging using fluorescent microscopy. Imaging for NBIA4 abnormalities by ICC for protein markers such as C19orf12, or using dyes such as a Lysotracker, or by uptake through the endocytic compartment of fluorescent dextran or other markers is also performed. Imaging for autophagy marker accumulation due to defective function with the lysosome, such as for LC3, can also be performed. Western blotting and /or ELISA is used to quantify abnormal accumulation of these markers. Also, the accumulation of glycolipid substrates and products of PANK2 is measured using standard approaches.

Therapeutic endpoints (e.g., reduction of PKAN-associated pathology) are measured in the context of expression of transduction for the AAV vectors, to confirm and quantify activity and function. Pantothenate can also be quantified using ELISA measures or by standard Pantothenate activity assays.

Example 3: In Vivo Assays Using Mutant Mice

This example describes in vivo assays of AAV vectors using mutant mice. In vivo studies of AAV vectors as above in mutant mice are performed using assays described, for example by Liou et al. (2006) *J. Biol. Chem.* 281(7): 4242-4523, Sun et al. (2005) J. Lipid Res. 46:2102-2113, and Farfel-Becker et al. (2011) *Dis. Model Mech.* 4(6): 746-752.

The intrathecal or intraventricular delivery of vehicle control and AAV vectors (e.g., at dose of 2x10^11 vg/mouse) are performed using concentrated AAV stocks, for example at an injection volume between 5-10uL. Intraparenchymal delivery by convection enhanced delivery is performed.

Treatment is initiated either before onset of symptoms, or subsequent to onset. Endpoints measured are the accumulation of substate in the CNS and CSF, accumulation of pantothenate kinase enzyme by ELISA and of enzyme activity, motor and cognitive endpoints, and iron accumulation.

Example 4: Chemical Models of Disease

This example describes in vivo assays of AAV vectors using a chemically-induced mouse model of PKAN disease (e.g., the PANKi mouse). In vivo studies of these AAV vectors are performed in a chemically-induced mouse of PKAN disease, for example as described by Ben Mamoun et al. (2021) *TREMOR Jor.* 1:12-13

Intrathecal or intraventricular delivery of vehicle control and AAV vectors (e.g., at a dose of 2x10^11 vg/mouse) are performed using concentrated AAV stocks, for example with injection volume between 5-10 μL. Intraparenchymal delivery by convection enhanced delivery is performed. Peripheral delivery is achieved by tail vein injection.

Treatment is initiated either before onset of symptoms or after onset. Endpoints measured are the accumulation of substrate in the CNS and CSF, accumulation of iron by ELISA and of other enzyme activity, motor and cognitive endpoints, pantothenate dysfunction.

Example 5: Clinical Trials in PKAN disease and other neurodegenerative diseases.

In some embodiments, patients have certain forms of PKAN disease that can lead to parkinsonism, dystonia, dementia and death. This Example describes clinical trials to assess the safety and efficacy of rAAvs as described by the disclosure, in patients having PKAN disease, parkinsonism, and/or dementia.

Clinical trials of such vectors for treatment of PKAN disease, parkinsonism, and/or dementia are performed using a study design similar to that described in Kurian et al. (2021) *Tremor Jor.* 51 38-41.

Example 6: Gene Therapy of PKAN Disease and/or Parkinson's disease in Subjects Having Mutations in PKAN2

This example describes administration of a recombinant adeno-associated virus (rAAV) encoding PKAN2 to a subject having PKAN and or Parkinson's disease characterized by mutation of PKAN2 gene.

The rAAV vector insert contacts the CBA promoter element (CBA), consisting of four parts: the CMV enhancer (CMVE), CBA promoter (CBAp), Exon1, and intron (int) to constitutively express the codon optimized coding sequence of (CDS) of human PKAN2. The 3' region also contains a Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) followed by a bovine Growth Hormone poly(A) signal (bGH polyA) tail. The flanking ITRs allow for the correct packaging of the intervening sequences. Two variants of the 5'ITR sequence (FIG. 7, inset box, bottom sequence) were evaluated; these variants have several nucleotide differences within the 20-nucleotide "D" region of the ITR, which is believed to impact the efficiency of packaging and expression. The rAAV product contains the "D" region of the ITR, which is believed to impact the efficiency of packaging and expression. The rAAv product contains the "D" domain nucleotide sequence shown in FIG 7 (inset box, top sequence). A variant vector, harbors a mutant "D" domain (termed an "S" domain herein, with the nucleotide changes shown by shadowing), performed similarly in preclinical studies. The backbone contains the gene confer resistance to kanamycin as well as a stuffer sequence to prevent reverse packaging. A schematic depicting the rAAV vector is show in FIG 8. The rAAv vector is packaged into an rAAv using AAV9 serotype capsid proteins.

PKAN2-rAAV is administered to a subject as a single dose via a fluoroscopy guided sub-occiptal injection into the cisterna magna (intracisternal magna; ICM). One embodiment of a dosing regime study is as follows:

A single dose of rAAV is administered to patients (N=12) at one of two dose levels (3el3 vg(low dose); 1e14 vg (high dose), etc.) which are determined based on the results of nonclinical pharmacology and toxicology studies. Initial studies were conducted in a chemical mouse model involving daily delivery of PANKi, a reversible pantothenate kinase inhibitor to assess the efficacy and safety of the rAAV vector and a variant rAAV S-variant construct (as described further below). Additionally, initial studies were performed in a genetic mouse model, which carries a homozygous PKAN2 mutation and exhibits claval hypertrophy. Additional dose-ranging studies in mice and nonhuman primates(NHPs) are conducted to further evaluate vector safety and efficacy.

Two slightly different versions of the 5' inverted terminal repeat (ITR) in the A domain within the 145 bp 5'ITR is thought to be necessary for optimal viral vector production, but mutations within the "D" domain have also been reported to increase transgene expression in some cases. Thus, in addition to the viral vector, which harbors an intact "D" domain, a second vector form with a D domain (termed an "S" domain herein) was also evaluated. Both rAAV and variant rAAV express the same transgene. While both vectors produced virus that was efficacious in

vivo as detailed below, the rAAV which contains a wild-type “D” domain, was selected for further development.

To establish the PANKi model of pantothenate kinase deficiency, juvenile mice were dosed with PANKi, the inhibitor of pantothenate kinase activity. Mice were given PANKi by IP injection daily, starting at postnatal day 8 (P8). Three different PANKi doses (25 mg/kg, 37.5 mg/kh, 50 mg/kg) and PBS were tested to establish a model that exhibits a behavioral pehotype (FIG. 9) Higher doses of PANKi led to lethality in a dose dependent manner. All mice treated with 50 mg/kg PANKi died by P23, and 6 of the 8 mice treated with 37.5 mg/kg PANKi died by p27. There was no lethality in mice treated with 25 mg/kg PANKi. Whereas PANKi-injected mice showed no general motor deficits in the open field assay(traveling the same distance and at the same velocity as mice given placebo), PANKi-treated mice exhibited a motor coordination and balance deficit as measured by the rotarod assay.

Mice surviving to the end of the study were sacrificed on the day after their last PANKi dose (p27, “Day 1”) or after three days of Pantothenate Kinase withdrawal (p29, “Day 3”). Lipid analysis was performed on the cortex of mice given 25 mg/kg PANKi to evaluate the accumulation of pantothenate kinase substrates in both the Day 1 and Day 3 cohorts. N-alkylpantothenamide levels were significantly accumulated in the PANKi-treated mice compared to the controls, consistent with Pantothenate Kinase insufficiency.

Based on the study described above, the 25 mg/kg PANki dose was selected since it produced behavioral deficits without impacting survival. To Achieve widespread PANK2 distribution throughout the brain and transgene expression during treatment, rAAV or excipient was delivered by intracerebroventricular (ICV) injection at postnatal day 4 (P4). Followed by daily IP PANKi or placebo treatment initiated at P(8) (Fig. 10).

PANKi-treated mice that received rAAV performed statistically significantly better on the rotard than those that received excipient (FIG 11). Mice in the variant vector gtreatment group did not differ from excipient treated mice in terms of other behavioral measures, such as the total distance traveled during testing (FIG.11).

At the completion of the in-life study, half of the mice were sacrificed the day after the last PANKi dose (p36 “Day 1”) or after three days of Pantothenate Kinase withdrawal (p38, “Day 3”) for biochemical analysis (FIG 12). Using a fluorometric enzyme assay performed in biological triplicate, Pantothenate Kinase activity was assessed in the cortex. PKAN activity was increased in mice that were treated with PKAN2-rAAV, while PANKi treated mice reduced Pantothenate Kinase activity. Additionally, mice that received both PANki and PANK2 – rAAV had Pantothenate Kinase activity levels that were similar to the placebo group, indicating that delivery of rAAV is able to overcome the inhibition of pantothenate kinase activity induced by PANKi treatment. Lipid analysis was performed on the motor cortex of the mice to examine levels of the substrate N-alkylpantothenamide. The lipid accumulated in the brains of mice given PANKi, and rAAV treatment significantly reduced substrate accumulation.

Lipid levels were negatively correlated with both pantothenate kinase activity and performance on the Rotarod across treatment groups. The increased pantothenate kinase activity after rAAV administration was associated with the substrate reduction and enhanced motor function (FIG 13). As shown in FIG 14, preliminary biodistribution was assessed by vector genome presence, as measured by qPCR (with>100 vector genomes per 1 μg genomic DNA defined as positive). Mice that received PKAN2-rAAV, both with and without PANKi, were positive for rAAV vector genomes in the cortex, indicating that ICV delivery results in rAAV delivery to the cortex. Additionally, vector genomes were detected in the liver, few in spleen, and none in the heart, kidney or gonads. For all measures, there was no statistically significant difference between the Day 1 and Day 3 groups.

A larger study in the PANKi model further explored efficacious doses of PANK2-rAAV in the PANKi model. Using the 2mg/kg PANKi dose model, excipient or PANK2-rAAV was delivered via ICV at p3, and dailly IP PBS or PANKi treatment initiated at p8. Given the similarity between the groups with and without Pantothenate Kinase withdrawal observed in the previous studies, all mice were sacrificed one day after the final PANKi dose (p38-40). The effect of three different rAAV doses was assessed, resulting in the following five groups, with 10 mice (5M/5F) per group:

Excipient ICV+PBS IP
Excipient ICV+25 mg/kg PANKi IP
3.2e9 vg (2.13e10 vg/g brain) rAAV ICV+25 mg/kg PANKi IP
1.0e10 vg (6.67e10 vg/g brain) rAAV ICV+25 mg/kg PANKi IP
3.2e10 vg (2.13e11 vg/g brain) rAAV ICV+25 mg/kg PANKi IP

The highest dose of rAAV rescued the PANKi treatment-related failure to gain weight at P37. Additionally, this dose resulted in a statistically significant increase in performance on the rotarod and tapered beam compared to the Excipient+PANKi treated group (FIG. 15) Lethality was observed in several groups, including both excipient-treated and rAAV-treatment groups (Excipient+PBS: 0 , Excipient _25 mg/kg PANKi: 1;3.2e9 vg rAAV + 25mg/kg PANKi: 4; 1.0e10vg rAAV + 25 mg/kg PANKi: 0; 3.2e10 vg rAAV +25 mg/kh PANKi: 3)

At the completion of the in-life study, mice were sacrificed for biochemical analysis (fig 16). Pantothenate Kinase activity in the cortex was assessed in biological triplicates by a fluorometric assay. PANKi-treated mice showed reduced Pantothenate Kinase activity whereas mice that received a high rAAV dose showed a statistically significant increase in Pantothenate Kinase activity compared to PANKi treatment. PANKi-treated mice also had accumulation of the substrate N-alkylpantothenamide, which is rescued by administration of a high dose rAAV.

In addition to the established chemical PANKi model, PANK2-rAAV is also evaluated in the dPANK/fbl genetic model. These mice exhibit motor strength, coordination, and balance deficits, as evidenced by their performance in the beam walk, rotarod, and wire hand assays. Typically, the lifespan of these mice is less than 22 weeks. In an initial study 3 μl of maximal

titer virus was delivered by ICV at p23, with a final dose of 2.4 e10 vg (6.0e10 vg/g brain). With 6 mice per group the treatment groups were:

WT+Excipient ICV
dPANK/fbl+Excipient ICV
dPANK/fbl+2.4e10 vg(6.0e10 vg/g brain) rAAV ICV

Motor performance by the beam walk test was assessed 4 weeks post r-AAV delivery. The group of mutant mice that received PANK2-rAAV showed a trend towards fewer total slips and fewer slips per speed when compared to mutant mice treated with excipient, restoring motor function to near WT levels (FIG. 17). Since the motor Phenotypes become more severe as these mice age, their performance on this and other behavioral tests is assessed at later time points. At the completion of the in-life study, lipid levels, Pantothenate Kinase activity, and biodistribution are assessed in these mice.

Additional lower doses of rAAV are currently being tested using the PANKi model, corresponding to .03x, .1x and 1x the proposed phase 1 high clinical dose. Each group includes 10 mice (5M/5F) per group:

Excipient ICV
Excipient ICV+25 mg/kg PANki IP
3.28 vg (2.13e9 vg/g brain) rAAV ICV+25 mg/kg PANKi IP
1.0e9 vg (6.67e9 vg/g brain) rAAV ICV+25 mg/kg PANKi IP
1.e10 vg (6.67e10 vg/g brain) rAAV ICV+25 mg/kg PANKi IP.

In addition to motor phenotypes, lipid levels and pantothenate kinase activity are assessed in the cortex. Tim course of treatments and analyses are also performed.

A larger dose ranging study was initiated to evaluate efficacy and safety data. 10 mice (5m/5F per group) were injected with 10 μl of rAAV. Using an allometric brain weight calculation, the doses correlate to 0.15x, 4.4x and 14.5x the proposed phase 1 high clinical dose. The injection groups consist of:

WT+Excipient ICV
dPANK/fbl +Excipient ICV

dPANK/fbl+4.3e9 vg (1.1e10 vg/g brain) rAAV ICV
dPANK/fbl+4.3e10 vg(1.1e11 vg/g/brain) rAAV ICV
dPANK/fbl+1.3e11 vg (3.2e11 vg/g brain) rAAV ICV
dPANK/fbl+4.3e11 vg (1.1e12 vg/g brain) rAAV ICV

A summary of nonclinical studies in the PANKi model are shown in Table 3 below.

TABLE 3

Summary of Results in PANKi Mouse

Test Material	Study Number	Dose Cohort	Model Behavioral Changes: Tapered Rotarod	Tapered Beam	Open Field	Open Lipids	Open Enzyme	BD Brain	BD Liver
PANK2-rAAV	PRV-2018-005 Dose-ranging rAAV in PANki Model	3.2e9 vg (2.13e10 vg/g brain)	NS	NS	NS	NS	NS	+	–
		1.10e10 vg (6.67e10 vg/g brain)	T	NS	NS	T/S	NS	+	+
		2.3e10 vg (2.13e11 vg/g brain)	S	S	NS	S	S	+	+
variant PANK2 rAAV	PRV-2018-005 Dose-ranging rAAV in PANKi Model	8.8e9 vg (5.9e10 vg/g brain)	S	N/A	NS	S	S	+	+

Note

that positive biodistribution is defined as >100 vg/1 μg genomic DNA.

Abbreviations: BD = biodistribution; NS = nonsignificant; T = trend; S = significant; N/A = not applicable; + = positive; – = negative.

Example 9: In Vitro Analysis of rAAV Vectors

A pilot study was performed to assess in vitro activity of rAAV vectors encoding Phospholipase A2 ,PLA2g6, and c190rf12, alone or in combination with PANK2 and or/or one inhibitory RNAs. Vectors tested include those shown in TABLE 4. “Opt” refers to a nucleic acid sequence codon optimized for expression in mammalian cells (e.g., human cells). FIG 18 shows representative data indicating that transfection of HEK293 cells with each of the constructs resulted in overexpression of the corresponding gene product compared to mock transfected cells.

TABLE 4

ID	Promoter	Inhibitory RNA	Promoter	Transgene
I00015	JL_intronic	SCNA	JetLong	Opt-PLA2g6 PANK2
I00039	—	—	JetLong	OpPLA2g6 C19orf12
I00046	—	—		Opt-PSAP

Example 10: ITR "D" Sequence Placement and Cell Transduction

The effect of placement of ITR "D" sequence on cell transduction of rAAV vectors was investigated. HEK 293 cells were transduced with PANki-encoding rAAVs have 1) wild-type ITRs (e.g., "D sequences proximal to the transgene insert and distal to the terminus of the ITR) or 2) ITRs with the "D" sequence located on the "outside" of the vector (e.g., "D" sequence located proximal to the terminus of the ITR and distal to the transgene insert), as shown in FIG 19. Surprisingly, data indicate that rAAVs having the "D" sequence located in the "outside" to position retain the ability to be packaged and transduce cells efficiently (FIG 20).

Example 11: In Vitro Toxicity Studies

Fifty (50) mice were administered PANk2- encoding rAAVs via a 4 μl intraventricular (ICV) injection on post-natal day 3. All mice received daily intraperitoneal (IP) injections of a Pantothenate Kinase inhibitor, PANki, depending on the treatment group, from post-natal day 8 to the end of the study. Animals were euthanized 24 hours after their last IP dose. After euthanasia, target tissues were harvested, drop fixed in chilled 5% paraformaldehyde, and stored at 3 degrees C., then sent for histopathological processing and evaluation. There were three(3) early death animals over the course of the study, which were not sent or analyzed.

Tissues from the forty-seven, (47) animals euthanized at 38-40 days were trimmed processed, and embedded in paraffin blocks. They were then sectioned at -5 μm, stained with hematoxlyin and eosin (H&E) and affixed to slides for evaluation.

There were no histopathlogic findings or evidence of toxicity due to treatment with the rAAVs. In the mice treated with Pantothenate Kinase inhibitor (PANKi), there were findings in the central nervous system (CNS) that included glial scars and neuronal necrosis in the cerebral cortex, and neuronal necrosis in the brain stem and thoracic spinal cord. High dose rAAV treatment resulted in a notable reduction in the incidence of these CNS findings, while the low and mid dose virus had a dose dependent reduction in the incidence of these CNS findings, while the low and mid dose virus had a dose dependent reduction in the incidence of glial scars in the cerebral cortex, with equivocal effects on the other CNS findings.

EQUIVALENTS

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and or structures for performing the functions and or obtaining the results and or one or more of the advantages described herein, and each of such variations and or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teaching of the present invention is/are used. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is therefore to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, and/or methods, if such features, systems, articles, materials, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and

disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specially identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to "And and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A without B (optionally included elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both" when preceded by the terms of exclusivity, such as "either", "one of," "only one of," or "exactly one of" "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims the phrase "at least one" in reference to a list of one or more elements, should be understood to mean at least one element selected from any or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, to whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or equivalently, at least one of A or B," or, equivalently " at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A and at least one, optionally including more than one, B (and optionally including other elements); etc.

In the claims, as well as in the specifications above, all transitional phrases such as "compromising," "including," "carrying," "having", "containing", "involving", "holding", and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Use of ordinal terms such as “first,” ‘second,” “third,” etc. in the claims to modify a claim element does not by itself connote any priority, precedence or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

SEQUENCES

In some embodiments, an expression cassette encoding one or more gene products (e.g., a first second and/or third gene product) comprises or consists of (or encodes a peptide having) a sequence set forth in an of SEQ ID Nos: 1,2 3, 4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24, or 25.

Number of SEQ ID NOS: 13

SEQ ID NO 1
Length:8161
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

```
1 gctctggctg gactgccgcg gaggaggcga gaaggaatcc gacgctgggg ggcttgctcg
       61 ggcggcagcg actgctgctg cggatgggag ggggccggct cggcgcgccc atggagcgcc
      121 acggcagggc ttccgccacc tccgtctcgt cggctgggga gcaggcggcc ggggaccccg
      181 aagggcggcg gcaggagcca ctgcggcgcc gggcgagcag cgcgtcggtg cccgcggtcg
      241 gggcctcggc tgagggcacg aggcgggatc gactgggctc ttacagcggc cccacctcgg
      301 tctcccgcca gcgcgtcgaa agcctgagga aaaagcggcc gcagatggga tctcttatgt
      361 tgcccaggct ggtcttgaat tcctcctggt ctcaagcgat cttcccactt cggcctccca
      421 aagtgctggg atatagacgg gagccattgt gcccgacctg gcagtcgtct actaagagct
      481 ttggtaactc attagcctcg tggctctgag tttttccatg gtttggactg gatatcggtg
      541 gaactctggt caagctggta tattttgaac ccaaagacat cactgctgaa gaagaagagg
      601 aagaagtgga aagtcttaaa agcattcgga agtacctgac ctccaatgtg gcttatgggt
      661 ctacaggcat tcgggacgtg cacctcgagc tgaaggacct gactctgtgt ggacgcaaag
      721 gcaatctgca ctttatacgc tttcccactc atgacatgcc tgcttttatt caaatgggca
      781 gagataaaaa cttctcgagt ctccacactg tcttttgtgc cactggaggt ggagcgtaca
      841 aatttgagca ggatttttctc acaataggtg atcttcagct ttgcaaactg gatgaactag
      901 attgcttgat caaaggaatt ttatacattg actcagtcgg attcaatgga cggtcacagt
      961 gctattactt tgaaaaccct gctgattctg aaaagtgtca gaagttacca tttgatttga
     1021 aaaatccgta tcctctgctt ctggtgaaca ttggctcagg ggttagcatc ttagcagtat
     1081 attccaaaga taattacaaa cgggtcacag gtactagtct tggaggagga actttttttg
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1141 gtctctgctg tcttcttact ggctgtacca cttttgaaga agctcttgaa atggcatctc
1201 gtggagatag caccaaagtg gataaactag tacgagatat ttatggaggg gactatgaga
1261 ggtttggact gccaggctgg gctgtggctt caagctttgg aaacatgatg agcaaggaga
1321 agcgagaggc tgtcagtaaa gaggacctgg ccagagcgac tttgatcacc atcaccaaca
1381 acattggctc aatagcaaga atgtgtgccc ttaatgaaaa cattaaccag gtggtatttg
1441 ttggaaattt cttgagaatt aatacgatcg ccatgcggct tttggcatat gctttggatt
1501 attggtccaa ggggcagttg aaagcacttt tttcggaaca cgagggttat tttggagctg
1561 ttggagcact ccttgagctg ttgaagatcc cgtgatcatt acctggggag gggttcctga
1621 aaccttccac aatgggatct gtggactttc attttttaa gagacttact caatttcatg
1681 actgtactac ctgaaacaaa gtgagaaagg acaggtgtat ttttctaagt catcaagata
1741 aatccttaag aattcagtct aaattagcaa ccaggaagga aaaatatatt aaaaacaaca
1801 aaaaagtggc acatgtccag gcagtgtgag gatttgctgt atataagttg cctgctttgt
1861 atttttgaaa tctctgcatc actcattgga agtgcttctg aagagagctg ctctgtgttc
1921 agttgactgg ttttgtgtcc tgtttgaact tgctgaatgt aaggcaggct actatgcgtt
1981 ataatctaat cacaatttgt caatatggtc ttggcaatca tctgtgcatt actctggttt
2041 gcattaagcc tgtgtgtgaa cttactgtaa aacatgtttt atttcaaggt tctgcaaaat
2101 taattgggca ggttaattgt gtacctgaaa cttaacaagc agtttttgga agggcagttc
2161 cacgttactt tacactaaat cctgggaatg aatgtctgga gagacagggc ttcttttctt
2221 ggcagaattt ccttgggtag tgggcggact gttctgatgc aagtgggtac actggcagaa
2281 gcagcacaat ctggaggact aacatgagtc agcagggcca ctagaattga cacccccтca
2341 ggggatcagg gagaatctgg tttttctttc aggctaagta gaaaaggaag aactgaagtg
2401 tctgtcaaag aactcatggg gagtgggggg tccttcaggg cactgtggca cttgggaggt
2461 gagtctcaga agactgcctg aggagagtgc caggctgcag gtgctcttgg taggggctga
2521 agcttttctt tctgctttgc agaataattt caaaagcctt tcccaaggag agaatgcacc
2581 ttttatatct gaggtcttaa gtggtgccca tattgctgtt gaaggcaagg atgggcctgt
2641 acaaagcagc cttggaccag gagggaaaac cccaaggctc tgcagcctcc ctgctcccct
2701 ccgtttaagg ctgtggtcag ggagggatgg gcagggtgcc agccgcatgg aggcagcaga
2761 gacacccgtg ttccagctgg ggcaagggga actgatatgt gggctgtggg ctgttccact
2821 aggcgtggcc acctctgcac atgggccttg gcacttagga aatatgggtg ctgctgcatt
2881 gggaccttgt gtgggtgcag agcaggatgg ctgctgcgtg gagggtaatt ttgtcccctg
2941 gagagtgatt ggtgaccctc ttccctgctg ctgctgtttg caggctcctg gcctatacta
3001 cctctgacgc cctgatcctg agttcctctg ccctcctctg ctgccagcag cgctgttggt
3061 ggcctcaccc tcctccagta cccaggcacc aaagatggca ccaccatcct cctgcttgta
3121 ccacagggtg ctgctccctg ggccaactcc tgtgaacttg ctcacttccc atggagaccg
3181 aggccagggc ctgcccctct gaggcccctg ttttgtggtc ctgctgcctc tgctgtgctg
3241 cgttcaggag gttgtccact gctcgacgtg ggtcaccatg cagcccttgc tgttcccaca
3301 atttaacaga aaaggaaact gaacggatga gtaaggctgc ccagggtcac tcagctggaa
3361 aagagtagag ctggactcaa acccgggact gtctgtctct ggtgcctgag ttgggaagac
3421 gctgttgatg tccccttctg cctcgctttg agcttcctcc tccgtgggcc ctccccctac
3481 tctagagaaa ctagtccatt ccctcatgtt ccaccttgtg ctcagtaaac cctgtcactc
3541 cacagcaggg tttttgagac agtctcgctg tgtcacccag gtggagtgca gtggggcgat
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3841 agggttcttg ataggccacc agtgaatccc atgccaccga atctggcatt catttcacag
3901 cctcatctca gttctcactc agcagagccc tcttgcctgt cctgccaccc aggctactaa
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4021 ctccccaagc agtcattagc atcaccttga ctgatttcca gggaacactt caaccacccc
4081 ttacctctga gctccagccc cgagacactg ccttctctct gcatttgttg atctctgtgc
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4381 tggagagatg cttagagggc tgtgttggga agtggtgact ggatacaggg gagggaggag
4441 agatacaaga gggcacctgg gctcatttgt ggactacgtg attttgagac tccaaataag
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4561 caaggggat ttcatgagtt ctaaggtagg gtgagaaaaa taaccgagag ttgagcagtg
4621 acggaggaag ctgcaaagaa aacgactgct cagggagagg ctggccaggg gcctgtggct
4681 caggggactg agaagaggag ctctctaggc atctgaattg aggttgccca caattggttg
4741 caagatcgtg gctggccttg atggaagctg ggaagccact cggtcctagt gaggggtggt
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4861 atttccaggc ctgggatata gctaggggct tggggtgggg gcaactcttt gcagggtggt
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5281 cctggtgctg gcgttcacgt ggaagactgc ttatctgtat ccagctgttt gcagagctca
5341 gggaggccag ggggcttcag actccactgt actctgcatt ccaggattct ctgttctctg
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5461 caggaagggc aggggcttcc atcacaatca ctcagtacta gttaccagct ctgatagaat
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5581 ccctctgttc aaaggcatat gcacaacact atttttcaaa atttgctttt atttacacgt
5641 ttgagtcaat tacatgcatg ttttattttt gaggagtgat tagcaaaatg cattaaaatt
5701 ttaaaatagc aattaaatat acaaaaatat agcttacaaa aaactgcgaa tgtttaaaaa
5761 tattctgctg cagaattttt agtgtacaaa gacgtctatg aaacctgagg ttcagcattt
5821 cataaacctt tttttgaggg agttacacaa tacctagtga ggaaacccga gggccaaact
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6001 cctgctcccc cgaccggggg cctgggaggg aagggcagat ggagaggaac ggtgtgcagt
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6121 ccccaaagca caaacaagtc tgctgacact ggtggggccc ttacagtcac agaagtaagg
6181 actggatggt aagccaaagg tgctgatgct tggtcaaaaa gtggagtaca tctttgcatc
6241 ttgtaacaat ttgggctcta cagctgaatt ggcaaagtaa ggtttactac acaagcccct
6301 ctttttcttc tgatatcccc actcaaatgg aagcacactc cccagcacat ggggatccct
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6541 agaatttttt ctcaagtttt gggttagggc accagttgca attttaatcc aggggaggat
6601 ctgggctgct gctgtagggg gcaagtacac cagggaggtg ggtgtggaca gaggcactgg
6661 gcagctgctg ggaagcgagg cacaagtctg tgcccctact cccagggctg cctggaggaa
6721 gcagctctaa ctttcatcag cggggacaaa aatgaactta aactaaaaaa attattggcc
6781 gggcgcagtg gctcacgcct gtaatcccag cactttggga ggtcaaggca ggtgcatcac
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6961 atggcgtgaa cctgggaggc ggagcttgca gtgggccaag atcgtgccac tgcattccag
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7081 ttgttggggt aatttctgtt gtcaaatgga aaacaaggca taaagggaaa attctgtagg
7141 aagcattttc ttttcttgtg tgttttttg agacggagtc tcgctctgtt gcccaggctg
7201 gagtgcagtg gggatcttgg ctcactgcaa cttctgtctc ccgggttcaa tcaattctcc
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7381 aaggtgatcc gcctgcctcg gcctcccaaa gtgctgggat gttgggatta caggcgtgag
7441 ccatcgcgcc cgaccagaag cattttcttt tcaaagtgtg tgcacagagt gaggaggcca
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7561 caggtgtgct ggtgtgcacc tgtagacgca gctacagact gaggcgggag gatcacttga
7621 gtccaggaag ttgaggctgc agtgagctac aatggtgcca ctgcactcca gtctgggtaa
7681 caagaccctg tctcaacaac aacgaaaagt tgggagggaa agggagtagc tccatgaact
7741 gacgctggac attcaggcat atccacatga gactcactgt gcacttgctc agtagctaag
7801 atgtctgccc cacaaaatag aagccttcag ttgctgctgc tcaaactcct gcatcaaaga
7861 cacaagagct tgcctaagtg accacaacag ttgcagccac acatcaccac tggtcctcac
7921 ttggaactgg ccaaggtagg ggaggggcca gcagctgtcc aggcacctgc agaggctgct

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7981 cagagctggg agaggcctgt gccactgctg tcagctacag agcctgatct tgagcatcct
8041 gtcgcagata gaaagtcaat cagaaaaatc tggttgtgct cttggattag ctgggcattt
8101 ggggtaaggg agggactgca gattctgatt tgtacagaaa actaaaattt cagtatgttg
8161 caataaaatg aaaatatgtc ttactcaa
```

SEQ ID NO 2
Length:541
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

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1 mrrlgpfhpr vhwaappsls sglhrllflr gtripssttl spprhdslsl dggtvnpprv
   61 reptgreafg pspassdwlp arwrngrggr prarlcsgwt aaeearrnpt lggllgrqrl
  121 llrmgggrlg apmerhgras atsvssageq aagdpegrrq eplrrrassa svpavgasae
  181 gtrrdrlgsy sgptsvsrqr veslrkkrpl fpwfgldigg tlvklvyfep kditaeeeee
  241 eveslksirk yltsnvaygs tgirdvhlel kdltlcgrkg nlhfirfpth dmpafiqmgr
  301 dknfsslhtv fcatgggayk feqdfltigd lqlckldeld clikgilyid svgfngrsqc
  361 yyfenpadse kcqklpfdlk npyplllvni gsgvsilavy skdnykrvtg tslgggtffg
  421 lcclltgctt feealemasr gdstkvdklv rdiyggdyer fglpgwavas sfgnmmskek
  481 reavskedla ratlititnn igsiarmcal neninqvvfv gnflrintia mrllayaldy
  541 wskgqlkalf sehegyfgav gallellkip
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SEQ ID NO 3
Length:3001
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

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1 gtttgtttgc ggaagtagga ggaagtagaa gtgctgagta agccgagaca gaggggggaag
   61 acggtggggc ctccccacct gccccgcaga agatgcagtt ctttggccgc ctggtcaata
  121 ccttcagtgg cgtcaccaac ttgttctcta acccattccg ggtgaaggag gtggctgtgg
  181 ccgactacac ctcgagtgac cgagttcggg aggaagggca gctgattctg ttccagaaca
  241 ctcccaaccg cacctgggac tgcgtcctgg tcaaccccag gaactcacag agtggattcc
  301 gactcttcca gctggagttg gaggctgacg ccctagtgaa tttccatcag tattcttccc
  361 agctgctacc cttctatgag agctcccctc aggtcctgca cactgaggtc ctgcagcacc
  421 tgaccgacct catccgtaac caccccagct ggtcagtggc ccacctggct gtggagctag
  481 ggatccgcga gtgcttccat cacagccgta tcatcagctg tgccaattgc gcggagaacg
  541 aggagggctg cacacccctg cacctggcct gccgcaaggg tgatggggag atcctggtgg
  601 agctggtgca gtactgccac actcagatgg atgtcaccga ctacaaggga gagaccgtct
  661 tccattatgc tgtccagggt gacaattctc aggtgctgca gctccttgga aggaacgcag
  721 tggctggcct gaaccaggtg aataaccaag ggctgacccc gctgcacctg gcctgccagc
  781 tggggaagca ggagatggtc cgcgtgctgc tgctgtgcaa tgctcggtgc aacatcatgg
  841 gccccaacgg ctaccccatc cactcggcca tgaagttctc tcagaagggg tgtgcggaga
  901 tgatcatcag catggacagc agccagatcc acagcaaaga cccccgttac ggagccagcc
  961 ccctccactg ggccaagaac gcagagatgg cccgcatgct gctgaaacgg ggctgcaacg
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1021 tgaacagcac cagctccgcg gggaacacgg ccctgcacgt ggcggtgatg cgcaaccgct
1081 tcgactgtgc catagtgctg ctgacccacg gggccaacgc ggatgcccgc ggagagcacg
1141 gcaacacccc gctgcacctg gccatgtcga aagacaacgt ggagatgatc aaggccctca
1201 tcgtgttcgg agcagaagtg gacaccccga atgactttgg ggagactcct acattcctag
1261 cctccaaaat cggcagacaa ctacaggatc tcatgcacat ctcacgggcc cggaagccag
1321 cgttcatcct gggctccatg agggacgaga agcggaccca cgaccacctg ctgtgcctgg
1381 atggaggagg agtgaaaggc ctcatcatca tccagctcct catcgccatc gagaaggcct
1441 cgggtgtggc caccaaggac ctgtttgact gggtggcggg caccagcact ggaggcatcc
1501 tggccctggc cattctgcac agtaagtcca tggcctacat gcgcggcatg tactttcgca
1561 tgaaggatga ggtgttccgg ggctccaggc cctacgagtc ggggcccctg gaggagttcc
1621 tgaagcggga gtttggggag cacaccaaga tgacggacgt caggaaaccc aaggtgatgc
1681 tgacagggac actgtctgac cggcagccgg ctgaactcca cctcttccgg aactacgatg
1741 ctccagaaac tgtccgggag cctcgtttca accagaacgt taacctcagg cctccagctc
1801 agccctcaga ccagctggtg tggcgggcgg cccgaagcag cggggcagct cctacttact
1861 tccgacccaa tgggcgcttc ctggacggtg ggctgctggc caacaacccc acgctggatg
1921 ccatgaccga gatccatgag tacaatcagg acctgatccg caagggtcag gccaacaagg
1981 tgaagaaact ctccatcgtt gtctccctgg ggacagggag gtccccacaa gtgcctgtga
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2101 ccaaggaact gggcaagatg gtggtggact gttgcacgga tccagacggg cgggctgtgg
2161 accgggcacg ggcctggtgc gagatggtcg gcatccagta cttcagattg aacccccagc
2221 tggggacgga catcatgctg gatgaggtca gtgacacagt gctggtcaac gccctctggg
2281 agaccgaggt ctacatctat gagcaccgcg aggagttcca gaagctcatc cagctgctgc
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2401 tgccaggcca agcccagcca ctgccctccc gggcagatct gggcccaggc acctctgagt
2461 ccatagacca ggcctgggag aatgccaagc tgcctgcccg aggctggtcc tgaaggcctg
2521 tctcccacta accccgcctt ccagcacttt ctgtcattcc aggctgggaa agtctagagc
2581 ccccttggc ccctttccct gactgtcaag gacaactgac tcccccatca gctcaaacat
2641 taagggtacc cgggcacaac cgtacccctg cccccagccc cagcctccct gagggcctgc
2701 cgggctgcct ctgccccagc ccccagcaag ggcactccca ggcttcctgg tgggtgcagc
2761 ccactccctc tgccctctgc tccgttccct gggggctggg actaaagaaa tgggtgtccc
2821 ccaccccatc agctgggaaa gcccaggccg caggagtggg atgcccgttg gactttgccc
2881 ctcacactgg cccagcccct cacactgccc caccccgaga accctcagct ctcaaaggtc
2941 actcctggga gtttcttctt cccaatggaa gtggcttaag agccaaaact gaaataaatc
3001 atttggattc aagttca
```

SEQ ID NO 4
Length:3001
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

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   1 gtttgtttgc ggaagtagga ggaagtagaa gtgctgagta agccgaggtg agtgacctcg
  61 cgggtgggcg gggcctgggg gtccgttccc caacttcctc ggcgctccgg actcccaagt
 121 ctccgccgga ccctcctttg gatattcctc gtgtctccga ttctgagagg gggaagacgg
 181 tggggcctcc ccacctgccc cgcagaagat gcagttcttt ggccgcctgg tcaatacctt
 241 cagtggcgtc accaacttgt tctctaaccc attccgggtg aaggaggtgg ctgtggccga
 301 ctacacctcg agtgaccgag ttcgggagga agggcagctg attctgttcc agaacactcc
 361 caaccgcacc tgggactgcg tcctggtcaa ccccaggaac tcacagagtg gattccgact
 421 cttccagctg gagttggagg ctgacgccct agtgaatttc catcagtatt cttcccagct
 481 gctacccttc tatgagagct cccctcaggt cctgcacact gaggtcctgc agcacctgac
 541 cgacctcatc cgtaaccacc ccagctggtc agtggcccac ctggctgtgg agctagggat
 601 ccgcgagtgc ttccatcaca gccgtatcat cagctgtgcc aattgcgcgg agaacgagga
 661 gggctgcaca cccctgcacc tggcctgccg caagggtgat ggggagatcc tggtggagct
 721 ggtgcagtac tgccacactc agatggatgt caccgactac aagggagaga ccgtcttcca
 781 ttatgctgtc cagggtgaca attctcaggt gctgcagctc cttggaagga acgcagtggc
 841 tggcctgaac caggtgaata accaagggct gaccccgctg cacctggcct gccagctggg
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 961 caacggctac cccatccact cggccatgaa gttctctcag aaggggtgtg cggagatgat
1021 catcagcatg gacagcagcc agatccacag caaagacccc cgttacggag ccagcccct
1081 ccactgggcc aagaacgcag agatggcccg catgctgctg aaacggggct gcaacgtgaa
1141 cagcaccagc tccgcgggga acacggccct gcacgtggcg gtgatgcgca accgcttcga
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1261 caccccgctg cacctggcca tgtcgaaaga caacgtggag atgatcaagg ccctcatcgt
1321 gttcggagca gaagtggaca ccccgaatga ctttggggag actcctacat tcctagcctc
1381 caaaatcggc agacaactac aggatctcat gcacatctca cgggcccgga agccagcgtt
1441 catcctgggc tccatgaggg acgagaagcg gacccacgac cacctgctgt gcctggatgg
1501 aggaggagtg aaaggcctca tcatcatcca gctcctcatc gccatcgaga aggcctcggg
1561 tgtggccacc aaggacctgt ttgactgggt ggcgggcacc agcactggag gcatcctggc
1621 cctggccatt ctgcacagta agtccatggc ctacatgcgc ggcatgtact ttcgcatgaa
1681 ggatgaggtg ttccggggct ccaggcccta cgagtcgggg cccctggagg agttcctgaa
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1801 agggacactg tctgaccggc agccggctga actccacctc ttccggaact acgatgctcc
1861 agaaactgtc cgggagcctc gtttcaacca gaacgttaac ctcaggcctc cagctcagcc
1921 ctcagaccag ctggtgtggc gggcggcccg aagcagcggg gcagctccta cttacttccg
1981 acccaatggg cgcttcctgg acggtgggct gctggccaac aaccccacgc tggatgccat
2041 gaccgagatc catgagtaca atcaggacct gatccgcaag ggtcaggcca acaaggtgaa
2101 gaaactctcc atcgttgtct ccctggggac agggaggtcc ccacaagtgc ctgtgacctg
2161 tgtggatgtc ttccgtccca gcaacccctg ggagctggcc aagactgttt ttggggccaa
2221 ggaactgggc aagatggtgg tggactgttg cacggatcca gacgggcggg ctgtggaccg
2281 ggcacgggcc tggtgcgaga tggtcggcat ccagtacttc agattgaacc cccagctggg
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2581 agaccaggcc tgggagaatg ccaagctgcc tgcccgaggc tggtcctgaa ggcctgtctc
2641 ccactaaccc cgccttccag cactttctgt cattccaggc tgggaaagtc tagagccccc
2701 tttggccccc ttccctgact gtcaaggaca actgactccc ccatcagctc aaacattaag
2761 ggtacccggg cacaaccgta cccctgcccc cagccccagc ctccctgagg gcctgccggg
2821 ctgcctctgc cccagccccc agcaagggca ctcccaggct tcctggtggg tgcagcccac
2881 tccctctgcc ctctgctccg ttccctgggg gctgggacta aagaaatggg tgtcccccac
2941 cccatcagct gggaaagccc aggccgcagg agtgggatgc ccgttggact ttgcccctca
3001 cactggccca gcccctcaca ctgccccacc ccgagaaccc tcagctctca aaggtcactc
3061 ctgggagttt cttcttccca atggaagtgg cttaagagcc aaaactgaaa taaatcattt
3121 ggattcaagt tca
```

SEQ ID NO 5
Length:121
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

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1 mtimvedimk llcslsgerk mkaavkhsgk galvtgamaf vgglvggppg lavggavggl
       61 lgawmtsgqf kpvpqilmel ppaeqqrlfn eaaaiirhle wtdavqltal vmgsealqqq
      121 llamlvnyvt kelraeiqyd d
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SEQ ID NO 6
Length:7561
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

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1 cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc
       61 gggcgacctt tggtcgcccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca
      121 actccatcac taggggttcc tgcggccaat tcagtcgata actataacgg tcctaaggta
      181 gcgatttaaa tacgcgctct cttaaggtag ccccgggacg cgtcaattgc atgaagaatc
      241 tgcttagggt taggcgtttt gctgccacct gacgtcggca gtgaaaaaaa tgctttattt
      301 gtgaaatttg tgatgctatt gctttatttg taaccattat aagctgcaat aaacaagtta
      361 acaacaacaa ttgcattcat tttatgtttc aggttcaggg ggaggtgtgg gaggtttttt
      421 aaagcaagta aaacctctac aaatgtggta tggctgatta tgatcctcta gactgcagac
      481 tagttcattg aaagtacaag ttctctgaac cagctttttt gtagagagat gttatgcttt
      541 cctgggcaca agacattggc agcttgtaaa gttcgtccat tccgtgtgtt atcccagccg
      601 ccgcagtagt ttgattctta acgagcgcaa cgatcctcgc tgcttcgttc ttatccttga
      661 tgccaagctt ctgcatgtcg gggatcacag cggtggggtg gtcagcccac tcgaaggccg
      721 ccttggagat cctgtcccac ttctgcacct gcttcccctg cgtcacggtc aaggtcaccc
      781 cgtgctggct gttgaacccc gcgtccaccg tgcgggacac ggtgttgaac cgcagcccct
      841 cgcacaccat gaccaccagc ttcaccagct tgctcttcgt gtcggcctgt gggtcagccg
      901 ccatggtgaa ttctccaggc gatctgacgg ttcactaaac gagctctgct tatataggcc
      961 tcccaccgta cacgccacct cgacatactc gagtttactc cctatcagtg atagagaacg
     1021 tatgaagagt ttactcccta tcagtgatag agaacgtatg cagactttac tccctatcag
     1081 tgatagagaa cgtataagga gtttactccc tatcagtgat agagaacgta tgaccagttt
     1141 actccctatc agtgatagag aacgtatcta cagtttactc cctatcagtg atagagaacg
     1201 tatatccagt ttactcccta tcagtgatag agaacgtata agctttaggc gtgtacggtg
     1261 ggcgcctata aaagcagagc tcgtttagtg aaccgtcaga tcgcctggag caattccaca
     1321 acacttttgt cttataccaa ctttccgtac cacttcctac cctcgtaaag tcgacaccgg
     1381 ggcccagatc tatgaagttc actgaaatct tccccgtgga ggacgcgaac tacccttaca
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7261 gtattaccgc ctttgagtga gctgataccg ctcgccgcag ccgaacgacc gagcgcagcg

```
 7321 agtcagtgag cgaggaagcg gaagagcgcc tgatgcggta ttttctcctt acgcatctgt
 7381 gcggtatttc acaccgcata tggtgcactc tcagtacaat ctgctctgat gccgcatagt
 7441 taagccagta tacactccgc tatcgctacg tgactgggtc atggctgcgc cccgacaccc
 7501 gccaacaccc gctgacgcgc cctgacgggc ttgtctgctc ccggcatccg cttacagaca
 7561 agctgtgacc gtctccggga gctgcatgtg tcagaggttt tcaccgtcat caccgaaacg
 7621 cgcgaggcag ctgtggaatg tgtgtcagtt agggtgtgga aagtccccag gctccccagc
 7681 aggcagaagt atgcaaagca tgcatctcaa ttagtcagca accaggtgtg gaaagtcccc
 7741 aggctcccca gcaggcagaa gtatgcaaag catgcatctc aattagtcag caaccatagt
 7801 cccgccccta actccgccca tcccgccccc aactccgccc agttccgccc attctccgcc
 7861 ccatggctga ctaatttttt ttatttatgc agaggccgag gccgcctcgg cctctgagct
 7921 attccagaag tagtgaggag gcttttttgg aggcctaggc ttttgcaaaa agcttcacgc
 7981 tgccgcaagc actcagggcg caagggctgc taaaggaagc ggaacacgta gaaagccagt
 8041 ccgcagaaac ggtgctgacc ccggatgaat gtcagctact gggctatctg gacaagggaa
 8101 aacgcaagcg caaagagaaa gcaggtagct tgcagtgggc ttacatggcg atagctagac
 8161 tgggcggttt tatggacagc aagcgaaccg gaattgccag ctggggcgcc ctctggtaag
 8221 gttgggaagc cctgcaaagt aaactggatg gctttcttgc cgccaaggat ctgatggcgc
 8281 aggggatcaa gatcgatctg atcaagagac aggatgagga tcgtttcgca tgattgaaca
 8341 agatggattg cacgcaggtt ctccggccgc ttgggtggag aggctattcg gctatgactg
 8401 ggcacaacag acaatcggct gctctgatgc cgccgtgttc cggctgtcag cgcaggggcg
 8461 cccggttctt tttgtcaaga ccgacctgtc cggtgccctg aatgaactgc aggacgaggc
 8521 agcgcggcta tcgtggctgg ccacgacggg cgttccttgc gcagctgtgc tcgacgttgt
 8581 cactgaagcg ggaagggact ggctgctatt gggcgaagtg ccggggcagg atctcctgtc
 8641 atctcacctt gctcctgccg agaaagtatc catcatggct gatgcaatgc ggcggctgca
 8701 tacgcttgat ccggctacct gcccattcga ccaccaagcg aaacatcgca tcgagcgagc
 8761 acgtactcgg atggaagccg gtcttgtcga tcaggatgat ctggacgaag agcatcaggg
 8821 gctcgcgcca gccgaactgt tcgccaggct caaggcgcgc atgcccgacg gcgaggatct
 8881 cgtcgtgacc catggcgatg cctgcttgcc gaatatcatg gtggaaaatg gccgcttttc
 8941 tggattcatc gactgtggcc ggctgggtgt ggcggaccgc tatcaggaca tagcgttggc
 9001 tacccgtgat attgctgaag agcttggcgg cgaatgggct gaccgcttcc tcgtgcttta
 9061 cggtatcgcc gctcccgatt cgcagcgcat cgccttctat cgccttcttg acgagttctt
 9121 ctgagcggga ctctggggtt cgaaatgacc gaccaagcga cgcccaacct gccatcacga
 9181 gatttcgatt ccaccgccgc cttctatgaa aggttgggct tcggaatcgt tttccgggac
 9241 gccggctgga tgatcctcca gcgcggggat ctcatgctgg agttcttcgc ccaccccggg
 9301 ctcgatcccc tcgcgagttg gttcagctgc tgcctgaggc tggacgacct cgcggagttc
 9361 taccggcagt gcaaatccgt cggcatccag gaaaccagca gcggctatcc gcgcatccat
 9421 gcccccgaac tgcaggagtg gggaggcacg atggccgctt tggtccggat ctttgtgaag
 9481 gaaccttact tctgtggtgt gacataattg gacaaactac ctacagagat ttaaagctct
 9541 aaggtaaata taaaattttt aagtgtataa tgtgttaaac tactgattct aattgtttgt
 9601 gtattttaga ttccaaccta tggaactgat gaatgggagc agtggtggaa tgcctttaat
 9661 gaggaaaacc tgttttgctc agaagaaatg ccatctagtg atgatgaggc tactgctgac
 9721 tctcaacatt ctactcctcc aaaaaagaag agaaaggtag aagaccccaa ggactttcct
 9781 tcagaattgc taagtttttt gagtcatgct gtgtttagta atagaactct tgcttgcttt
 9841 gctatttaca ccacaaagga aaaagctgca ctgctataca agaaaattat ggaaaaatat
 9901 tctgtaacct ttataagtag gcataacagt tataatcata acatactgtt ttttcttact
 9961 ccacacaggc atagagtgtc tgctattaat aactatgctc aaaaattgtg tacctttagc
10021 tttttaattt gtaaaggggt taataaggaa tatttgatgt atagtgcctt gactagagat
10081 cataatcagc cataccacat ttgtagaggt tttacttgct tta
```

SEQ ID NO 8
Length:601
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

```
  1 mgkiennerv ilnvggtrhe tyrstlktlp gtrlallass eppgdcltta gdklqpsppp
 61 lsppprappl spgpggcfeg gagncssrgg rasdhpgggr efffdrhpgv fayvlnyyrt
121 gklhcpadvc gplfeeelaf wgidetdvep ccwmtyrqhr daeealdife tpdliggdpg
181 ddedlaakrl giedaaglgg pdgksgrwrr lqprmwalfe dpyssraarf iafaslffil
241 vsittfclet heafnivknk tepvingtsv vlqyeietdp altyvegvcv vwftfeflvr
301 ivfspnklef iknllniidf vailpfylev glsglsskaa kdvlgflrvv rfvrilrifk
361 ltrhfvglrv lghtlrastn efllliifla lgvlifatmi yyaervgaqp ndpsasehtq
421 fknipigfww avvtmttlgy gdmypqtwsg mlvgalcala gvltiampvp vivnnfgmyy
481 slamakqklp rkrkkhippa pqassptfck telnmacnst qsdtclgkdn rllehnrsvl
541 plphgamhln lihkkqlfkk ngclhfkgln skqcyqvttv qevsrhyhpq kgspsdalvp
601 etkteegkhv sy
```

SEQ ID NO 9
Length:481
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

```
  1 marelsesta ldaqstedqm ellvikveee eagfpsspdl gsegsrerfr gfrypeaagp
 61 realsrlrel crqwlqpemh skeqilellv leqfltilpg nlqswvreqh pesgeevvvl
121 leylerqlde papqvsgvdq gqellcckma lltpapgsqs sqfqlmkall khesvgsqpl
181 qdrvlqvpvl ahggccredk vvasrltpes qgllkvedva ltltpewtqq dssqgnlcrd
241 ekqenhgslv slgdekqtks rdlppaeelp ekehgkisch lrediaqipt caeageqegr
301 lqrkqknatg grrhichecg ksfaqssgls khrrihtgek pyeceecgka figssalvih
361 qrvhtgekpy eceecgkafs hssdlikhqr thtgekpyec ddcgktfsqs csllehhrih
421 tgekpyqcsm cgkafrrssh llrhqrihtg dknvqepeqg eawksrmesq lenvetpmsy
481 kcnecersft qntgliehqk ihtgekpyqc nacgkgftri sylvqhqrsh vgknilsq
```

SEQ ID NO 10
Length:361
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

```
  1 mlvmaprtvl lllsaalalt etwagshsmr yfytsvsrpg rgeprfisvg yvddtqfvrf
 61 dsdaaspree prapwieqeg peywdrntqi ykaqaqtdre slrnlrgyyn qseagshtlq
121 smygcdvgpd grllrghdqy aydgkdyial nedlrswtaa dtaaqitqrk weaareaeqr
181 raylegecve wlrrylengk dkleradppk thvthhpisd heatlrcwal gfypaeitlt
241 wqrdgedqtq dtelvetrpa gdrtfqkwaa vvvpsgeeqr ytchvqhegl pkpltlrwep
301 ssqstvpivg ivaglavlav vvigavvaav mcrrkssggk ggsysqaacs dsaqgsdvsl
361 ta
```

SEQ ID NO 11
Length:2581
Type: DNA
Organism: Artificial Sequence

Feature:
Other Information: Synthetic Polynucleotide

```
1 gtggctcgct tcgccgcgct ccctccttcc ccgccttcca tacctccccg gctccgctcg
       61 gttcctggcc accccgcagc ccctgcccag gtgccatggc cgcattgtac cgccctggcc
      121 tgcggtgagt gacccccggc ccggggccca cccgcacctt ccgctgcgct cgccccctcg
      181 gggctgccag tggcgctctc ctgctctcag cctccgccag gtttcccatc ctaggcggag
      241 gcgggcaggg gcgactgctg tgggtccagc ctcccgcgcc gcgcgtctct tgggagggca
      301 gccggccggt gctcctcgtt tccgcctgca cctccccttc tctgcctcgc tcgcctctga
      361 ccgcgcgatc tctatctgcc actctcagaa cttcctctct ctcctcgctc ctctctgctg
      421 agccaggtct ccgcatatcc tcctttcctt cccagatacc tccctcggac ctctaacggg
      481 ctctcagcca gcgccccagg gtacttcgag aggcagcagg gccctgggga caagggctta
      541 actggcatgg gctgagcccc ttgggctggc catcatgccg tagcatccag accctgcgag
      601 tgcttagtgg agatctgggc cagcttccca ctggcattcg agattttgta gagcacagtg
      661 cccgcctgtg ccaaccagag ggcatccaca tctgtgatgg aactgaggct gagaatactg
      721 ccacactgac cctgctggag cagcagggcc tcatccgaaa gctccccaag tacaataact
      781 gctggctggc ccgcacagac cccaaggatg tggcacgagt agagagcaag acggtgattg
      841 taactccttc tcagcgggac acggtaccac tcccgcctgg tggggcccgt gggcagctgg
      901 gcaactggat gtccccagct gatttccagc gagctgtgga tgagaggttt ccaggctgca
      961 tgcagggccg caccatgtat gtgcttccat tcagcatggg tcctgtgggc tccccgctgt
     1021 cccgcatcgg ggtgcagctc actgactcag cctatgtggt ggcaagcatg cgtattatga
     1081 cccgactggg gacacctgtg cttcaggccc tgggagatgg tgactttgtc aagtgtctgc
     1141 actccgtggg ccagcccctg acaggacaag gggagccagt gagccagtgg ccgtgcaacc
     1201 cagagaaaac cctgattggc cacgtgcccg accagcggga gatcatctcc ttcggcagcg
     1261 gctatggtgg caactccctg ctgggcaaga agtgctttgc cctacgcatc gcctctcggc
     1321 tggcccggga tgagggctgg ctggcagagc acatgctgat cctgggcatc accagccctg
     1381 cagggaagaa gcgctatgtg gcagccgcct tccctagtgc ctgtggcaag accaacctgg
     1441 ctatgatgcg gcctgcactg ccaggctgga aagtggagtg tgtgggggat gatattgctt
     1501 ggatgaggtt tgacagtgaa ggtcgactcc gggccatcaa ccctgagaac ggcttctttg
     1561 gggttgcccc tggtacctct gccaccacca atcccaacgc catggctaca atccagagta
     1621 acactatttt taccaatgtg gctgagacca gtgatggtgg cgtgtactgg gagggcattg
     1681 accagcctct tccacctggt gttactgtga cctcctggct gggcaaaccc tggaaacctg
     1741 gtgacaagga gccctgtgca catcccaact ctcgattttg tgccccggct cgccagtgcc
     1801 ccatcatgga cccagcctgg gaggccccag agggtgtccc cattgacgcc atcatctttg
     1861 gtggccgcag acccaaaggg gtacccctgg tatacgaggc cttcaactgg cgtcatgggg
     1921 tgtttgtggg cagcgccatg cgctctgagt ccactgctgc agcagaacac aaagggaaga
     1981 tcatcatgca cgacccattt gccatgcggc ccttttttgg ctacaacttc gggcactacc
     2041 tggaacactg gctgagcatg gaagggcgca agggggccca gctgccccgt atcttccatg
     2101 tcaactggtt ccggcgtgac gaggcagggc acttcctgtg gccaggcttt ggggagaatg
     2161 ctcgggtgct agactggatc tgccggcggt tagaggggga ggacagtgcc cgagagacac
     2221 ccattgggct ggtgccaaag gaaggagcct tggatctcag cggcctcaga gctatagaca
     2281 ccactcagct gttctccctc cccaaggact tctgggaaca ggaggttcgt gacattcgga
     2341 gctacctgac agagcaggtc aaccaggatc tgcccaaaga ggtgttggct gagcttgagg
     2401 ccctggagag acgtgtgcac aaaatgtgac ctgaggccct agtctagcaa gaggacatag
     2461 caccctcatc tgggaatagg gaaggcacct tgcagaaaat atgagcaatt tgatattaac
     2521 taacatcttc aatgtgccat agaccttccc acaaagactg tccaataata agagatgctt
     2581 atctatttta
```

SEQ ID NO 12
Length:3721
Type: DNA
Organism: Artificial Sequence
Feature:
Other Information: Synthetic Polynucleotide

```
   1 attcgggggc gcgagctgcc ccaggtgagc cgtggctcag gtccggagcg cggtcgggac
  61 acagcgcctc taggagaaag cctggaaggc gctccggggg tacccagagc tcttagcggg
 121 ccggcagcat gtgcggggcc caaggtcttc cgggctctgc gtgggggtcg ctgcggctgg
 181 agaccaggga gggggatgga aacggggttg gaaagtaatc ccacccgagc agtaaatgga
 241 aatgttttct aacatataaa aacctacaga agaagaaaat aattttctgg atcaaattag
 301 aagtctgtat tatattgatg tctccagatt caaatatatt agaaagcagc cgtggagaca
 361 accatcttca ttttgggaga aataactaaa gcccgcctca agcattagaa ctacagacaa
 421 accctgatgc gacctctcca gattgtccca agtcgattga tttcccagct atattgtggc
 481 ctgaagcctc cagcgtccac acgaaaccag atttgcctga aaatggctcg gccaagttca
 541 acacaaggct gagagaaatt aagacggtca cggtggaaca gaacagaggc ctcagaaata
 601 acactacaca tctacaacca tctgatcttt gacaaacctg acaaaaacaa gcagcctatc
 661 tctggaaggg atgtgagttc aggtatggca gattttcgaa agtttttgc aaaagcaaag
 721 cacatagtca tcatctcagg agctggtgtt agtgcagaaa gtggtgttcc gaccttcaga
 781 ggagctggag gttattggag aaaatggcaa gcccaggacc tggcgactcc cctggccttt
 841 gcccacaacc cgtcccgggt gtgggagttc taccactacc ggcgggaggt catggggagc
 901 aaggagccca acgccgggca ccgcgccata gccgagtgtg agacccggct gggcaagcag
 961 ggccggcgag tcgtggtcat cacccagaac atcgatgagc tgcaccgcaa ggctggcacc
1021 aagaaccttc tggagatcca tggtagctta tttaaaactc gatgtacctc ttgtggagtt
1081 gtggctgaga attacaagag tccaatttgt ccagctttat caggaaaagg tgctccagaa
1141 cctggaactc aagatgccag catcccagtt gagaaacttc cccggtgtga agaggcaggc
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1261 ctggaggagg ttgacagaga gctcgcccac tgtgatttat gtctagtggt gggcacttcc
1321 tctgtggtgt acccagcagc catgtttgcc ccccaggtgg ctgccagggg cgtgccagtg
1381 gctgaattta acacggagac caccccagct acgaacagat tcaggtttca tttccaggga
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1561 aatggtctta tgggtggtga gctgagtact gaacaatcta aaaatagcct ctgattccct
1621 cgctggaatc caacctgttg ataagtgatg ggggtttaga agtagcaaag agcacccaca
1681 ttcaaaagtc acagaactgg aaagttaatt catattattt ggtttgaact gaaacgtgag
1741 gtatctttga tgtgtatggt tggttattgg gagggaaaaa ttttgtaaat tagattgtct
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1861 aaaaacccta ctattctgat ttttgcacaa gttttagtgg aaaataaaat cacactctac
1921 agtaggtaat ttattgtata aagacattac cccacgatat ggctttatta gggacttttt
1981 ttttttttt ttgagacaga gtttcactct tgttgcccag gctggagtgc agtggtgcga
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2281 gggcttttta accttgtttg gctacattac ctcagtgaac aaggggaagc tcacaacagg
2341 aactcacaca aagaaggaag agaacagtac caacaaggta gaacatcacg atgagaagaa
2401 agaatgatgc aaatatgtgg acccccagga taaacacagc tgcccaaaat ttagctctgg
2461 ccccccttaga tgggggaagt caacagtgaa gtgtttctca gcatcataaa ggcagaagct
2521 gtggttcctg tggggcctgc caaccgttcc cagatgctga actctgctgg gagttttttc
2581 catgggactt taaaaaatga tgcccttagg ttgggccaga cctctgttaa cttcagtagg
```

2641 gatggcacca ggttcaagag gccaaagaag agacctggag ctagtgaagg aaacataggg
2701 tttatttggg gaaccttaca gggtggtcca gtggccgcgg gctggacaga actgcaacca
2761 cttataaaaa gcatgcagtt tacatagcac tttcactcag caccctcccc tcagcagcct
2821 ccacgtggca accctcactt cttaagttat tgctgtcaga tgcatctgcc atacagggtc
2881 attctcaggg gatgcttaag ttatttctgt caggtacatc ttccatacac tttactacct
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3001 aggggccttg gaatgcctgc tgtccagcag gtgtcacagg cctgactggg aggcatggcc
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3121 catcacctca gccccttgca aaattgcatt taatgttaca cccttggctt ttgtagaaac
3181 aggcaacaag acactatcat ataaaacttt gtactgcatt gcaaggcata acctttaata
3241 aatcccagtg gtcctttgtg tagggaacgg ggatgctcat agcctatggg gcggctggag
3301 aaccagtcag ggaccctgag ggctcgatgt acactttaca tgggtgggcc aaggagttta
3361 cactgagggc actggtaata cctgtaatag tcttatagta cttataaagc agttttgcac
3421 ataaaatacc atcatgcact tataagtttg ttcttgggct gctccaagtt aactttattc
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3541 tgactaaggc ctagagattt caaaactgtt ctttgcaatt cctctcatac tgaacctctg
3601 gttcagcagc tttttttgtt gttgttgttt tcaagtttta tcatttttgt tcctatttgg
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3721 aatacaatat atgtgtaaga ta

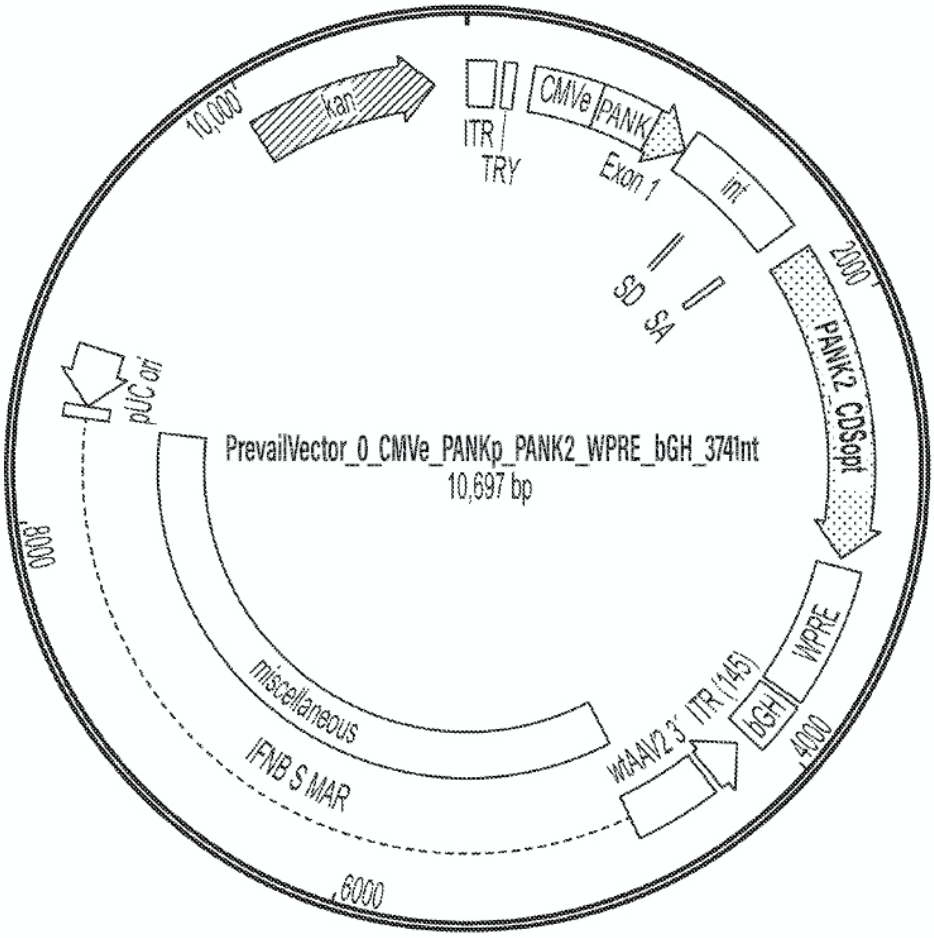

10,000
kan
ITR /
TRY
CMVe
PANK
Exon 1
int
SD
SA
2000
PANK2_CDSopt
WPRE
bGH
4000
wtAAV2 3' ITR(145)
6000
IFNB S MAR
miscellaneous
8000
pUC ori
PrevailVector_0_CMVe_PANKp_PANK2_WPRE_bGH_3741nt
10,697 bp

FIG. 1

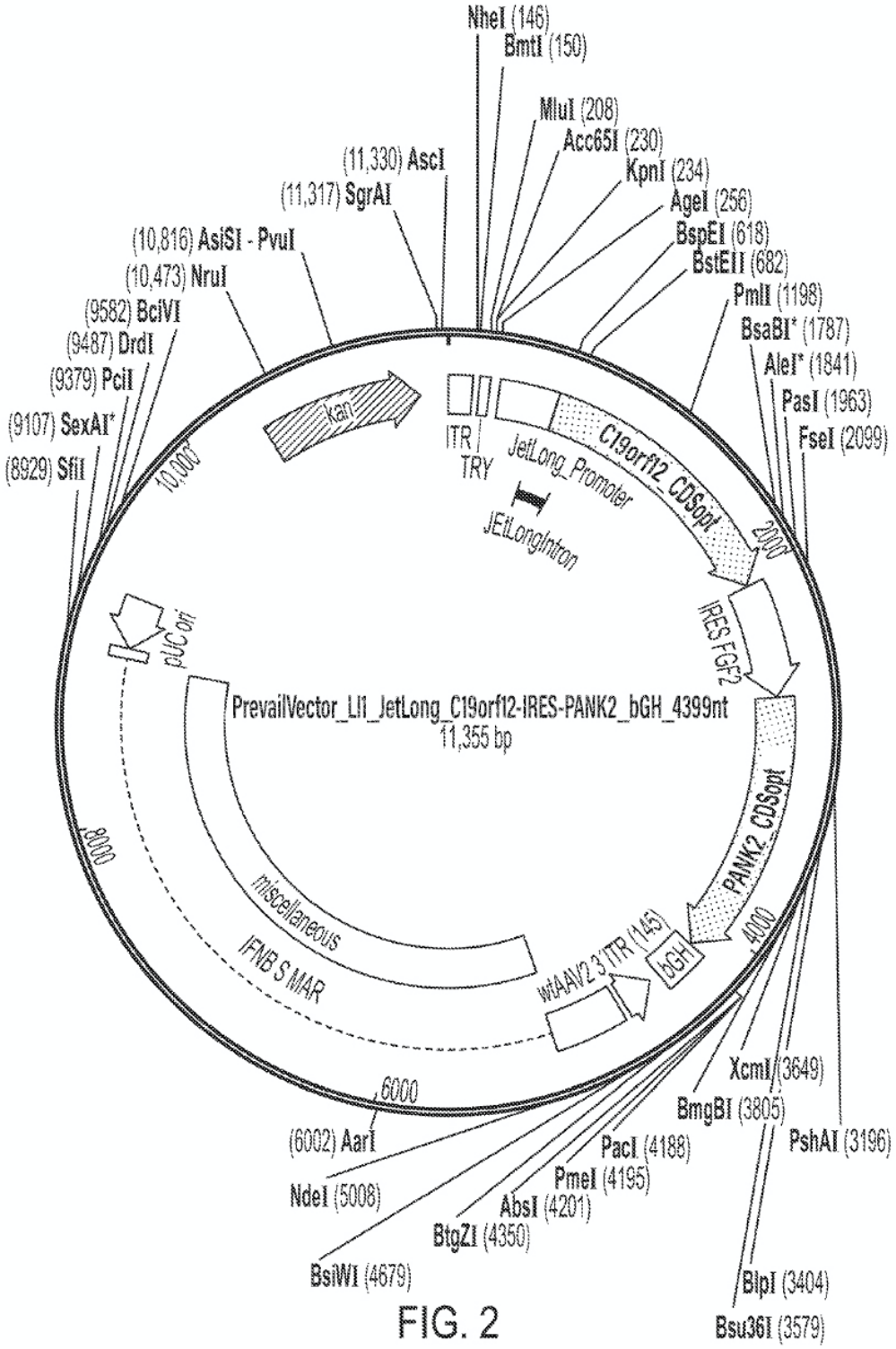
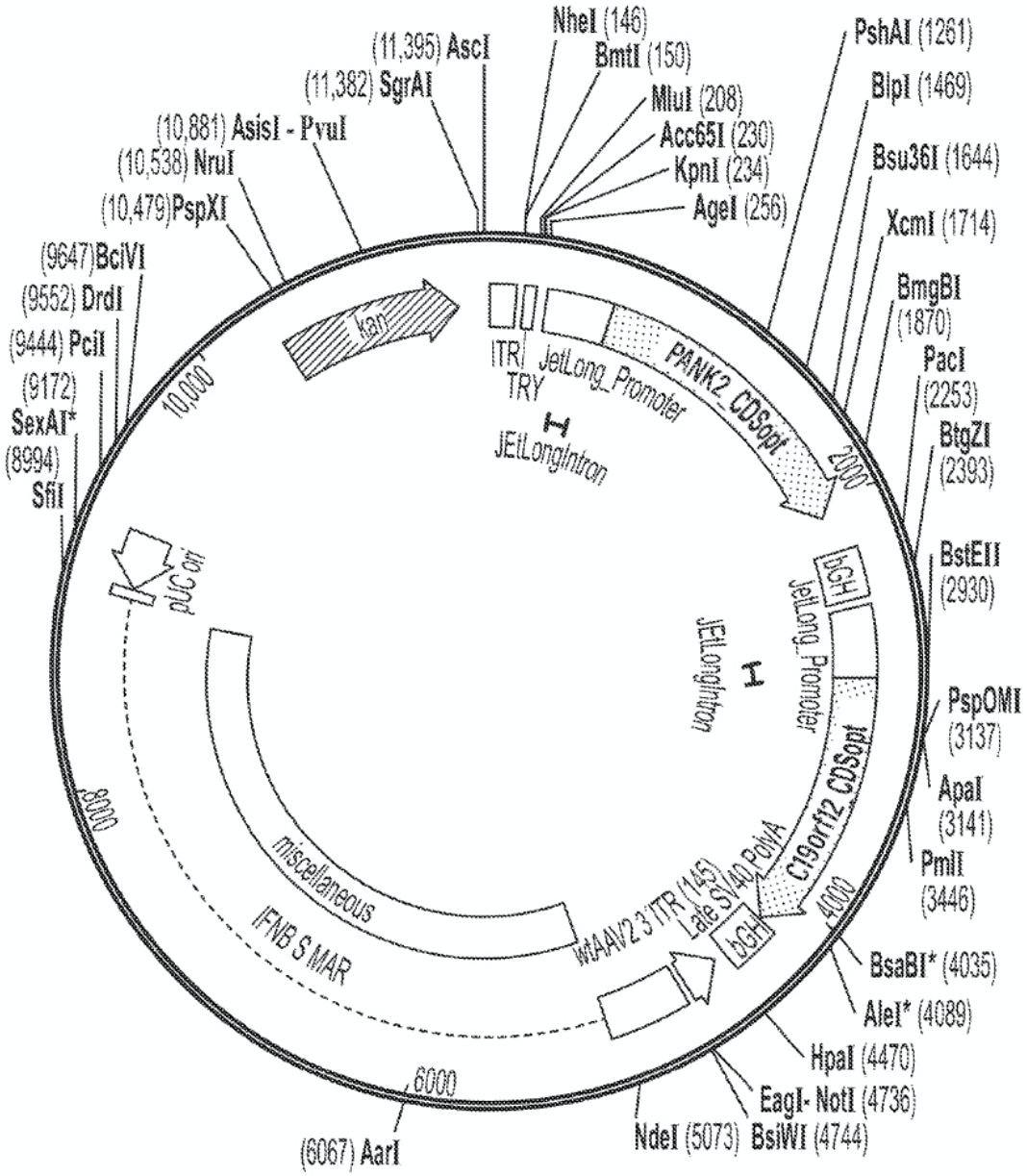

NheI (146)
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
BstEII (682)
PmlI (1198)
BsaBI* (1787)
AleI* (1841)
PasI (1963)
FseI (2099)
(11,330) AscI
(11,317) SgrAI
(10,816) AsiSI - PvuI
(10,473) NruI
(9582) BciVI
(9487) DrdI
(9379) PciI
(9107) SexAI*
(8929) SfiI
Kan
ITR
TRY
JetLong_Promoter
C19orf12_CDSopt
JEtLongIntron
2000
IRES FGF2
pUC ori
PrevailVector_LI1_JetLong_C19orf12-IRES-PANK2_bGH_4399nt
11,355 bp
10,000
8000
6000
4000
miscellaneous
IFNB S MAR
wtAAV2 3'ITR (145)
bGH
PANK2_CDSopt
XcmI (3649)
BmgBI (3805)
PshAI (3196)
(6002) AarI
NdeI (5008)
PacI (4188)
PmeI (4195)
AbsI (4201)
BtgZI (4350)
BsiWI (4679)
BlpI (3404)
Bsu36I (3579)

FIG. 2



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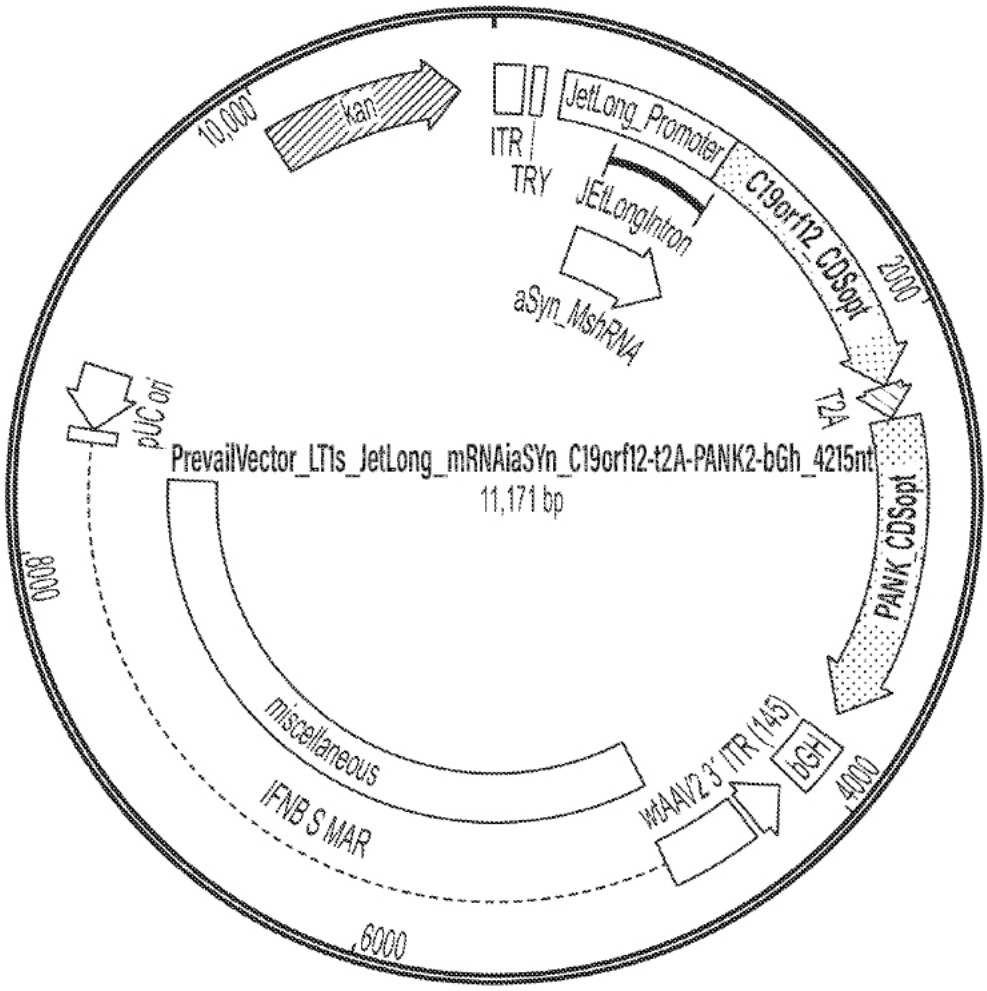
11,420 bp

FIG. 3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Kan
ITR
TRY
JetLong_Promoter
C19orf12_CDSopt
2000
JETLongIntron
aSyn_MshRN4
T2A
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PrevailVector_LT1s_JetLong_mRNAiaSYn_C19orf12-t2A-PANK2-bGh_4215nt
11,171 bp
PANK_CDSopt
8000
miscellaneous
IFNB S MAR
wtAAV2 3' ITR (145)
bGH
4000
6000

FIG. 4

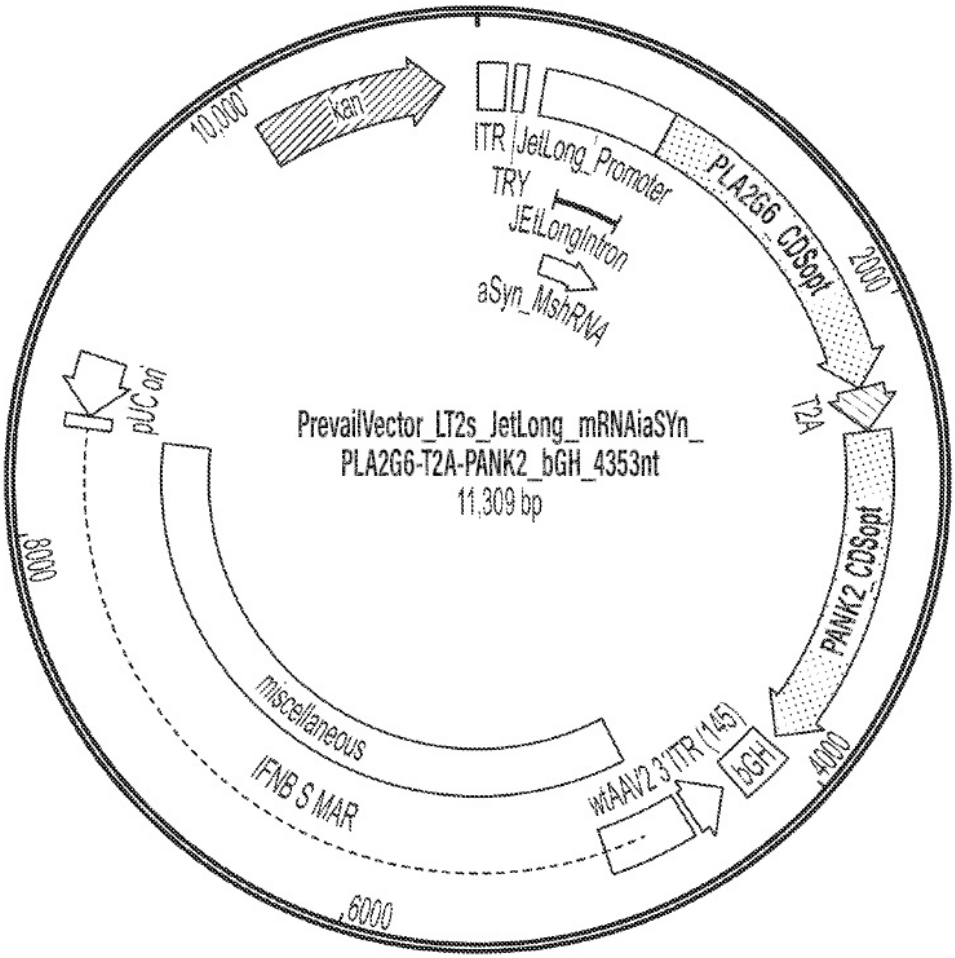

10,000
Kan
ITR
JetLong_Promoter
TRY
JEtLongIntron
aSyn_MshRNA
PLA2G6_CDSopt
2000
T2A
PANK2_CDSopt
4000
bGH
wtAAV2 3'ITR (145)
6000
IFNB S MAR
miscellaneous
8000
pUC on
PrevailVector_LT2s_JetLong_mRNAiaSYn_
PLA2G6-T2A-PANK2_bGH_4353nt
11,309 bp

FIG. 5

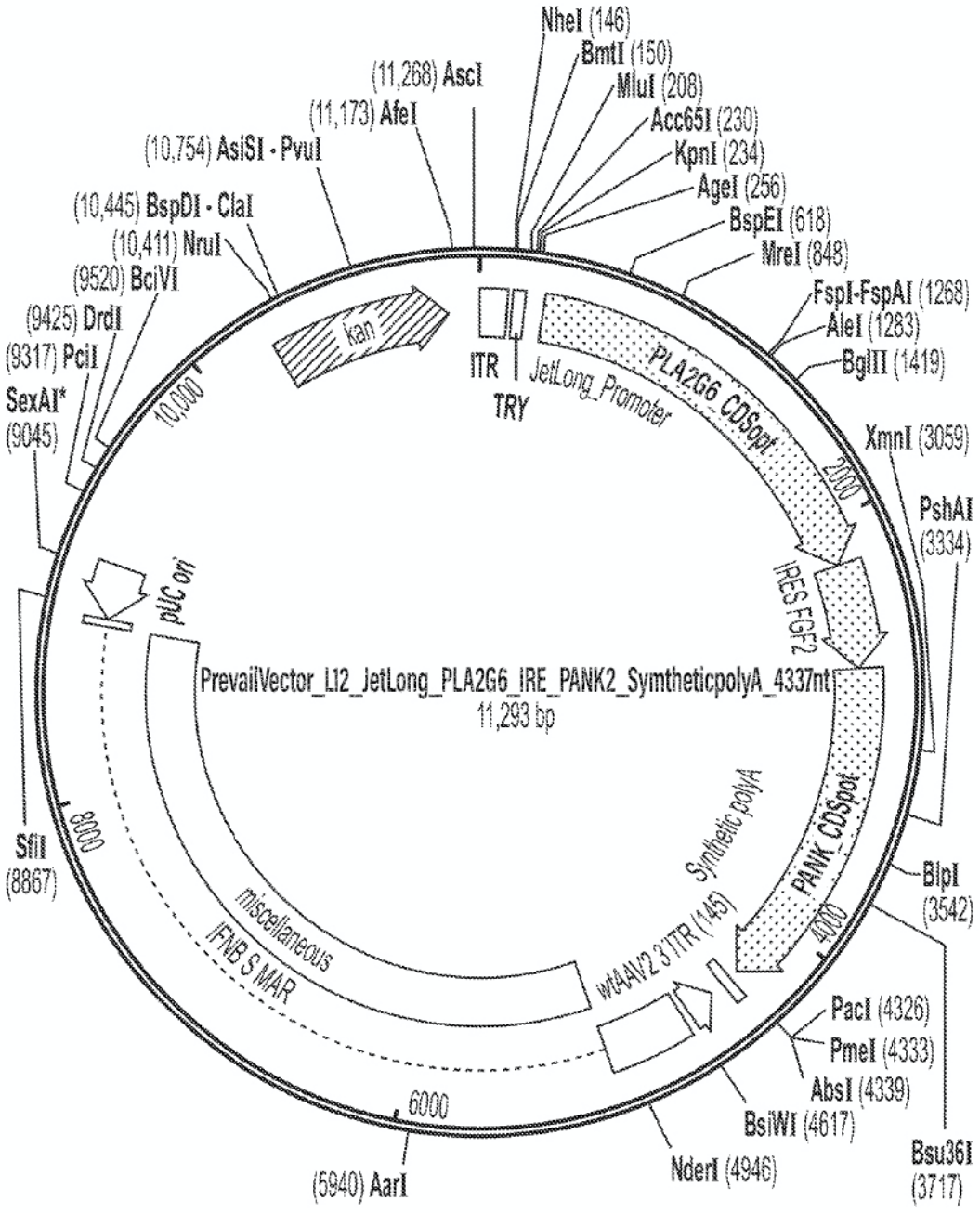

NheI (146)
BmtI (150)
MluI (208)
Acc65I (230)
KpnI (234)
AgeI (256)
BspEI (618)
MreI (848)
FspI-FspAI (1268)
AleI (1283)
BglII (1419)
XmnI (3059)
PshAI
(3334)
BlpI
(3542)
PacI (4326)
PmeI (4333)
AbsI (4339)
BsiWI (4617)
NdeI (4946)
Bsu36I
(3717)
(5940) AarI
SfiI
(8867)
SexAI*
(9045)
(9317) PciI
(9425) DrdI
(9520) BciVI
(10,411) NruI
(10,445) BspDI - ClaI
(10,754) AsiSI - PvuI
(11,173) AfeI
(11,268) AscI
kan
ITR
TRY
JetLong_Promoter
PLA2G6_CDSopt
IRES FGF2
PANK_CDSopt
Synthetic polyA
wtAAV2 3'ITR (145)
miscellaneous
IFNB S MAR
pUC ori
PrevailVector_LI2_JetLong_PLA2G6_IRE_PANK2_SymtheticpolyA_4337nt
11,293 bp
2000
4000
6000
8000
10,000

FIG. 6

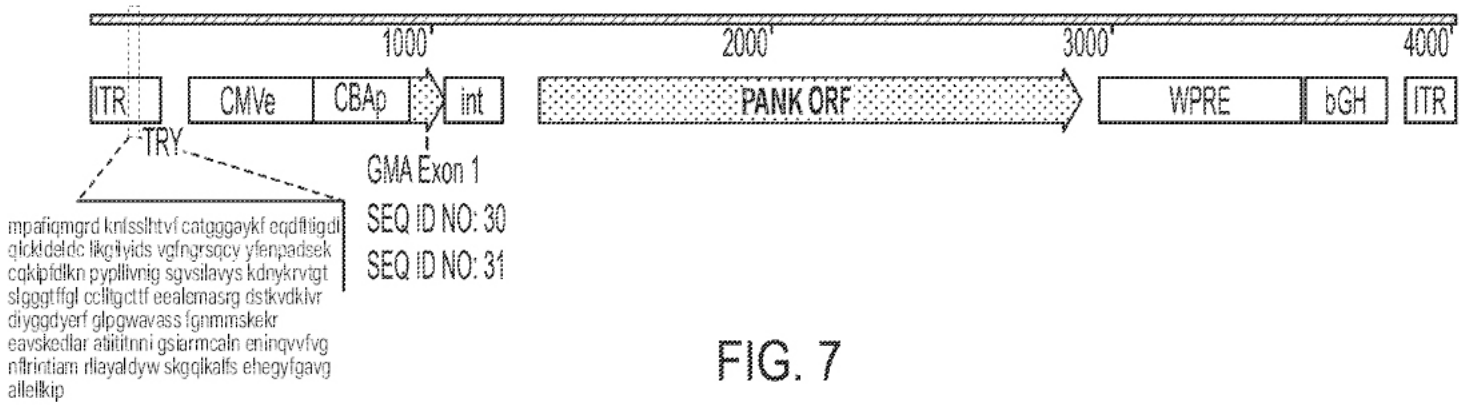

1000'
2000'
3000'
4000'
ITR
CMVe
CBAp
int
PANK ORF
WPRE
bGH
ITR
TRY
GMA Exon 1
SEQ ID NO: 30
SEQ ID NO: 31
mpafiqmgrd knfsslhtvf catgggaykf eqdftigdl
qickldeldc likgflyids vgfngrsqcy yfenpadsek
cqkipfdlkn pypllvnig sgvsllavys kdnykrvtgt
slgggtffgl ccltgcttf eealcmasrg dstkvdklvr
diyggdyerf glpgwavass fgnmmskekr
eavskedlar atititnni gsiarmcaln eninqvvfvg
nftrintam rlayaldyw skgqikalfs ehegyfgavg
allelkip

FIG. 7

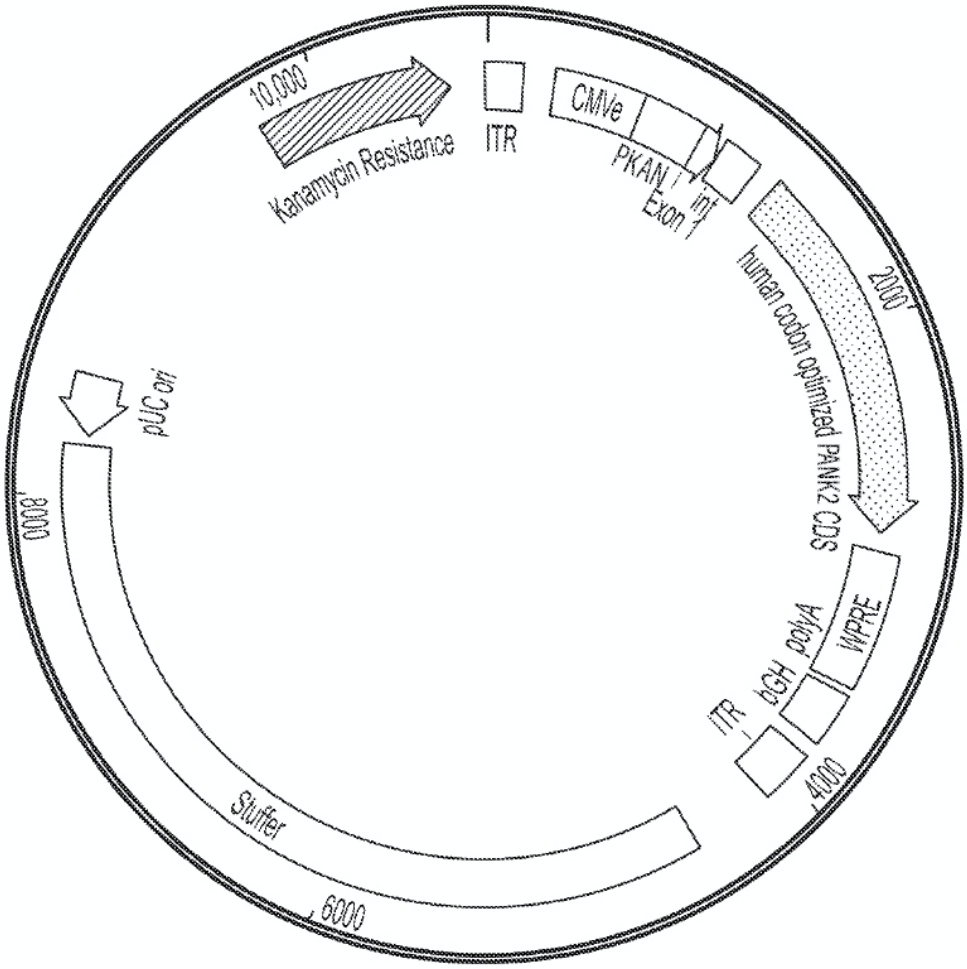

10,000
Kanamycin Resistance
ITR
CMVe
PKAN
Exon 1
INT
2000
human codon optimized PANK2 CDS
WPRE
hGH polyA
ITR
4000
6000
Stuffer
8000
pUC ori

FIG. 8

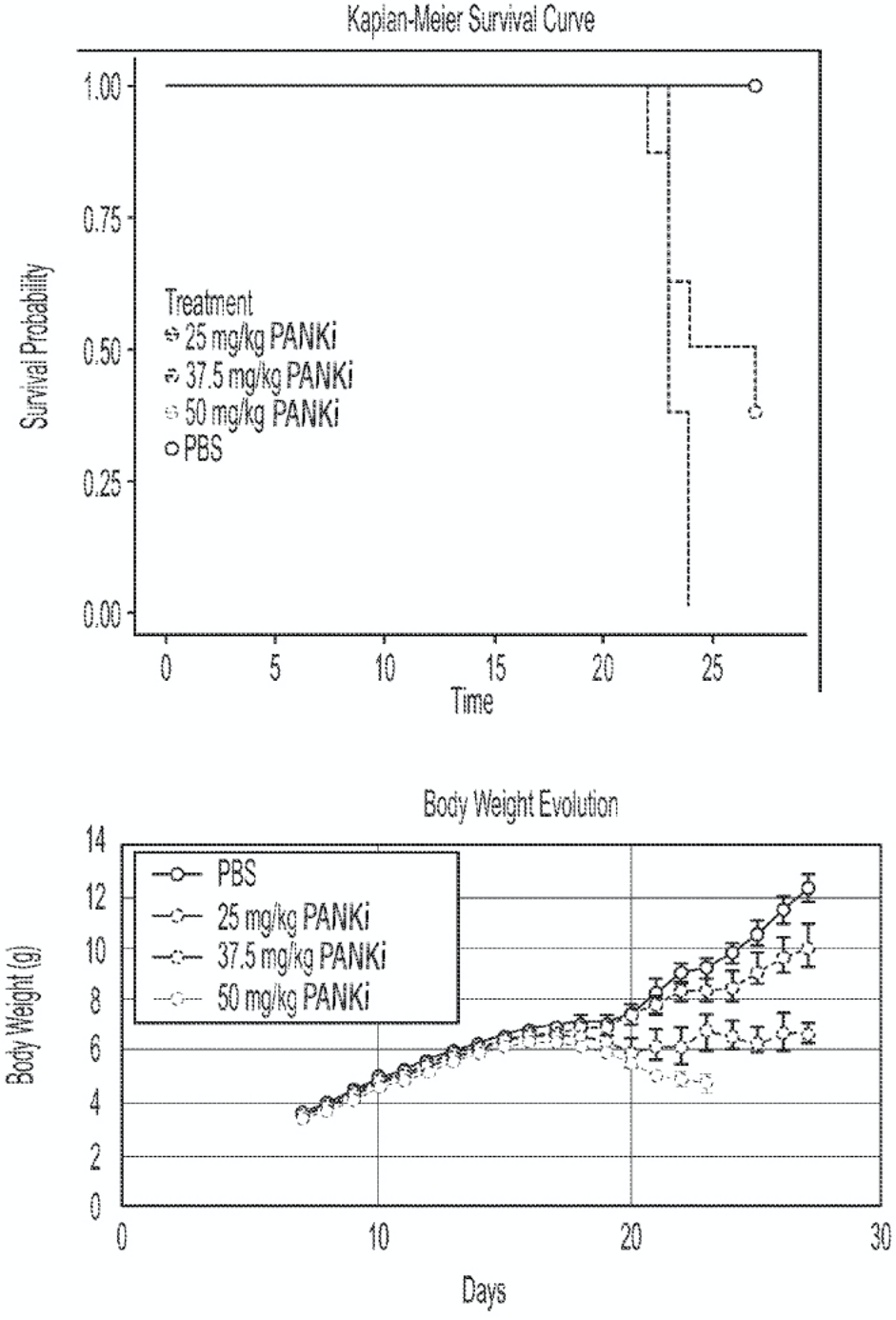

Kaplan-Meier Survival Curve
Survival Probability
1.00
0.75
0.50
0.25
0.00
Treatment
25 mg/kg PANKi
37.5 mg/kg PANKi
50 mg/kg PANKi
PBS
0
5
10
15
20
25
Time
Body Weight Evolution
Body Weight (g)
14
12
10
8
6
4
2
0
PBS
25 mg/kg PANKi
37.5 mg/kg PANKi
50 mg/kg PANKi
0
10
20
30
Days

FIG. 9

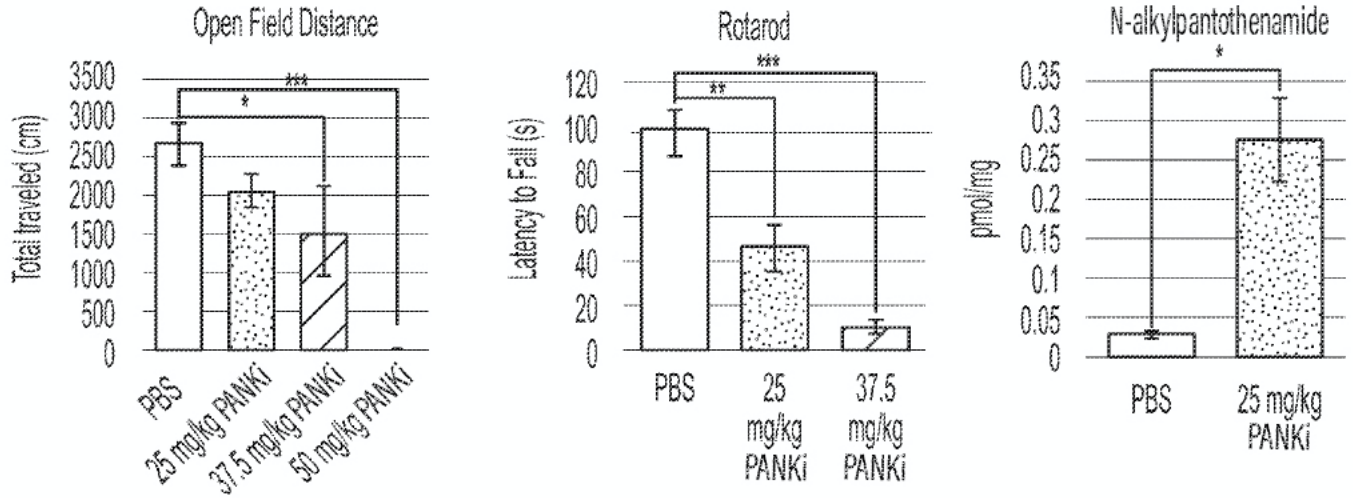
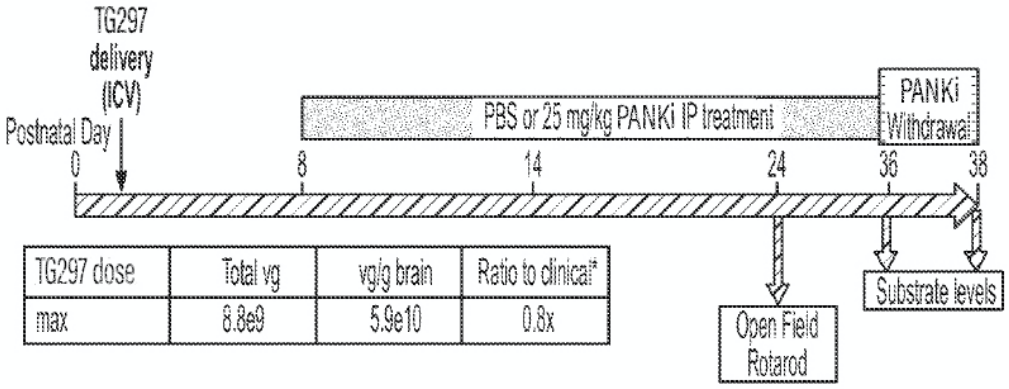

Open Field Distance
Total traveled (cm)
3500
3000
2500
2000
1500
1000
500
0
***
*
PBS
25 mg/kg PANKi
37.5 mg/kg PANKi
50 mg/kg PANKi
Rotarod
Latency to Fall (s)
120
100
80
60
40
20
0
***
**
PBS
25 mg/kg PANKi
37.5 mg/kg PANKi
N-alkylpantothenamide
pmol/mg
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
*
PBS
25 mg/kg PANKi

FIG. 9 cont.



TG297 dose	Total vg	vg/g brain	Ratio to clinical*
max	8.8e9	5.9e10	0.8x

FIG. 10

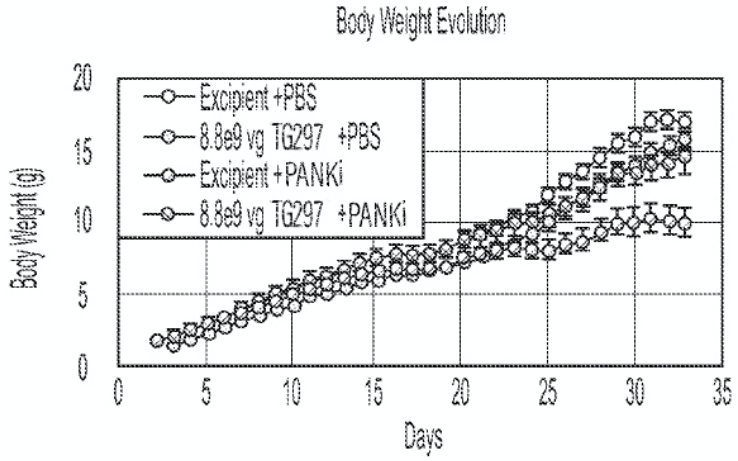


FIG. 11

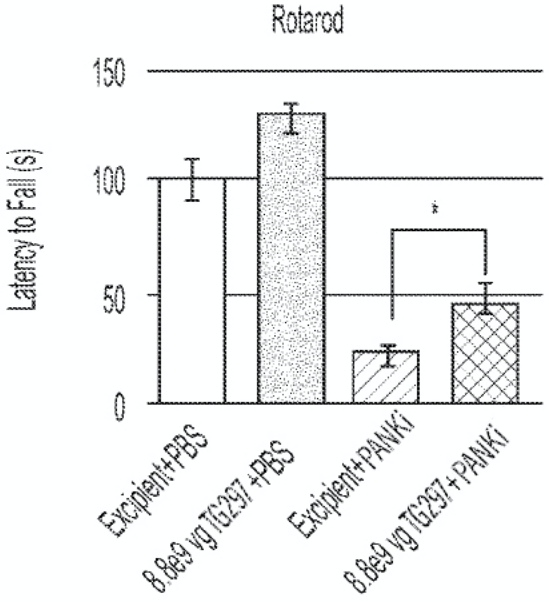


FIG. 11 cont.

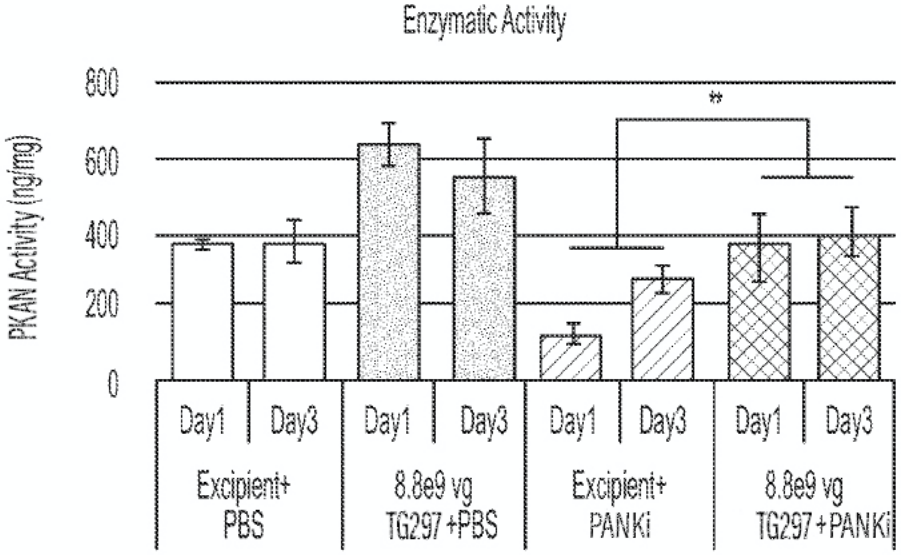
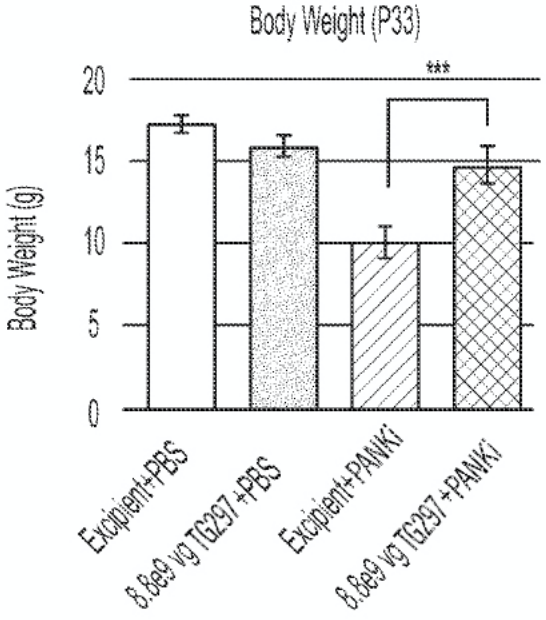


FIG. 12


Body Weight (P33)
***
20
15
10
5
0
Body Weight (g)
Excipient+PBS
8.8e9 vg TG297 +PBS
Excipient+PANKi
8.8e9 vg TG297 +PANKi

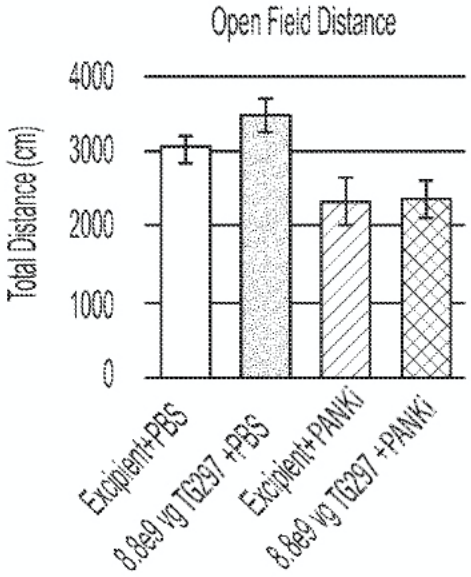

Open Field Distance
4000
3000
2000
1000
0
Total Distance (cm)
Excipient+PBS
8.8e9 vg TG297 +PBS
Excipient+PANKi
8.8e9 vg TG297 +PANKi

FIG. 11 cont.

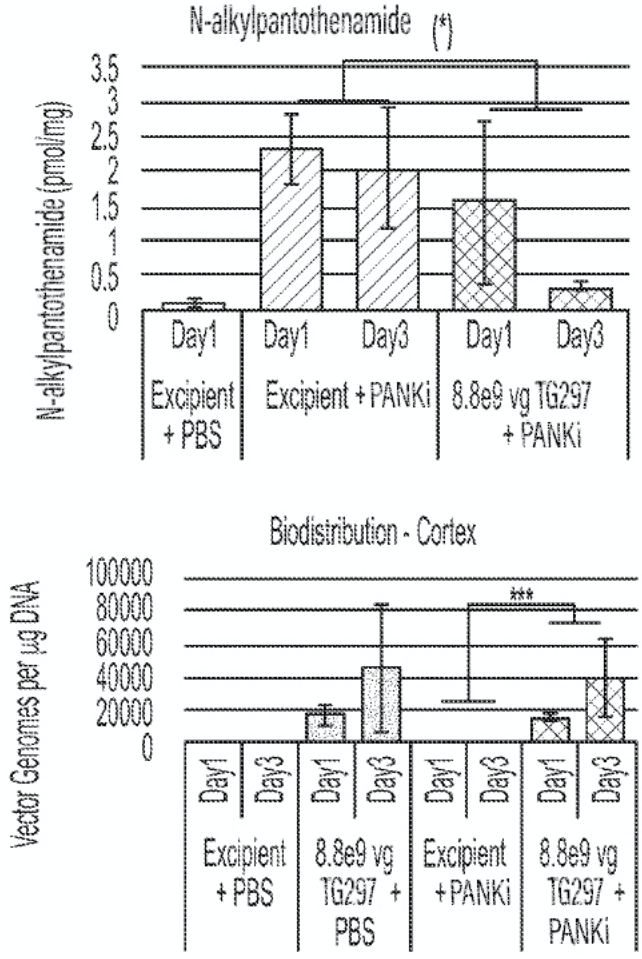

N-alkylpantothenamide (*)
N-alkylpantothenamide (pmol/mg)
3.5
3
2.5
2
1.5
1
0.5
0
Day1
Day1
Day3
Day1
Day3
Excipient + PBS
Excipient + PANKi
8.8e9 vg TG297 + PANKi
Biodistribution - Cortex
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
***
Day1
Day3
Day1
Day3
Day1
Day3
Day1
Day3
Excipient + PBS
8.8e9 vg TG297 + PBS
Excipient + PANKi
8.8e9 vg TG297 + PANKi

FIG. 12 cont.

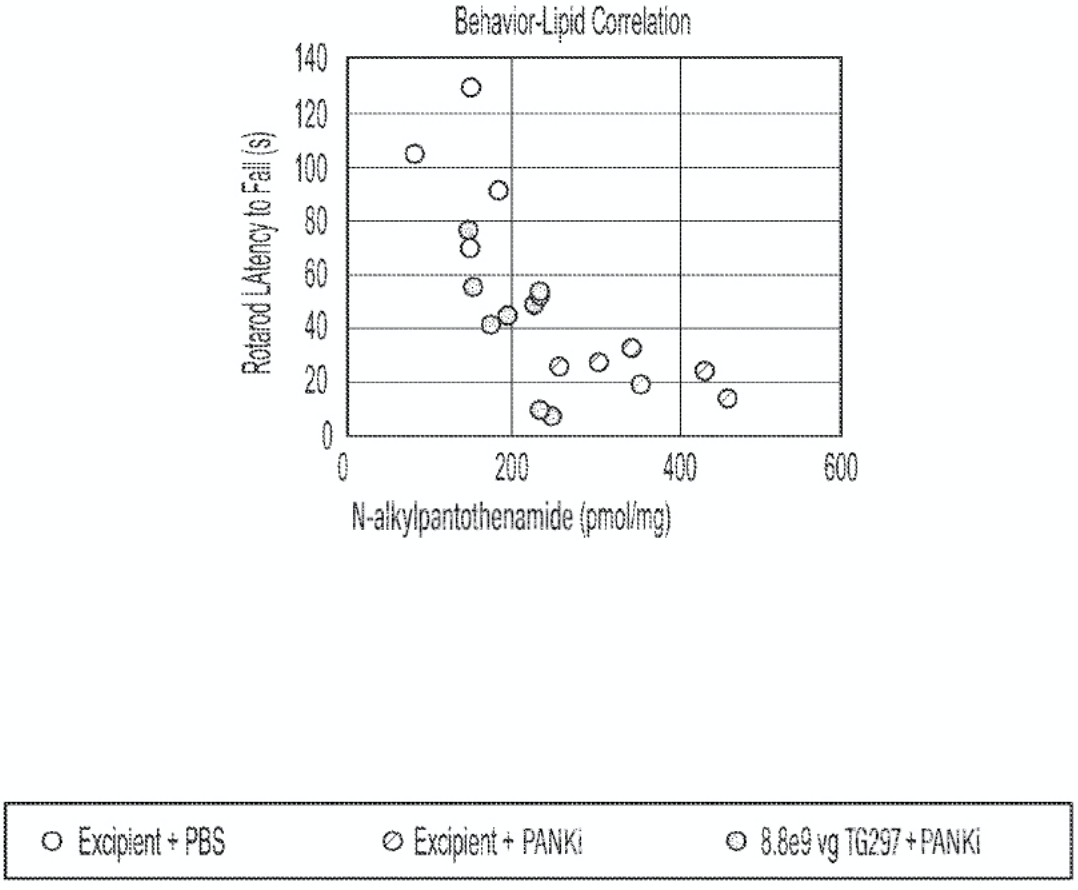

Behavior-Lipid Correlation
Rotarod Latency to Fall (s)
140
120
100
80
60
40
20
0
0
200
400
600
N-alkylpantothenamide (pmol/mg)
Excipient + PBS
Excipient + PANKi
8.8e9 vg TG297 + PANKi

FIG. 13

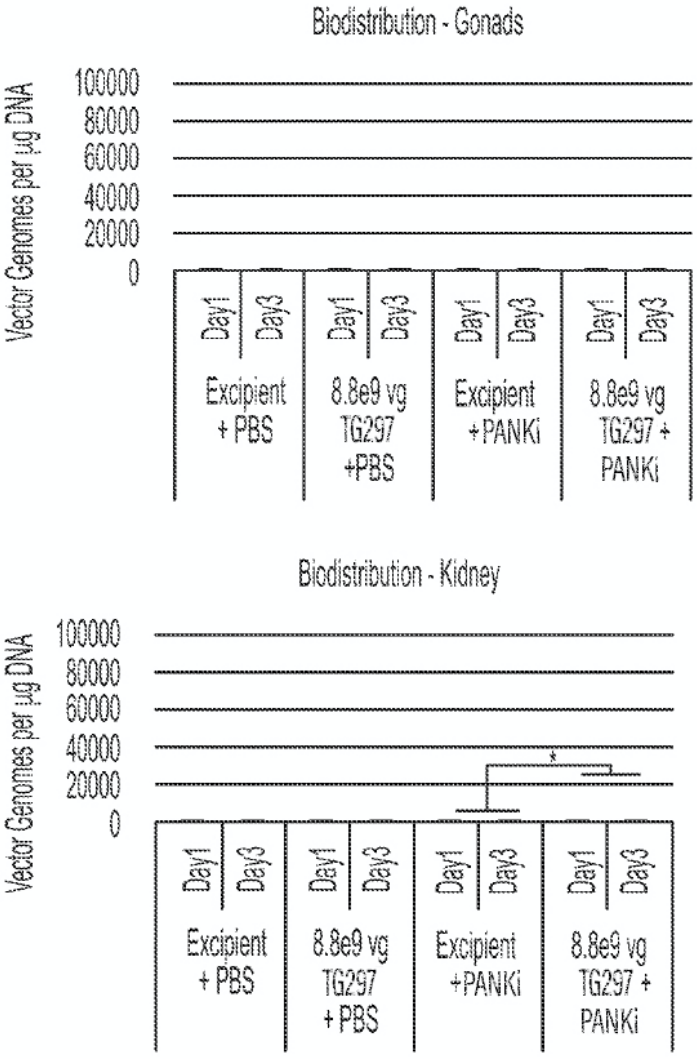

Biodistribution - Gonads
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
Day1
Day3
Excipient + PBS
8.8e9 vg TG297 +PBS
Excipient +PANKi
8.8e9 vg TG297 + PANKi
Biodistribution - Kidney
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
*
Day1
Day3
Excipient + PBS
8.8e9 vg TG297 + PBS
Excipient +PANKi
8.8e9 vg TG297 + PANKi

FIG. 14 cont.

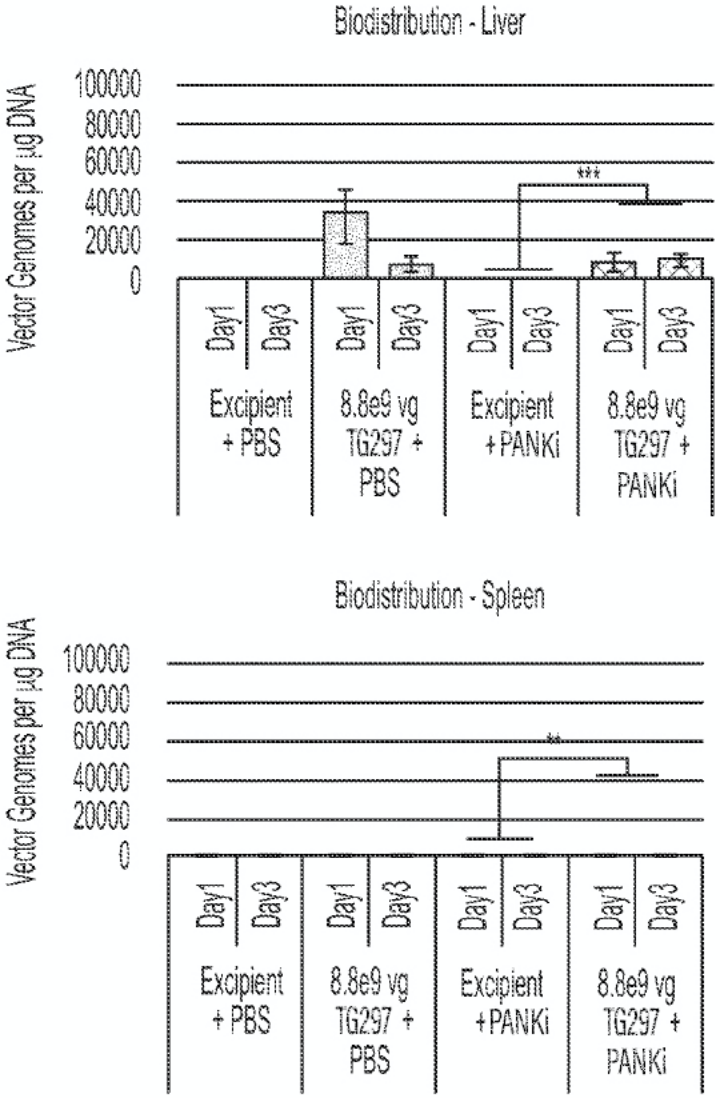

Biodistribution - Liver
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
***
Day1
Day3
Excipient + PBS
8.8e9 vg TG297 + PBS
Excipient + PANKi
8.8e9 vg TG297 + PANKi
Biodistribution - Spleen
Vector Genomes per µg DNA
100000
80000
60000
40000
20000
0
**
Day1
Day3
Excipient + PBS
8.8e9 vg TG297 + PBS
Excipient + PANKi
8.8e9 vg TG297 + PANKi

FIG. 14

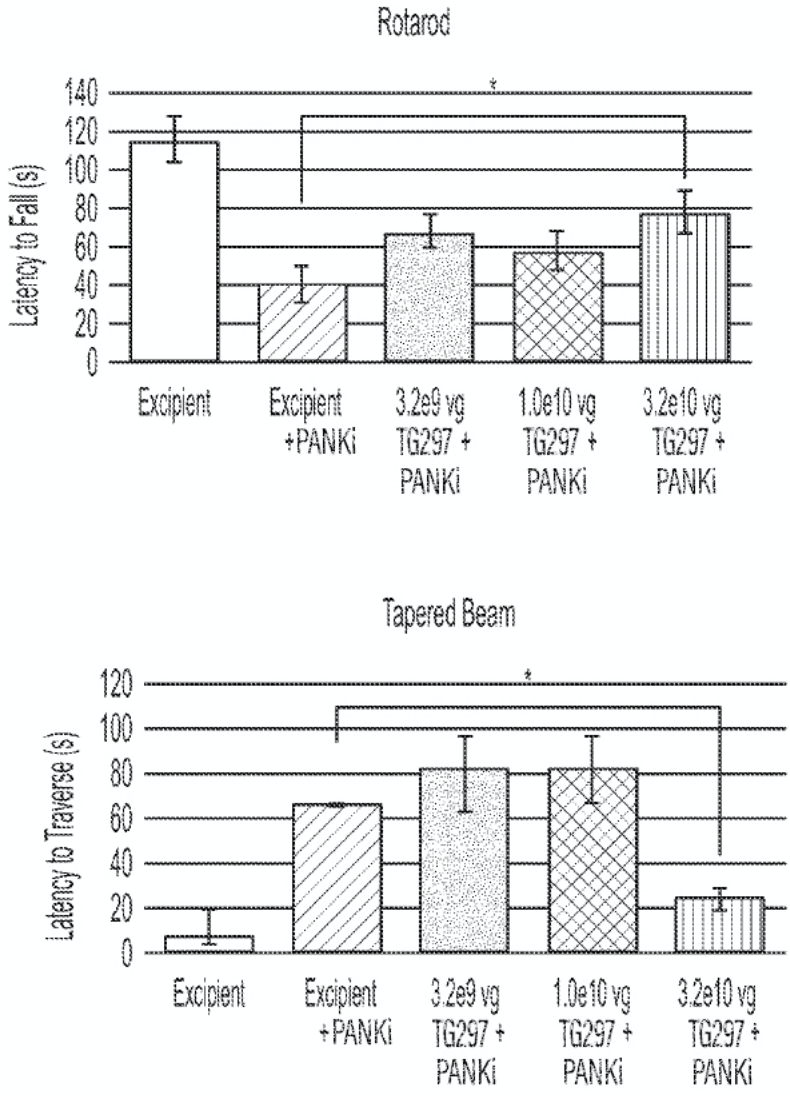

Rotarod
Latency to Fall (s)
140
120
100
80
60
40
20
0
*
Excipient
Excipient +PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi
Tapered Beam
Latency to Traverse (s)
120
100
80
60
40
20
0
*
Excipient
Excipient +PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi

FIG. 15 cont.

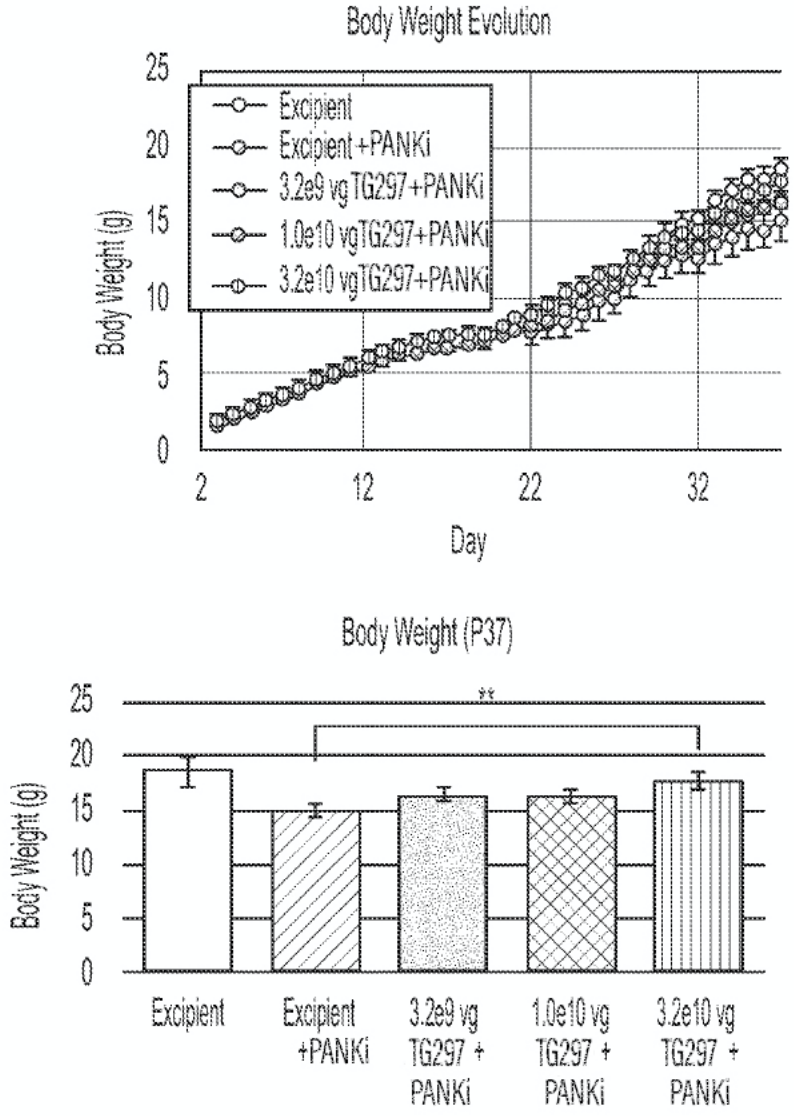

Body Weight Evolution
Excipient
Excipient +PANKi
3.2e9 vg TG297+PANKi
1.0e10 vg TG297+PANKi
3.2e10 vg TG297+PANKi
Body Weight (g)
Day
25
20
15
10
5
0
2
12
22
32
Body Weight (P37)
Body Weight (g)
**
Excipient
Excipient +PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi

FIG. 15

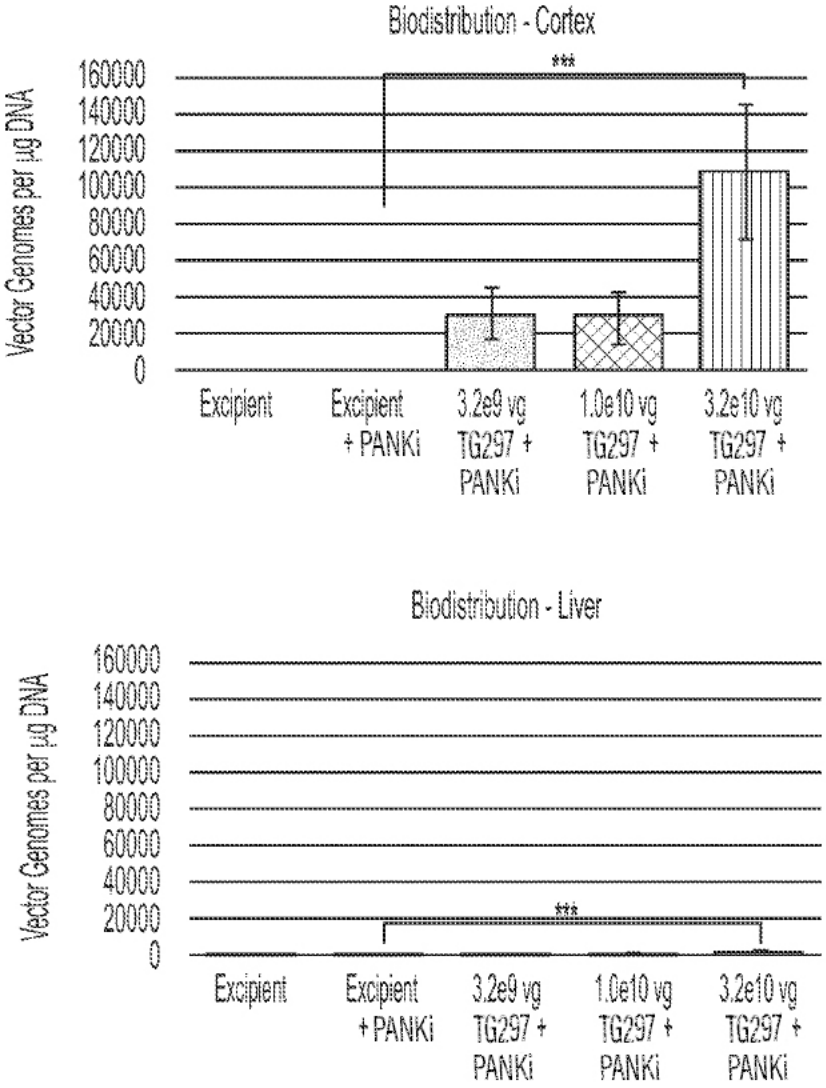

Biodistribution - Cortex
Vector Genomes per µg DNA
160000
140000
120000
100000
80000
60000
40000
20000
0
***
Excipient
Excipient + PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi
Biodistribution - Liver
Vector Genomes per µg DNA
160000
140000
120000
100000
80000
60000
40000
20000
0
***
Excipient
Excipient + PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi

FIG. 16 cont.

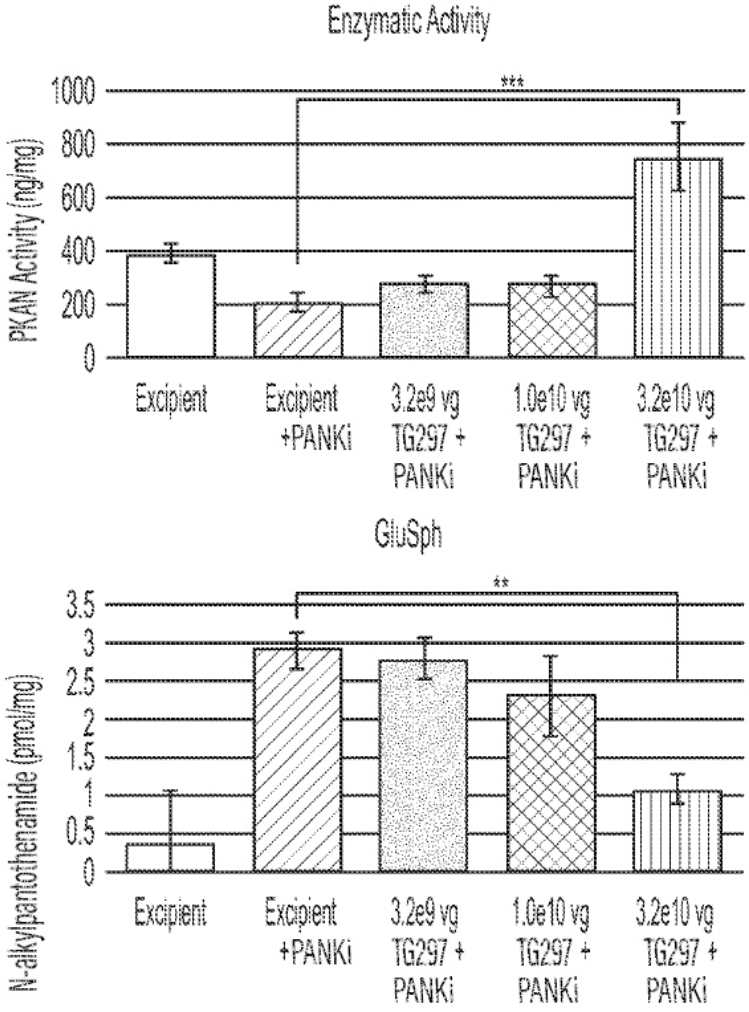
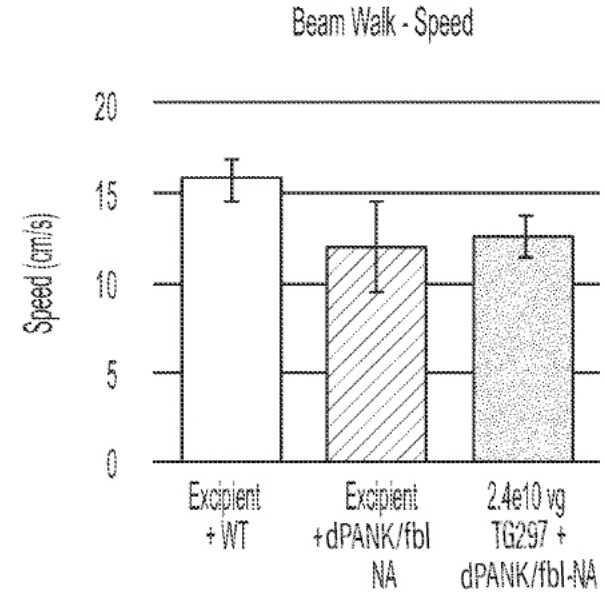

Enzymatic Activity
PKAN Activity (ng/mg)
1000
800
600
400
200
0
***
Excipient
Excipient +PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi
GluSph
N-alkylpantothenamide (pmol/mg)
3.5
3
2.5
2
1.5
1
0.5
0
**
Excipient
Excipient +PANKi
3.2e9 vg TG297 + PANKi
1.0e10 vg TG297 + PANKi
3.2e10 vg TG297 + PANKi

FIG. 16


Beam Walk - Speed
Speed (cm/s)
20
15
10
5
0
Excipient + WT
Excipient + dPANK/fbl NA
2.4e10 vg TG297 + dPANK/fbl-NA

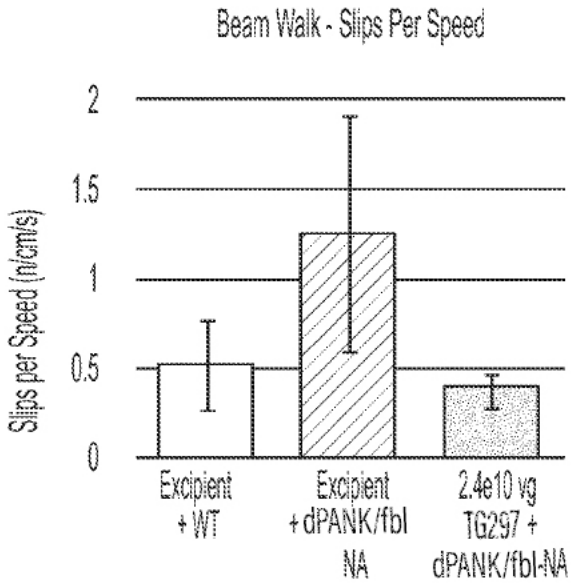
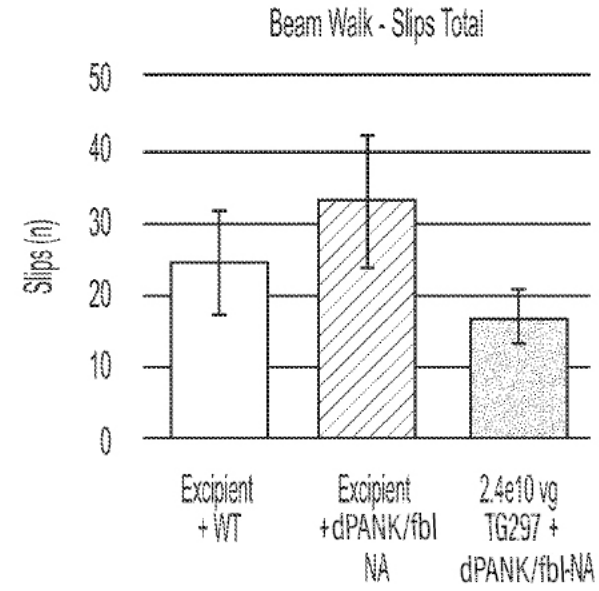

Beam Walk - Slips Per Speed
Slips per Speed (n/cm/s)
2
1.5
1
0.5
0
Excipient + WT
Excipient + dPANK/fbl NA
2.4e10 vg TG297 + dPANK/fbl-NA

FIG. 17 cont.


Beam Walk - Slips Total
Slips (n)
50
40
30
20
10
0
Excipient + WT
Excipient + dPANK/fbl NA
2.4e10 vg TG297 + dPANK/fbl-NA

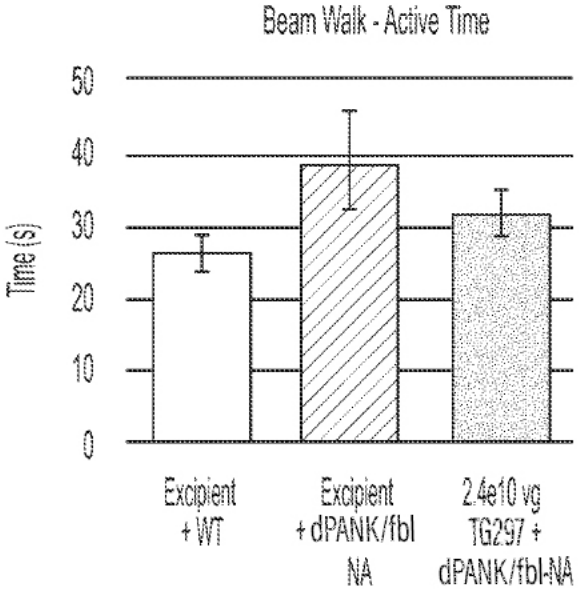

Beam Walk - Active Time
Time (s)
50
40
30
20
10
0
Excipient + WT
Excipient + dPANK/fbl NA
2.4e10 vg TG297 + dPANK/fbl-NA

FIG. 17

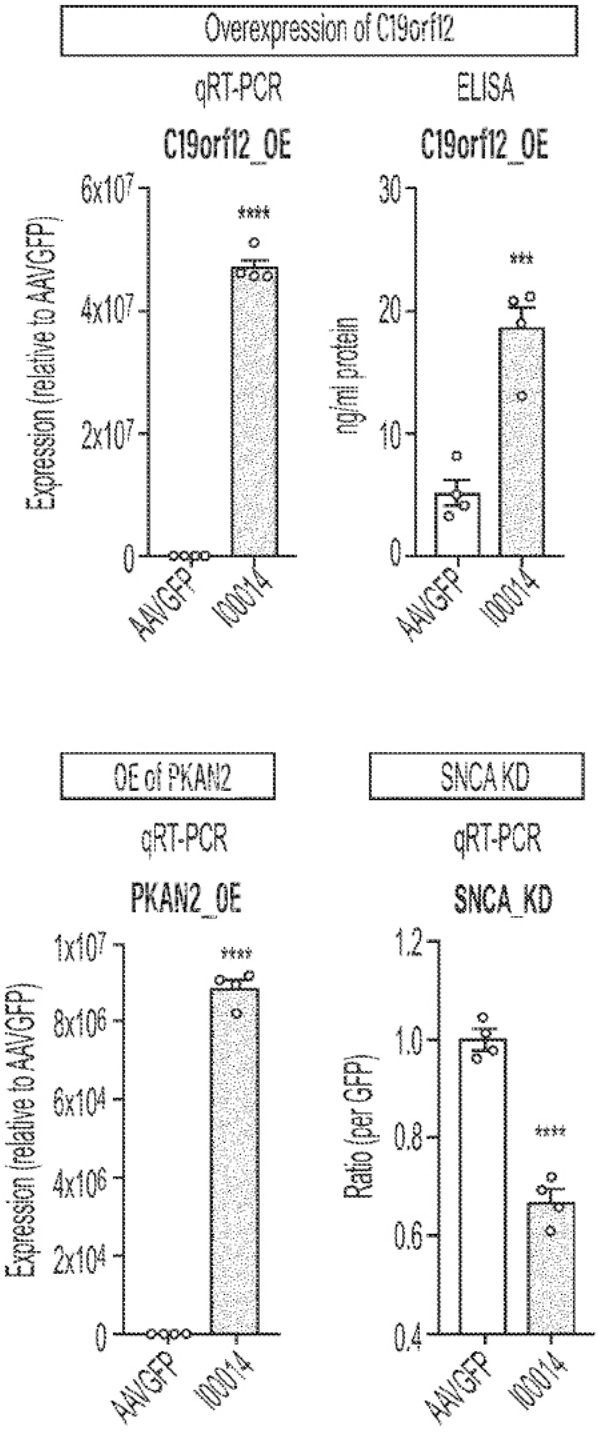

Overexpression of C19orf12
qRT-PCR
C19orf12_OE
Expression (relative to AAVGFP)
6x10^7
4x10^7
2x10^7
0
****
AAVGFP
I00014
ELISA
C19orf12_OE
ng/ml protein
30
20
10
0
***
AAVGFP
I00014
OE of PKAN2
qRT-PCR
PKAN2_OE
Expression (relative to AAVGFP)
1x10^7
8x10^6
6x10^4
4x10^6
2x10^4
0
****
AAVGFP
I00014
SNCA KD
qRT-PCR
SNCA_KD
Ratio (per GFP)
1.2
1.0
0.8
0.6
0.4
****
AAVGFP
I00014

FIG. 18 cont.

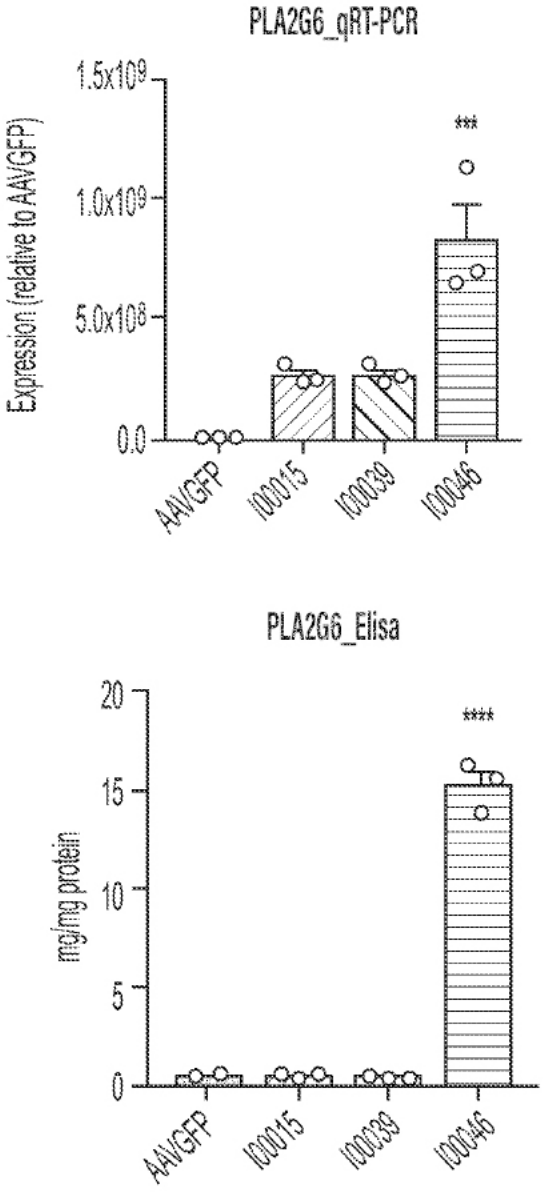

PLA2G6_qRT-PCR
Expression (relative to AAVGFP)
1.5x10^9
1.0x10^9
5.0x10^8
0.0
***
AAVGFP
I00015
I00039
I00046
PLA2G6_Elisa
mg/mg protein
20
15
10
5
0
****
AAVGFP
I00015
I00039
I00046

FIG. 18

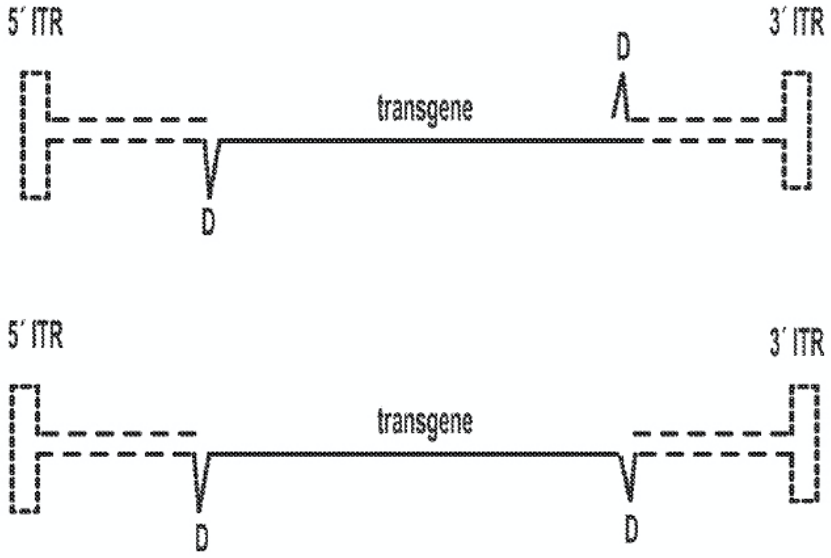

5′ ITR
3′ ITR
D
transgene
D
5′ ITR
3′ ITR
transgene
D
D

FIG. 19

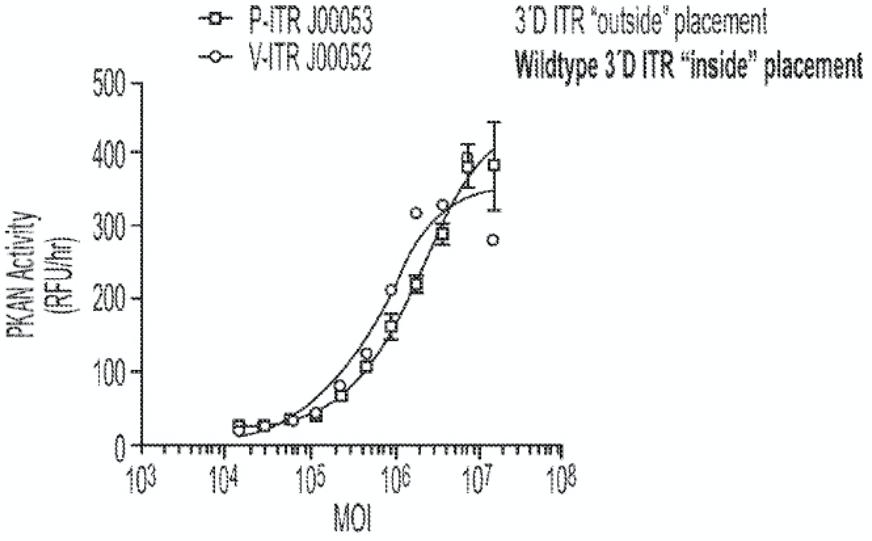

P-ITR J00053
V-ITR J00052
3′D ITR "outside" placement
Wildtype 3′D ITR "inside" placement
PKAN Activity (RFU/hr)
500
400
300
200
100
0
10³
10⁴
10⁵
10⁶
10⁷
10⁸
MOI

FIG. 20