RECOMBINANT ADENO-ASSOCIATED VIRUS FOR TREATMENT OF GRN-ASSOCIATED ADULT-ONSET NEURODEGENERATION

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of and priority to U.S. Provisional Application Nos. 62/809,329, filed February 22, 2019, 62/923,812, filed October 21, 2019, and 62/969,108, filed February 2, 2020, which are incorporated herein by reference in their entireties for all purposes.

BACKGROUND OF THE INVENTION

Frontotemporal dementia (FTD) is a fatal neurodegenerative disease that typically presents in the sixth or seventh decade of life with deficits in executive function, behavior, speech, or language comprehension. These symptoms are associated with a characteristic pattern of brain atrophy affecting the frontal and temporal cortices. Patients universally exhibit a progressive course, with an average survival of 8 years from symptom onset (Coyle-Gilchrist IT, et al. Neurology. 2016;86(18):1736-43).

FTD is highly heritable, with approximately 40% of patients having a positive family history (Rohrer JD, et al. Neurology. 2009;73(18):1451-6.). In 5-10% of FTD patients, pathogenic loss-of-function mutations can be identified in the granulin (GRN) gene encoding progranulin (PGRN), a ubiquitous lysosomal protein (Rohrer JD, et al. Neurology. 2009;73(18):1451-6). GRN mutation carriers exhibit rapid and widespread brain atrophy and may present with clinical features of other neurodegenerative diseases, such as progressive supranuclear palsy, corticobasal syndrome, Parkinson's disease, dementia with Lewy bodies, or Alzheimer's disease (Le Ber I, et al. Brain: a journal of neurology. 2008;131(3):732-46.). GRN mutations are inherited in an autosomal dominant fashion with greater than 90% penetrance by age 70 (Gass J, et al. Human molecular genetics. 2006;15(20):2988-3001). While inheritance of a single GRN mutation causes FTD and other late-onset neurodegenerative diseases, patients with homozygous loss-of-function mutations present much earlier in life with neuronal ceroid lipofuscinosis (NCL, Batten disease), characterized by accumulation of autofluorescent material (lipofuscin) in the lysosomes of neurons, rapid cognitive decline and retinal degeneration (Smith Katherine R, et al. American Journal of Human Genetics. 2012;90(6):1102-7). Though patients heterozygous for GRN mutations have much later symptom onset, they ultimately develop

lysosomal storage lesions in the brain and retina identical to those of NCL patients, and likewise experience progressive neurodegeneration (Ward ME, et al. Science Translational Medicine. 2017;9(385); Gotzl JK, et al. Acta neuropathologica. 2014;127(6):845-60). Progranulin was recently found to play a critical role in lysosomal function by promoting lysosome acidification and serving as a chaperone for lysosomal proteases including cathepsin D (CTSD) (Beel S, et al. Human molecular genetics. 2017 Aug 1;26(15):2850-2863; Tanaka Y, et al. Human molecular genetics. 2017;26(5):969-88). Mutations in the gene encoding CTSD also result in an NCL phenotype, supporting common pathophysiology related to deficient lysosomal protease activity (Siintola E, et al. Brain: a journal of neurology. 2006;129(Pt 6):1438-45).

There are currently no disease modifying therapies for adult-onset neurodegeneration caused by GRN haploinsufficiency. Disease management includes supportive care and off-label treatments aimed at reducing disease-associated behavioral, cognitive, and/or movement symptoms (Tsai and Boxer, 2016, J Neurochem. 138 Suppl 1:211-21). Further, more patients may be reached at an earlier stage with screening individuals with a family history of dementia, which is currently not indicated in view of the lack of treatment. Thus, this disease spectrum represents an area of high unmet medical need.

What are needed are treatments for adult-onset neurodegenerative disorders associated with GRN haploinsufficiencies, and for the symptoms associated therewith.

SUMMARY OF THE INVENTION

A recombinant AAV (rAAV) suitable for use in treating neurodegeneration caused by progranulin (PGRN) - related frontotemporal dementia (FTD) and other adult-onset neurodegenerative disorders associated with GRN haploinsufficiencies are provided. The rAAV comprises an adeno-associated virus 1 capsid and a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats (ITRs), a coding sequence encoding a human progranulin, and regulatory sequences which direct expression of the progranulin. In certain embodiments, the vector genome comprises an AAV 5' inverted terminal repeat (ITR), a human PGRN coding sequence and regulatory elements which direct its expression, and an AAV 5' ITR.

A pharmaceutical composition comprising an aqueous liquid and a recombinant AAV (rAAV) is also provided. In certain embodiments, the aqueous liquid comprises an artificial cerebrospinal fluid with a surfactant, which are suitable for intrathecal administration.

A method of treating a human patient with progranulin - related frontotemporal dementia (FTD) neurodegeneration or another adult onset neurodegeneration disease caused by GRN haploinsufficiency is provided. The method comprises delivering a rAAV including a human progranulin coding sequence to the central nervous system (CNS). The recombinant adeno-associated virus (rAAV) having an adeno-associated virus 1 (AAV1) capsid, said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

A rAAV1.hPGRN for use in a method for treating a human patient with PGRN-FTD or another adult onset neurodegeneration disease caused by GRN haploinsufficiency is provided. The method comprises administering to the CNS a rAAV having an adeno-associated virus 1 capsid encoding human progranulin which targets ependymal cells. The rAAV1 further comprises a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin in the ependymal cells. In one embodiment, a secretable human progranulin is expressed following delivery of the rAAV1 gene therapy.

A method for treating a human patient with brain lesions associated with progranulin - related frontotemporal dementia (FTD) neurodegeneration or another adult onset neurodegeneration disease caused by GRN haploinsufficiency is provided. The method comprises administering to the central nervous system (CNS) a recombinant adeno-associated virus (rAAV) having an adeno-associated virus 1 (AAV1) capsid, said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

In certain embodiments, the methods provided herein may further comprises (a) non-invasively assessing the patient for reduction in retinal storage lesions as a predictor of reduction of brain lesions, (b) performing magnetic resonance imaging to assess brain volume, and/or (c) measuring concentration of progranulin concentration in the CSF.

These and other aspects of the invention will be apparent from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a linear map of an AAV1.hPGRN vector genome. The AAV1.CB7.CI.hPGRN.rBG vector genome comprises a coding sequence for human PGRN under the control of the ubiquitous CB7 promoter, which is composed of a hybrid between a CMV IE enhancer and a chicken β-actin promoter. Abbreviations: BA, β-actin; bp, base pairs; CMV IE, cytomegalovirus immediate-early; ITR, inverted terminal repeats; PolyA, polyadenylation; rBG, rabbit β-globin.

FIG. 2 is a linear vector map of a cis plasmid carrying the vector genome.

FIG. 3A – FIG. 3D provide a natural history of lipofuscin accumulation and hexosaminidase activity in brains of $GRN^{-/-}$ mice. $GRN^{-/-}$ mice (KO) or $GRN^{+/+}$ (WT) controls were sacrificed at the ages indicated (n = 10 per time point). Unstained brain sections were imaged for autofluorescent material (lipofuscin) in hippocampus, thalamus and frontal cortex, and lipofuscin deposits were quantified by three blinded reviewers and averaged (FIG. 3A – FIG. 3C). Lipofuscin counts are expressed relative to the total area of the region of interest. Hexosaminidase activity was measured in brain samples and normalized to total protein concentration (FIG. 3D). Values are expressed as a ratio to wild-type controls.

FIG. 4A – FIG. 4G show correction of lysosomal pathology in brains of young adult $GRN^{-/-}$ mice by AAV-mediated PGRN expression. $GRN^{-/-}$ mice (KO) or $GRN^{+/+}$ (WT) controls were treated with a single ICV injection of vehicle (PBS) or an AAVhu68 vector expressing human PGRN (10^{11} GC) at 2 months of age (n = 10 per group). Animals were sacrificed 60 days after injection, and human PGRN was measured in CSF (FIG. 4A) and frontal lobes of the brain (FIG. 4B) by ELISA. Hexosaminidase activity was measured in brain samples (FIG. 4C). Brain PGRN concentration and Hex activity were normalized to total protein. Unstained brain sections were imaged for autofluorescent material (lipofuscin) in hippocampus, thalamus and frontal cortex. Lipofuscin deposits in hippocampus, thalamus and frontal cortex were quantified by a blinded reviewer (FIG. 4D – FIG. 4G). Lipofuscin counts are expressed per high power field. N = 10 per group except KO+PBS hippocampus n=8. *$p<0.05$, **$p<0.005$, ***$p<0.001$,

****p<0.0001, one-way ANOVA followed by Tukey's multiple comparisons test. Hexosaminidase activity was measured in serum (FIG. 4G).

FIG. 5A – FIG. 5D show correction of lysosomal pathology in brains of aged $GRN^{-/-}$ mice by AAV-mediated expression of human PGRN. $GRN^{-/-}$ mice (KO) or $GRN^{+/+}$ (WT) controls were treated with a single ICV injection of vehicle (PBS) or an AAVhu68 vector expressing human PGRN (10^{11} GC) at 7 months of age. Animals were sacrificed 4 months after injection. Hexosaminidase activity was measured in brain samples (FIG. 5A) and lipofuscin deposits were quantified in hippocampus, thalamus and cortex by a blinded reviewer (FIG. 5B – FIG. 5D). Lipofuscin counts are expressed per high power field. *p<0.05, **p<0.005, one-way ANOVA followed by Tukey's multiple comparisons test.

FIG. 6A – FIG. 6C show correction of brain microgliosis in aged $GRN^{-/-}$ mice by AAV-mediated PGRN expression. $GRN^{-/-}$ mice (KO) or $GRN^{+/+}$ (WT) controls were treated with a single ICV injection of vehicle (PBS) or an AAVhu68 vector expressing human PGRN (10^{11} GC) at 7 months of age. Animals were sacrificed 4 months after injection, and brain sections were stained for CD68. CD68 positive areas in images of hippocampus, thalamus and frontal cortex was quantified using ImageJ software by a blinded reviewer (FIG. 6A – FIG. 6C). Areas are expressed per high power field. *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001, one-way ANOVA followed by Tukey's multiple comparisons test.

FIG. 7A – FIG. 7C show human PGRN expression in rhesus macaques following AAV delivery. Adult rhesus macaques were administered AAV1, AAV5, or AAVhu68 vectors (3 x 10^{13} GC) expressing human PGRN from a chicken beta actin promoter by ICM injection on study day 0 (n = 2 per vector). Two additional macaques were administered an AAVhu68 vector expressing hPGRN from a ubiquitin C promoter (AAVhu68 V2). ICM injection was performed under fluoroscopic guidance. After confirming needle placement by fluoroscopy and CSF return injection of contrast material demonstrated distribution within the cisterna magna. Displacement of the contrast was apparent during subsequent vector infusion. Human PGRN was measured in CSF of treated macaques (FIG. 7A) and of healthy adult human subjects (FIG. 7B) and in the serum of treated macaques (FIG. 7C) by ELISA. In FIG. 7A and FIG. 7B, the dotted lines refer to limit of quantification for hPGRN in CSF at 1:5 dilution.

FIG. 8A – FIG. 8C show the immune response to human PGRN in vector treated nonhuman primates. Following ICM administered of AAV1, AAV5 or AAVhu68 vectors (3 x 10^{13} GC) expressing human PGRN from a chicken beta actin promoter or a ubiquitin C promoter

(AAVhu68 V2) CSF was collected weekly for analysis of chemistry and cytology. Increased leukocyte counts (predominantly small lymphocytes) were evident in most animals (FIG. 8A). Antibody responses to human PGRN were measured by ELISA in CSF (FIG. 8B) and serum (FIG. 8C) of animals treated with AAVhu68 or AAV1. Antibodies against human PGRN were not evaluated in serum or CSF samples from animals treated with AAV5.

FIG. 9 shows levels of brain transduction following ICM administration of AAV1 and AAVhu68 vectors to nonhuman primates. Adult rhesus macaques were administered 3 x 10^{13} GC AAVhu68 (n = 2) or AAV1 (n = 2) vectors expressing GFP from a chicken beta actin promoter by ICM injection. Animals were necropsied 28 days after vector administration, and sections of five regions of the right hemisphere of the brain were analyzed by GFP immunohistochemistry or immunofluorescence with staining for GFP and DAPI. Costaining with markers of specific cell types (NeuN, GFAP and Olig2) allowed for quantification of transduced, astrocytes, and oligodendrocytes. Mean transduction of each cell type was calculated for all sampled brain regions. Error bars = SEM of the five sections.

FIG. 10 provides a table showing percent neuron, astrocyte and oligodendrocyte transduction following ICM administration of AAV1 (animal ID 1826 and 2068) and AAVhu68 (animal ID 1518 and 2076) vectors to nonhuman primates. Adult rhesus macaques were administered 3 x 10^{13} GC AAVhu68 (n = 2) or AAV1 (n = 2) vectors expressing GFP from a chicken beta actin promoter by ICM injection on study day 0. Animals were necropsied 28 days after vector administration, and sections of five regions of the right hemisphere of the brain were analyzed by GFP immunofluorescence with costaining for specific cell types (NeuN, GFAP and Olig2). Total cells of each cell type and the number of GFP expressing cells of each type were quantified using HALO software. The percentage of each cell type transduced is shown for each region. For some animals two sections were analyzed from region 5.

FIG. 11A – FIG. 11B show results from median sensory nerve conduction studies in non-human primates. The Y-axis represents latencies from onset to peak amplitude (peak latency) measured over time (x-axis) following administration of three different doses (3 x 10^{12} GC, 1 x 10^{13} GC, or 3 x 10^{13} GC of rAAV1.hPGRN or a vehicle control).

FIG. 12A – FIG. 12D show results from neuropathological studies of dorsal root ganglia (DRG), median nerve, and spinal cord (SC) in non-human primates (NHP), 90 days after treatment with rAAV1.hPGRN.

FIG. 13 shows a manufacturing process flow diagram for vector production. *Abbreviations*: AEX, anion exchange; CRL, Charles River Laboratories; ddPCR, droplet digital polymerase chain reaction; DMEM, Dulbecco's modified Eagle medium; DNA, deoxyribonucleic acid; FFB, final formulation buffer; GC, genome copies; HEK293, human embryonic kidney 293 cells; ITFFB, intrathecal final formulation buffer; PEI, polyethylenimine; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFF, tangential flow filtration; USP, United States Pharmacopeia; WCB, working cell bank.

FIG. 14 shows a manufacturing process flow diagram for vector formulation. Manufacturing process for producing GTP-205 drug product. *Abbreviations:* Ad5, adenovirus serotype 5; AUC, analytical ultracentrifugation; BDS, bulk drug substance; BSA, bovine serum albumin; CZ, Crystal Zenith; ddPCR, droplet digital polymerase chain reaction; E1A, early region 1A (gene); ELISA, enzyme-linked immunosorbent assay; FDP, final drug product; GC, genome copies; HEK293, , human embryonic kidney 293 cells; ITFFB, intrathecal final formulation buffer; KanR, kanamycin resistance (gene); MS, mass spectrometry; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCID50 50% tissue culture infective dose; UPLC, ultra-performance liquid chromatography; USP, United States Pharmacopeia.

DETAILED DESCRIPTION OF THE INVENTION

A recombinant AAV (rAAV) suitable for use in treating neurodegenerative conditions associated with GRN-haploinsufficiencies, such as progranulin (PGRN) - related frontal temporal dementia (FTD), and compositions containing the same are provided. In certain preferred embodiments, the rAAV comprises an adeno-associated virus 1 capsid and a vector genome packaged in the AAV capsid, the vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin. In certain embodiments, the vector genome comprises an AAV 5' inverted terminal repeat (ITR), a human PGRN coding sequence and regulatory elements which direct its expression, and an AAV 3' ITR. A pharmaceutical composition comprising an aqueous liquid and a recombinant AAV (rAAV) is also provided. In certain embodiments, the aqueous liquid comprises an artificial cerebrospinal fluid with a surfactant, which are suitable for intrathecal administration. Also provided are methods for treating a human patient with PGRN-FTD and/or treating a patient with brain lesions associated with PGRN-FTD. In certain embodiments,

provided are methods for treating or reducing microgliosis in a patient. The method comprises delivering a rAAV.PGRN to the central nervous system (CNS). In certain embodiments, the methods provided herein may further comprise monitoring treatment by (a) non-invasively assessing the patient for reduction in retinal storage lesions as a predictor of reduction of brain lesions, (b) performing magnetic resonance imaging to assess brain volume, and/or (c) measuring concentration of PGRN concentration in the CSF. Optionally, concentration of PGRN in plasma may be measured.

As used herein, the terms "AAV.hPGRN" or "rAAV.hPGRN" are used to refer to a recombinant adeno-associated virus which has an AAV capsid having therewithin a vector genome comprising a human progranulin coding sequence under the control of regulatory sequences. Specific capsid types may be specified, such as, e.g., AAV1.hPGRN, which refers to a recombinant AAV having an AAV1 capsid; AAVhu68.hPGRN, which refers to recombinant AAV having an AAVhu68 capsid; AAV5.hPGRN refers to a recombinant AAV having an AAV5 capsid.

A "recombinant AAV" or "rAAV" is a DNAse-resistant viral particle containing two elements, an AAV capsid and a vector genome containing at least non-AAV coding sequences packaged within the AAV capsid. Unless otherwise specified, this term may be used interchangeably with the phrase "rAAV vector". The rAAV is a "replication-defective virus" or "viral vector", as it lacks any functional AAV rep gene or functional AAV cap gene and cannot generate progeny. In certain embodiments, the only AAV sequences are the AAV inverted terminal repeat sequences (ITRs), typically located at the extreme 5' and 3' ends of the vector genome in order to allow the gene and regulatory sequences located between the ITRs to be packaged within the AAV capsid.

As used herein, a "vector genome" refers to the nucleic acid sequence packaged inside the rAAV capsid which forms a viral particle. Such a nucleic acid sequence contains AAV inverted terminal repeat sequences (ITRs). In the examples herein, a vector genome contains, at a minimum, from 5' to 3', an AAV 5' ITR, coding sequence(s), and an AAV 3' ITR. ITRs from AAV2, a different source AAV than the capsid, or other than full-length ITRs may be selected. In certain embodiments, the ITRs are from the same AAV source as the AAV which provides the rep function during production or a transcomplementing AAV. Further, other ITRs may be used. Further, the vector genome contains regulatory sequences which direct expression of the gene product. Suitable components of a vector genome are discussed in more detail herein.

Therapeutic Protein and Coding Sequence:

The rAAV includes a coding sequence for human progranulin (hPGRN) protein or a variant thereof which performs one or more of the biological functions of hPGRN. The coding sequence of this protein is engineered into the vector genome for expression in the central nervous system (CNS).

HuPGRN1 is most commonly characterized by the 593 amino acid sequence of GenBank NP_002078, which is reproduced in SEQ ID NO: 1. This sequence contains a signal peptide at positions 1 to 17, with the secreted progranulin protein or secreted granulin(s) comprising amino acids 18 to about 593. This protein may be cleaved into 8 chains: granulin 1 (aka granulin G: about aa 58 about amino acid 113), granulin 2 (about amino acids 123 to about 179), granulin 3 (about amino acid 206 to about amino acid 261), granulin 4 (about amino acid 281 to about amino acid 336), granulin 5 (about amino acid 364 to about amino acid 417), granulin 6 (about amino acid 442 to about amino acid 496), and granulin 7 (about amino acid 518 to about amino acid 573), with reference to the numbering of SEQ ID NO: 1. In certain embodiments, a heterologous signal peptide may be substituted for the native signal peptide. However, other embodiments, may encompass progranulin with an exogenous signal peptide (e.g., a human IL2 leader). See, also, e.g., www.signalpeptide.de/index.php?m=listspdb_mammalia. Thus, fusion proteins containing progranulin and/or fragments thereof are contemplated. Such fusion proteins may encompass one or more of active GRN (e.g., GRN 1, 2, 3, 4, 4, 6, or 7) in various combinations with each other, or one or more of these peptides may be combined with the full-length PGRN or another protein or peptide (e.g., another active protein or peptide and/or a signal peptide exogenous to human PGRN).

The vector genome is engineered to carry the coding sequence for this protein and to express the protein in human cells, and particularly, in the central nervous system. In certain embodiments, the coding sequence may be the native sequence, found in GenBank: NM_002087.3, which is reproduced in SEQ ID NO: 2.

In certain embodiments, the coding sequence is provided in SEQ ID NO: 3. Certain other embodiments will encompass a coding sequence which is within 95% to 99.9% or 100% identity to SEQ ID NO: 3, including values therebetween. In some embodiments, the coding sequence is codon optimized for better therapeutic outcome, e.g., enhanced expression in mammalian cells. Identity may be assessed over the coding sequence for the full-length progranulin with the signal

(leader) sequence, over the progranulin without the signal (leader) sequence, or over the length of the coding sequence for a fusion protein as defined herein. In certain embodiments, the coding sequence is provided in SEQ ID NO: 3. Certain other embodiments will encompass a coding sequence which is within 95% to less than 100% identity to SEQ ID NO: 4. Identity may be assessed over the coding sequence for the full-length progranulin with the signal (leader) sequence, over the progranulin without the signal (leader) sequence, or over the length of the coding sequence for a fusion protein as defined herein.

Suitably, these coding sequences encode the full-length progranulin. However, other embodiments, may encompass the active granulin chain with a heterologous signal peptide (e.g., a human IL2 leader). See, also, e.g., www.signalpeptide.de/index.php?m=listspdb_mammalia.

In certain embodiments, fragments of the coding sequences for human PGRN (e.g., SEQ ID NO: 3 or SEQ ID NO: 4), or a sequence about 95% to 99.9% or 100% identical thereto, may be utilized. Such fragments may encode the active human GRN (aa 18-593), or a fusion peptide comprising a heterologous signal peptide with the active human GRN. In certain embodiments, one or more of the coding sequences for one or more of active GRN (e.g., GRN 1, 2, 3, 4, 4, 6, or 7) may be included in the vector genome in various combinations with each other, or one or more of these peptides may be combined with the full-length PGRN or another coding sequence.

Without wishing to be bound by theory, it is believed that AAV-mediated PGRN expression in a subset of cells in the CNS (e.g., ependymal cells) provides a depot of secreted protein. The secreted PGRN protein (and/or one or more GRN(s)) is taken up by other cells via sortilin or mannose-6-phosphate receptors where it is subsequently trafficked to the lysosome. In certain embodiments, the secreted protein is progranulin. In certain embodiments, the secreted protein is a granulin. In certain embodiments, the secreted protein includes a mixture of progranulin and granulin(s).

In certain embodiments, in addition to the progranulin coding sequence, another non-AAV coding sequence may be included, *e.g.*, a peptide, polypeptide, protein, functional RNA molecule (*e.g.*, miRNA, miRNA inhibitor) or other gene product, of interest. Useful gene products may include miRNAs. miRNAs and other small interfering nucleic acids regulate gene expression via target RNA transcript cleavage/degradation or translational repression of the target messenger RNA (mRNA). miRNAs are natively expressed, typically as final 19-25 non-translated RNA products. miRNAs exhibit their activity through sequence-specific interactions with the 3′ untranslated regions (UTR) of target mRNAs. These endogenously expressed

miRNAs form hairpin precursors which are subsequently processed into a miRNA duplex, and further into a "mature" single stranded miRNA molecule. This mature miRNA guides a multiprotein complex, miRISC, which identifies target site, *e.g.*, in the 3′ UTR regions, of target mRNAs based upon their complementarity to the mature miRNA.

In certain embodiments, the expression cassette further comprises one or more miRNA target sequences that repress expression of hPGRN in dorsal root ganglion (drg). In certain embodiments, the expression cassette comprises at least two tandem repeats of drg-specific miRNA target sequences, wherein the at least two tandem repeats comprise at least a first miRNA target sequence and at least a second miRNA target sequence which may be the same or different. In certain embodiments, the tandem miRNA target sequences are continuous or are separated by a spacer of 1 to 10 nucleic acids, wherein said spacer is not an miRNA target sequence. In certain embodiments, there are at least two drg-specific miRNA target sequences located at 3' to the hPGRN coding sequence. In certain embodiments, the start of the first of the at least two drg-specific miRNA tandem repeats is within 20 nucleotides from the 3' end of the hPGRN-coding sequence. In certain embodiments, the start of the first of the at least two drg-specific miRNA tandem repeats is at least 100 nucleotides from the 3' end of the hPGRN coding sequence. In certain embodiments, the miRNA tandem repeats comprise 200 to 1200 nucleotides in length. In certain embodiments, there are at least two drg-specific miRNA target sequences located at 5' to the hPGRN coding sequence. In certain embodiments, at least two drg-specific miRNA target sequences are located in both 5' and 3' to the hPGRN coding sequence. In certain embodiments, the miRNA target sequence for the at least first and/or at least second miRNA target sequence for the expression cassette mRNA or DNA positive strand is selected from (i) AGTGAATTCTACCAGTGCCATA (miR183, SEQ ID NO: 32); (ii) AGCAAAAATGTGCTAGTGCCAAA (SEQ ID NO: 33), (iii) AGTGTGAGTTCTACCATTGCCAAA (SEQ ID NO: 34); and (iv) AGGGATTCCTGGGAAAACTGGAC (SEQ ID NO: 35). In certain embodiments, two or more consecutive miRNA target sequences are continuous and not separated by a spacer. In certain embodiments, two or more of the miRNA target sequences are separated by a spacer and each spacer is independently selected from one or more of (A) GGAT; (B) CACGTG; or (C) GCATGC. In certain embodiments, the spacer located between the miRNA target sequences may be located 3' to the first miRNA target sequence and/or 5' to the last miRNA target sequence. In certain embodiments, the spacers between the miRNA target sequences are the same. See, US

Provisional Patent Application No. 62/783,956, filed December 21, 2018 and International Patent Application No. PCT/US19/67872, filed February 12, 2020, which are hereby incorporated by reference.

AAV1

AAVhu68 which is from Clade F can be used to produce vectors which target and express hPGRN in the CNS. However, it was unexpectedly observed that AAV1-mediated PGRN delivery provided superior PGRN expression in the CNS than AAVhu68, even though comparable plasma concentrations were observed. The inventors have discovered that intrathecal delivery of rAAV1.PGRN is an attractive route of delivery for the therapies described herein. Thus, in particularly desirable embodiments, an AAV1 capsid is selected.

In certain embodiments, a composition is provided which comprises an aqueous liquid suitable for intrathecal injection and a stock of rAAV having a AAV capsid which preferentially targets ependymal cells, wherein the rAAV further comprises a vector genome having a PGRN coding sequence for delivery to the central nervous system (CNS). In certain embodiments, the composition is formulated for sub-occipital injection into the cisterna magna (intra-cisterna magna). In certain embodiments, the rAAV is administered via a computed tomography- (CT-) guided rAAV injection. In certain embodiments, the patient is administered a single dose of the composition.

An AAV1 capsid refers to a capsid having AAV vp1 proteins, AAV vp2 proteins and AAV vp3 proteins. In particular embodiments, the AAV1 capsid comprises a pre-determined ratio of AAV vp1 proteins, AAV vp2 proteins and AAV vp3 proteins of about 1:1:10 assembled into a T1 icosahedron capsid of 60 total vp proteins. An AAV1 capsid is capable of packaging genomic sequences to form an AAV particle (e.g., a recombinant AAV where the genome is a vector genome). Typically, the capsid nucleic acid sequences encoding the longest of the vp proteins, i.e., VP1, is expressed *in trans* during production of an rAAV having an AAV1 capsid are described in, e.g., US Patent 6,759,237, US Patent 7,105,345, US Patent 7,186,552, US Patent 8,637,255, and US Patent 9,567,607, which are incorporated herein by reference.

The capsid coding sequences are not present in the final assembled rAAV1.hPGRN. However, such sequences are utilized in production of a recombinant AAV. In certain embodiments, the AAV1 capsid coding sequence is any nucleic sequence which encodes the full-length AAV1 VP1 protein of SEQ ID NO: 26, or the VP2 or VP3 regions thereof. See, e.g., US

Patent 6,759,237, US Patent 7,105,345, US Patent 7,186,552, US Patent 8,637,255, and US Patent 9,567,607, which are incorporated herein by reference. In certain embodiments, the AAV1 capsid coding sequence is SEQ ID NO: 25. In some embodiments, the AAV1 capsid is a protein produced from the coding sequence of SEQ ID NO: 25 with or without post-translational modification. However, variants of this coding sequence may be engineered and/or other coding sequences may be backtranslated for a desired expression system using the AAV1 VP1, AAV1 VP2, and/or AAV VP3 amino acid sequence.

In certain embodiments, compositions comprising recombinant AAV1 have capsids in which AAV1 contain five amino acids which are highly deamidated (N57, N383, N512, and N718), based on the numbering of the primary sequence of the AAV1 VP1 reproduced in SEQ ID NO: 26.

AAV1 Modification								
Enzyme		Trypsin	Trypsin	Trypsin	Trypsin	Trypsin	Trypsin	Trypsin
% Coverage	N+1	97.6	84.2	92.4	87.4	90.4	85.2	88.9
N35+Deamidation	Q	9.5						
~N57+Deamidation	G	100.0	100.0	100.0	92.0	89.3	86.1	85.5
~N94+Deamidation	H				2.3	3.7	4.9	2.2
N113+Deamidation	L		5.6					
~N214+Deamidation	N				0.9	0.4	1.0	0.7
~N223+Deamidation	A	21.4		25.9				
N227+Deamidation	W	4.9		3.1				
~N253+Deamidation	H		29.7					
Q259+Deamidation	I	24.6		14.2				
~N269+Deamidation	D			21.6			5.2	
~N271+Deamidation	H	27.7						
N286+Deamidation	R	5.4		5.2				
~N302+Deamidation	**N**NN	43.7	48.6	18.8	12.4	28.7	16.3	11.9
~N303+Deamidation	N**N**N		50.8	19.3				
~N383+Deamidation	G	88.5	86.9	82.5	82.1	84.6	83.4	92.3

~N408+Deamidation	N	58.2	43.2	40.5	30.1	25.7	28.3	22.8
~N451+Deamidation	Q	20.5						
~Q452+Deamidation	S	1.7						
N477+Deamidation	W	4.4	3.1	39.7	1.2	1.3	1.1	1.8
~N496+Deamidation	**N**NN	1.1		69.9				
N512+Deamidation	G	93.7	100.0	100.0	100.0	100.0	100.0	97.3
N651+Deamidation	T	2.0	2.1	1.6	0.6			
N691+Deamidation	S			57.1				
~N704+Deamidation	Y		9.4					
N718+Deamidation	G	98.7	98.1	98.2	89.5	91.9	92.3	87.4

In certain embodiments, AAV1 is characterized by a capsid composition of a heterogenous population of VP isoforms which are deamidated as defined in the following table, based on the total amount of VP proteins in the capsid, as determined using mass spectrometry. In certain embodiments, the AAV capsid is modified at one or more of the following positions, in the ranges provided below, as determined using mass spectrometry. Residue numbers are based on the published AAV1 sequence, reproduced in SEQ ID NO: 26.

TABLE A

AAV1 Capsid Position Based on VP1 numbering	%
N35+Deamidation	1-15, 5-10
~N57+Deamidation	65-90, 70-95, 80-95, 75 - 100, 80-100, or 90-100
N113+Deamidation	0-8
~N223+Deamidation	0-30, 0, 20-28
N227+Deamidation	0, 1-5
~N253+Deamidation	0, 1-35
Q259+Deamidation	0, 10-25
~N269+Deamidation	0-25

TABLE A	
AAV1 Capsid Position Based on VP1 numbering	%
~N271+Deamidation	0-25
N286+Deamidation	2-10
~N302+Deamidation	10-50
~N303+Deamidation	0-55
~N383+Deamidation	65-90, 70-95, 80-95, 75 - 100, 80-100, or 90-100
~N408+Deamidation	30-65
~N451+Deamidation	0-25
~Q452+Deamidation	0-5
N477+Deamidation	0-45
~N496+Deamidation	0-75
N512+Deamidation	75 - 100, 80-100, 90-100
N651+Deamidation	0-3
N691+Deamidation	0, 1-60
~N704+Deamidation	0-10
N718+Deamidation	75 - 100, 80-100, 90-100

Suitable modifications include those described in the paragraph above labelled modulation of deamidation, which is incorporated herein. In certain embodiments, one or more of the following positions, or the glycine following the N is modified as described herein. In certain embodiments, an AAV1 mutant is constructed in which the glycine following the N at position 57, 383, 512 and/or 718 are preserved (i.e., remain unmodified). In certain embodiments, the NG at the four positions identified in the preceding sentence are preserved with the native sequence. Residue numbers are based on the published AAV1 VP1, reproduced in SEQ ID NO: 26. In certain embodiments, an artificial NG is introduced into a different position than one of the positions identified in the table above.

rAAV Vectors

As indicated above, recombinant AAV having an AAV1 capsid are the preferred vectors described herein for treatment of FTD. In certain embodiments, e.g., in the examples below (e.g., AAVhu68 or AAV5), other AAV capsids may be used to generate an rAAV. In certain embodiments, an AAV1 capsid may be selected and one or more of the elements of the vector genome comprising a hPGRN coding sequence may be substituted.

As used herein, an AAVhu68 capsid refers to a capsid as defined in WO 2018/160582, incorporated herein by reference. As described herein, a rAAVhu68 has a rAAVhu68 capsid produced in a production system expressing capsids from an AAVhu68 nucleic acid (e.g., SEQ ID NO: 30) which encodes the vp1 amino acid sequence of SEQ ID NO: 31, and optionally additional nucleic acid sequences, e.g., encoding a vp 3 protein free of the vp1 and/or vp2-unique regions. The rAAVhu68 resulting from production using a single nucleic acid sequence vp1 produces the heterogenous populations of vp1 proteins, vp2 proteins and vp3 proteins. More particularly, the AAVhu68 capsid contains subpopulations within the vp1 proteins, within the vp2 proteins and within the vp3 proteins which have modifications from the predicted amino acid residues in SEQ ID NO: 31. These subpopulations include, at a minimum, deamidated asparagine (N or Asn) residues. For example, asparagines in asparagine - glycine pairs are highly deamidated. In one embodiment, the AAVhu68 vp1 nucleic acid sequence has the sequence of SEQ ID NO: 30, or a strand complementary thereto, e.g., the corresponding mRNA or tRNA. In certain embodiments, the vp2 and/or vp3 proteins may be expressed additionally or alternatively from different nucleic acid sequences than the vp1, e.g., to alter the ratio of the vp proteins in a selected expression system. In certain embodiments, also provided is a nucleic acid sequence which encodes the AAVhu68 vp3 amino acid sequence of SEQ ID NO: 31 (about aa 203 to 736) without the vp1-unique region (about aa 1 to about aa 137) and/or vp2-unique regions (about aa 1 to about aa 202), or a strand complementary thereto, the corresponding mRNA or tRNA (about nt 607 to about nt 2211 of SEQ ID NO: 30). In certain embodiments, also provided is a nucleic acid sequence which encodes the AAVhu68 vp2 amino acid sequence of SEQ ID NO: 31 (about aa 138 to 736) without the vp1-unique region (about aa 1 to about 137), or a strand complementary thereto, the corresponding mRNA or tRNA (nt 411 to 2211 of SEQ ID NO: 30).

As used herein, an AAV5 capsid has a predicted amino acid sequence of SEQ ID NO: 29. In certain embodiments, the AAV5 capsid is expressed from a nucleic acid sequence of SEQ ID NO: 28.

Genomic sequences which are packaged into an AAV capsid and delivered to a host cell are typically composed of, at a minimum, a transgene and its regulatory sequences, and AAV inverted terminal repeats (ITRs). Both single-stranded AAV and self-complementary (sc) AAV are encompassed with the rAAV. The transgene is a nucleic acid coding sequence, heterologous to the vector sequences, which encodes a polypeptide, protein, functional RNA molecule (e.g., miRNA, miRNA inhibitor) or other gene product, of interest. The nucleic acid coding sequence is operatively linked to regulatory components in a manner which permits transgene transcription, translation, and/or expression in a cell of a target tissue.

The AAV sequences of the vector typically comprise the cis-acting 5′ and 3′ inverted terminal repeat sequences (See, e.g., B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155 168 (1990)). The ITR sequences are about 145 bp in length. Preferably, substantially the entire sequences encoding the ITRs are used in the molecule, although some degree of minor modification of these sequences is permissible. The ability to modify these ITR sequences is within the skill of the art. (See, e.g., texts such as Sambrook et al, "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J. Virol., 70:520 532 (1996)). An example of such a molecule employed in the present invention is a "cis-acting" plasmid containing the transgene, in which the selected transgene sequence and associated regulatory elements are flanked by the 5′ and 3′ AAV ITR sequences. In one embodiment, the ITRs are from an AAV different than that supplying a capsid. In one embodiment, the ITR sequences from AAV2. A shortened version of the 5' ITR, termed ΔITR, has been described in which the D-sequence and terminal resolution site (trs) are deleted. In other embodiments, the full-length AAV 5' and 3' ITRs are used. However, ITRs from other AAV sources may be selected. Where the source of the ITRs is from AAV2 and the AAV capsid is from another AAV source, the resulting vector may be termed pseudotyped. However, other configurations of these elements may be suitable.

In addition to the major elements identified above for the recombinant AAV vector, the vector also includes conventional control elements necessary which are operably linked to the transgene in a manner which permits its transcription, translation and/or expression in a cell transfected with the plasmid vector or infected with the virus produced by the invention. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

The regulatory control elements typically contain a promoter sequence as part of the expression control sequences, e.g., located between the selected 5' ITR sequence and the coding sequence. Constitutive promoters, regulatable promoters [see, e.g., WO 2011/126808 and WO 2013/04943], tissue specific promoters, or a promoter responsive to physiologic cues may be used may be utilized in the vectors described herein. The promoter(s) can be selected from different sources, e.g., human cytomegalovirus (CMV) immediate-early enhancer/promoter, the SV40 early enhancer/promoter, the JC polymovirus promoter, myelin basic protein (MBP) or glial fibrillary acidic protein (GFAP) promoters, herpes simplex virus (HSV-1) latency associated promoter (LAP), rouse sarcoma virus (RSV) long terminal repeat (LTR) promoter, neuron-specific promoter (NSE), platelet derived growth factor (PDGF) promoter, hSYN, melanin-concentrating hormone (MCH) promoter, CBA, matrix metalloprotein promoter (MPP), and the chicken beta-actin promoter. In addition to a promoter a vector may contain one or more other appropriate transcription initiation, termination, enhancer sequences, efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA for example WPRE; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. An example of a suitable enhancer is the CMV enhancer. Other suitable enhancers include those that are appropriate for desired target tissue indications. In one embodiment, the expression cassette comprises one or more expression enhancers. In one embodiment, the expression cassette contains two or more expression enhancers. These enhancers may be the same or may differ from one another. For example, an enhancer may include a CMV immediate early enhancer. This enhancer may be present in two copies which are located adjacent to one another. Alternatively, the dual copies of the enhancer may be separated by one or more sequences. In still another embodiment, the expression cassette further contains an intron, e.g, the chicken beta-actin intron. Other suitable introns include those known in the art, e.g., such as are described in WO 2011/126808. Examples of suitable polyA sequences include, e.g., SV40, SV50, bovine growth hormone (bGH), human growth hormone, and synthetic polyAs. Optionally, one or more sequences may be selected to stabilize mRNA. An example of such a sequence is a modified WPRE sequence, which may be engineered upstream of the polyA sequence and downstream of the coding sequence [see, e.g., MA Zanta-Boussif, et al, Gene Therapy (2009) 16: 605-619.

In one embodiment, the vector genome comprises: an AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a coding sequence for human PGRN(s) or a fusion protein comprising same, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: a AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a coding sequence for human PGRN or a fusion protein comprising same, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: a AAV 5' ITR, a promoter, an optional enhancer, an optional intron, a huPGRN coding sequence, a poly A, and an AAV 3' ITR. In certain embodiments, the vector genome comprises: an AAV2 5' ITR, an EF1a promoter, an optional enhancer, an optional promoter, huPGRN, an SV40 poly A, and an AAV2 3' ITR. In certain embodiments, the vector genome is AAV2 5' ITR, UbC promoter, optional enhancer, optional intron, huPGRN, an SV40 poly A, and an AAV2 3' ITR. In certain embodiments, the vector genome is AAV2 5' ITR, CB7 promoter, an intron, huPGRN, an SV40 poly A, and an AAV2 3' ITR. In certain embodiment, the vector genome is an AAV2 5' ITR, CB7 promoter, intron, huPGRN, a rabbit beta globin poly A, and an AAV2 3' ITR. See, e.g., SEQ ID NO: 22 (EF1a.huPGRN.SV40), SEQ ID NO: 23 (UbC.PI.huPGRN.SV40), or SEQ ID NO: 24 (CB7.CI.hPGRN1.rGB). The huPGRN coding sequences are selected from those defined in the present specification. See, e.g., SEQ ID NO: 3 or a sequence 95% to 99.9% identical thereto, or SEQ ID NO: 4 or a sequence 95% to 99.9% identical thereto, or a fragment thereof as defined herein. Illustrative sequences of vector elements used in the examples below are provided, e.g., in SEQ ID NO: 6 (rabbit globin polyA), AAV ITRs (SEQ ID NO: 7 and 8), human CMV IE promoter (SEQ ID NO: 9), CB promoter (SEQ ID NO: 10), a chimeric intron (SEQ ID NO: 11), UbC promoter (SEQ ID NO: 12), an EF-1a promoter (SEQ ID NO: 17), an intron (SEQ ID NO: 13), and an SV40 late poly A (SEQ ID NO: 14). Other elements of the vector genome or variations on these sequences may be selected for the vector genomes for certain embodiments of this invention.

Vector Production

For use in producing an AAV viral vector (*e.g.*, a recombinant (r) AAV), the expression cassettes can be carried on any suitable vector, *e.g.*, a plasmid, which is delivered to a packaging host cell. The plasmids useful in this invention may be engineered such that they are suitable for replication and packaging *in vitro* in prokaryotic cells, insect cells, mammalian cells, among

others. Suitable transfection techniques and packaging host cells are known and/or can be readily designed by one of skill in the art.

Methods for generating and isolating AAVs suitable for use as vectors are known in the art. *See* generally, *e.g.,* Grieger & Samulski, 2005, "Adeno-associated virus as a gene therapy vector: Vector development, production and clinical applications," *Adv. Biochem. Engin/Biotechnol.* 99: 119-145; Buning *et al.,* 2008, "Recent developments in adeno-associated virus vector technology," *J. Gene Med.* 10:717-733; and the references cited below, each of which is incorporated herein by reference in its entirety. For packaging a transgene into virions, the ITRs are the only AAV components required in *cis* in the same construct as the nucleic acid molecule containing the expression cassettes. The cap and rep genes can be supplied in *trans.*

In one embodiment, the expression cassettes described herein are engineered into a genetic element (*e.g.,* a shuttle plasmid) which transfers the immunoglobulin construct sequences carried thereon into a packaging host cell for production a viral vector. In one embodiment, the selected genetic element may be delivered to an AAV packaging cell by any suitable method, including transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion. Stable AAV packaging cells can also be made. Alternatively, the expression cassettes may be used to generate a viral vector other than AAV, or for production of mixtures of antibodies in vitro. The methods used to make such constructs are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. *See, e.g.,* Molecular Cloning: A Laboratory Manual, ed. Green and Sambrook, Cold Spring Harbor Press, Cold Spring Harbor, NY (2012).

The term "AAV intermediate" or "AAV vector intermediate" refers to an assembled rAAV capsid which lacks the desired genomic sequences packaged therein. These may also be termed an "empty" capsid. Such a capsid may contain no detectable genomic sequences of an expression cassette, or only partially packaged genomic sequences which are insufficient to achieve expression of the gene product. These empty capsids are non-functional to transfer the gene of interest to a host cell.

The recombinant adeno-associated virus (AAV) described herein may be generated using techniques which are known. *See, e.g.,* WO 2003/042397; WO 2005/033321, WO 2006/110689; US 7588772 B2. Such a method involves culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein; a functional rep gene; an expression cassette

composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the expression cassette into the AAV capsid protein. Methods of generating the capsid, coding sequences therefor, and methods for production of rAAV viral vectors have been described. *See, e.g.,* Gao, et al, Proc. Natl. Acad. Sci. U.S.A. 100 (10), 6081-6086 (2003) and US 2013/0045186A1.

In one embodiment, a production cell culture useful for producing a recombinant AAV is provided. Such a cell culture contains a nucleic acid which expresses the AAV capsid protein in the host cell; a nucleic acid molecule suitable for packaging into the AAV capsid, e.g., a vector genome which contains AAV ITRs and a non-AAV nucleic acid sequence encoding a gene product operably linked to sequences which direct expression of the product in a host cell; and sufficient AAV rep functions and adenovirus helper functions to permit packaging of the nucleic acid molecule into the recombinant AAV capsid. In one embodiment, the cell culture is composed of mammalian cells (e.g., human embryonic kidney 293 cells, among others) or insect cells (e.g., baculovirus).

Typically, the rep functions are from the same AAV source as the AAV providing the ITRs flanking the vector genome. In the examples herein, the AAV2 ITRs are selected and the AAV2 rep is used. The coding sequence is reproduced in SEQ ID NO: 27. Optionally, other rep sequences or another rep source (and optionally another ITR source) may be selected. For example, the rep may be, but is not limited to, AAV1 rep protein, AAV2 rep protein; or rep 78, rep 68, rep 52, rep 40, rep68/78 and rep40/52; or a fragment thereof; or another source. Optionally, the rep and cap sequences are on the same genetic element in the cell culture. There may be a spacer between the rep sequence and cap gene. Any of these AAV or mutant AAV capsid sequences may be under the control of exogenous regulatory control sequences which direct expression thereof in a host cell.

In one embodiment, cells are manufactured in a suitable cell culture (e.g., HEK 293) cells. Methods for manufacturing the gene therapy vectors described herein include methods well known in the art such as generation of plasmid DNA used for production of the gene therapy vectors, generation of the vectors, and purification of the vectors. In some embodiments, the gene therapy vector is an AAV vector and the plasmids generated are an AAV cis-plasmid encoding the AAV genome and the gene of interest, an AAV trans-plasmid containing AAV rep and cap genes, and an adenovirus helper plasmid. The vector generation process can include method steps such as initiation of cell culture, passage of cells, seeding of cells, transfection of

cells with the plasmid DNA, post-transfection medium exchange to serum free medium, and the harvest of vector-containing cells and culture media.

In certain embodiments, the manufacturing process for rAAV.hPGRN involves transient transfection of HEK293 cells with plasmid DNA. A single batch or multiple batches are produced by PEI-mediated triple transfection of HEK293 cells in PALL iCELLis bioreactors. Harvested AAV material are purified sequentially by clarification, TFF, affinity chromatography, and anion exchange chromatography in disposable, closed bioprocessing systems where possible.

The harvested vector-containing cells and culture media are referred to herein as crude cell harvest. In yet another system, the gene therapy vectors are introduced into insect cells by infection with baculovirus-based vectors. For reviews on these production systems, see generally, e.g., Zhang et al., 2009, "Adenovirus-adeno-associated virus hybrid for large-scale recombinant adeno-associated virus production," Human Gene Therapy 20:922-929, the contents of each of which is incorporated herein by reference in its entirety. Methods of making and using these and other AAV production systems are also described in the following U.S. patents, the contents of each of which is incorporated herein by reference in its entirety: 5,139,941; 5,741,683; 6,057,152; 6,204,059; 6,268,213; 6,491,907; 6,660,514; 6,951,753; 7,094,604; 7,172,893; 7,201,898; 7,229,823; and 7,439,065, which are incorporated herein by reference.

The crude cell harvest may thereafter be subject to additional method steps such as concentration of the vector harvest, diafiltration of the vector harvest, microfluidization of the vector harvest, nuclease digestion of the vector harvest, filtration of microfluidized intermediate, crude purification by chromatography, crude purification by ultracentrifugation, buffer exchange by tangential flow filtration, and/or formulation and filtration to prepare bulk vector.

A two-step affinity chromatography purification at high salt concentration followed anion exchange resin chromatography are used to purify the vector drug product and to remove empty capsids. These methods are described in more detail in International Patent Application No. PCT/US2016/065970, filed December 9, 2016, which is incorporated by reference herein. Purification methods for AAV8, International Patent Application No. PCT/US2016/065976, filed December 9, 2016, and rh10, International Patent Application No. PCT/US16/66013, filed December 9, 2016, entitled "Scalable Purification Method for AAVrh10", also filed December 11, 2015, and for AAV1, International Patent Application No. PCT/US2016/065974, filed December 9, 2016, for "Scalable Purification Method for AAV1", filed December 11, 2015, are all incorporated by reference herein.

To calculate empty and full particle content, VP3 band volumes for a selected sample (*e.g.*, in examples herein an iodixanol gradient-purified preparation where # of GC = # of particles) are plotted against GC particles loaded. The resulting linear equation (y = mx+c) is used to calculate the number of particles in the band volumes of the test article peaks. The number of particles (pt) per 20 µL loaded is then multiplied by 50 to give particles (pt) /mL. Pt/mL divided by GC/mL gives the ratio of particles to genome copies (pt/GC). Pt/mL–GC/mL gives empty pt/mL. Empty pt/mL divided by pt/mL and x 100 gives the percentage of empty particles.

Generally, methods for assaying for empty capsids and AAV vector particles with packaged genomes have been known in the art. See, e.g., Grimm et al., *Gene Therapy* (1999) 6:1322-1330; Sommer et al., Molec. Ther. (2003) 7:122-128. To test for denatured capsid, the methods include subjecting the treated AAV stock to SDS-polyacrylamide gel electrophoresis, consisting of any gel capable of separating the three capsid proteins, for example, a gradient gel containing 3-8% Tris-acetate in the buffer, then running the gel until sample material is separated, and blotting the gel onto nylon or nitrocellulose membranes, preferably nylon. Anti-AAV capsid antibodies are then used as the primary antibodies that bind to denatured capsid proteins, preferably an anti-AAV capsid monoclonal antibody, most preferably the B1 anti-AAV-2 monoclonal antibody (Wobus et al., *J. Virol.* (2000) 74:9281-9293). A secondary antibody is then used, one that binds to the primary antibody and contains a means for detecting binding with the primary antibody, more preferably an anti-IgG antibody containing a detection molecule covalently bound to it, most preferably a sheep anti-mouse IgG antibody covalently linked to horseradish peroxidase. A method for detecting binding is used to semi-quantitatively determine binding between the primary and secondary antibodies, preferably a detection method capable of detecting radioactive isotope emissions, electromagnetic radiation, or colorimetric changes, most preferably a chemiluminescence detection kit. For example, for SDS-PAGE, samples from column fractions can be taken and heated in SDS-PAGE loading buffer containing reducing agent (*e.g.*, DTT), and capsid proteins were resolved on pre-cast gradient polyacrylamide gels (*e.g.*, Novex). Silver staining may be performed using SilverXpress (Invitrogen, CA) according to the manufacturer's instructions or other suitable staining method, i.e. SYPRO ruby or coomassie stains. In one embodiment, the concentration of AAV vector genomes (vg) in column fractions can be measured by quantitative real time PCR (Q-PCR). Samples are diluted and digested with DNase I (or another suitable nuclease) to remove exogenous DNA. After inactivation of the

nuclease, the samples are further diluted and amplified using primers and a TaqMan™ fluorogenic probe specific for the DNA sequence between the primers. The number of cycles required to reach a defined level of fluorescence (threshold cycle, Ct) is measured for each sample on an Applied Biosystems Prism 7700 Sequence Detection System. Plasmid DNA containing identical sequences to that contained in the AAV vector is employed to generate a standard curve in the Q-PCR reaction. The cycle threshold (Ct) values obtained from the samples are used to determine vector genome titer by normalizing it to the Ct value of the plasmid standard curve. End-point assays based on the digital PCR can also be used.

In one aspect, an optimized q-PCR method is used which utilizes a broad-spectrum serine protease, e.g., proteinase K (such as is commercially available from Qiagen). More particularly, the optimized qPCR genome titer assay is similar to a standard assay, except that after the DNase I digestion, samples are diluted with proteinase K buffer and treated with proteinase K followed by heat inactivation. Suitably samples are diluted with proteinase K buffer in an amount equal to the sample size. The proteinase K buffer may be concentrated to 2-fold or higher. Typically, proteinase K treatment is about 0.2 mg/mL, but may be varied from 0.1 mg/mL to about 1 mg/mL. The treatment step is generally conducted at about 55 °C for about 15 minutes, but may be performed at a lower temperature (e.g., about 37 °C to about 50 °C) over a longer time period (e.g., about 20 minutes to about 30 minutes), or a higher temperature (e.g., up to about 60 °C) for a shorter time period (e.g., about 5 to 10 minutes). Similarly, heat inactivation is generally at about 95 °C for about 15 minutes, but the temperature may be lowered (e.g., about 70 to about 90 °C) and the time extended (e.g., about 20 minutes to about 30 minutes). Samples are then diluted (e.g., 1000-fold) and subjected to TaqMan analysis as described in the standard assay.

Additionally, or alternatively, droplet digital PCR (ddPCR) may be used. For example, methods for determining single-stranded and self-complementary AAV vector genome titers by ddPCR have been described. See, e.g., M. Lock et al, Hu Gene Therapy Methods, Hum Gene Ther Methods. 2014 Apr;25(2):115-25. doi: 10.1089/hgtb.2013.131. Epub 2014 Feb 14.

In brief, the method for separating rAAV particles having packaged genomic sequences from genome-deficient AAV intermediates involves subjecting a suspension comprising recombinant AAV viral particles and AAV capsid intermediates to fast performance liquid chromatography, wherein the AAV viral particles and AAV intermediates are bound to a strong anion exchange resin equilibrated at a high pH, and subjected to a salt gradient while monitoring

eluate for ultraviolet absorbance at about 260 and about 280. The pH may be adjusted depending upon the AAV selected. See, e.g., WO2017/160360 (AAV9), WO2017/100704 (AAVrh10), WO 2017/100676 (e.g., AAV8), and WO 2017/100674 (AAV1), which are incorporated by reference herein. In this method, the AAV full capsids are collected from a fraction which is eluted when the ratio of A260/A280 reaches an inflection point. In one example, for the Affinity Chromatography step, the diafiltered product may be applied to a Capture Select™ Poros-AAV2/9 affinity resin (Life Technologies) that efficiently captures the AAV2 serotype. Under these ionic conditions, a significant percentage of residual cellular DNA and proteins flow through the column, while AAV particles are efficiently captured.

Compositions

Provided herein are compositions containing at least one rAAV.hPGRN stock (e.g., an rAAV stock) and an optional carrier, excipient and/or preservative. An rAAV stock refers to a plurality of rAAV vectors which are the same, e.g., such as in the amounts described below in the discussion of concentrations and dosage units.

In certain embodiments, a composition comprises a virus stock which is a recombinant AAV (rAAV) suitable for use in treating progranulin - related frontal temporal dementia (FTD), said rAAV comprising: (a) an adeno-associated virus 1 capsid, and (b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin. In certain embodiments, the vector genome comprises a promoter, an enhancer, an intron, a human PGRN coding sequence, and a polyadenylation signal. In certain embodiments, the intron consists of a chicken beta actin splice donor and a rabbit β splice acceptor element. In certain embodiments, the vector genome further comprises an AAV2 5' ITR and an AAV2 3' ITR which flank all elements of the vector genome.

The rAAV.hPGRN, preferably suspended in a physiologically compatible carrier, may be administered to a human or non-human mammalian patient. In certain embodiments, for administration to a human patient, the rAAV is suitably suspended in an aqueous solution containing saline, a surfactant, and a physiologically compatible salt or mixture of salts. Suitably, the formulation is adjusted to a physiologically acceptable pH, e.g., in the range of pH 6 to 9, or pH 6.5 to 7.5, pH 7.0 to 7.7, or pH 7.2 to 7.8. As the pH of the cerebrospinal fluid is about 7.28 to about 7.32, or a pH of 7.2 to 7.4, for intrathecal delivery, a pH within this range may be desired;

whereas for intravenous delivery, a pH of about 6.8 to about 7.2 may be desired. However, other pHs within the broadest ranges and these subranges may be selected for other route of delivery.

In certain embodiments, the formulation may contain a buffered saline aqueous solution not comprising sodium bicarbonate. Such a formulation may contain a buffered saline aqueous solution comprising one or more of sodium phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium chloride and mixtures thereof, in water, such as a Harvard's buffer. The aqueous solution may further contain Kolliphor® P188, a poloxamer which is commercially available from BASF which was formerly sold under the trade name Lutrol® F68. The aqueous solution may have a pH of 7.2 or a pH of 7.4.

In another embodiment, the formulation may contain a buffered saline aqueous solution comprising 1 mM Sodium Phosphate (Na3PO4), 150 mM sodium chloride (NaCl), 3mM potassium chloride (KCl), 1.4 mM calcium chloride (CaCl2), 0.8 mM magnesium chloride (MgCl2), and 0.001% Kolliphor® 188. See, e.g., harvardapparatus.com/harvard-apparatus-perfusion-fluid.html. In certain embodiments, Harvard's buffer is preferred.

In other embodiments, the formulation may contain one or more permeation enhancers. Examples of suitable permeation enhancers may include, e.g., mannitol, sodium glycocholate, sodium taurocholate, sodium deoxycholate, sodium salicylate, sodium caprylate, sodium caprate, sodium lauryl sulfate, polyoxyethylene-9-laurel ether, or EDTA.

In another embodiment, the composition includes a carrier, diluent, excipient and/or adjuvant. Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the transfer virus is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (e.g., phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The buffer/carrier should include a component that prevents the rAAV, from sticking to the infusion tubing but does not interfere with the rAAV binding activity in vivo.

Optionally, the compositions may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, and parachlorophenol. Suitable chemical stabilizers include gelatin and albumin.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host. Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the present invention into suitable host cells. In particular, the rAAV vector delivered transgenes may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

In one embodiment, a composition includes a final formulation suitable for delivery to a subject, e.g., is an aqueous liquid suspension buffered to a physiologically compatible pH and salt concentration. Optionally, one or more surfactants are present in the formulation. In another embodiment, the composition may be transported as a concentrate which is diluted for administration to a subject. In other embodiments, the composition may be lyophilized and reconstituted at the time of administration.

A suitable surfactant, or combination of surfactants, may be selected from among non-ionic surfactants that are nontoxic. In one embodiment, a difunctional block copolymer surfactant terminating in primary hydroxyl groups is selected, e.g., such as Pluronic® F68 [BASF], also known as Poloxamer 188, which has a neutral pH, has an average molecular weight of 8400. Other surfactants and other Poloxamers may be selected, i.e., nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)), SOLUTOL HS 15 (Macrogol-15 Hydroxystearate), LABRASOL (Polyoxy capryllic glyceride), polyoxy 10 oleyl ether, TWEEN (polyoxyethylene sorbitan fatty acid esters), ethanol and polyethylene glycol. In one embodiment, the formulation contains a poloxamer. These copolymers are commonly named with the letter "P" (for poloxamer) followed by three digits: the first two digits x 100 give the approximate molecular mass of the polyoxypropylene core, and the last digit x 10 gives the percentage polyoxyethylene content. In one embodiment Poloxamer 188 is selected. The surfactant may be present in an amount up to about 0.0005 % to about 0.001% of the suspension.

The vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Optionally, routes other than intrathecal administration may be used, such as, e.g., direct delivery to a desired organ (*e.g.*, the liver (optionally via the hepatic artery), lung, heart, eye, kidney), oral, inhalation, intranasal, intratracheal, intraarterial, intraocular, intravenous, intramuscular, subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 25 to about 1000 microliters to about 100 mL of solution containing concentrations of from about 1 x 10^9 to 1 x 10^{16} genomes virus vector (to treat an average subject of 70 kg in body weight) including all integers or fractional amounts within the range, and preferably 1.0 x 10^{12} GC to 1.0 x 10^{14} GC for a human patient. In one embodiment, the compositions are formulated to contain at least 1x10^9, 2x10^9, 3x10^9, 4x10^9, 5x10^9, 6x10^9, 7x10^9, 8x10^9, or 9x10^9 GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{10}, 2x10^{10}, 3x10^{10}, 4x10^{10}, 5x10^{10}, 6x10^{10}, 7x10^{10}, 8x10^{10}, or 9x10^{10} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{11}, 2x10^{11}, 3x10^{11}, 4x10^{11}, 5x10^{11}, 6x10^{11}, 7x10^{11}, 8x10^{11}, or 9x10^{11} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{12}, 2x10^{12}, 3x10^{12}, 4x10^{12}, 5x10^{12}, 6x10^{12}, 7x10^{12}, 8x10^{12}, or 9x10^{12} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{13}, 2x10^{13}, 3x10^{13}, 4x10^{13}, 5x10^{13}, 6x10^{13}, 7x10^{13}, 8x10^{13}, or 9x10^{13} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{14}, 2x10^{14}, 3x10^{14}, 4x10^{14}, 5x10^{14}, 6x10^{14}, 7x10^{14}, 8x10^{14}, or 9x10^{14} GC per dose including all integers or fractional amounts within the range. In another embodiment, the compositions are formulated to contain at least 1x10^{15}, 2x10^{15}, 3x10^{15}, 4x10^{15}, 5x10^{15}, 6x10^{15}, 7x10^{15}, 8x10^{15}, or 9x10^{15} GC per dose including all integers or fractional amounts within the range. In one

embodiment, for human application the dose can range from $1x10^{10}$ to about $1x10^{12}$ GC per dose including all integers or fractional amounts within the range.

In certain embodiments, the dose is in the range of about 1×10^{9} GC/g brain mass to about 1×10^{12} GC/g brain mass. In certain embodiments, the dose is in the range of about 1×10^{10} GC/g brain mass to about 3.33×10^{11} GC/g brain mass. In certain embodiments, the dose is in the range of about 3.33×10^{11} GC/g brain mass to about 1.1×10^{12} GC/g brain mass. In certain embodiments, the dose is in the range of about 1.1×10^{12} GC/g brain mass to about 3.33×10^{13} GC/g brain mass. In certain embodiments, the dose is lower than 3.33×10^{11} GC/g brain mass. In certain embodiments, the dose is lower than 1.1×10^{12} GC/g brain mass. In certain embodiments, the dose is lower than 3.33×10^{13} GC/g brain mass.

In certain embodiments, the dose is about 1×10^{10} GC/g brain mass. In certain embodiments, the dose is about 2×10^{10} GC/g brain mass. In certain embodiments, the dose is about 2×10^{10} GC/g brain mass. In certain embodiments, the dose is about 3×10^{10} GC/g brain mass. In certain embodiments, the dose is about 4×10^{10} GC/g brain mass. In certain embodiments, the dose is about 5×10^{10} GC/g brain mass. In certain embodiments, the dose about 6×10^{10} GC/g brain mass. In certain embodiments, the dose is about 7×10^{10} GC/g brain mass. In certain embodiments, the dose about 8×10^{10} GC/g brain mass. In certain embodiments, the dose is about 9×10^{10} GC/g brain mass. In certain embodiments, the dose is about 1×10^{11} GC/g brain mass. In certain embodiments, the dose is about 2×10^{11} GC/g brain mass. In certain embodiments, the dose is about 3×10^{11} GC/g brain mass. In certain embodiments, the dose is about 4×10^{11} GC/g brain mass.

In certain embodiments, the dose is administered to humans as a flat dose in the range of about 1.44×10^{13} to 4.33×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 1.44×10^{13} to 2×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 3×10^{13} to 1×10^{14} GC of the rAAV. In certain embodiments, the dose is administered to humans as a flat dose in the range of about 5×10^{13} to 1×10^{14} GC of the rAAV.

In some embodiments, the compositions can be formulated in dosage units to contain an amount of AAV that is in the range of about 1×10^{13} to 8×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the range of about 1.44×10^{13} to 4.33×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the

range of about 3×10^{13} to 1×10^{14} GC of the rAAV. In some embodiments, the compositions can be formulated in dosage units to contain an amount of rAAV that is in the range of about 5×10^{13} to 1×10^{14} GC of the rAAV.

In certain embodiments, the rAAV is administered to a subject in a single dose. In certain embodiments, multiple doses (for example 2 doses) is desired.

The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed. The levels of expression of the transgene can be monitored to determine the frequency of dosage resulting in viral vectors, preferably AAV vectors containing the minigene. Optionally, dosage regimens similar to those described for therapeutic purposes may be utilized for immunization using the compositions of the invention.

As used herein, the terms "intrathecal delivery" or "intrathecal administration" refer to a route of administration for drugs via an injection into the spinal canal, more specifically into the subarachnoid space so that it reaches the cerebrospinal fluid (CSF). Intrathecal delivery may include lumbar puncture, intraventricular (including intracerebroventricular (ICV)), suboccipital/intracisternal, and/or C1-2 puncture. For example, material may be introduced for diffusion throughout the subarachnoid space by means of lumbar puncture. In another example, injection may be into the cisterna magna or via intraparenchymal delivery. In certain embodiments, the rAAV is administered via a computed tomography- (CT-) guided sub-occipital injection into the cisterna magna (intra-cisterna magna). In certain embodiments, the patient is administered a single dose.

As used herein, the terms "intracisternal delivery" or "intracisternal administration" refer to a route of administration for drugs directly into the cerebrospinal fluid of the cisterna magna cerebellomedularis, more specifically via a suboccipital puncture or by direct injection into the cisterna magna or via permanently positioned tube.

In certain embodiments, the stock of rAAV.hPGRN is formulated in intrathecal final formulation buffer (ITFFB; artificial CSF with 0.001% Pluronic F-68). The batch or batches are frozen, subsequently thawed, pooled if necessary, adjusted to the target concentration, sterile-filtered through a 0.22 μm filter, and vials are filled. In certain embodiments, the suspension comprising the formulation buffer the rAAV1.hPGRN is adjusted to a pH of 7.2 to 7.4.

In one embodiment, volumes for delivery of the doses of rAAV1.hPGRN provided herein and concentrations may be determined by one of skill in the art. For example, volumes of about 1

μL to 150 mL may be selected, with the higher volumes being selected for adults. Typically, for newborn infants a suitable volume is about 0.5 mL to about 10 mL, for older infants, about 0.5 mL to about 15 mL may be selected. For toddlers, a volume of about 0.5 mL to about 20 mL may be selected. For children, volumes of up to about 30 mL may be selected. For pre-teens and teens, volumes up to about 50 mL may be selected. In still other embodiments, a patient may receive an intrathecal administration in a volume of about 5 mL to about 15 mL are selected, or about 7.5 mL to about 10 mL. Other suitable volumes and dosages may be determined. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may vary depending upon the therapeutic application for which the recombinant vector is employed.

In certain embodiments, a composition comprises: rAAV.EF1a.huPGRN.SV40, rAAV.UbC.PI.huPGRN.SV40, or rAAVCB7.CI.hPGRN1.rGB. Compositions in which the rAAV capsid is AAVhu68, AAV5 or AAV1 are illustrated in the examples below. In particularly preferred embodiments, the rAAV is AAV1. In certain embodiments, the huPGRN coding sequences are selected from those defined in the present specification. See, e.g., SEQ ID NO: 3 or a sequence 95% to 99.9% identical thereto, or SEQ ID NO: 4 or a sequence 95% to 99.9% identical thereto, or a fragment thereof as defined herein. Illustrative sequences of vector elements used in the examples below are provided, e.g., in SEQ ID NO: 6 (rabbit globin polyA), AAV ITRs (SEQ ID NO: 7 and 8), human CMV IE promoter (SEQ ID NO: 9), CB promoter (SEQ ID NO: 10), a chimeric intron (SEQ ID NO: 11), UbC promoter (SEQ ID NO: 12), an EF-1a promoter (SEQ ID NO: 17), an intron (SEQ ID NO: 13), and an SV40 late poly A (SEQ ID NO: 14).

Uses

As used herein, a PGRN haploinsufficiency refers to patients with a mutation in the PGRN gene, which results in deficient PGRN and/or deficient GRN(s) levels. The target population for an rAAV1-PGRN therapy includes patients which have a PGRN haploinsufficiency and/or patients who otherwise have deficient PGRN or deficient GRN levels. In certain embodiments, the patient is heterozygous for a PGRN mutation. In yet another embodiment, the patient is homozygous from a PGRN mutation. In certain embodiments, the

patient is administered an immune suppression regimen in combination with rAAV1-mediated hPGRN therapy provided herein.

In certain embodiments, the rAAV1.PGRN is useful in treating patients having a GRN haploinsufficiency. Such patients may have been diagnosed with adult-onset neurodegeneration caused by GRN haploinsufficiency or may be pre-symptomatic. The rAAV1.PGRN can be administered as a single dose via a computed tomography- (CT-) guided sub-occipital injection into the cisterna magna (intra-cisterna magna [ICM]). A single dose is administered at a pre-determined dose level. The superior brain transduction achieved with a single ICM injection in NHPs resulted in the selection of this route of administration. In certain embodiments, administration of the vector into the ICM also results in reduced anti-PGRN T cell responses as compared to another route of administration (e.g. injection into the lateral ventricle). Once a common procedure, ICM injection (also known as suboccipital puncture) had previously been supplanted by lumbar-puncture. However, other dosing levels and routes of delivery may be selected and/or used in conjunction with this rAAV1-mediated hPGRN therapy.

In certain embodiments, the rAAV1-mediated therapy described herein may provide PGRN expression at about average, normal, physiological levels for a human without a GRN mutation (haploinsufficiency). However, the treatment may provide therapeutic effect even if the increase in PGRN expression is below normal levels, providing about 40% to 99% of normal average levels, e.g., 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or other values therebetween these ranges. In certain embodiments, this may result from an increased PGRN level of at least 5% to about 70%, or more, above the patient's expression levels prior to treatment. In certain embodiments, the treatment provides therapeutic efficacy where administration of rAAV1-mediated hPGRN results in elevated levels of PGRN in the CSF (e.g., 10-fold to 40-fold higher than normal levels).

In certain embodiments, efficacy is assessed by one or more of: increased levels of PGRN protein in CSF and/or changes in brain cortical thickness. In certain embodiment, efficacy of rAAV1-mediated therapy is assessed following administration of a single ICM dose as measured by one or more of: prolonged survival, and improvement on of clinical symptoms and daily functioning as assessed by the Mini-Mental State Exam (MMSE), Clinical Global Impression of Change (CGI-C), Frontal Assessment Battery (FAB), Frontotemporal Dementia Rating Scale (FRS), Frontal Behavioral Inventory (FBI), Unified Parkinson's Disease Rating Scale (UPDRS), verbal fluency testing, Clinical Dementia Rating for Frontotemporal Lobar Degeneration Sum of

Boxes (CDR-FTLD sb), and/or Neuropsychiatric Inventory (NPI). In certain embodiments, efficacy is demonstrated by improvement in CSF levels of neurofilament light chain (NFL), tau, phosphorylated tau, and inflammatory markers and/or increased Plasma levels of PGRN. In certain embodiments, efficacy is assessed by measuring a reduction or reversal in levels of microgliosis.

In certain embodiments, efficacy is measured by improvement in one or more of the clinical symptoms associated with GRN patients, including, e.g., behavioral deficits (disinhibition, apathy, loss of sympathy or empathy, compulsive or stereotyped behaviors, or hyperorality) and cognitive deficits (decline in executive function without a significant impact on episodic memory or visual-spatial skills).

In certain embodiments, improvement is observed in some other, more atypical symptoms, including psychiatric features (delusions, hallucinations, and obsessive behaviors) and/or other cognitive deficits (episodic memory impairment, apraxia, and visuospatial dysfunction). Assessment may be performed using FTDC criteria may be evaluated, including brain imaging for signs of frontal and/or temporal degeneration, an assessment of decline on a clinical rating scale (such as the Clinical Dementia Rating for Frontotemporal Lobar Degeneration [CDR-FTLD], Frontal Behavior Inventory [FBI], Neuropsychiatric Inventory [NPI], and Frontotemporal Dementia Rating Scale [FRS]), and, ultimately, genetic testing to confirm a pathogenic GRN mutation. Cerebrospinal fluid (CSF) biomarkers, including tau and amyloid-β, as well amyloid positron emission tomography (PET) imaging, may be used.

In certain embodiments, improvement is observed in *GRN* mutation carriers having primary progressive aphasia (PPA), which is characterized by symptoms related to speech and language. They may be diagnosed using guidelines based upon the Mesulam criteria, which distinguishes three clinical variants of PPA: semantic variant PPA (svPPA), nonfluent variant PPA (nfvPPA), and logopenic variant PPA (lvPPA) (Gorno-Tempini et al., (2011). "Classification of primary progressive aphasia and its variants." Neurology. 76(11):1006-14). nfvPPA presents with deficits in the ability to produce speech, and the core features include agrammatism in language production, effortful speech, and apraxia of speech. svPPA presents with deficits in the ability to understand the meanings of words, and the core features include impaired naming of words and single-word comprehension. lvPPA is characterized by difficulty finding the appropriate words while speaking, and is not accompanied by a decline in word comprehension. The core features of lvPPA are deficits in word retrieval and the capacity to

repeat sentences. *GRN* mutation carriers most commonly present with nfvPPA; however, they can have broader symptoms spanning the PPA clinical spectrum, resulting in a diagnosis of "PPA-not otherwise specified" (Gorno-Tempini et al., 2011; Woollacott and Rohrer, 2016).

A method of treating a human patient with a neurodegenerative condition associated with GRN haploinsufficiency is provided. In certain embodiments, this condition is progranulin-related frontotemporal dementia (FTD). The method comprises delivering a coding sequence for a progranulin to the central nervous system (CNS) via a recombinant adeno-associated virus (rAAV) having an adeno-associated virus 1 (AAV1) capsid, said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

A method for treating a human patient with brain lesions associated with progranulin-related frontal temporal dementia or another neurodegenerative condition associated with GRN haploinsufficiency is provided. The method comprises administering a coding sequence for a progranulin to the central nervous system (CNS) via a recombinant adeno-associated virus (rAAV) having an adeno-associated virus 1 (AAV1) capsid, said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

In certain embodiments, the methods provided herein may further comprise monitoring treatment by (a) non-invasively assessing the patient for reduction in retinal storage lesions as a predictor of reduction of brain lesions, (b) performing magnetic resonance imaging to assess brain volume, and/or (c) measuring concentration of progranulin in the CSF. Optionally, progranulin concentration in plasma may be assessed.

In certain embodiments, efficacy of an rAAV.hPGRN composition is assessed by one or more of the following primarily cognitive, primarily behavioral, or cognitive/other methods. The following describes suitable assessments.

Primarily Cognitive assessments include verbal fluency testing, clinical dementia ratio for FTLD, or mini-mental state exam (MMSE). Verbal fluency testing will likely be conducted by presenting the same picture/ photograph to each subject and asking for a verbal description. During the description, rate of speech (words/minute) will be counted, recorded and ultimately compared to rates reflective of neuro-typical adults. The CDR-FTLD is an extended version of

the classic CDR, which is historically used to rate the severity of Alzheimer's disease spectrum disorders. The assessment includes the original 6 domains of the CDR (memory, orientation, judgment and problem solving, community affairs, home and hobbies, personal care) as well as two additional domains: language and behavior, which allows for more sensitivity in detection of decline in FTLD. A rating of "0" indicates normal behavior or language, while scores of "1", "2" or "3" indicate mild to severe deficits. The 'sum of boxes', or the sum of the individual domain scores, is used to determine global dementia severity. The MMSE is an 11-question global cognitive assessment widely used in clinical and research practice. Questions such as "What is the year? Season? Date? Day of the week? Month?" are asked and one point is given for each correct answer, with maximum scores provided for each question. The maximum, total score is 30, with two cut-offs at scores of 24 and 27. These cutoffs are indicators of cognitive decline.

Primarily Motor assessments include, e.g., Unified Parkinson's Disease Rating Scale (UPDRS). The UPDRS is a 42-item, 4-part assessment of several domains related to Parkinsonism, such as Mentation, Behavior and Mood and Activities of Daily Living. Each item includes a rating scale typically ranging from 0 (typically indicating no impairment) to 4 (typically indicating the most severe impairment). The scores for each part are tallied to provide an indication of severity of the disease with a high score of 199 indicating the worst/ most total disability.

Primarily Behavioral assessments include, e.g., neuropsychiatric inventory (NPI) or Frontal Behavioral Inventory (FBI). The NPI is used to elucidate the presence of psychopathology in patients with disorders of the brain. Initially, it was developed for use in Alzheimer's disease populations; however, it may be useful to assess behavioral changes in other conditions. The assessment consists of 10 behavioral domains and 2 neurovegetative areas, within which there are 4 scores: frequency, severity, total and caregiver distress. The NPI total score is obtained by adding the domain scores of the behavioral domains, less the caregiver distress scores. The FBI is a 24-item assessment targeted to assess changes in behavior and personality associated specifically with bvFTD and to differentiate between FTD and other dementias. It is administered as a face-to-face interview with the primary caregiver, as patients with a bvFTD diagnosis generally do not have sufficient insight into these types of changes. It focuses on several behavioral and personality-related areas, scoring each question from 0 (none) to 3 (severe/most of the time). The total score provides insight into the severity of illness and can be used to assess change over time.

Other/ Both Cognitive and Motor assessments include, e.g., Columbia Suicide Severity Rating Scale (C-SSRS), Clinical Global Impression of Change (CGI-C), Frontal Assessment Battery (FAB), and/or Frontotemporal Dementia Rating Scale (FDR). The C-SSRS is a 3-part scale measuring Suicidal Ideation, Intensity of Ideation and Suicidal Behavior through questions evaluating suicidal ideation and behavior. The outcome of this assessment is composed of a suicidal behavior lethality rating taken directly from the scale, a suicidal ideation score and a suicidal ideation intensity ranking. An ideation score greater than 0 may indicate the need for intervention, based on the assessment guidelines. The intensity rating has a range of 0 to 25, with 0 representing no endorsement of suicidal ideation. The CGI-C is one of three parts of a brief, widely used assessment composed of 3 items that are clinician-observer rated. The CGI-C is rated on a 7 point scale, ranging from 1 (very much improved) to 7 (very much worse) starting from enrollment in the study, whether or not any improvement is due entirely to treatment. The FAB is a brief assessment to assist in differentiating between dementias with a frontal dysexecutive phenotype and of Alzheimer's type. It is particularly useful in mildly demented patients (MMSE > 24). The assessment consists of 6 parts, addressing cognitive, motor and behavioral areas, with a total score of 18 and higher scores indicating better performance. The FDR is a brief staging assessment for patients with frontotemporal dementia that detects differences in disease progression for FTD subtypes over time. This brief interview is conducted with the primary caregiver and consists of 30 items which are categorized as occurring Never, Sometimes or Always. A percentage score is then calculated and converted to a logit score and, ultimately, a severity score. The severity score will range from Very Mild to Profound.

Other measures of efficacy include, increased survival term from the point of diagnosis, following onset of symptoms, is a measure of efficacy. Currently, patients diagnosed with neurodegeneration caused by *GRN* mutations have a life expectancy of 7–11 years from symptom onset. Another measure of efficacy is stabilization and/or increase of atrophy in the thickness of the middle frontal cortex and parietal regions, which are the most commonly affected brain regions across all clinical presentations in the target population. This may be assessed using MRI or other imaging techniques. Still other assessments include biochemical biomarkers. Levels of PGRN protein in the CSF and plasma are measured as a readout of AAV transduction, and are expected to increase in patients following administration of rAAV1.hPGRN. In other embodiments, CSF levels of neurofilament light chain (NFL), tau, phosphorylated tau, and other

inflammatory markers are assessed. In certain embodiments, modulation and/or a decrease of these biomarkers levels correlates to efficacy.

Although the examples below focus on treatment of certain conditions associated with heterozygous GRN haploinsufficiencies, in certain embodiments, the vectors and compositions described herein may be used in treatment of other diseases, e.g., diseases associated with homozygous mutation of the GRN gene such as neuronal ceroil lipofuscinosis, cancer (e.g., ovarian, breast, adrenal, and/or pancreatic cancer), atherosclerosis, type 2 diabetes, and metabolic diseases.

As used herein, the term Computed Tomography (CT) refers to radiography in which a three-dimensional image of a body structure is constructed by computer from a series of plane cross-sectional images made along an axis.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid, or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology is over full-length sequence, or an open reading frame thereof, or another suitable fragment which is at least 15 nucleotides in length. Examples of suitable fragments are described herein.

The terms "sequence identity" "percent sequence identity" or "percent identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the genome, the full-length of a gene coding sequence, or a fragment of at least about 500 to 5000 nucleotides, is desired. However, identity among smaller fragments, *e.g.* of at least about nine nucleotides, usually at least about 20 to 24 nucleotides, at least about 28 to 32 nucleotides, at least about 36 or more nucleotides, may also be desired. Similarly, "percent sequence identity" may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length and may be up to about 700 amino acids. Examples of suitable fragments are described herein.

The term "substantial homology" or "substantial similarity," when referring to amino acids or fragments thereof, indicates that, when optimally aligned with appropriate amino acid insertions or deletions with another amino acid (or its complementary strand), there is amino acid sequence identity in at least about 95 to 99% of the aligned sequences. Preferably, the homology

is over full-length sequence, or a protein thereof, *e.g.*, a cap protein, a rep protein, or a fragment thereof which is at least 8 amino acids, or more desirably, at least 15 amino acids in length. Examples of suitable fragments are described herein.

By the term "highly conserved" is meant at least 80% identity, preferably at least 90% identity, and more preferably, over 97% identity. Identity is readily determined by one of skill in the art by resort to algorithms and computer programs known by those of skill in the art.

Generally, when referring to "identity", "homology", or "similarity" between two different adeno-associated viruses, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences. "Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. In the examples, AAV alignments are performed using the published AAV9 sequences as a reference point. Alignments are performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Examples of such programs include, "Clustal Omega", "Clustal W", "CAP Sequence Assembly", "MAP", and "MEME", which are accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™, a program in GCG Version 6.1. Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Multiple sequence alignment programs are also available for amino acid sequences, *e.g.*, the "Clustal Omega", "Clustal X", "MAP", "PIMA", "MSA", "BLOCKMAKER", "MEME", and "Match-Box" programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. *See, e.g.*, J. D. Thomson et al, Nucl. Acids. Res., "A comprehensive comparison of multiple sequence alignments", 27(13):2682-2690 (1999).

It is to be noted that the term "a" or "an" refers to one or more. As such, the terms "a (or "an"), "one or more," and "at least one" are used interchangeably herein.

The words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. The words "consist", "consisting", and its variants, are to be interpreted exclusively, rather than inclusively. While various embodiments in the specification are presented using "comprising" language, under other circumstances, a related embodiment is also intended to be interpreted and described using "consisting of" or "consisting essentially of" language.

As used herein, the term "about" means a variability of 10 % (±10%, e.g., ±1, ±2, ±3, ±4, ±5, ±6, ±7, ±8, ±9, ±10, or values therebetween) from the reference given, unless otherwise specified.

As used herein, "disease", "disorder" and "condition" are used interchangeably, to indicate an abnormal state in a subject.

Unless defined otherwise in this specification, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art and by reference to published texts, which provide one skilled in the art with a general guide to many of the terms used in the present application.

The term "expression" is used herein in its broadest meaning and comprises the production of RNA or of RNA and protein. With respect to RNA, the term "expression" or "translation" relates in particular to the production of peptides or proteins. Expression may be transient or may be stable.

As used herein, an "expression cassette" refers to a nucleic acid molecule which comprises a coding sequence, promoter, and may include other regulatory sequences therefor, which cassette may be delivered via a genetic element (*e.g.*, a plasmid) to a packaging host cell and packaged into the capsid of a viral vector (*e.g.,* a viral particle). Typically, such an expression cassette for generating a viral vector contains the coding sequence for the gene product described herein flanked by packaging signals of the viral genome and other expression control sequences such as those described herein.

As used herein, the term "operably linked" refers to both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest.

The term "heterologous" when used with reference to a protein or a nucleic acid indicates that the protein or the nucleic acid comprises two or more sequences or subsequences which are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid. For example, in one embodiment, the nucleic acid has a promoter from one gene arranged to direct the expression of a coding sequence from a different gene. Thus, with reference to the coding sequence, the promoter is heterologous.

The term "translation" in the context of the present invention relates to a process at the ribosome, wherein an mRNA strand controls the assembly of an amino acid sequence to generate a protein or a peptide.

The following examples are illustrative only and are not intended to limit the present invention.

EXAMPLES

Abbreviations	Description
A	Absorbance
aa	Amino Acids
AAV	Adeno-Associated Virus
AAV1	Adeno-Associated Virus Serotype 1
AAV2	Adeno-Associated Virus Serotype 2
AAV5	Adeno-Associated Virus Serotype 5
AAVhu68	Adeno-Associated Virus Serotype hu68
ACMG	American College of Medical Genetics
AD	Alzheimer's Disease
AD & FDM	Alzheimer's Disease and Frontotemporal Dementia Mutation Database
Ad5	Adenovirus Serotype 5
AE	Adverse Events
AEX	Anion Exchange
AmpR	Ampicillin Resistance (gene)
ANOVA	Analysis of Variance
ARTFL	Advancing Research and Treatment for Frontotemporal Lobar Degeneration
AUC	Analytical Ultracentrifugation
BA	β-Actin
BCA	Bicinchoninic Acid
BDS	Bulk Drug Substance
BMCB	Bacterial Master Cell Bank
bp	Base Pairs

Abbreviations	Description
BRF	Batch Record Form
BSA	Bovine Serum Albumin
BSE	Bovine spongiform encephalopathy
BSC	Biological Safety Cabinet
bvFTD	Behavioral Variant Frontotemporal Dementia
BWCB	Bacterial Working Cell Bank
C9orf72	Chromosome 9 Open Reading Frame 72 (gene, human)
cap	Capsid (gene)
CB7	Chicken β-Actin Promoter and CMV enhancer
CBC	Complete Blood Count
CBER	Center for Biologics Evaluation and Research
CBS	Corticobasal Syndrome
CDR-FTLD sb	Clinical Dementia Rating (CDR) Scale for Frontotemporal Lobar Degeneration Sum of Boxes
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CGI-C	Clinical Global Impression of Change
CI	Chimeric Intron
CMC	Chemistry Manufacturing and Controls
CMO	Contract Manufacturing Organization
CMV IE	Cytomegalovirus Immediate-Early Enhancer
CNS	Central Nervous System
COA	Certificate of Analysis
CPE	Cytopathic Effects
CRL	Charles River Laboratories
CRO	Contract Research Organization
CSF	Cerebrospinal Fluid
C-SSRS	Columbia-Suicide Severity Rating Scale
CT	Computed Tomography
CTL	Cytotoxic T Lymphocyte
CTSD	Cathepsin D
ddPCR	Droplet Digital Polymerase Chain Reaction
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle Medium
DMF	Drug Master File
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DP	Drug Product
DRG	Dorsal Root Ganglia
DS	Drug Substance
E1A	Early Region 1A (gene)
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay

Abbreviations	Description
ELISpot	Enzyme-Linked Immunospot
EU	Endotoxin Units
F	Female
F/U	Follow-Up
FAB	Frontal Assessment Battery
FBI	Frontal Behavioral Inventory
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FDP	Filled Drug Product
FFB	Final Formulation Buffer
FIH	First-in-Human
FRS	Frontotemporal Dementia Rating Scale
FTD	Frontotemporal Dementia
FTLD	Frontotemporal Lobar Degeneration
FTDC	International Behavioral Variant FTD Criteria Consortium
GC	Genome Copies
GENFI	Genetic Frontotemporal Dementia Initiative
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GRN	Granulin Precursor (gene, human)
Grn	Granulin Precursor (gene, mouse)
GTP	Gene Therapy Program
HCDNA	Host Cell Deoxyribonucleic Acid
HCP	Host Cell Protein
HEK293	Human Embryonic Kidney 293
HEX	Hexosaminidase (protein)
hPGRN	Human Progranulin
hPGRN v2	Human Progranulin version 2
ICH	International Conference on Harmonization
ICM	Intra-Cisterna Magna
ICP	Intracranial Pressure
ICV	Intracerebroventricular
IDS	Iduronate-2-Sulfatase
IDUA	Iduronidase
IFN-γ	Interferon Gamma
IND	Investigational New Drug
IT	Intrathecally
ITFFB	Intrathecal Final Formulation Buffer
ITR	Inverted Terminal Repeat
IU	Infectious Unit
IV	Intravenous
KanR	Kanamycin Resistance (gene)
kb	kilobases
KO	Knockout

Abbreviations	Description
LAL	Limulus Amoebocyte Lysate
LBD	Lewy Body Dementia
LEFFTDS	Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects
LFTs	Liver Function Tests
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LP	Lumbar Puncture
LTFU	Long-Term Follow-Up
lvPPA	Logopenic Variant Primary Progressive Aphasia
M	Male
MAPT	Microtubule-Associated Protein Tau (gene, human)
MBR	Master Batch Record
MCB	Master Cell Bank
MED	Minimum Effective Dose
MMSE	Mini-Mental State Exam
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MS	Mass Spectrometry
MTD	Maximum Tolerated Dose
N	Number of Subjects or Animals
N/A	Not Applicable
NAbs	Neutralizing Antibodies
NCL	Neuronal Ceroid Lipofuscinosis
nfvPPA	Nonfluent Variant Primary Progressive Aphasia
NFL	Neurofilament Light Chain
NGS	Next-Generation Sequencing
NHP	Non-Human Primate
NHS	Natural History Study
NPI	Neuropsychiatric Inventory
NSAID	Non-Steroidal Anti-Inflammatory Drug
OL	Open-Label
PBS	Phosphate-Buffered Saline
PD	Parkinson's Disease
PEI	Polyethylenimine
PES	Polyethersulfone
PET	Positron Emission Tomography
PGRN	Progranulin (protein)
PI	Principal Investigator
POC	Proof-of-Concept
PolyA	Polyadenylation
PPA	Primary Progressive Aphasia
PSP	Progressive Supranuclear Palsy
QA	Quality Assurance
QC	Quality Control

Abbreviations	Description
qPCR	Quantitative Polymerase Chain Reaction
rAAV	Recombinant Adeno-Associated Virus
ROA	Route of Administration
rcAAV	Replication-Competent Adeno-Associated Virus
rBG	Rabbit β-Globin
rDNA	Ribosomal Deoxyribonucleic Acid
rep	Replicase (gene)
RNA	Ribonucleic Acid
SA	Single Arm
SAE	Serious Adverse Events
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SOP	Standard Operating Procedure
SRT	Safety Review Trigger
ssDNA	Single-Stranded Deoxyribonucleic Acid
svPPA	Semantic Variant Primary Progressive Aphasia
TBD	To Be Determined
$TCID_{50}$	50% Tissue Culture Infective Dose
TDP-43	TAR DNA-Binding Protein 43 (protein)
TE	Tris-EDTA
TFF	Tangential Flow Filtration
UbC	Ubiquitin C
UCSF	University of California at San Francisco
UPenn	University of Pennsylvania
UPenn-GTP	University of Pennsylvania Gene Therapy Program
UPDRS	Unified Parkinson's Disease Rating Scale
UPLC	Ultra-Performance Liquid Chromatography
US	United States
USP	United States Pharmacopeia
WCB	Working Cell Bank
WT	Wild Type

Example 1: Materials and Methods

Vectors

An engineered human PGRN cDNA was cloned into an expression construct containing a chicken beta actin promotor with cytomegalovirus early enhancer, a chimeric intron, and a rabbit beta-globin polyadenylation sequence (FIG. 1). A second engineered human PGRN cDNA was cloned into an expression construct containing the human ubiquitin C promoter. The expression constructs were flanked by AAV2 inverted terminal repeats. Adeno-associated virus serotypes 1, 5 and human 68 (AAVhu68) were generated from this construct by triple transfection of HEK293

cells and iodixanol purification as previously described (Lock M, et al. Hum Gene Ther. 2010;21(10):1259-71).

Animal procedures

All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Breeding pairs of GRN knockout mice were purchased from The Jackson laboratory (stock #013175), and a colony was maintained at the University of Pennsylvania. Wild type C57BL/6 (stock #000664) served as controls. In the first study, mice 2 months of age were anesthetized with isoflurane and injected in the lateral cerebral ventricle (ICV) with $1x10^{11}$ vector genome copies (GC) in a volume of 5 µL. 60 days post injection mice were euthanized by exsanguination under ketamine/xylazine anesthesia and death was confirmed by cervical dislocation. In the second study, mice were treated at 7 months of age and sacrificed at 11 months of age. At the time of necropsy serum was collected by cardiac puncture and CSF was collected by suboccipital puncture with a 32-gauge needle connected to polyethylene tubing. Serum and CSF samples were immediately frozen on dry ice and stored at -80 degrees until analysis. The frontal cortex was collected for biochemistry and was immediately frozen on dry ice, while the rest of the brain was fixed in 10% formalin for histology.

3-4-year-old rhesus macaques were purchased from Covance. For vector administration, animals were sedated with intramuscular dexmedetomidine and ketamine, and administered a single intra-cisterna magna (ICM) injection of 3×10^{13} GC of an AAV vector in 1 mL artificial CSF. Needle placement was verified via myelography using a fluoroscope (OEC9800 C-Arm, GE), as previously described (Katz N, et al. Hum Gene Ther Methods. 2018 Oct;29(5):212-219). Animals were euthanized by barbiturate overdose. Collected tissues were immediately frozen on dry ice or fixed in 10% formalin for histology.

Histology and imaging

Mouse brains were fixed in 10% formalin, cryo-preserved in sucrose, embedded in optimal cutting temperature (OCT) compound and cryostat sectioned. Low magnification images of autofluorescent material (lipofuscin) of regions of interest were taken. Lipofuscin deposits were quantified in a blinded manner, using Image J software. Nonhuman primate tissues were fixed in 10% formalin, paraffin embedded and stained with Hematoxylin and Eosin (H&E). Slides were reviewed by a board-certified veterinary pathologist (ELB). For animals treated with GFP vectors, brain sections were stained with antibodies against olig2, GFAP, or NeuN. All

sections were co-stained with DAPI and an antibody against GFP, followed by fluorescent secondary antibodies. Slides were scanned on a Leica Aperio Versa 200 slide scanner and downloaded from eSlide Manager to be analyzed on HALO imaging software (Indica Labs). Five regions of the right hemisphere were sampled for each animal, and cells with each cell type marker were quantified. Cells were detected by adjusting the following settings: "minimum nuclear intensity", "nuclear size", "nuclear segmentation aggressiveness", and "minimum nuclear roundness", under the nuclei detection tab. Then, criteria were defined for each individual dye to further identify cells and generate a quantitative total cell count for each marker. Settings were determined empirically based on the sensitivity and reliability of detection the desired cell type; in some cases, settings such as NeuN detection in cytoplasm did not reflect the true intracellular localization of the marker yet provided greater specificity and sensitivity of detection. All cells detected by automated means were manually verified. For neurons, under the "dye 1" tab, the "nucleus positive threshold" and "cytoplasm positive threshold" were adjusted to detect only cells with NeuN present in both the nucleus and cytoplasm. For astrocytes, DAPI and GFAP markers were selected and both had to be present in the nucleus and cytoplasm of a cell for it to be included in the count. For oligodendrocytes, a cell was counted if DAPI and olig2 were both present in the nucleus, but not the cytoplasm of the cell. For colocalization, the same settings were used, however GFP was included as an additional dye in the nucleus for neurons, and in both the nucleus and the cytoplasm for astrocytes. Cells that did not express all selected markers were eliminated from the generated results table by "masking" them using the nucleus or cytoplasm "mask" function. Because of the scarcity of GFP positive cells colocalized with olig2, transduced oligodendrocytes were manually counted. In some cases, blood vessels or portions of the choroid plexus exhibited autofluorescence and were manually outlined and excluded using the "scissors" tool. The resulting values were expressed as percentages of GFP positive cells for each cell type marker.

Sample preparation for Hexosaminidase (Hex) assay

Serum was used directly in the Hex activity assay while brain samples were homogenized in lysis buffer (0.2 % Triton-X100, 0.9% NaCl, pH 4.0), followed by three freeze-thaw cycles and clarification by centrifugation. Protein concentrations were determined by Bradford assay. Hex activity measurements were performed as previously described (Hinderer C, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2014;22(12):2018-27).

ELISA

Human PGRN was measured using a DuoSet ELISA kit (R&D # DY2420) with minor modifications. Briefly, high binding polystyrene ELISA plates were coated over night at 4ºC with 5ug/ml human PGRN capture antibody diluted in phosphate buffered saline (PBS). After washing, plates were blocked with 1% bovine serum albumin (BSA) in PBS for 2 hours, followed by sample incubation for one hour. Human and nonhuman primate CSF was diluted 1:5, while murine CSF samples were diluted 1:40 in PBS. Brain samples were diluted to 2mg/ml total protein concentration in lysis buffer. Bound antibody was detected with biotinylated Mouse anti-Human Progranulin antibody and Streptavidin-HRP. Plates were developed using tetramethylbenzidine substrate for 20 minutes then reaction was stopped with 2 N sulfuric acid, before absorbance measurement at 450 nm.

Neutralizing antibody assay

Neutralizing antibodies against AAVhu68 were evaluated as previously described (Calcedo R, et al. J Infect Dis. 2009;199(3):381-90).

Statistics

Comparisons of Hex enzymatic activity, lipofuscin counts and CD68+ area in wild type, GRN knockout and AAV-treated GRN knockout mice, were preformed using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test.

Example 2: AAV-mediated delivery of a human GRN transgene in a murine disease model

Recombinant AAV vectors having AAVhu68 capsids expressing human PGRN (SEQ ID NO: 3) under the control of a CB7 promoter and chimeric intron (CB7.CI.hPGRN.rBG) were produced using published triple transfection technques as described, e.g., WO 2018/160582.

We evaluated AAV-mediated delivery of a human GRN transgene in a GRN knockout mouse model. Mice heterozygous for GRN mutations ($GRN^{+/-}$) do not exhibit pathological hallmarks of GRN-related neurodegenerative disease, likely because the mouse lifespan does not allow for development of the sequelae of GRN haploinsufficiency, which first manifest after several decades in humans. In contrast, complete PGRN deficiency in $GRN^{-/-}$ mice recapitulates several early hallmarks of GRN haploinsufficieny in humans, such as impaired lysosomal function, accumulation of autofluorescent lysosomal storage material (lipofuscin), and activation

of microglia, though $GRN^{-/-}$ mice do not exhibit neuron loss even up to two years of age (Lui H, et al. Cell. 2016;165(4):921-35; Ward ME, et al. Sci Transl Med. 2017 Apr 12;9(385):pii: eaah5642). Both $GRN^{+/-}$ and $GRN^{-/-}$ mice have been reported to exhibit behavior abnormalities, but findings have been inconsistent between groups (Ahmed Z, et al. Am J Pathol. 2010;177(1):311-24; Wils H, et al. The Journal of Pathology. 2012;228(1):67-76; Ghoshal N, et al. Neurobiology of Disease. 2012;45(1):395-408; Filiano AJ, et al. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(12):5352-61; Yin F, et al. The FASEB Journal. 2010;24(12):4639-47). Similarly, some reports have indicated reduced survival in $GRN^{-/-}$ mice, whereas others have found that $GRN^{-/-}$ mice have a normal lifespan, consistent with our experience (Ahmed Z, et al. Am J Pathol. 2010;177(1):311-24; Wils H, et al. The Journal of Pathology. 2012;228(1):67-76). Although $GRN^{-/-}$ mice do not exhibit overt neurodegeneration or neurological signs, the remarkable biochemical and histological similarities to GRN haploinsufficiency in humans make them a potentially informative model to evaluate novel therapies. We therefore focused our analyses on these biochemical and histological findings in $GRN^{-/-}$ mice.

The aim of this study was to assess whether delivery of the human GRN gene to the brain can eliminate existing lysosomal storage material and normalize lysosome function in $GRN^{-/-}$ mice. In response to lysosomal storage, cells upregulate expression of lysosomal enzymes, which can be used as biomarkers for lysosomal storage diseases (Hinderer C, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2014;22(12):2018-27; Gurda BL, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(2):206-16; Karageorgos LE, et al. Experimental Cell Research. 1997;234(1):85-97). We evaluated the activity of the lysosomal enzyme hexosaminidase in brain tissue from $GRN^{-/-}$ and $GRN^{+/+}$ mice of different ages, as well as lipofuscin deposits in the cortex, hippocampus and thalamus (FIG. 3A – FIG. 3D). Elevated hexosaminidase activity was evident throughout life, whereas lipofuscin exhibited progressive accumulation. Lipofuscin was apparent as early as 2 months of age, consistent with previous findings (Klein ZA, et al. Neuron. 2017;95(2):281-96 e6). Our initial studies were performed with an AAV vector based on the natural isolate AAVhu68, which is closely related to the clade F isolate AAV9. We treated $GRN^{-/-}$ mice at 2–3 months of age with an intracerebroventricular (ICV) injection of either an AAVhu68 vector expressing human GRN or vehicle (PBS) (N=10 per group). In addition, a cohort of wild type mice was injected with vehicle (N=10). The ICV ROA (involving injection of AAV vector directly into the CSF of the

cerebral ventricles) was used because the small size of the 2-month-old mouse makes it challenging to reliably administer vector via the ICM route, which is the ROA that is used for the NHP study and the proposed FIH clinical trial. Previous studies demonstrated that ICV administration of AAVhu68 at the dose selected for this study (10^{11} GC) results in transduction limited to brain regions near the injected ventricle, making this a useful system to evaluate whether global improvements in brain lesions can be achieved through secretion of PGRN by a small population of cells.

Two months after vector administration the animals were euthanized, and brain, CSF and serum were collected. Quantification of human PGRN protein levels in the brain confirmed transduction in the AAV-treated group (FIG. 4A – FIG. 4F). PGRN is a secreted protein that can be measured in the CSF, and is reduced in the CSF of human GRN mutation carriers (Lui H, et al. Cell. 2016;165(4):921-35; Meeter LH, et al. Dement Geriatr Cogn Dis Extra. 2016;6(2):330-40). We therefore evaluated PGRN protein levels in the CSF of AAV-treated $GRN^{-/-}$ mice, which revealed an average CSF concentration of 14 ng/mL, while in vehicle-treated groups, human PGRN was below detection levels (FIG. 4A – FIG. 4F). Expression of PGRN was accompanied by normalization of lysosomal enzyme expression, with Hex activity levels returning to near normal levels in the brains of AAV-treated $GRN^{-/-}$ mice (FIG. 4A – FIG. 4F).

After confirming PGRN expression in the brains of $GRN^{-/-}$ mice, we assessed whether PGRN expression reduced the number of lipofuscin deposits in the hippocampus, thalamus and cortex. For that purpose, unstained fixed brain sections were mounted on cover glass and autofluorescent lipofuscin was imaged and quantified in a blinded manner. AAV treated $GRN^{-/-}$ mice exhibited reduced lipofuscin in all brain regions compared to vehicle treated $GRN^{-/-}$ mice, and similar levels to age-matched wild-type controls (FIG. 4A – FIG. 4F).

The initial proof of concept study demonstrated the therapeutic activity of AAV-mediated PGRN expression in mice treated at an early age, when storage material has just begun to appear in the brain. We subsequently evaluated the impact of gene transfer in older mice with more severe pre-existing pathology. In this study, 7-month-old $GRN^{-/-}$ mice received a single ICV injection of an AAVhu68 vector expressing human PGRN or vehicle and were sacrificed at 11 months of age. In addition to extensive brain lipofuscin deposits (FIG. 5A – FIG. 5D) 11-month-old $GRN^{-/-}$ mice exhibited extensive microgliosis, similar to patients with FTD caused by GRN mutations (FIG. 6A – FIG. 6C) (Ahmed Z, et al. Journal of neuroinflammation. J Neuroinflammation. 2007 Feb 11;4:7). GRN gene transfer reduced brain Hex activity and

lipofuscin deposits in aged mice similar to the findings in younger animals (FIG. 5A – FIG. 5D). In addition, the size and number of microglia was normalized in the brains of treated mice (FIG. 6A – FIG. 6C).

Cumulatively, ICV delivery of an AAV vector expressing human PGRN to the brain of $GRN^{-/-}$ mice cleared lipofuscin aggregates and almost fully normalized lysosomal enzymatic activity, demonstrating that PGRN gene delivery can effectively correct key aspects of the underlying pathophysiology of GRN-related neurodegenerative diseases.

Example 3: AAV-mediated GRN gene transfer in nonhuman primates

The findings in $GRN^{-/-}$ mice demonstrate that delivery of an AAV vector into the CSF can achieve sufficient brain transduction to produce therapeutic levels of PGRN and prevent or reverse biochemical and histological findings associated with PGRN deficiency. In order to translate this approach to humans, studies were carried out in nonhuman primates using ICM delivery, a clinically relevant route of vector administration. Intrathecal AAV delivery via injection into the cisterna magna is a minimally invasive approach that results in more extensive brain transduction than administration by lumbar puncture (Hinderer C, et al. Molecular therapy Methods & clinical development. 2014;1:14051). NHPs aged 3–10 years old were utilized because this age is representative of the intended adult patient population. Rhesus macaques (N=2 per group) were administered a single image-guided ICM injection of an AAV1, AAV5, or AAVhu68 vector expressing human *GRN* from a transgene termed hPGRN (SEQ ID NO: 3) under the control of a chicken BA promoter and CMV IE enhancer (referred to as the CB7 promoter). An additional group was treated with an AAVhu68 vector carrying a different engineered transgene sequence (hPGRN v2, SEQ ID NO: 4) expressed from a ubiquitin C promoter (UbC). Human progranulin levels in CSF were measured weekly, and animals were sacrificed 35 days after injection for analysis of histopathology. Preliminary safety analyses were performed, including daily cage-side observations, serial physical exams, complete blood counts, serum chemistry panels, CSF chemistry and cytology, and a full necropsy with microscopic evaluation of brain and spinal cord. The treatment groups are summarized in the table below.

Capsid	Transgene	Promoter	Species	N	ROA	Dose	Injection Volume
AAVhu68	hPGRN	CB7	Adult rhesus macaque	2	ICM	3.00 x 10^{13} GC	1 mL
AAVhu68	hPGRN v2	UbC		2			
AAV1	hPGRN	CB7		2			
AAV5	hPGRN	CB7		2			

Robust PGRN expression was detected in the CSF of all NHPs following vector administration (FIG. 7A). The two animals treated with the AAVhu68 vector exhibited CSF human PGRN levels up to 10-fold greater than those of healthy human controls, and similar to the levels that reversed lysosomal abnormalities in the brains of $GRN^{-/-}$ mice. AAV5 treatment yielded roughly equivalent CSF expression levels to AAVhu68. Expression of human PGRN was greatest in the animals treated with an AAV1 vector, reaching more than 40-fold normal human levels. CSF and plasma samples from the NHPs treated with AAVhu68 and AAV1 vectors were tested for antibodies to human PGRN. All four animals developed antibodies to the human transgene product (FIG. 8A – FIG. 8C), which may explain the declining expression levels at the end of the study. The onset of the anti-human PGRN antibody response in CSF correlated with transgene expression levels, peaking earlier in the AAV1 group.

ICM AAV delivery was well-tolerated in all treatment groups. No treatment-related abnormalities were identified on daily observations, physical exams, complete blood counts, or serum chemistry panels. Similar to other ICM AAV studies utilizing a xenogenic transgene (Hordeaux J, et al. Mol Ther Methods Clin Dev. 2018;10(79-88), CSF analysis revealed an asymptomatic lymphocytic pleocytosis beginning 7–21 days after injection for all vector serotypes, mirroring the antibody response to the transgene product (FIG. 8A – FIG. 8C). CSF cell counts declined from peak levels but remained elevated at the time of necropsy for most animals. Histopathology of the brain and spinal cord was evaluated for the AAV1- and AAVhu68- treated groups. Findings were similar to previous ICM AAV studies (Hordeaux J, et al. Mol Ther Methods Clin Dev. 2018;10:79-88; Hordeaux J, et al. Mol Ther Methods Clin Dev. 2018;10:68-78), with occasional minimal lymphocytic infiltrates identified in the meninges and choroid plexus, and degeneration of sensory neurons and their associated axons in some dorsal

root ganglia (DRG) and spinal cord sections. As in previous ICM AAV studies, the sensory neuron findings were typically minimal to mild in severity and not associated with clinical signs (Gurda BL, et al. Molecular therapy : the journal of the American Society of Gene Therapy. 2016;24(2):206-16; Hordeaux J, et al. Mol Ther Methods Clin Dev. 2018;10:79-88; Hordeaux J, et al. Mol Ther Methods Clin Dev. 2018;10:68-78). No vector-related abnormalities we noted in the brain parenchyma of any animal.

Differing patterns of CNS transduction following ICM administration of AAV1 and AAVhu68 vectors to nonhuman primates

The markedly higher PGRN expression in the CSF of NHPs treated with an AAV1 vector led us to further explore differences in the transduction patterns of AAV1, AAV5, and AAVhu68 vectors. NHPs were administered a single ICM injection of an AAV1, AAV5 or AAVhu68 vector (3×10^{13} GC, n = 2 per vector) expressing a GFP reporter gene. Animals were sacrificed 28 days after injection for histological analysis of brain transduction.

Immunohistochemistry revealed diffuse, patchy transduction throughout the brains of NHPs treated with AAV1 and AAVhu68 vectors (not shown). Minimal transduction was evident in brain of animals that received the AAV5 vector. In order to more precisely characterize differences in transduction between AAV1 and AAVhu68, a semi-automated method was developed to quantify transduced cells in sections collected from multiple brain regions. Using sections stained with fluorescently labeled antibodies against GFP and markers of specific cell types, the total numbers of neurons, oligodendrocytes and astrocytes were quantified by NeuN, olig2 and GFAP staining, respectively, followed by quantification of GFP expressing cells of each type (FIG. 9, FIG. 10). AAV1 and AAVhu68 each transduced less than one percent of each cell type in all regions examined. Transduction of neurons was nearly equivalent between the two vectors, whereas AAVhu68 appeared to transduce modestly greater numbers of astrocytes and oligodendrocytes.

The roughly equivalent brain transduction observed with AAV1 and AAVhu68 vectors was unexpected, given the dramatically higher CSF PGRN levels achieved with AAV1. Ependymal cell transduction was evaluated by immunohistochemistry in multiple regions of the lateral ventricle and fourth ventricle of animals treated with AAVhu68 and animal RA1826 treated with AAV1. Interestingly, multiple brain sections from an AAV1 treated animal (RA1826) that contained portions of the ventricular system demonstrated extensive transduction

of the ependymal cells that line the ventricles, which was not observed in either AAVhu68 treated animal (not shown). An average of 48% of ependymal cells were transduced across all sampled regions, including the frontal, temporal and occipital horn of the lateral ventricle and the fourth ventricle. In contrast, only 1-2% of ependymal cells were transduced in the same brain regions of the animals that were given the AAVhu68 vector. Only small segments of one lateral ventricle were evaluable in the second AAV1-treated animal, which showed approximately 1% ependymal cell transduction, though the analysis was limited to the small sampled region. These findings suggest that highly transduced ependymal cells in AAV1-treated animals could be the source of high levels of PGRN in the CSF, given that the transduction of other cells types appeared similar between the two serotypes. The bystander effect mediated by secreted PGRN makes FTD caused by GRN mutations exceptionally amenable to AAV gene therapy. Since extracellular PGRN can be taken up by neurons, the high CSF PGRN levels achieved with the AAV1 vector - apparently mediated by robust ependymal cell transduction - makes AAV1 an ideal choice for GRN gene therapy.

Example 4: Recombinant AAV1.PGRN

rAAV1.PGRN is produced by triple plasmid transfection of HEK293 cells with: 1) the AAV *cis* plasmid (termed pENN.AAV.CB7.CI.hPGRN.rBG.KanR) encoding the transgene cassette flanked by AAV ITRs, 2) the AAV *trans* plasmid (termed pAAV2/1.KanR) encoding the AAV2 *rep* and AAV1 *cap* genes, and 3) the helper adenovirus plasmid (termed pAdΔF6.KanR). The size of the rAAV1.PGRN packaged vector genome is 4129 bases.

A. AAV Vector Genome Plasmid Sequence Elements

A linear map of the vector genome from the *cis* plasmid, termed pENN.AAV.CB7.CI.hPGRN.rBG.KanR (p4862). See, FIG. 2.

The *cis* plasmid contains the following vector genome sequence elements:

1. Inverted Terminal Repeat (ITR): The ITRs are identical, reverse complementary sequences derived from AAV2 (130 base pairs [bp], GenBank: NC_001401) that flank all components of the vector genome. The ITRs function as both the origin of vector DNA replication and the packaging signal for the vector genome when AAV and adenovirus helper

functions are provided in *trans*. As such, the ITR sequences represent the only *cis* sequences required for vector genome replication and packaging.

2. Human Cytomegalovirus Immediate-Early Enhancer (CMV IE): This enhancer sequence obtained from human-derived CMV (382 bp, GenBank: K03104.1) increases expression of downstream transgenes.

3. Chicken β-Actin Promoter (BA): This ubiquitous promoter (282 bp, GenBank: X00182.1) was selected to drive transgene expression in any CNS cell type.

4. Chimeric Intron (CI): The hybrid intron consists of a chicken β-actin splice donor (973 bp, GenBank: X00182.1) and rabbit β-globin splice acceptor element. The intron is transcribed, but removed from the mature mRNA by splicing, bringing together the sequences on either side of it. The presence of an intron in an expression cassette has been shown to facilitate the transport of mRNA from the nucleus to the cytoplasm, thus enhancing the accumulation of the steady level of mRNA for translation. This is a common feature in gene vectors intended for increased levels of gene expression.

5. Coding sequence: The engineered cDNA of the human *GRN* gene encodes human PGRN (hPGRN) protein, which is implicated in lysosomal function and other nervous system roles (1785 bp, GenBank: NM_002087.3; 593 amino acids [aa], GenBank: NP_002078).

6. Rabbit β-Globin Polyadenylation Signal (rBG PolyA): The rBG PolyA signal (127 bp, GenBank: V00882.1) facilitates efficient polyadenylation of the transgene mRNA in *cis*. This element functions as a signal for transcriptional termination, a specific cleavage event at the 3′ end of the nascent transcript and the addition of a long polyadenyl tail.

B. AAV1 Trans Plasmid: pAAV2/1.KanR (p0069)

The AAV2/1 *trans* plasmid is pAAV2/1.KanR (p0069). The pAAV2/1.KanR plasmid is 8113 bp in length and encodes four wild type AAV2 replicase (Rep) proteins required for the replication and packaging of the AAV vector genome. pAAV2/1.KanR also encodes three wild type AAV1 virion protein capsid (Cap) proteins, which assemble into a virion shell of the AAV serotype 1 (AAV1) to house the AAV vector genome. The AAV1 *cap* genes contained on pAAV2/1.KanR were isolated from a simian source.

To create the pAAV2/1.KanR construct, a 3.0- kilobase (kb) fragment from p5E18(2/2), a 2.3-kb fragment from pAV1H, and a 1.7-kb fragment from p5E18(2/2) were incorporated to form pAAV2/1 (p0001), which contains AAV2 *rep* and AAV1 *cap* in an

ampicillin resistance (AmpR) cassette (referred to in the literature as p5E18[2/1]). This cloning strategy also relocated the AAV *p5* promoter sequence (which normally drives *rep* expression) from the 5′ end of *rep* to the 3′ end of *cap,* leaving behind a truncated *p5* promter upstream of *rep*. This truncated promoter serves to down-regulate expression of *rep* and, consequently, maximize vector production (Xiao et al., (1999) Gene therapy vectors based on adeno-associated virus type 1. J Virol. 73(5):3994-4003).

To generate pAAV2/1.KanR for clinical product manufacturing, the ampicillin resistance (*AmpR*) gene in the backbone sequence of pAAV2/1 was replaced with the kanamycin resistance (*KanR*) gene. All component parts of the *trans* plasmids have been verified by direct sequencing.

C. Adenovirus Helper Plasmid: pAdDeltaF6(KanR)

Plasmid pAdDeltaF6(KanR) was constructed in the laboratory of Dr. James M. Wilson and colleagues at the University of Pennsylvania and is 15,774 bp in size. The plasmid contains the regions of adenovirus genome that are important for AAV replication; namely, *E2A, E4*, and *VA* RNA (the adenovirus E1 functions are provided by the HEK293 cells). However, the plasmid does not contain other adenovirus replication or structural genes. The plasmid does not contain the *cis* elements critical for replication, such as the adenoviral ITRs; therefore, no infectious adenovirus is expected to be generated. The plasmid was derived from an E1, E3-deleted molecular clone of Ad5 (pBHG10, a pBR322-based plasmid). Deletions were introduced into Ad5 to eliminate expression of unnecessary adenovirus genes and reduce the amount of adenovirus DNA from 32 kb to 12kb). Finally, the ampicillin resistance gene was replaced by the kanamycin resistance gene to create pAdeltaF6(KanR). The *E2, E4*, and *VAI* adenoviral genes that remain in this plasmid, along with *E1*, which is present in HEK293 cells, are necessary for AAV vector production. Vector is produced according to the following flow charts shown in FIG. 13 and FIG. 14.

The final product should have a pH in the range of 6.2 to 7.7, as determined by USP <791>, and an osmolaity conent of 260 to 320 mOsm/kg as determined by USP <785>, and a GCtiter of greater than or equal to 2.5×10^{13} GC/mL as determined by ddPCR (Lock et al, (2014). "Absolute determination of single-stranded and self-complementary adeno-associated viral vector genome titers by droplet digital PCR." Hum Gene Ther Methods. 25(2):115-25.

EXAMPLE 5: Identification of the Minimum Effective Dose of rAAV1.PGRN in the $GRN^{-/-}$ Mouse Model

• Dose • (GC/g brain mass)	• Mouse (GC)	• NHP (GC)	• Human (GC)
3.33×10^{11}	1.30×10^{11}	3.00×10^{13}	4.33×10^{14}
1.11×10^{11}	4.40×10^{10}	1.00×10^{13}	1.44×10^{14}
3.33×10^{10}	1.30×10^{10}	3.00×10^{12}	4.33×10^{13}
1.11×10^{10}	4.40×10^{9}	-	1.44×10^{13}

The impact of different doses of rAAV1.PGRN on CNS lesions in the *Grn*$^{-/-}$ mouse model is assessed as follows. Efficacy is assessed by the extent of reduction in brain storage pathology (lipofuscin) which serves as a quantitative outcome measure that is directly linked to disease pathophysiology. In addition, terminal blood collection for complete blood counts and serum chemistry panels as well as histopathology of target organs are included to identify disease-specific toxicity that may not be detected in the NHP toxicology study. Mice are treated at 5–6 months of age, when extensive lipofuscin storage is present to replicate the disease state in aged subjects with *GRN* haploinsufficiency. *Grn*$^{-/-}$ mice receive one of four doses of rAAV1.PGRN (1.30×10^{11} GC, 4.40×10^{10} GC, 1.30×10^{10} GC, or 4.40×10^{9} GC) or vehicle (ITFFB [artificial CSF with 0.001% Pluronic F-68]) by ICV injection (N=15 per group). *Grn*$^{+/+}$ mice treated with vehicle (N=15) serve as normal controls. Animals are sacrificed 90 days after treatment, brains are harvested and sectioned, and lipofuscin lesions are quantified. The MED is determined by the dose that shows significant reduction in brain storage lesions relative to vehicle-treated Grn-/- mice and significance is determined using one-way ANOVA followed by Tukey's multiple comparisons test (alpha = 0.05), if applicable.

EXAMPLE 6: Toxicology Study in Non-Human Primates

rAAV1.CB7.CI.hPGRN.rBG, is a non-replicating recombinant adeno-associated (AAV) vector consisting of serotype AAV1, which contains an engineered human progranulin (PGRN) cDNA under the control of a chicken beta actin promoter with cytomegalovirus enhancer and a rabbit beta globin polyadenylation sequence flanked by AAV serotype 2 inverted terminal repeats formulated in Intrathecal Final Formulation Buffer (ITFFB). The ddPCR titer was 2.04×10^{13} GC/mL. The pH of the ITFFB was adjusted to pH 7.4 to maximize the solubility of the test article product. Control article(s) were prepared on the day of dosing. Diluted articles are kept on wet ice or at 2-8°C until dosing on the same day.

A 90-day GLP-compliant safety study in adult rhesus macaques to investigate the toxicology of rAAV1.PGRN following ICM administration was performed. NHPs aged 3–10 years old were utilized for this study because this age is representative of our intended adult human patient population. The 90-day evaluation period was selected because this allows sufficient time for a secreted transgene product to reach stable plateau levels following ICM AAV administration. Rhesus macaques received either one of three dose levels – 3.00×10^{12} GC total, 1.00×10^{13} GC total, or 3.00×10^{13} GC total (N=3 per dose) of rAAV1.hPGRN – or vehicle (ITFFB; N=2). Dose levels were selected to be equivalent to those that are to be evaluated in the planned minimum effective dose (MED) study when scaled by brain mass (assuming 0.4 g for the mouse brain and 90 g for the rhesus macaque brain). Baseline neurologic examinations, clinical pathology (cell counts with differentials, clinical chemistriess, and a coagulation panel), CSF chemistry, and CSF cytology were performed. After rAAV1.PGRN or vehicle administration, the animals were monitored daily for signs of distress and abnormal behavior.

Blood and CSF clinical pathology assessments and neurologic examinations were performed on a weekly basis for 30 days following rAAV1.PGRN or vehicle administration, followed by every 30 days thereafter. At baseline and at each 30-day time point thereafter, anti-AAV1 neutralizing antibodies (Nabs) and cytotoxic T lymphocyte (CTL) responses to AAV1 and the rAAV1.PGRN transgene product were assessed by an interferon gamma (IFN-γ) enzyme-linked immunospot (ELISpot) assay.

Species	*Macaca mulatta*
Synonym	Rhesus Macaques
Age of animals at initiation of study	3-8 years
Weight of animals at initiation of study	3.0-10.0 kg
Number of animals used	11 Total 6 Males 5 Females

Group Designation	**1**	**2**	**3**	**4**
rAAV1.PGRN/ ITFFB	Vehicle (ITFFB)	rAAV1.PGRN	rAAV1.PGRN	rAAV1.PGRN
rAAV1.PGRN Dose (GC)	N/A	$3.0x10^{12}$	$1.0x10^{13}$	$3.0x10^{13}$
Number of Macaques	2	3	3	3
Sex	Both	Both	Both	Both
ROA	ICM	ICM	ICM	ICM
Administration Volume (mL)	Up to 2.0	Up to 2.0	Up to 2.0	Up to 2.0
Necropsy Day	90±5	90±5	90±5	90±5

Ninety days after rAAV1.PGRN or vehicle administration, animals were euthanized and tissues were harvested for a comprehensive microscopic histopathological examination. In addition, lymphocytes were harvested from the liver, spleen, and bone marrow to evaluate the presence of T cells reactive to both the capsid and transgene product in these organs at the time of necropsy.

Vector biodistribution was evaluated by qPCR in tissue samples. Vector genomes were also quantified in serum and CSF samples. Vector excretion was evaluated by analysis of vector genomes detected in urine and feces. These analyses were performed only for the highest dose cohort because previous studies had demonstrated that the pattern of vector distribution after ICM

administration is dose-independent, and higher vector doses yield greater overall signal in the qPCR-based biodistribution assay, allowing for the most sensitivity in the detection of vector deposition in target tissues (Hordeaux et al., 2018b).

Nerve Conduction Velocity Assessment

Animals were sedated with a combination of ketamine/dexmedetomidine. Sedated animals were placed in lateral or dorsal recumbency on a procedure table, with heat packs to maintain body temperature. Electronic warming devices were not recommended due to potential for interference with electrical signal acquisition.

For sensory nerve conduction studies (NCS), the stimulator probe was positioned over the median nerve with the cathode closest to the recording site, and two needle electrodes inserted subcutaneously on digit II at the level of the distal phalanx (reference electrode) and proximal phalanx (recording electrode), while the ground electrode was placed proximal to the stimulating probe (cathode). A pediatric stimulator was used. The elicited responses were differentially amplified and displayed on the monitor. The initial acquisition stimulus strength was set to 0.0 mA in order to confirm a lack of background electrical signal. In order to find the optimal stimulus location, the stimulus strength was increased up to 10.0 mA, and a train of stimuli was generated while the probe was moved along the median nerve until the optimal location was found as determined by a definitive waveform. Keeping the probe at the optimal location, the stimulus strength was progressively increased in a step wise fashion until the peak amplitude response was no longer increasing. Each stimulus response was recorded and saved in the software. Up to 10 maximal stimuli were averaged and reported for the median nerve. The distance (cm) from the recording site to the stimulation cathode was measured and entered into the software and the conduction velocity was calculated using the onset latency of the response and the distance (cm). Both the conduction velocity and the average of the sensory nerve action potential (SNAP) amplitude were reported. The median nerve was tested bilaterally. All raw data generated by the instrument were retained as part of the study file.

FIG. 11A and FIG. 11B show results from the median sensory nerve conduction studies in NHPs. No effects on median sensory nerve conduction were observed at the doses administered. Preliminary histological analysis showed findings primarily within the DRG, TRG, dorsal white matter tracts of the spinal cord and peripheral nerves (FIG. 12A – FIG. 12D). These findings consisted of neuronal degeneration within the DRG/TRG and axonal degeneration (i.e.,

axonopathy) within the dorsal white matter tracts of the spinal cord and peripheral nerves. Overall, these findings were observed across all treated groups; however, the incidence and severity tended to be higher in individual animals from the mid and high-dose groups at both time points.

Given the severity of *GRN*-related neurodegenerative disease, the benefit/risk profile for ICM administration of rAAV1.PGRN is expected to remain favorable .

Example 7: HumanTrial

A First-in-Human FIH Phase 1/2 dose escalation study of a single administration of rAAV1.hPGRN in patients with adult-onset neurodegenerative disease caused by mutations in the GRN gene (see table below) is performed. rAAV1.hPGRN is designed to replace the GRN gene. This FIH study evaluates safety and tolerability as well as collect preliminary data on efficacy. Up to 12 symptomatic heterozygous GRN mutation carrier patients are treated with rAAV1.hPGRN and initially followed for a period of 2 years (24 months), with continued long-term follow-up for 5 years post-dose. This study provides data to support initiation of a registrational study which utilizes the Phase 1/2 maximum tolerated dose (MTD). This registrational study evaluates the effect of rAAV1.hPGRN on clinical outcomes and biomarkers relevant to the disease. All trials include administration of a single ICM dose of rAAV1.hPGRN in adult patients with GRN-associated neurodegenerative disease.

Protocol(s) Title:	A Phase 1/2 Open-Label, Multi-Center Dose Escalation Study to Assess the Safety and Tolerability of a Single Dose of rAAV1.hPGRN Delivered into the Cisterna Magna (ICM) of Adult Patients with Adult-Onset Neurodegenerative Disease Caused by Heterozygous Mutations in the Granulin Precursor (*GRN*) Gene
Number of Subjects:	Up to 12 evaluable subjects
Objectives:	*Primary:* • To assess the safety and tolerability of rAAV1.hPGRN through 2

	years (24 months) following administration of a single ICM dose through evaluation of: o Adverse events (AEs) and serious adverse events (SAEs) o Vital signs and physical examinations o Electrocardiograms (ECGs) o Sensory nerve conduction studies o Laboratory assessments (serum chemistry, hematology, coagulation studies, liver function tests [LFTs], urinalysis, and CSF chemistry and cytology) o Immunogenicity of the vector and transgene product *Secondary (exploratory efficacy):* • To assess the pharmacodynamics and biological activity of rAAV1.hPGRN over 2 years (24 months) following administration of a single ICM dose based on the following endpoints: o Effect of rAAV1.hPGRN on levels of PGRN protein in CSF o Effect of rAAV1.hPGRN on changes in brain cortical thickness
	• To assess the efficacy of rAAV1.hPGRN through 2 years (24 months) following administration of a single ICM dose as measured by: o Effect of rAAV1.hPGRN on survival o Effect of rAAV1.hPGRN on clinical symptoms and daily functioning as assessed by the Mini-Mental State Exam (MMSE), Clinical Global Impression of Change (CGI-C), Frontal Assessment Battery (FAB), Frontotemporal Dementia Rating Scale (FRS), Frontal Behavioral Inventory (FBI), Unified Parkinson's Disease Rating Scale (UPDRS), verbal fluency testing, Clinical Dementia Rating Scale for Frontotemporal Lobar Degeneration sum of boxes (CDR-FTLD sb), and Neuropsychiatric Inventory (NPI) *Exploratory:* • To further assess the pharmacodynamic effects of rAAV1.hPGRN

	through 2years (24 months) following administration of a single ICM dose as measured by: o CSF levels of neurofilament light chain (NFL), tau, phosphorylated tau, and inflammatory markers o Plasma levels of PGRN
Study Design:	This is a Phase 1/2, FIH, multi-center, open-label, single-arm, dose escalation study of rAAV1.hPGRN administered by a single ICM injection in adult subjects with adult-onset neurodegenerative disease caused by *GRN* haploinsufficiency. Safety and tolerability, pharmacodynamics, and clinical efficacy is assessed over 2 years, and all subjects are followed through 5 years post-administration of rAAV1.hPGRN for the long-term evaluation of safety and tolerability, pharmacodynamics, and clinical outcomes. The study consists of a screening phase to determine eligibility of each potential subject from approximately Day –35 to Day –7. After confirmation of subject eligibility and participation in the study, the subject undergoes baseline assessments, which will include brain magnetic resonance imaging (MRI), LP for CSF collection, blood draw, urine collection, vitals, ECG, a physical exam, and clinical assessments. Baseline assessments occur between Day –6 and Day 0 (inclusive) prior to administration of rAAV1.hPGRN. During the treatment phase, subjects are admitted to the hospital on the morning of Day 1. Subjects receive a single ICM dose of rAAV1.hPGRN on Day 1 and remain in the hospital for at least 24 hours after dosing for observation. Subsequent study visits occur at Day 7, Day 14, Day 30, and 6 months after dosing, followed by every 6 months for the first 2 years after dosing. LTFU visits occur for an additional 3 years at a frequency of every 12 months, through 5 years post-dosing.
	The study consists of the following three cohorts administered rAAV1.hPGRN as a single ICM injection: • Cohort 1 (Low Dose): Three eligible subjects are sequentially

	enrolled and administered the low dose of rAAV1.hPGRN with a 4 week safety observation period between each subject. If no safety review triggers (SRTs) are observed, all available safety data are evaluated by a safety board 4 weeks after the third subject in Cohort 1 is administered rAAV1.hPGRN. • Cohort 2 (High Dose): If the decision is made to proceed, three eligible subjects are sequentially enrolled and administered the high dose of hAAV1.hPGRN with a 4 week safety observation period between each subject. If no SRTs are observed, all available safety data are evaluated by the safety board 4 weeks after the third subject Cohort 2 is administered rAAV1.HPGRN. • Cohort 3 (MTD): Pending a positive recommendation by the safety board, 6 additional subjects are enrolled and administered the MTD dose of rAAV1.hPGRN. Dosing is not staggered with a 4 week safety observation period between each subject. Cumulatively, a total enrollment of 9 subjects in either the high- or low-dose cohort, and 12 subjects in total (across all doses) is anticipated.
Inclusion Criteria:	1. ≥ 35 years and ≤ 75 years of age at enrollment 2. Confirmed clinical diagnosis of adult-onset neurodegenerative disease caused by heterozygous GRN mutation 3. Confirmation of GRN mutation as causal by one of the following criteria: a. Mutation classified as pathogenic by the Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB) (AD&FTDMDB and Cruts, (2019). "Alzheimer Disease & Frontotemporal Dementia Mutation Database." Retrieved January 16, 2019, from www.molgen.ua.ac.be/ADmutations/default.cfm?MT=1&ML=1&Page=MutByQue ry&Query=tblContexts.GeneSymbo-

	l%20In%20(%27GRN%27)&Selection=Gene%20In %20(GRN).) following the guidelines published by the American College of Medical Genetics (ACMG) (Richards et al., (2015). "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology." Genet Med. 17(5):405-24) b. Classified as "pathogenic nature unclear" in the AD&FDMDB or "variant of uncertain significance" by ACMG guidelines, and the patient has a family history of the same or related neurodegenerative disease 4. CDR-FTLD global score > 0.5 and ≤ 1
Exclusion Criteria:	1. Inability to provide full consent or the lack of a legally authorized caregiver with adequate contact who can provide consent 2. Contraindication to MRI, ICM delivery, or LP (e.g., local infection, thrombocytopenia, coagulopathy, elevated intracranial pressure ([ICP] due to a space-occupying lesion) 3. Classification of the *GRN* mutation as "not pathogenic," "likely benign variant," or "benign variant" in the AD&FDMDB 4. Immunocompromised patients 5. Patients with a positive test result for human immunodeficiency virus (HIV) or Hepatitis C 6. Other malignancies or chronic CNS disorders not caused by *GRN* mutation 7. Medications that, in the opinion of the investigator, may pose a risk to the patient, such as immunosuppressive medications or systemic corticosteroids. Non-steroidal anti-inflammatory drug (NSAID) use acceptable if on a stable dose for 30 days prior to screening and agrees to remain on same dose for duration of trial 8. Malignant neoplasia (except localized skin cancer) or a documented

	history of hereditary cancer syndrome. Subjects with a prior successfully treated malignancy and a sufficient follow-up to exclude recurrence (based on oncologist opinion) can be included after discussion and approval by the Sponsor or designee 9. Any concurrent disease that, in the opinion of the investigator, may cause cognitive impairment unrelated to *GRN* mutations, including neurosyphilis, hydrocephalus, stroke, small vessel ischemic disease, uncontrolled hypothyroidism, or vitamin deficiency 10. For females of childbearing potential, a positive urine confirmed by serum pregnancy test at the screening visit, a positive urine confirmed by serum result on Day 1 prior to administration of the investigational product, or unwillingness to have additional pregnancy tests during the study 11. For men and women of childbearing potential, unwillingness to use a medically accepted method of double-barrier contraception (such as a condom/diaphragm used with spermicide) or engage in abstinence from the date of screening to 52 weeks after vector administration 12. Any condition (e.g., history of any disease, evidence of any current disease, any finding upon physical examination, or any laboratory abnormality) that, in the opinion of the investigator, would put the subject at undue risk or would interfere with evaluation of the investigational product or interpretation of subject safety or study results 13. Any acute illness requiring hospitalization within 30 days of treatment

Route of Administration and Procedure

rAAV1.hPGRN as a single dose is administered on Day 1 to subjects via CT-guided sub-occipital injection into the cisterna magna. On Day 1, a syringe containing 5.6 mL of

rAAV1.hPGRN at the appropriate titer is prepared by the Investigational Pharmacy associated with the study and delivered to the procedure room.

Prior to study drug administration, the subject is anesthetized, intubated, and the injection site is prepped and draped using sterile technique. An LP is performed to remove a predetermined volume of CSF, after which iodinated contrast is IT injected to aid in visualization of relevant anatomy of the cisterna magna. IV contrast may be administered prior to or during needle insertion as an alternative to the IT contrast. The decision to use IV or IT contrast is at the discretion of the interventionalist performing the procedure. A spinal needle (22–25 G) is advanced into the cisterna magna under fluoroscopic guidance. A larger introducer needle may be used to assist with needle placement. After confirmation of needle placement, the extension set is attached to the spinal needle and allowed to fill with CSF. At the discretion of the interventionalist, a syringe containing contrast material may be connected to the extension set and a small amount injected to confirm needle placement in the cisterna magna. After the needle placement is confirmed, the syringe containing rAAV1.hPGRN is connected to the extension set. The syringe contents are slowly injected over 1–2 minutes, delivering a volume of 5.0 mL.

Safety assessments, including collection of adverse events (AEs), physical/neurologic examinations, vital signs, clinical laboratory (serum chemistry, hematology, coagulation, LFTs, urinalysis), ECGs, nerve conduction studies, and CSF cytology and chemistry (cell counts, protein, glucose) are performed at the times indicated in the study schedule.

No statistical comparisons are planned for safety evaluations; all results are descriptive only. Data are listed and summary tables are produced.

Statistical comparisons are performed for secondary and exploratory endpoints. Measurements at each time point are compared to baseline values for each subject, as well as natural history data from healthy volunteers and GRN patients with comparable cohort characteristics where available for each endpoint. All data are presented in subject data listings. Categorical variables are be summarized using frequencies and percentages, and continuous variables are summarized using descriptive statistics (number of non-missing observations, mean, standard deviation, median, minimum, and maximum). Graphical displays are presented as appropriate.

The early clinical presentation of adult-onset neurodegeneration caused by GRN haploinsufficiency is heterogeneous. This heterogeneity results in a variety of diagnoses with additional symptoms emerging as the disease progresses. Because patients typically decline

rapidly following symptom onset, patients with any diagnosis of neurodegeneration may be treated, as long as they have a confirmed pathogenic heterozygous GRN mutation. Patients may be screened utilizing the CDR-FTLD global score. This rating scale is designed to assess disease severity in patients with an FTLD spectrum diagnosis. Due to the overlap in FTLD-related symptomology for those with other GRN-related diagnoses, and the number of domains captured in the CDR-FTLD global score (memory, orientation, judgment and problem solving, community affairs, home and hobbies, personal care, behavior, and language), this scale can be used for diagnoses. A CDR-FTLD global score greater than 0.5 (which includes mildly symptomatic patients) and less than or equal to 1 would permit treatment of symptomatic patients at an early stage of neurodegeneration in which the benefits of gene therapy are likely to be maximized. Treating patients at this early stage permits the subsequent detection of changes or stabilization in disease progression and delays in the onset of additional symptoms. However, the requirement of a minimum 0.5 CDR-FTLD global score in this population precludes participation of motor-prominent diagnoses, as the scale is not optimized to capture deficiencies in motor phenotypes.

This gene therapy is not expected to result in PGRN expression above physiological levels. Using rAAV1.hPGRN doses in nonclinical NHP studies that are higher than the doses that would be used in the FIH trial, PGRN was found to be expressed in serum at close to normal levels following rAAV1.hPGRN ICM administration. Because subjects enrolled in the FIH trial would initially have circulating PGRN levels at approximately 30% of normal, it is expected that circulating serum PGRN levels may be restored to normal. Patients' bloodwork is screened through complete blood count (CBC) panels, and patients are monitored for tumors via MRI with gadolinium contrast of the brain and upper spine for 5 years at the follow-up time points.

In addition to measuring safety and tolerability as primary endpoints, secondary and exploratory efficacy endpoints were chosen for this study based on the current literature and in consultation with leading clinicians specializing in the study of *GRN*-related neurodegeneration. These endpoints track clinical outcomes and disease biomarkers with the goal of identifying appropriate endpoints for a subsequent registrational trial.

Because neurodegeneration caused by *GRN* mutations ultimately results in death, the impact of rAAV1.hPGRN on patient survival is also an efficacy endpoint for this study. However, the duration of the study and sample size may not be sufficient to demonstrate a survival benefit because most patients have a life expectancy of 7–11 years from symptom onset.

The effect of rAAV1.hPGRN on clinical symptoms and daily functioning of patients is assessed. Because of the phenotypic heterogeneity displayed by the target patient population, functional and clinical scales that capture the progression of symptoms expressed across the range of clinical presentations are employed. The proposed study utilizes the FAB, FRS, MMSE, CGI-C, NPI, and FBI to measure changes over time. These scales primarily measure cognition, language, neuropsychological behaviors, and capabilities related to daily function which inform on the progression or stabilization of the various clinical presentations of the disease. UPDRS is also included to capture changes in motor symptoms. In the FIH, these efficacy assessments are exploratory in nature and intended to capture over time the ability of the rAAV.hPGRN to stabilize the decline in symptoms. Data from the FIH on the rate of further decline across the various clinical parameters in the patients with different clinical presentations are used to further inform the selection of appropriate endpoints and define clinically meaningful changes for the registrational trial.

Each clinical scale is briefly described below:

Clinical Scales Primarily Measuring Cognitive Function

CDR-FTLD: The CDR-FTLD is an extended version of the classic clinical dementia rating (CDR) scale, which is historically used to rate the severity of AD spectrum disorders. The assessment includes the original six domains of the CDR (memory, orientation, judgment and problem solving, community affairs, home and hobbies, personal care). It also includes the two additional domains of language and behavior, which allows for more sensitivity in the detection of decline in FTLD spectrum patients. A rating score of 0 indicates normal behavior or language, while scores of 1, 2, or 3 indicate mild to severe deficits. For the CDR-FTLD sum of boxes (CDR-FTLD sb) represents the sum of the individual domains, and is used to determine global dementia severity.

MMSE: The MMSE is an 11-question global cognitive assessment widely used in clinical and research practice. Questions such as "What is the year? Season? Date? Day of the week? Month?" are asked, and one point is given for each correct answer, with maximum scores provided for each question. The maximum total score is 30, with two cut-offs at scores of 24 and 27. These cutoffs are indicators of cognitive decline.

Verbal Fluency Testing: Although not one of the proposed exploratory efficacy endpoints, verbal fluency testing is performed throughout the FIH trial. This is likely to be conducted by presenting the same picture to each subject and asking for a verbal description. During the description, rate of speech (words/minute) is counted, recorded, and ultimately compared to rates reflective of neuro-typical adults.

Clinical Scales Primarily Measuring Motor Function

UPDRS: The UPDRS is a 42-item, four-part assessment of several domains related to parkinsonism, such as mentation, behavior and mood, and activities of daily living. Each item includes a rating scale ranging from 0 (indicating no impairment) to 4 (indicating the most severe impairment). The scores for each part are tallied to provide an indication of severity of the disease with a high score of 199 indicating the most severe disability.

Clinical Scales Primarily Measuring Behavior

NPI: The NPI is used to elucidate the presence of psychopathology in patients with disorders of the brain. Initially, it was developed for use in AD populations; however, it is believed to be useful for assessing behavioral changes in other conditions. The assessment consists of 10 behavioral domains and two neurovegetative areas, within which there are four scores: frequency, severity, total distress, and caregiver distress. The NPI total score is obtained by adding the domain scores of the behavioral domains and subtracting the caregiver distress scores.

FBI: The FBI is a 24-item assessment of changes in behavior and personality associated specifically with bvFTD and to differentiate between bvFTD and other dementias. It is administered as a face-to-face interview with the primary caregiver because patients with a bvFTD diagnosis generally do not recognize these types of changes. It focuses on several behavioral and personality-related areas, scoring each question from 0 (none) to 3 (severe/most of the time). The total score typically correlates with the severity of illness and can be used to assess change over time.

Clinical Scales Measuring both Cognitive and Motor Function

CGI-C: The CGI-C is one of three parts of a brief, widely used assessment. It is composed of three items that are clinician-rated. The CGI-C is rated on a 7-point scale, ranging

from 1 (very much improved) to 7 (very much worse) starting from enrollment in the study, whether or not any improvement is due entirely to treatment.

FAB: The FAB is a brief assessment to assist in differentiating between dementias with a frontal dysexecutive phenotype and those of an AD type. It is particularly useful in mildly demented patients (MMSE > 24). The assessment consists of six parts that address cognitive, motor, and behavioral areas. A total score of 18 or higher indicates better performance.

FDR: The FDR is a brief staging assessment for patients diagnosed with a frontotemporal dementia (FTD) subtype (i.e., bvFTD or any of the PPA subtypes). The FDR detects differences in disease progression for FTD over time. This brief interview is conducted with the primary caregiver and consists of 30 items, which are categorized as occurring "never," "sometimes," or "always." A percentage score is then calculated and converted to a logit score and, ultimately, a severity score. The severity score ranges from "very mild" to "profound."

Columbia Suicide Severity Rating Scale (C-SSRS): Although the C-SSRS score is not an exploratory efficacy endpoint for the FIH, this assessment is performed throughout the study. The C-SSRS is a three-part scale measuring suicidal ideation, intensity of ideation, and suicidal behavior. The outcome of this assessment is composed of a suicidal behavior lethality rating taken directly from the scale, a suicidal ideation score, and a suicidal ideation intensity ranking. An ideation score greater than 0 may indicate the need for intervention based on the assessment guidelines. The intensity rating has a range of 0 to 25, with 0 representing no endorsement of suicidal ideation.

As an additional exploratory endpoint, patient survival is assessed. However, patients diagnosed with neurodegeneration caused by *GRN* haploinsufficiency have a life expectancy of 7–11 years from symptom onset. As such, the duration of the study, sample size, and the inclusion of subjects who are in the early phase of disease may not be sufficient to demonstrate a survival benefit.

Administration of rAAV1.hPGRN stabilize the decline in the atrophy (neuronal cell loss) primarily in the frontal and temporal cortical lobes, and whole brain volume over time caused by GRN-associated haploinsufficiency. MRI may be used to track changes in the thickness of the middle frontal cortex and parietal regions.

Biochemical biomarkers are also assessed. Levels of PGRN protein in the CSF and plasma are measured as a readout of AAV transduction, and increase in patients following

administration of rAAV1.hPGRN. CSF levels of neurofilament light chain (NFL), tau, phosphorylated tau, and other inflammatory markers are also be tracked. NFL is considered a general indicator of neuronal loss or damage. Tau and phosphorylated-tau are associated with pathology seen in AD, PD, and some forms of FTD.

Patients' bloodwork is screened through complete blood count (CBC) panels, and patients are monitored for tumors via MRI with gadolinium contrast of the brain and upper spine for 5 years at the follow-up time points.

A single administration of rAAV1.hPGRN is safe and tolerable following administration. A single administration of rAAV1.hPGRN improves survival, and/or reduces disease progression as assessed by clinical symptoms and daily functioning of patients. Treatment slows of loss of neurocognitive function.

(Sequence Listing Free Text)

The following information is provided for sequences containing free text under numeric identifier <223>.

SEQ ID NO	Free Text under <223>
3	<223> Engineered human PGRN1 coding sequence
4	<223> engineered hPGRN2 coding sequence <220> <221> CDS <222> (1)..(1779)
5	<223> Synthetic Construct
6	<223> rabbit globin polyA
7	<223> 3' AAV ITR
8	<223> 5' AAV ITR
9	<223> human CMV IE enhancer
10	<223> CB promoter
11	<223> chimeric intron
12	<223> UbC promoter
13	<223> intron
14	<223> SV40 late polyA

15	<223> Ampicillin resistance gene
16	<223> COL E1 origin
17	<223> EF-1a promoter
18	<223> F1 ori
19	<223> Kanamycin resistance gene
20	<223> P5 promoter
21	<223> LacZ promoter
22	<223> EF1a.huPGRN.SV40 <220> <221> repeat_region <222> (1)..(130)
23	<223> Ubc.PI.huPGRN.SV40
24	<223> CB7.CI.hPGRN1.rBG <220> <221> misc_feature <222> (1)..(130) <223> 5' ITR <220> <221> misc_feature <222> (198)..(579) <223> CMV IE enhancer <220> <221> misc_feature <222> (582)..(863) <223> chicken beta-actin promoter <220> <221> misc_feature

	<222> (958)..(1930) <223> chimeric intron <220> <221> misc_feature <222> (1942)..(3726) <223> hPGRN <220> <221> misc_feature <222> (3787)..(3913) <223> rabbit beta globin poly A <220> <221> misc_feature <222> (4002)..(4131) <223> 3' ITR
25	<223> AAV1 VP1 gene <220> <221> CDS <222> (1)..(2208)
26	<223> Synthetic Construct
27	<223> AAV2 rep
28	<223> AAV5 capsid VP1 gene <220> <221> CDS <222> (1)..(2172)
29	<223> Synthetic Construct
30	<223> AAVhu68 VP1 capsid

	<220> <221> CDS <222> (1)..(2211)
31	<223> Synthetic Construct
32	<223> miRNA target sequence
33	<223> miRNA target sequence
34	<223> miRNA target sequence
35	<223> miRNA target sequence

All documents cited in this specification are incorporated herein by reference. US Provisional Patent Application No. 62/809,329, filed February 22, 2019, US Provisional Patent Application No. 62/923,812, filed October 21, 2019, and US Provisional Patent Application No. 62/969,108, filed February 2, 2020, are incorporated by reference in their entireties, together with their sequence listings. The sequence listing filed herewith named "18-8663PCT_ST25.txt" and the sequences and text therein are incorporated by reference. While the invention has been described with reference to particular embodiments, it will be appreciated that modifications can be made without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS:

1. A recombinant AAV (rAAV) comprising:

(a) an AAV capsid from adeno-associated virus 1, and

(b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats (ITRs), a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

2. The rAAV according to claim 1, wherein the coding sequence encodes the human progranulin protein of SEQ ID NO: 1.

3. The rAAV according to claim 1 or 2, wherein the progranulin coding sequence is SEQ ID NO: 3 or a sequence at least 95% to 99.9% identical thereto.

4. The rAAV according to any one of claims 1 to 3, wherein the vector genome comprises a promoter, an enhancer, an intron, a human progranulin coding sequence, and a polyadenylation signal.

5. The rAAV according to any one of claims 1 to 4, wherein the intron consists of a chicken beta actin splice donor and a rabbit β splice acceptor element.

6. The rAAV according to any one of claims 1 to 5, wherein the AAV inverted terminal repeats (ITRs) are an AAV2 5' ITR and an AAV2 3' ITR which flank the progranulin coding sequence and regulatory sequences.

7. The rAAV according to any one of claims 1 to 6, wherein the vector genome comprises (a) an EF-1a promoter and an SV40 late poly A, (b) a UbC promoter, an intron, and an SV40 late poly A, or (c) a CB7 promoter, a chimeric intron, and a rabbit globin poly A.

8. The rAAV according to claim 7, wherein the vector genome comprises a sequence of SEQ ID NO: 24.

9. A pharmaceutical composition comprising an aqueous liquid suitable for intrathecal administration and a recombinant AAV (rAAV) suitable for use in treating neurodegeneration caused by GRN-haploinsufficiency, said rAAV comprising:

(a) an AAV capsid from adeno-associated virus 1, and

(b) a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats (ITRs), a coding sequence for human progranulin, and regulatory sequences which direct expression of the progranulin.

10. The composition according to claim 9, wherein the coding sequence encodes the human progranulin protein of SEQ ID NO: 1.

11. The composition according to claim 9 or 10, wherein the progranulin coding sequence is SEQ ID NO: 3 or a sequence at least 95% to 99.9% identical thereto over at least amino acid 18-593 of SEQ ID NO: 3.

12. The composition according to any one of claims 9 to 11, wherein the vector genome comprises a promoter, an enhancer, an intron, the human progranulin coding sequence, and a polyadenylation signal.

13. The composition according to any one of claims 9 to 12, wherein the intron consists of a chicken beta actin splice donor and a rabbit β splice acceptor element.

14. The composition according to any one of claims 9 to 13, wherein AAV inverted terminal repeats (ITRs) are an AAV2 5' ITR and an AAV2 3' ITR which flank the progranulin coding sequence and the regulatory sequences of the vector genome.

15. The composition according to any one of claims 9 to 14, wherein the vector genome comprises (a) an EF-1a promoter and an SV40 late poly A, (b) a UbC promoter, an intron, and an SV40 late poly A, or (c) a CB7 promoter, a chimeric intron, and a rabbit globin poly A.

16. The rAAV according to claim 15, wherein the vector genome comprises a sequence of SEQ ID NO: 24.

17. The composition according to any one of claims 9 to 16, wherein the composition comprises an artificial cerebrospinal fluid with a surfactant.

18. The composition according to claim 17, wherein the surfactant is Pluronic F-68.

19. The recombinant AAV (rAAV) according to any of claims 1 to 8 or the pharmaceutical composition according to any one of claims 9 to 18 for use in a method for treating a patient having adult-onset neurodegeneration caused by GRN-haploinsufficiency.

20. Use of the recombinant AAV (rAAV) according to any of claims 1 to 8 or the pharmaceutical composition according to any one of claims 9 to 18 in the manufacture of a medicament for treating a patient having adult-onset neurodegeneration caused by GRN-haploinsufficiency.

21. The use according to claim 19 or 20, wherein the composition is formulated to be administered a dose of 1×10^{10} GC/g brain mass to 3.33×10^{11} GC/g brain mass of the rAAV intrathecally.

22. The use according to any one of claims 19 or 20, wherein the patient is a human adult and is administered a dose of 1.44×10^{13} to 4.33×10^{14} GC of the rAAV.

23. The use according to any one of clams 19 to 22, wherein the rAAV or composition is administrable in a regimen further comprising one or more of (a) non-invasively assessing the patient for reduction in retinal storage lesions as a predictor of reduction of brain lesions, (b) performing magnetic resonance imaging to assess brain volume, and/or (b) measuring concentration of progranulin concentration in the CSF.

24. The use according to any one of claim 19 to 23, wherein the rAAV comprising the coding sequence for progranulin is delivered intrathecally, via intracerebroventricular delivery, or via intraparenchymal delivery.

25. The use according to any one of claims 19 to 23, wherein the composition is administered as a single dose via a computed tomography- (CT-) guided sub-occipital injection into the cisterna magna (intra-cisterna magna).

26. The use according to any one of claims 19 to 25, wherein the patient has progranulin - frontotemporal dementia.

27. A method of treating a human patient with adult onset neurodegeneration caused by granulin (GRN) haploinsufficiency, comprising delivering to the central nervous system (CNS) a recombinant adeno-associated virus (rAAV) having an AAV capsid of adeno-associated virus 1 (AAV1), said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a human progranulin coding sequence, and regulatory sequences which direct expression of the progranulin.

28. A method of treating a human patient with adult onset neurodegeneration caused by granulin (GRN) haploinsufficiency, comprising administering to the central nervous system (CNS) a recombinant adeno-associated virus (rAAV) having an AAV capsid of adeno-associated virus 1 (AAV1) which targets ependymal cells, said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal repeats, a human progranulin coding sequence, and regulatory sequences which direct expression of the progranulin in the ependymal cells.

29. A method for treating a human patient with brain lesions associated with adult-onset neurodegeneration caused by granulin haploinsufficiency, the method comprising administering to the central nervous system a recombinant adeno-associated virus (rAAV) having an AAV capsid of adeno-associated virus 1 (AAV1), said rAAV further comprising a vector genome packaged in the AAV capsid, said vector genome comprising AAV inverted terminal

repeats, a human progranulin coding sequence, and regulatory sequences which direct expression of the progranulin.

30. The method according to any one of claims 27 to 29, wherein the patient is administered an rAAV according to any of claims 1 to 8 or a pharmaceutical composition according to any one of claims 9 to 18.

31. The method according to any one of claims 27 to 30, wherein the patient is administered a dose of 1×10^{10} GC/g brain mass to 3.33×10^{11} GC/g brain mass of the rAAV intrathecally.

32 The method according to any one of claims 27 to 30, wherein the patient is a human adult and is administered a dose of 1.44×10^{13} to 4.33×10^{14} GC of the rAAV.

33. The method according to any one of claims 27 to 32, further comprising one or more of (a) non-invasively assessing the patient for reduction in retinal storage lesions as a predictor of reduction of brain lesions, (b) performing magnetic resonance imaging to assess brain volume, and/or (c) measuring concentration of progranulin concentration in the CSF.

34. The method according to any one of claim 27 to 33, wherein the rAAV comprising the progranulin coding sequence is delivered intrathecally, via intracerebroventricular delivery, or via intraparenchymal delivery.

35. The method according to any one of claims 27 to 33, wherein the rAAV is administered as a single dose via a computed tomography- (CT-) guided sub-occipital injection into the cisterna magna (intra-cisterna magna).

36. The method according to any of claims 27 to 35, wherein the patient has progranulin-related frontotemporal dementia (FTD).