An Open-label Dose Escalation Study of an Adeno-associated Virus Vector (scAAV2-P1ND4v2) for Gene Therapy of Leber's Hereditary Optic Neuropathy (LHON) caused by the G11778 mutation in mitochondrial DNA.

NCT02161380

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1) **Protocol Title**

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SHORT TITLE: Leber's hereditary optic neuropathy (LHON): Gene Therapy Clinical Trial

IND NO.: 15941
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- LHON: Gene Therapy Clinical Trial is registered on the Clinical Trials.gov website (http://clinicaltrials.gov). Clinicaltrials.gov number: NCT02161380

2) **IRB Review History***

N/A

3) **Objectives***

Our goal is to test safety.

The **purpose** of this dose-escalation study is to assess the safety and tolerability of scAAV2-P1ND4v2 (abbreviated as AAV-ND4) gene replacement therapy in subjects confirmed with the G11778A mutation in mtDNA responsible for Leber's Hereditary Optic Neuropathy. Ocular and systemic toxicity will be assessed following vector administration to determine if there are adverse changes that may be associated with vector administration.

This first-in-man (FIM) clinical trial will assess safety, tolerability, and potentially efficacy of a single intravitreal injection in patient groups reflecting the acute, presymptomatic and chronic stages and manifestation of the LHON disease.

Aim 1. To Evaluate the Safety of AAV-ND4 in subjects with long-standing bilateral acuity loss (Group 1). Phase I will consist of an open-label, unilateral single-dose, intravitreal injection of AAV-ND4 per subject in a dose-escalation study investigating the safety of four vector doses (Low: 1.18 $x10^9$ vg*, Medium: 5.81.x 10^9vg*(*based on CHOP titers as recommended by FDA), High: 2.40$x10^{10}$ vg and Higher: 1$x10^{11}$ vg) in subjects with molecularly confirmed G11778A-mutated mitochondrial DNA. The bilateral chronic group will include patients with ≥12 months since onset in one eye and at least 6 months onset in the more recently affected eye. Both eyes must have acuity reduced to ≤ 35 letters. If both eyes have ≥12 months since onset, the eye with worse visual acuity will be

injected. If both eyes have the same acuity the eye with longest onset will be injected. If one eye has <12 months and >6month since onset and the difference in acuity between the eyes is ≤10 letters, the eye with ≥12 months will be injected. If eyes have an acuity difference >10 letters, the eye with worse acuity will be injected.

Aim 2. To Evaluate the Safety of AAV-ND4 in subjects with acute (<12 months) bilateral loss of visual acuity to ≤ 35 ETDRS letters (Snellen = 20/200) (Group 2). The eye with worse acuity will be injected.

Aim 3. To Evaluate the Safety of AAV-ND4 in subjects with unilateral loss of acuity to ≤35 ETDRS letters, but who have "good acuity" ≥ 70 letters (20/40 Snellen) in the contralateral eye (Group 3). The eye with better acuity will be selected for injection.

For each group, administration of study drug will follow an adaptive plan to identify the maximum tolerated dose. If none of the three subjects at a given dose level develops a major toxicity, the next cohort will receive the next higher dose. We will wait a minimum of 6 weeks between injections of the same dose in successive subjects, and will wait until 3 months after the last subject is injected with this dose before moving to the cohort receiving the next higher dose. We will test the lowest dose first in 3 subjects of Aim I, before moving on to testing this low dose in Aim 2, then lastly in Aim 3. Safety will be monitored for loss of 15 letters in Aims 1 and 2, but a difference of 15 letters worse in the treated eyes relative to untreated eyes for Aim 3. If acuity, and visual field testing demonstrate no toxicity in Aims 1 and 2, we will proceed with Aim 3.

The **objective** of this proposed clinical trial is to evaluate the safety of mitochondrially targeted ND4 gene therapy with the adenoassociated viral vector in appropriate LHON patients in a Phase I clinical trial. If warranted by the results of the Phase I trial, another aim will be to obtain a preliminary estimate of the therapy's efficacy. We provide a summary of the extensive preclinical testing in relevant rodent animal models to support the efficacy of the approach proposed in this clinical trial.

The **primary hypothesis** being tested is there will be no systemic toxicity or loss of vision to No Light Perception in the injected eye.

4) Background*

Background and Significance

Leber's Hereditary Optic Neuropathy (LHON) is a maternally inherited form of bilateral optic neuropathy causing severe and permanent visual loss in young adults, usually between the second and fourth decades of life. LHON

usually presents as a loss of central vision that typically progresses over weeks without pain, until bilateral central scotomas and visual loss remain. Visual loss may occur in both eyes simultaneously, or in one eye with the fellow eye affected weeks to months later. The deterioration of vision may progress over several months to a maximum loss of function which stabilizes at visual acuities worse than 20/200.

Characterization of the hereditary nature of LHON led early researchers to postulate that it was a disorder of mitochondrial inheritance. Molecular analysis has confirmed that the basic etiology results from a point mutation in mitochondrial DNA (mtDNA). Three primary mutations at nucleotide pair 3460, 11778, and 14484 are generally agreed to be the primary causes of LHON, accounting for 98% of LHON cases. All primary LHON mutations alter mtDNA-encoded protein subunits, which comprise NADH-ubiquinone oxidoreductase (complex I) of the mitochondrial respiratory chain. Therefore, it is generally hypothesized that a dysfunctional respiratory chain impairs ganglion cell function leading to ganglion cell layer and optic nerve damage resulting in vision loss. While the relationship between genotype and phenotype appears straightforward, the selective clinical presentation observed in LHON patients indicates a complex pathogenesis. Although a primary LHON mutation is necessary for the expression of the disease, it is unclear how the mutations interact with other genetic and/or epigenetic factors to cause tissue specific disease in only select individuals.

To date an effective treatment for LHON has not been identified. Anecdotal reports of treatment efficacy have been difficult to interpret given the potential for spontaneous recovery in certain genotypes. Treatments including systemic steroids, hydroxycobalamin, cyanide antagonists, and naturally occurring cofactors (e.g., coenzyme Q10, succinate and idebenone) have not produced definitive evidence of treatment or prevention of visual loss.

We have taken an alternative approach for correcting point mutations in mitochondrial protein-coding genes—by expressing these genes in the nucleus in conjunction with an N-terminal targeting sequence for mitochondrial import. Most mitochondrial proteins are in fact made this way and are transported into the organelles after their translation on cytoplasmic polyribosomes. The mechanism of their import and assembly has been a popular research topic for over 20 years. There are two major obstacles to correcting mitochondrial mutations in this way. First, mitochondrial translation employs a partially different genetic code than the nuclear-cytoplasmic system, and second, some mitochondrial proteins may be too hydrophobic to be imported and properly assembled outside

the mitochondria. The first obstacle can be addressed by completely recoding mitochondrial genes so that they can be expressed on cytoplasmic ribosomes, an approach coined “allotopic expression”. This process involves using overlapping oligonucleotides to rebuild the genes in the universal genetic code, choosing codons that permit high-level translation ~ or using in situ mutagenesis to change codons that differ between the mitochondria and nucleus. The second problem is more difficult.

Our investigative team headed by [redacted] was the first group to overcome the deficiency in oxidative phosphorylation of LHON cells by constructing a ''nuclear version'' of the mitochondrial gene (ND4) then targeted the cytoplasmically synthesized protein to the mitochondria by using a targeting sequence appended to the reading frame (allotopic expression). When delivered to cultured cells containing a mutant ND4 respiratory function was restored. Recently our group showed in vivo allotopic ND4 expression is safe in rodents and primates suggesting it may be useful in LHON patients.

In previous work, we have made major strides towards determining the pathogenesis and testing a treatment for LHON. First, we discovered that G11778A ND4 mutant cells have a severe reduction in ATP synthesis, even though mild reductions in complex I activity appear insufficient to induce disease. Since no technology existed to introduce DNA directly into mitochondria, we overcame this deficiency in oxidative phosphorylation by constructing a "nuclear version" of the mitochondrial gene then targeted the cytoplasmically synthesized protein to the mitochondria by using a targeting sequence appended to the reading frame (allotopic expression). When delivered to cultured cells containing a mutant ND4, respiratory function was restored. Next, we created a bona fide animal model for LHON. Using site directed mutagenesis of the nuclear version of ND4 we replaced the codon for arginine to that for histidine at amino acid 340. Injection of this construct into the mouse visual system disrupted mitochondrial cytoarchitecture, elevated reactive oxygen species, induced swelling of the optic nerve head and induced apoptosis, with a progressive demise of ganglion cells in the retina and their axons comprising the optic nerve. In contrast, ocular expression of the wild-type human ND4 subunit appeared safe, suggesting that it may be useful for treatment of patients with LHON. Here by using the scientific and clinical knowledge acquired to date, we will begin the journey towards genetic therapy for human optic neuropathy and mitochondrial disease.

In summary, LHON is a worthy target for mitochondrial gene therapy that we are proposing here by allotopic expression of a normal ND4 gene. While

other experimental approaches such as importing genes from other species, changing the ratio of heteroplasmy with specific restriction endonucleases, selecting for respiratory function or regeneration (in muscle), none of these techniques is directly applicable to the treatment of LHON caused by 100% mutated mtDNA. We propose to apply gene therapy to a human disease caused by mutated mtDNA. Success here may have implications well beyond these initially targeted patients and organ systems besides the eye.

rAAV Human Clinical Trials: General and Specific Experience

The NIH Genetic Modification Clinical Research Information System (GeMCRIS®) (http://www.gemcris.od.nih.gov/) currently lists 9 active clinical trial protocols involving rAAV gene transfer in a number of different diseases, including ophthalmic disorders. Administration of the vector is by a wide variety of different routes, such as aerosolized inhalation, intramuscular, intrahepatic, intracerebral and intraocular injections.

Specific background for this proposed protocol includes the human rAAV studies performed at the University of Florida. The University of Florida (UF) Powell Gene Therapy Center's core facilities and investigators have substantial experience in current Good Manufacturing Practice (cGMP) production of rAAV vectors and in the performance of Phase I clinical trials of rAAV vectors in humans. Among the first human trials with rAAV for any disease were those performed by T.R. Flotte, which began in November, 1995, with Dr. Flotte as PI at Johns Hopkins University and continued by the Hopkins group and the Flotte group (later at the University of Florida), beginning in July,1996. The several Phase I and II clinical studies (using rAAV2 instilled in nose or maxillary sinus or by aerosol administration), completed or in progress, in cystic fibrosis (CF) show no significant vector-related toxicity. These studies have included individuals from ages 12 to 43 years and they have ranged in severity of their underlying CF lung disease. Overall, vector administration to the respiratory tract has been very well-tolerated in these human subjects and there is clear evidence of the feasibility of gene transfer. There is a trend to indicate that the vector is biologically active for the correction of chloride secretion. Further relevant background to the current proposal is the human study at the University of Florida using a rAAV2-CB-hRPE65 vector with intraocular administration to clinical subjects [56]. The production and purification protocol of the cGMP vector for the proposed LHON clinical trial will be essentially identical to that used for the currently active Phase I trial of rAAV2-CB-hRPE65 vector with 2 differences. Here we used a self complementary AAV containing both positive and negative strands with 3 tyrosine to

phenylalanine modifications in the VP3 AAV capsid to increase the speed and efficiency of transgene expression. Unlike RPE 65 mutations causing Leber's congenital amaurosis that have slowly progressive visual loss since childhood, LHON presents with acute visual loss. Therefore, speed and efficiency of transgene delivery are critical in LHON.

A summary of findings from preclinical studies

Preclinical studies of **scAAV2-P1ND4v2** by intraocular delivery have included proof-of-principle studies in a mouse model, and safety studies to evaluate the potential for toxicity and the biodistribution of intravitreal rAAV-ND4 in normal rats and normal non-human primates.

Proof-of-Principle Studies:

To demonstrate the safety and efficacy of the gene therapy vector to be used in the Leber's hereditary optic neuropathy gene therapy (LHON) clinical trial, we modified the ND4 subunit of complex I in the nuclear genetic code for import into mitochondria. The protein was targeted into the organelle by agency of a targeting sequence. The gene was packaged into adenoassociated viral (AAV) vectors, then vitreally injected into rodents, primates and ex vivo human eyes that were tested for expression and integration by immunohistochemistry and blue native polyacrylamide gel electrophoresis. Animals were followed by serial fundus photography, OCT, multifocal or pattern ERGs. Rescue of visual loss was tested in rodent eyes also injected with a mutant G11778A ND4 homologue responsible for most cases of LHON. FLAG-tagged human ND4 expressed in almost all mouse retinal ganglion cells a week after injection and it integrated into the rodent complex I. Furthermore, in mouse eyes also injected with a mutant ND4, wild-type ND4 prevented defective ATP synthesis, suppressed visual loss, reduced apoptosis of retinal ganglion cells and prevented demise of axons in the optic nerve. ND4 injected into the ex vivo human eye expressed in most retinal ganglion cells. Primate(s) vitreally injected with untagged wild-type ND4 and followed for 3 months had no serious adverse reactions.

Thus, expression of our tagged ND4 vector in the ex vivo human eye, safety of the test article, rescue of the LHON mouse model and the severe irreversible loss of retinal ganglion cells in LHON support clinical testing in our patients with mutated G11778A mitochondrial DNA.

Safety Studies (Rodent):

A GLP compliant 1-month and 3-month dose based toxicity and biodistribution study of scAAV2-P1ND4v2 vector delivered by intravitreal injection into the eyes of normal rodents was performed at the University of Florida, Powell Gene Therapy Center Toxicology Core Facility.

The test article (vector) used in this study is the same as that planned for clinical investigation. The study consisted of 4 experimental groups. The animals were injected intravitreally with either Balanced Salt Solution (vehicle control group) or scAAV2-P1ND4v2 at a total dose of 6.56×10^6, 6.56×10^7 and 1.97×10^8 vector genomes. Each arm consisted of 20 animals, which included 10 males and 10 females, half were sacrificed 30 days post injection and the remaining half were sacrificed 90 days post injection (+/-3 days). The primary safety endpoint of this study was the histopathology examination and biodistribution determination of the Skeletal muscle (quadriceps), Diaphragm, Heart, Cerebrum, Lung, Spleen, Liver, Kidney, Pancreas, Gonads, Jejunum, Parotid Gland, Mesenteric Lymph node, Un-injected eye with optic nerve (PCR only) and Injected eye with optic nerve (histopathology only). Clinical assessments were also measured by CBC and serum chemistry analysis. Clinical observations, including body weights, were also performed.

Intravitreal administration of vector genomes was well tolerated. There were no gross or microscopic findings that were related to administration of the test article. Daily clinical observations and weekly body weight measurements showed no test article-related trends.

Minimal test-article related clinical chemistry changes were observed in male rats in all vector dose groups, which was characterized by transient increases in alkaline phosphatase activity on Day 30. No vector or treatment related trends were seen in the complete blood count analyses. Vector genomes were not present in any non-target tissues and blood.

Safety Studies (Primate)

A 3-month dose based toxicity and biodistribution study of scAAV2-P1ND4v2 vector delivered by intravitreal injection into the eyes of normal non-human primates was performed at the Oregon National Primate Research Center at Oregon Health & Science University.

The test article (vector) used in this study is the same as that planned for clinical investigation. Primates were injected intravitreally with either Balanced Salt Solution (vehicle control group) or scAAV2-P1ND4v2 at a total dose of 2.46×10^{10} vector genomes in one eye (group 1) or both eyes (group 2). Each group consisted of 3 animals. Primates were sacrificed 90-117 days post injection. The primary safety endpoint of this study was the histopathology examination and biodistribution determination of brain, heart, lung, liver, pancreas, kidney, spleen, jejunum, gonads, skeletal muscle (quadriceps), and lymph nodes (mandibular, mesenteric and tracheobronchial). Also evaluated by histopathology were eyes, colon, sternum and bone marrow. Also evaluated for biodistribution were ocular

anterior chamber fluid and vitreous; specific brain structures evaluated for biodistribution included optic chiasm, optic tract, lateral geniculate nucleus, superior colliculus, visual cortex (occipital lobe), thalamus and cerebellum.

All major organs and were negative (≤ 100 copies/ug genomic DNA) at the time of sacrifice, with the exceptions of low levels in the spleen of the 2 vector-injected animals in group 2 and in the mandibular lymph nodes of one animal in each group. At sacrifice, there were no gross or microscopic findings that were related to administration of the test article.

In vivo, ocular status was evaluated by retinal color fundus photography, retinal ocular coherence tomography (OCT) imaging and intraocular pressure measurements. Retinal function was also assessed by multifocal electroretinography. Clinical assessments included differential CBC and serum chemistry analysis a multiple time points. Clinical observations, including body weights, were also performed.

Intravitreal administration of vector genomes was well tolerated. Retinal evaluations by retinal photography and OCT showed no retinal abnormalities and only mild and transient post-injection vitreal changes in some cases. Multifocal electroretinography showed no treatment-related changes in retinal function.

No test-article related clinical chemistry changes were observed in monkeys in both groups, and no vector or treatment related trends were seen in the complete blood count analyses. Daily clinical observations detected no test article-related findings, and body weight measurements were stable or gradually increasing in all animals. Assessments of ocular health by imaging and by clinical electrophysiology showed no test article-related abnormalities.

Conclusions from Safety Studies

Studies in rats and non-human primates described above suggest that intravitreal administration of scAAV2-P1ND4v2 at doses of up to 2.46 x 10^{10} vector genomes:

(a) Do not lead to any acute or long term ocular pathology,

(b) Do not lead to any systemic toxicity by histopathological analysis of all major organs groups, and

(c) Do not lead to significant vector spread (migration) beyond the eye/optic nerve (target site) in the vector-injected eye (treatment).

All the safety data at hand shows no observed adverse effect level of up to 2.46 x 10^{10} vector genomes in primates. This suggests that translation of

scAAV2-P1ND4v2 to human trials should be safe. However, we plan to take a conservative approach to dosing in man by starting the clinical investigation with a vector dose equivalent to 1 log unit below this level – at 5.0 x 10^9 vector genomes.

Summary of the known and potential risks and benefits to human subjects

This clinical trial is the first human study of scAAV2-P1ND4v2 so the risks associated with vector administration are not precisely known. Based on our preclinical studies of scAAV2-P1ND4v2 in normal rats and normal non-human primates there is a high likelihood that intravitreal administration of scAAV2-P1ND4v2 will be safe in humans. Safety of rAAV2 vectors in humans is also supported by previous and on-going clinical trials. Most relevant are studies performed at the University of Florida's Powell Gene Therapy Center. These trials include several Phase I and II clinical studies in individuals with cystic fibrosis with alpha-1-antitrypsin deficiency, with RPE65-associated retinal disease. Overall, to date, results indicate no significant vector-related toxicity and demonstrate the feasibility of gene transfer. Potential risks associated with scAAV2-P1ND4v2 and the study procedures are discussed below.

Specifically associated with intravitreal-injections of scAAV2-P1ND4v2, there is a risk of degeneration of lens and cataract formation in the injected eye. Detachment of the retina is also possible.

There is a potential risk of vector spread to other organs. In our safety studies, all major organs of rats and primates were essentially negative (≤ 100 copies/ug genomic DNA) for vector. Our biodistribution results after scAAV2-P1ND4v2 (intravitreal) administration to rats and non-human primates indicate only limited vector spread outside the injected eye and no evidence of a vector dose-related effect. The risks of vector spread and germline transmission (all gonad samples were negative) are therefore presumed to be low in the planned human trial.

There is a risk that subjects may develop an immune response to the vector. Our neutralizing antibody assay data from scAAV2-P1ND4v2 administration in large animals suggests that the risk of adverse immune response in humans is minimal. It is possible, but unlikely, that scAAV2-P1ND4v2 could interact with other viruses with which the subject comes in contact, forming a new virus that could produce new side effects.

In preclinical studies, there was minimal ocular inflammation which subsided with time after administration; in one animal of the non-human primate studies. Administration of corticosteroids and antibiotics

following intravitreal injection of the study agent is planned for this clinical trial to minimize the risk of local inflammation. There is potential that vision of subjects could be further reduced. Based on preclinical studies in animal models and in normal animals, the risk of further visual impairment due to scAAV2-P1ND4v2 is believed to be low.

There is a chance that the study agent could damage the DNA in the cells of a subject's retina. In the unlikely event that this occurred, it could put the subject at risk for developing retinal tumors in the future. In animal and human studies to date, cancer has not developed and therefore, the risk is considered to be low. In our preclinical work with the AAV vector, the majority of vector DNA appears to persist as episomal rather than integrated into genomic DNA, which makes it very unlikely that mutagenesis and tumor development will occur.

Intravitreal administration of scAAV2-P1ND4v2, as mentioned above, carries a risk of retinal cell loss from administration trauma. There is also a risk of cataract development that could require additional surgery. There is a risk of retinal tears or retinal detachment and there is the possibility that these could lead to worse vision. Exogenous intraocular infection (endophthalmitis) is rare but can occur. The most common pathogens can be treated but poor vision (in patients with better vision than those entering this study) can result from infection despite successful antibiotic treatment. Intraocular hemorrhage is rare in patients without bleeding diathesis. Vitreous hemorrhage is benign and usually resolves spontaneously. Choroidal or subretinal hemorrhage is usually self-limited and could affect vision (or potential vision) significantly. Temporary increases in intraocular pressure can occur following ocular/retinal injections and are treatable with topical or systemic medications to lower eye pressure. Risk of anesthesia, which is local (retrobulbar) is low. There is a small risk of penetration of the eye or optic nerve from retrobulbar injection and also the possibility of hemorrhage, which can be decompressed if necessary Patients have complained of feeling a burning or painful sensation in the injected eye after the anesthesia wears off. In addition to these risks, there may be other longer-term risks which are not known.

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Benefits: Due to the initial, safety establishing nature of this investigation, there is no anticipated benefit to the subjects in this trial.

Description of and Justification for the Route of Administration, Dosage, Dosage Regimen, and Treatment Period(s):

The route of administration, dosage, dosage regimen, and treatment period(s) proposed in this protocol is based on several considerations. The preclinical studies have been performed with the clinical vector. Only single injections have been made across the rodent and primate studies with a few exceptions. The dose delivered has varied as different parameters such as transfection efficiency and toxicity were explored.

Using naïve rodents we found that injections into uninjured ocular tissue are well tolerated. Doses of up to 1.97×10^8 vector genomes showed no observable effects of toxicity. There were no gross or microscopic findings that were related to administration of the vector. Daily clinical observations and weekly body weight measurements showed no vector-related trends. Minimal test-article related clinical chemistry changes were observed in male rats in all vector dose groups, which was characterized by transient increases in alkaline phosphatase activity on Day 30. No vector or treatment related trends were seen in the complete blood count analyses. Vector genomes were not present in any non-target tissues and blood.

In normal non-human primates, observed for up to 3-4 months post treatment, intravitreal administration of vector genomes (of up to 2.46×10^{10} vector genomes) in one or both eyes was well tolerated. No ocular or retinal abnormalities were observed and only mild and transient post-injection vitreal changes were observed. No vector related clinical chemistry changes were observed, and no vector or treatment related trends were seen in the complete blood count analyses. Daily clinical observations detected no vector-related findings, and body weight measurements were stable or gradually increasing in all animals. Assessments of ocular health by imaging and by clinical electrophysiology showed no vector-related abnormalities. At sacrifice, there were no gross or microscopic findings that were related to administration of the vector. Vector genomes were not present in any non-target tissues, with the exceptions of low levels in spleen of the 2 vector-injected animals and in the mandibular lymph nodes of two other animals.

The frequent study visits and safety assessments in year's 1-3 post-injection and the long-term follow-up will assure substantial opportunity for observation for adverse events and efficacy signals.

Use of Adeno-associated Virus (AAV) and Recombinant AAV

AAV is a parvovirus with a 4.7 kb single-stranded DNA genome. It was discovered as a laboratory contaminant of adenovirus cultures and was

subsequently found to require adenovirus or another helper virus to replicate under most circumstances. At least 9 AAV serotypes (1-9) have been isolated and cloned currently. None of the AAV serotypes has been associated with any human disease. AAV serotype 2 binds to cells via a heparin sulfate proteoglycan receptor. Once attached, AAV entry is dependent upon the presence of a co-receptor, which may consist of either the fibroblast growth factor receptor (FGF-R) or the α_v-β_5 integrin molecule.

The AAV life cycle is quite unusual. Cells infected with AAV and a helper virus (or another adjunctive agent, such as UV irradiation or treatment with genotoxic agents) will undergo productive replication of AAV prior to cell lysis, which is induced by the helper rather than by AAV. Human cells infected with AAV alone, however, will become persistently infected. This latency pathway results in colinear integration of AAV sequences within the host cell genome, within a specific site on human chromosome 19, the AAVS1 site. While this site is not strictly homologous to AAV, it contains consensus elements required for binding and nicking by the AAV Rep protein, a non-structural protein that is also involved in productive replication and in transcriptional regulation of the virus. Once AAV is integrated, it will remain stable within infected cells for prolonged periods of time, up to 100 passages. Episomal forms of the virus may also be present for extended periods in some circumstances. If latently infected cells are subsequently infected with a helper virus, the genome will be excised and productive AAV replication and packaging will ensue.

Recombinant AAV (rAAV) vectors have been developed by replacement of the viral coding sequences with a transgene of interest. The ITR sequences must be retained in rAAV since these serve as origins for viral DNA replication and contain the packaging signals. The maximum packaging capacity of rAAV is approximately 5kb, including the ITRs, the transgene, its promoter, and polyadenylation signal. The full exploitation of rAAV for gene transfer has been limited in the past primarily by the packaging and purification process. In particular, contamination of rAAV vector preparations with wild-type AAV has been found to alter the biological behavior of the vector, and limitations on the titers and infectivity of the vectors have limited their widespread use in the past. Advances in the packaging and purification technology have resulted in a dramatic improvement in the expression levels that have been achievable in vivo.

rAAV vectors are uniquely suitable for in vivo gene transfer for genetic and metabolic disorders, since they are non-toxic, highly efficient when used at high titers, relatively non-immunogenic, and very stable in their pattern of expression. The utility of rAAV vectors for in vitro and in vivo gene

transfer has now been well established. There appear to be important tissue specific differences in rAAV effects. However, rAAV vectors have been found to be particularly efficient for gene transfer into terminally differentiated cells such as neurons, myofibers, and photoreceptor cells. Gene transfer to the bronchial epithelium has also been demonstrated as has rAAV transduction of hepatocytes. Very high dosages of rAAV-CB-hAAT (alpha 1 antitrypsin) vectors given via the portal vein to adult mice or via superficial temporal vein to neonatal mice will result in long-term expression of high levels of hAAT. In fact, a single dose of $3x10^{11}$i.u. of rAAV2-CB-AAT vector given intraportal to young adult mice resulted in expression levels in excess of 2 mg/ml (well above the threshold for therapeutic effect of 0.5 to 0.8mg/ml) that were sustained for over 1 year without any detectable pathology. This same study showed that rAAV persisted primarily as high MW episomes and was not associated with tumorigenesis.

We have shown that intravitreal injections of AAV serotype 2 efficiently transduces the cell type, retinal ganglion cells affected in LHON (Koilkonda et al 2014), that wild-type ND4 does not lead to ocular toxicity. In fact it prevents visual, RGC and optic nerve axonal loss due to the effects of an AAV carrying the mutant ND4 allele}

Our group has performed specific preclinical toxicology and biodistribution studies with intravitreal scAAV2-P1ND4V2.

5) **Inclusion and Exclusion Criteria***

Subjects with LHON with the G11778A mutation confirmed by genetic testing during screening evaluation will be classified into three (3) groups based on long-standing (chronic), recent onset (acute bilateral) or acute unilateral (presymptomatic eye) disease manifestation.

The study population will include , males and females 15 years old or older diagnosed with G11778A-associated Leber's Hereditary Optic Neuropathy.

Inclusion Criteria

1. Age15 or older
2. Patients with LHON and the G11778A mitochondrial DNA mutation. A previous CLIA certified genetic lab result showing the LHON G11778A mutation will be accepted for inclusion;
3. Ability to perform tests of visual and retinal function;
4. Ability to comply with research procedures;
5. Able and willing to provide informed consent before undergoing any study related procedures.

6. Good general health as based on the investigator's assessment of the history, physical examination and laboratory testing performed at the baseline examination.

Exclusion Criteria

1. Unwilling or unable to give consent,
2. Unable or unlikely to return for scheduled protocol visits
3. Pregnant or nursing women or unwillingness for subject with childbearing potential to use contraception during the first year of the study.
4. Optic disc drusen on exam or in previous history.
5. Ocular diseases or visual dysfunction conditions other than refractive error (e.g. amblyopia, glaucoma, etc.) in the eye selected for the injection.
6. Previous eye surgery in the eye selected for injection.
7. Aspartate transaminase (AST)/alanine transaminase (ALT) >5.0 x upper limit of normal (ULN); Total bilirubin >3 x ULN; Hemoglobin < 8 g/dL; neutrophil count <1.0 x 109/L; or platelet count < 50 x 109/L

a) Any laboratory screening test that meets the abnormality criteria stated above can be repeated once between Baseline one to Baseline 2.

8. Type I diabetes or the presence of diabetic retinopathy
9. History of neurodegenerative conditions (e.g. multiple sclerosis, neuromyelitis optica, Parkinson disease)
10. History of autoimmune conditions (e.g. systemic lupus erythematosus)
11. Systemic diseases having ocular manifestations likely to confound assessment of study resultsHistory of cancer within five years other than localized basal or squamous cell carcinoma not near the orbital area. Patients with a prior history of cancer will need documentation from their cancer specialist that the cancer was cured at least 5 years before study entry.
12. Allergy to pupil dilating drops or narrow angles precluding safe dilation.
13. No Light Perception (NLP) vision in either eye.

, "No special populations are to be included in this research, such as children 14 years old or younger, prisoners, or pregnant women.

- **Exclude:** All subjects who are unable to consent
- **Exclude:** Children 14 years old or younger
- **Exclude:** Pregnant women

- **Exclude:** Prisoners

6) **Number of Subjects***

Number of Study Centers: One (1), Bascom Palmer Eye Institute, University of Miami, Miami, FL.

For each group patient recruitment will consist of 3 cohorts, until the last cohort which will consist of both Group I and III individuals.

Table 1 Proposed Sample Size

	Low-dose ($1.18x10^9$ vg)*	Medium dose ($5.81x10^9$ vg)*	High dose ($2.40x10^{10}$ vg)	Higher dose ($1x10^{11}$vg)
Group I	3	3	3	
Group II	3	3		3
Group III	3	3		
Group I And** Group III				3 3

*=based on CHOP titers as recommended by FDA

As summarized above, dose escalation will follow the adaptive Phase 1 plan of Rubinstein and Simon (http://linus.nci.nih.gov/techreport/phaseIctd.pdf). At each dose, escalation will occur until 2 of 3 (or 6) injected eyes manifest the safety endpoint. (See reference for detailed description of dose escalation strategy) Table 2 of the reference contains operating characteristics of this approach. Following the algorithm of Rubinstein and Simon with three doses, the sample size could range between 3 and 18 for each group. As an example, if the true probabilities of noteworthy safety events in the proposed three doses were 0.05, 0.25, 0.5, respectively, the most likely sample sizes for each group would range between N=9 with probability 20%, N=12 with probability 54%, or N=15, with probability 22%. These sample sizes could potentially be sufficient to observe a treatment effect when compared in a matched fashion to the uninjected eyes in groups 1 and 3.

**Escalation from high dose group I to higher dose group II was previously approved. Since chronic bilateral group I individuals are considered at lower risk than group II, there are no additional safety concerns to injecting group I intermixed with group III individuals, after the 3rd group II injection and 3 month safety period. In this combined cohort, each of the 6 injections will be spaced at least 6 weeks apart. Ordering of participants will be determined by their presentation to the study and PI discretion, with the aim of ensuring injection of the more difficult to recruit group III individuals.

7) **Study-Wide Recruitment Methods* N/A for Multi-center trial**

Number of Study Centers: One (1), Bascom Palmer Eye Institute, University of Miami, Miami, FL.

Phase I. A review of the LHON patients currently being seen by [redacted] and [redacted] with long-standing severe vision loss of 20/200 or worse, shows that we have 23 patients who would be eligible for the Phase 1 study in Groups 1 and 2. We expect that the availability of a potential treatment will draw more patients in groups 2 and 3. Thus, we should have a sizable pool of patients for the Phase 1 safety study of the AAV-ND4 vector.

Individuals who have been diagnosed with G11778A mutation associated Leber's hereditary Optic Neuropathy and who are eligible based on inclusion and exclusion criteria will be invited to participate in this clinical trial. A complete physical examination and clinical chemistries will be performed at a pre-treatment, screening evaluation to further determine eligibility. Subjects will be considered enrolled when they sign the Informed Consent.

Enrollment Procedures:

Eligibility will be assessed at a study team meeting prior to the baseline 2 exam. This meeting must include at least one MD coinvestigator in addition to the PI. Other participants may include clinical staff, biostatistics center staff, and the CRORS office. Minutes will be recorded during these meetings and a sign in sheet will be kept. If the injecting retina specialist is unable to attend the assessment meeting, they will be asked to review and sign off on the eligibility criteria checklist prior to the injection. If the patient meets all inclusion and exclusion criteria a request will be forwarded to the research pharmacist for Study Drug dilution.

8) **Study Timelines***

- The duration of an individual subject's participation in the study**:** 10 years
- The duration anticipated to enroll all study subjects**:** 3 years
- The estimated date for the investigators to complete this study (complete primary analyses): 5 years after first patient has received administration of study agent.

9) Study Endpoints*

Assessment of Primary Endpoint – Toxicity

The **primary safety endpoint** in this trial is the standard ocular examination. Toxicity will also be assessed by measurement of vision, hematology and serum chemistries, assays for vector genomes, reported subject history of any symptoms and adverse events.

Ocular. Routine eye examinations will be performed before and after vector administration, and surgeons administering the injections will be alert to injection administration related complications. The clinical ocular examinations for toxicity will include slit lamp examination, measurements of intraocular pressure (applanation tonometry or Tono-pen) and gonioscopy (as needed in cases of increased intraocular pressure to examine for anterior chamber abnormalities). Some potential ocular toxicities are included in Table 6-6 below. These are modifications of the Ocular/Visual Adverse Events tables of the Common Terminology Criteria for Adverse Events v3.0 (CTCAE), considering the LHON patient population and the circumstances of the intervention. Direct and indirect ophthalmoscopy and biomicroscopy will be the mainstay of the retinal examinations. Fundus photography (with infrared illumination) will also be taken to document fundus appearance.

Systemic. Physical examination at regularly-scheduled time points will be performed by a medical practitioner to allow detection of and response to any acute symptoms. Complete blood count with differential, prothrombin time (with INR), partial thromboplastin time, serum chemistries, including measures of hepatocellular integrity and of renal function (including BUN and creatinine), and a urinalysis will be performed at baseline visit (serving also as a pre-injection evaluation) and on post-treatment evaluations. This testing will help detect toxicity in a number of organ systems. For research purposes measurements of neutralizing antibodies to human ND4 and AAV capsid components and cellular assays will be performed prior to and after vector administration as markers of the immune response. At each contact with the subject, information will be obtained on adverse events by specific questioning. Systemic toxicity will be graded based on the scale included in Table 6-6 and 6-7.

Table 6-6: Ocular toxicity scale

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Keratitis (corneal inflammation / corneal ulceration)	Abnormal ophthalmologic changes only; intervention not indicated	Symptomatic; medical intervention indicated	Symptomatic; surgical intervention indicated	Perforation or blindness (worse than baseline visual function)*
Glaucoma	Elevated intraocular pressure (EIOP) warranting topical agent for intervention	EIOP requiring topical agents and oral agents for intervention	EIOP warranting operative intervention	EIOP uncontrolled, resulting in blindness (worse than baseline visual function)*
Cataract	Mild change from baseline, detected on ophthalmologic exam; not warranting intervention	Moderate change from baseline, detected on exam; not warranting intervention	Severe change from baseline, detected on exam; not warranting intervention	Severe change warranting operative intervention*
Uveitis	Mild anterior uveitis not warranting intervention	Moderate anterior uveitis warranting medical intervention	Severe posterior or pan-uveitis warranting medical intervention	Severe uveitis warranting operative intervention and threatening ocular integrity*
Vitreous hemorrhage	Mild hemorrhage detected on ophthalmologic exam; not warranting intervention	Moderate hemorrhage not warranting intervention	Severe hemorrhage warranting vitrectomy	Uncontrolled vitreous hemorrhage, threatening ocular integrity

Table 6-6: Ocular toxicity scale

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Retinal detachment	Localized and intervention not indicated	Not resolving but intervention not yet indicated	Operative intervention indicated	Operative failure threatening ocular integrity*

*

For group III, due to the natural history of LHON where visual loss is expected in the asymptomatic eye, a vision-related SAE will be considered a decrease in acuity in the injected eye to 0.3 logMAR units (15-letter equivalent) worse than the worst acuity measured in the uninjected fellow eye at the current or any prior study visit.

Table 6-7: Systemic toxicity scale

Grade	Definition
1	Mild toxicity, usually transient, requiring no special treatment and generally not interfering with usual daily activities.
2	Moderate toxicity which may be ameliorated by simple therapeutic maneuvers, and impairs usual activities.
3	Severe toxicity which requires therapeutic intervention and interrupts usual activities. Hospitalization may or may not be required.
4	Life-threatening toxicity which requires hospitalization.

Evaluation of Secondary Endpoint – Visual Function Changes

Visual function will be quantified prior to and after vector administration in order to determine whether vector administration affects visual

function. The main measure of visual function will include best corrected ETDRS visual acuity.

10) Procedures Involved*

Method of Assigning Subjects to Treatment Groups:

Subjects enrolled in the study will be assigned to disease groups based on disease manifestation and screening evaluation. All subjects in the study will receive active treatment (one injection of scAAV2-P1ND4v2) at the Low, Medium or High dose as explained in section 6, Number of Subjects.

Study Visits:

Table 6-4 outlines the schedule of planned study evaluations. Subjects will be enrolled and undergo baseline assessments prior to Day 0. Study agent administration which will occur on Day 0 and details of the procedure are included below.

Table 6-4 Study Visit Summary Procedures

Time point	Baseline		Day									Year			
	B1	B2	0 ▼	1	7	30	60	90	180	270	365	1.5	2	3	4 -10
Informed consent/assent	■														
Blood test for G11778A mutation	■														
History and physical exam	■			■		■		■			■				
Electrocardiogram, chest X-ray	■														
Pregnancy test	■	■													
Vital signs monitoring		■	■	■											
Hematology, chemistry, urinalysis	■		■	■	■	■		■			■		■	■	
Coagulation Panel		■													
AAV antibody and specific reactivity (ASR) measurement	■				■			■			■				
Peripheral blood PCR	■+			■+	■+										
Visual Acuity	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
Perimetry	■	■		■	■	■	■	■	■	■	■	■	■	■	
PERG	■	■		■	■	■	■	■	■	■	■	■	■	■	
Neuro-ophthalmic Exam	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
OCT Imaging	■	■			■		■				■		■	■	

Fundus photography	■			■	■	■	■				■		■	■			
Quality of life questions (NEI VFQ-25)	■						■		■		■						
Adverse event recording	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		
Long-term follow-up															■	■	

Baseline visits are no more than 1 month before day 0, date of vector administration;

[+] testing will continue until two consecutive samples test negative

Detailed Study Procedures

Informed Consent

The use of the informed consent process will ensure that subjects are adequately informed about the study procedures and associated risks and benefits. An investigator will also provide complete explanation of the study to potential participants and will ensure that all questions are answered prior to decisions about participation. Participation in this clinical trial is completely voluntary and great care will be taken to ensure that individuals do not feel coerced to participate. Subjects will be able to terminate their participation at any time without prejudice. In order to assess the voluntary nature of participation in the study, a decision monitor will speak with all participants prior to beginning the study procedures.

Complete History

A detailed medical history will be obtained from each subject during the first baseline visit to ensure that exclusion from participation is not indicated.

Physical Examination

During baseline and post-vector administration evaluations, physical examinations will be performed. These exams will assess occurrence of systemic adverse events.

Electrocardiogram, Chest X-ray

12-lead electrocardiogram and chest X-ray will be performed at a baseline visit as part of the pre-vector administration evaluation.

Hematology, Chemistries, Urinalysis, Coagulation Panel

Complete blood count, with platelet count, serum chemistries including BUN, creatinine, electrolytes, liver function and coagulation panel tests

will be obtained. Blood samples will be collected at baseline visit (serving also as a pre-vector administration evaluation) and during post-vector administration evaluations. This testing will help detect toxicity in a number of organ systems. In order to be eligible to participate, all women of childbearing potential must have two negative pregnancy tests within the month prior to study agent administration.

Vital Signs Monitoring

Following vector administration, subjects will be closely observed and vital signs (e.g. blood pressure, pulse, respiration, and temperature) will be monitored. Subjects will be discharged after IOP is confirmed to be less than 30 mm.

AAV Antibody and Antigen-specific Reactivity (ASR) Measurement (For Research Only)

Measurements of neutralizing antibody titers to AAV2 capsid components and ASR assays will be performed at a baseline visit and during post-vector administration evaluations as markers of any systemic immune response to vector administration.

Human ND4 Antibody (For Information Only)

Currently, there is no human ND4 antibody available for detection of transgene expression. In preclinical studies, we used the FLAG tagged ND4 for immunodetection. However, FLAG is not included in the clinical vector. Therefore, on a For Information Only basis, detection of antibodies against the human ND4 transgene will be attempted at a baseline visit and during post-vector administration evaluations as markers of a systemic immune response to the transgene ND4. Serum will be reacted against Western blots of human mitochondrial proteins before and after administration of scAAV2-P1ND4v2. Bands within this size range of P1ND4v2 (approximately 50 kilodaltons) that develop post treatment will be excised and submitted for identification by mass spectroscopy to determine if they are against human ND4.

Peripheral Blood Q-PCR (For Research Only)

To detect vector spread, peripheral blood analysis for vector DNA by Q-PCR will be performed at a baseline visit and following vector administration. If positive (>100 vg) testing will continue until two consecutive samples test negative.

Visual Acuity

Visual acuity is an important outcome variable. Visual acuity is measured before pupil dilation or any other technique that could affect vision.

Refraction is performed prior to formal measurement of ETDRS visual acuity testing at all visits.

Subjective Refraction:

The use of a phoropter or trial frame is permitted to determine best-corrected visual acuity. The left eye is occluded first. An approximate beginning refraction may be determined by retinoscopy, automated refraction, or a subjective refraction from a prior visit. The sphere is refined first. The cylinder is then refined, first the axis followed by the power. Finally, the sphere is rechecked. The right eye is then occluded, and the procedure is repeated for the left eye.

If the patient wears contact lenses and has glasses also, he or she is instructed not to wear the contact lenses on the day of the examination. In the event that the patient either has no glasses or has forgotten the instructions and reported for the assessment wearing contact lenses, the contact lenses are removed and at least thirty minutes allowed to elapse before subjective refraction and visual acuity testing is performed.

ETDRS Visual Acuity:

The logmar visual acuity testing has been adapted from the Early Treatment of Diabetic Retinopathy (ETDRS). The logmar visual acuity scale facilitates statistical analysis and simplifies quantification of acuity at various distances. The right eye is tested with ETDRS logmar chart 1, and then the left eye is tested with ETDRS logmar chart 2. Each chart is hidden from view until the eye being examined is ready for testing.

The room illumination should be at a level of 50 to 100 foot candles, and between 50 and 125 foot candles should illuminate the ETDRS visual acuity chart. The distance from the patient's eye to the visual acuity chart is exactly 4.0 meters. The patient may sit or stand, but he or she is not allowed to lean forward or backward so a constant testing distance is maintained. After proper instruction, refraction, and placement of the appropriate lenses in a trial frame, the left eye is occluded and testing is begun with the right eye. The patient is instructed that the chart has letters only and no numbers. If the patient forgets this information and reads a number, he or she is reminded that the chart contains only letters and the examiner requests a letter in lieu of the number. Each letter that is identified correctly is circled on the ETDRS Visual Acuity Worksheet. The patient is advised to read slowly, so as to achieve the best identification of each letter. When the patient says he or she cannot read a letter, he or she is encouraged to guess. The patient should be encouraged to fix eccentrically if this improves the visual acuity, but care must be taken to ensure that the fellow eye remains covered.

Eyes reading fewer than 20 letters correctly at a test distance of 4.0 meters are tested at 1.0 meter. Before testing at 1.0 meter, +0.75 sphere is added to the 4.0 meter correction already in the trial frame to compensate for the closer testing distance. The patient is asked to read only the first six lines at 1.0 meter, so the maximum score attainable at that distance is 30. Correctly identified letters are circled on the ETDRS Visual Acuity Worksheet. If the patient's visual acuity is so poor that he or she cannot read the largest letter at 1.0 meter, assess his or her ability to count fingers. After testing of the right eye is completed, chart 1 is replaced by chart 2 and the procedure is repeated for the left eye.

Each letter read correctly and circled on the ETDRS Visual Acuity Worksheet is scored as one point. The score for each line (which is zero if no letters were read correctly) and the total score is recorded after testing is completed. If testing at 1.0 meter is not required (i.e. 20 or more letters were seen with testing at 4.0 meters), 30 points are automatically scored for the 1.0 meter test. The total score, equaling the sum of the 4.0 meter and 1.0 meter scores, is recorded on the data form.

Testing for Finger Counting:

After proper instruction and refraction, the examiner's hand is viewed at a distance of two feet from the patient's eye. The fellow eye is closed and completely occluded by the palm of the patient's or assistant's hand. The examiner presents a random number of fingers to the patient. The patient is asked to indicate the number of fingers seen. If the number of fingers shown is correctly identified on four or more of five presentations, vision is recorded as count fingers. If the number of fingers presented cannot be identified on four or more of five presentations, test for hand motions.

Testing for Hand Motions:

In testing for hand motion, the examiner's hand is viewed with all fingers extended and separated at a distance of two feet from the patient's eye. The fellow eye is closed and completely occluded by the palm of the patient's or assistant's hand. The patient's glasses are not to be worn. The examiner's hand is presented in a random order under three conditions: stationary, moving back and forth horizontally, and moving up and down vertically. The speed of movement is approximately one complete cycle of movement (up and down or back and forth) per second. The patient is instructed that the examiner's hand will be presented in one of these conditions. He or she is asked to respond to the question, "what is my hand doing now?" with either, "still", "back and forth", or "up and down". The process is repeated five times. It is considered a correct response if the patient states the hand is still or he or she cannot see it while it is

stationary, and he or she is able to recognize movement and identify its direction. If hand motions are correctly identified on four or more of five presentations, vision is recorded as hand motions. If hand motions cannot be identified on four or more of five presentations, test for light perception.

Testing for Light Perception:

Light perception is tested using the same complete occlusion of the fellow eye with no other bright lights visible from the patient's position. The patient's glasses are not worn. The light of an indirect ophthalmoscope is directed into the eye from a distance of 2 feet for one or two seconds, and then turned away. The patient is asked to report "on" when he or she sees the light, and "off" when it disappears. The process is repeated five times in a nonrhythmic fashion. The visual acuity is recorded as light perception if the patient responds correctly four or more out of five times.

Perimetry

Visual field assessment is an important outcome measure. Quantitative automated perimetry is performed using the Humphrey Field Analyzer. Visual field testing is performed before any technique that could affect vision. A visual field should be attempted in any eye that has sufficient vision to permit finger counting at two feet. Eyes with poor central vision may have an intact, off-center island of vision which may be measured with perimetry.

A 30-2 threshold test is performed in all patients using a size III white stimulus. Visual field testing is performed with the standard Swedish Interactive Thresholding Algorithm (SITA) threshold strategy; the same testing strategy must be used throughout the duration of the study. The pupil diameter should be 3 mm or greater before visual field testing is undertaken, and this may require pharmacologic dilation. Standardized refraction is performed to determine the patient's distance refraction and best-corrected visual acuity prior to visual field testing. The age appropriate plus lens is added to the distance refraction. Patient education is provided, and the instrument is set up for the test. The technician should monitor fixation and patient responses during testing. Two HVF 30-2 SITA STANDARD will be done at baseline with a minimum of 30 minutes to 4 days between tests.

Pattern Electroretinography (PERG)

The pattern electroretinogram (PERG) is a special kind of ERG that is generated when a high-contrast patterned stimulus (alternating stripes or checkerboards) is viewed on a TV monitor. The primary generators of the PERG are retinal ganglion cell (RGC), whereas the traditional flash ERG is

generated in the outer retina. The PERG — but not the ERG — is altered in conditions that impair RGC function, and is abolished when RGC are degenerated. Therefore, the PERG has been extensively employed for detection and monitoring of RGC function in glaucoma and optic nerve diseases. Recently, a user-friendly protocol for PERG recording (PERGLA) has been developed at the Bascom Palmer Eye Institute, and it has been implemented in a commercially available instrument (Lace Elettronica, Italy). As this protocol uses skin electrodes, is fast and reproducible, it has been rapidly adopted by many investigators for longitudinal studies — including LHON — and for testing the effect of treatments. In this proposal we will use the PERGLA protocol.

A small patch (approximately 1 cm^2) of the skin of the central forehead, of the temples, and lower eyelids is gently cleansed with electrode skin preoperative preparation pads (containing 70% isopropyl alcohol and pumice) to reduce skin impedance at less than 5000 Ohm. Five skin electrode cups (9 mm diameter) are filled with conductive jelly and taped (surgical tape, 1.5 × 1.5 cm) over the cleansed skin. The central forehead electrode is placed 3 cm above the bridge of the nose. The temporal electrodes are placed 3 cm lateral to the lateral canthus. For lower-eyelid electrodes, the upper margin of the electrode is kept 5 mm below eyelashes. The subject leans his or her chin and forehead over a chin rest placed in front of a television display, placed at 30 cm distance from the subject's eyes. To record the PERG, the subject fixates with both eyes on a target at the center of the pattern display. The television display is contained within a Ganzfeld bowl to provide a fixed amount of background adaptation (4 cd/m2). The pupils are undilated.

Neuro-ophthalmic Examination

Ocular Motility:

The range of duction of each eye is assessment in the 9 cardinal gazes. Alternate cover test is used to detect ocular misalignment in the primary gaze if no duction defects are detected. If duction is impaired in one or both eyes, alternate cover test is also performed in the cardinal gazes.

Measurements of Intraocular Pressure:

Measurement of intraocular pressure will be done by either the Tono-pen instrument or by Goldmann applanation. The Tono-pen has a self-calibration program. If it fails to calibrate another instrument that does will be used. The calibration of the Goldmann applanation tonometer is checked every 3 months, as described in the Haag-Streit Goldmann Applanation Tonometer Operator's Manual. The intraocular pressure of the right eye is always tested first. Following instillation of a drop of 0.5%

proparacaine, a fluorescein strip is placed near the lateral canthus in the lower conjunctival sac. Once the lacrimal fluid has been sufficiently colored, the fluorescein strip is removed. Alternatively, one drop of premixed fluorescein and anesthetic may be instilled. The patient's head is properly positioned in the chin rest and against the forehead rest without leaning forward or straining. Any tight-fitting neckwear is loosened. The patient is asked to look straight ahead at a distant object or fixation target. If it is necessary to hold the eyelids open, the investigator holds the eyelids open against the orbital rim taking care not to apply any pressure on the globe. The patient is instructed not to hold his or her breath. If corneal astigmatism is greater than 3.0 diopters, the prism is rotated so that the axis of the minus cylinder on the prism graduation corresponds to the red mark on the prism holder. The investigator looks through the slit lamp and gently brings the tip of the prism in contact with the center of the cornea. The mires should be well focused, centered horizontally, and positioned vertically so that they are of equal circumference above and below the horizontal dividing line. If the mires are narrower than approximately one tenth their diameter, the investigator instills additional fluorescein. The investigator adjusts the measuring drum until the inner borders of the two mires just touch each other. If pulsation is present, the measuring drum is adjusted until the mires separate a given distance during systole and overlap the same distance during diastole. The investigator removes the prism from the cornea and records the measurement. After testing of the right eye is complete, testing of the left eye follows the same technique.

Biomicroscopy

Examination of the anterior segment using slit lamp biomicroscopy is performed at the Qualifying Assessment to document the status of the eye before therapy, and at all follow-up examinations to detect any changes in ocular status during the course of the study which may be attributable to the disease or treatment. Slit lamp biomicroscopy may be performed with any commercially available instrument, and it is used in a standard fashion starting anteriorly and working posteriorly. Standardizing subjective grading of lenticular opacities is difficult. However, it is expected that subjective grading by each investigator is relatively reproducible.

Lids:

The lids are examined for signs of blepharitis, including scales and crusting of the lashes, inspissated Meibomian glands, and erythema of the lid margin.

Conjunctiva:

The conjunctiva is examined for scarring from prior trauma or cicatrizing disease (e.g. Stevens Johnson syndrome, ocular pemphigoid). The presence and extent of conjunctival scarring may be evaluated using a cotton tipped applicator to test the mobility of the conjunctiva.

Cornea:

The cornea is examined to evaluate the epithelium, stroma, and endothelium. The techniques of diffuse illumination, scleral scatter, and retroillumination may be used. The presence of corneal epithelial or stromal edema is noted.

Anterior Chamber:

Before fluorescein instillation or pupillary dilation, the degree of anterior chamber cell and flare is determined. Eyes with chronic or recurrent uveitis are excluded from the study. Careful assessment of the anterior chamber depth is made after therapy. If the anterior chamber is shallow, the central anterior chamber depth is measured relative to the corneal thickness. The appropriate gradation of $\geq$ 3 CT, $\geq$ 2 CT, $\geq$ 1 CT, < 1 CT, or lens-cornea touch is documented.

Iris:

Before pupillary dilation, the pupillary iris is examined at high magnification for the presence of neovascularization. If rubeosis iridis is present, the eye is ineligible for the study.

Lens:

After pupillary dilation, the investigator assesses the lens and grades any cataract present as mild, moderate, or severe.

Fundoscopic Examination:

After pupil dilation with appropriate mydriatics, the vitreous will be assessed for determination of cell and flare and posterior vitreous detachment. The optic nerve and posterior pole are examined at the slit lamp using a Volk 90 diopter, 78 diopter, or 60 diopter lens. A head-mounted indirect ophthalmoscope and hand held condensing lens (20 diopter or 28 diopter Nikon aspheric lens) is used to evaluate the retinal periphery.

Ocular Coherence Tomography (OCT)

Optical Coherence Tomography (OCT) is a noninvasive imaging technique that can be used to measure the thickness of retinal nerve fiber layer (RNFL). The pupils should be dilated to help insure optimal quality scans. OCT should be performed after all other visual function testing. A spectral-domain Cirrus HD-OCT instrument (Carl Zeiss Meditec, Dublin, CA) will be

used to obtain circular peripapillary scans (Fast RNFL protocol). This will include three 3.4 mm diameter retinal scans that will be averaged to provide the RNFL thickness at 256 points spaced along the circumference of the circular scan in each eye after dilation. The software provided with the instrument calculates average NFL thickness and compares it to a database of age-matched control subjects, for patients 15 years of age or older giving a percentile score relative to this control database. The average and sector (divided into nasal, temporal, inferior, and superior quadrants) measurements obtained for each patient's affected and unaffected eye will be considered abnormal if ranked below the 5th percentile. OCT testing will be performed by a trained technician. Macular OCT will be performed to assess for the presence of any cystoid macular edema or macular alteration. Macular OCT also provides RNFL data in the macular region.

The patient will be seated at the instrument and his/her chin is positioned on the chinrest. The following scans will be acquired on each eye: at least one acceptable 200x200 macular cube and one acceptable 200x200 optic disc cube. Each scan will be repeated until 2 similar images are obtained and are deemed acceptable.

The right eye will be imaged first.

The operator will choose the type of scan to be performed from the available menu at the top of the screen. The patient will be asked to fixate on the internal fixation target. In cases of poor central vision, the patient may be unable to fixate on the internal fixation target. In these cases, the external fixation target should be presented to the fellow eye, and used to position the fovea centrally (macular cube) and nerve centrally (Optic Disc cube). The pupil is aligned in the center of the video image and the focus is adjusted by using the arrows to move the chinrest.

The LSO fundus image is centered and focused.

The OCT scan image is enhanced.

The image is acquired clicking on the capture button.

The operator will review all Cirrus HD-OCT scans as soon as they are acquired to ensure acceptable image quality, according to the criteria described above. If the image is deemed acceptable it will be saved, if not the operator will try again.

The steps above are repeated to complete all the scans required.

When the process is completed the operator will repeat the procedure to image the left eye.

If more than one dataset is saved for a given eye, scan type, and session, the dataset with the best image quality will be used for analysis. Scans may be exported from the instrument to perform additional analysis using custom software.

Fundus Photography

Using an infrared camera to avoid excess visible light exposure, fundus photographs of two standard fields of the fundus will be taken at a baseline visit and at post-vector administration evaluations to document fundus appearance.

The following descriptions of the standard fields assume that there are two cross hairs in the camera ocular, one vertical and the other horizontal intersecting in the center of the ocular.

Field 1: Stereoscopic pairs of photos of the disc (2X): Center the temporal edge of the optic disc at the intersection of the cross hairs in the ocular.

Field 2: Stereoscopic pairs of photos of the posterior pole (45 degrees): Center the macula near the intersection of the cross hairs in the ocular. The intersection of cross hairs should be placed about 1/8 - 1/4 disc diameter (DD) above the center of Macula.

Quality of Life Questionnaire (NEI VFQ-25)

The Clinical Coordinator for the study will administer the National Eye Institute's Visual Function Questionnaire (NEI VFQ-25) to all patients enrolled in the study at pre-injection, and Month 2, 6 and 12. A special note will be made of patients' responses to the questions concerning ocular pain. The Biostatistics Center at Bascom Palmer Eye Institute has extensive experience in analyzing data from the NEI VFQ-25 in patients with several diagnoses, including glaucoma, low vision, and age-related macular degeneration.

Detailed Study Agent Administration Procedures

One-time intravitreal injection in a single eye will be administered.

Procedure for Intravitreal Injection

Patients in this trial will undergo unilateral intravitreal injection. All intravitreal injections will be performed by [redacted] who have extensive experience in this procedure. The injection protocol is as follows:

- All intravitreal procedures shall be performed using aseptic technique and standard precautions protocol.
- Preparations for the injection should not begin until the ophthalmologist is present within the unit.
- A“time out” immediately prior to the procedure will be conducted by two persons as a final verification of the correct patient, procedure and site. The following equipment will be in the treatment room used for the injections.

    1. 1.0 cc syringe containing Study Drug Solution (Low, Medium, High or Higher dose)
    2. Dilating Drops
    3. Sterile eye pad
    4. Alcohol swap
    5. Sterile caliper
    6. Sterile eye lid speculum
    7. Optional-intravitreal kit with disposable calipers and lid speculum
    8. Tonopen with cover
    9. Lidocaine 4% vial
    10. Betadine 5% vial
    11. Betadine swab
    12. Medication to be injected
    13. 30 and 32 gauge needles
    14. Antibiotic drops
    15. Proparacaine drops
    16. Sterile cotton tip applicators
    17. Gloves
    18. Needle “box” holder
    19. Intravitreal Injection Form

20. Patient Discharge Instructions Form (Patient Education Assessment)

21. Vital signs monitor

Adequate documentation of the procedure will be done.

1. The procedure used to prepare the eye for intravitreal injection will be documented on the Intravitreal Injection Form.
2. The person who performs the eye prep will write or check mark the exact Procedure used to prepare the eye.
3. The physician performing the intravitreal injection will document the Name of the drug given, the dose and amount of the drug given, and the Location of the injection on the Intravitreal Injection Form.

The following procedure will be carefully followed:

1. The physician writes an order for the intravitreal injection.

2. The physician obtains an informed consent from the patient.

3. The patient's identity is confirmed using two patient identifiers. (Name and Date of Birth)

4. The Nurse/Injection Specialist will initiate documentation by utilizing the Intravitreal Injection form.

5. The Nurse/Injection specialist will assess the patient's desire to learn barriers of learning, any physical or cognitive limitations and complete the Patient Education Assessment.

6. The Nurse/Injection Specialist will explain the procedure to the patient.

7. The patient's blood pressure and pulse are monitored by the nurse/injection specialist. Abnormal findings are immediately reported to the patient's physician.

8. The Nurse/Injection specialist will verify the correct procedure and site (right or left eye) with the patient, the order and the consent form.

9. The Nurse/Injection Specialist will verify and mark the site with skin marker.

10. The Nurse/Injection Specialist will dilate the eye to be injected if not already dilated.

11. The Nurse/Injection Specialist will recline patient and prepare the patient for injection.

12. The Nurse/Injection Specialist will wash his/her hands and immediately put on gloves.

13. The Physician removes the 1 cc syringes prepared by the Sylvester Investigation Drug Service, containing Study drug from the transport container.

14. The Nurse/Injection Specialist will instill one drop of Proparacaine on the eye designated to receive an intravitreal injection.

15. The Nurse/Injection Specialist will instill one drop of Lidocaine 4% on the designated to receive an intravitreal injection.

16. The Nurse/Injection Specialist will instill one drop of Betadine 5% on the eye designated to receive an intravitreal injection.

17. The Nurse/Injection Specialist will instruct the patient to close his/her eye for a moment.

18. The Nurse/Injection Specialist will repeat steps #15-17, two more times.

19. The Nurse/Injection Specialist will use a Betadine swab to clean the eye lashes and lids, of the eye designated to receive an intravitreal injection. The eye will be swabbed from inner canthus to outer canthus.

20. The Nurse/Injection Specialist will wipe the designated eye with sterile eye pad.

21. The Nurse/Injection Specialist will place the eye lid speculum on the eye designated to receive an intravitreal injection.

22. The Nurse/Injection Specialist will saturate a sterile cotton tip applicator with drops of Lidocaine 4% without touching the applicator.

23. The Nurse/Injection Specialist will utilize the cotton tip applicator, saturated with lidocaine 4% drops, to apply pressure on the area to be injected. (i.e. 6:00, 7:00, 5:00 O'clock etc.)

24. The Nurse/ Injection Specialist will repeat steps 22-23 two more times. Apply A drop of Lidocaine 4% to the cornea between each cotton tip applicator pressure application.

25. The Nurse/Injection Specialist will call the physician and continue steps 22- 23 until the physician enters the room.

26. As the physician enters the room, the nurse/injection specialist will instill one drop of Betadine 5% to the area of the eye designated for injection and where pressure is being applied.

27. **TIME OUT**- The Nurse/Injection Specialist and Physician will conduct the final verification of the patient information, physician order, procedure and/or drug and eye designated to receive an intravitreal injection.

28. OPTIONAL- The Physician will use a sterile caliper to measure the area to be injected.

29. The Physician performs an anterior chamber paracentesis to remove 0.15 to 0.2 ml in order to reduce risk of reflux after injection using a 3 cc syringe mounted with a 32-gauge needle. The anterior chamber fluid will be stored for future analysis. The syringe is immediately discarded in the biohazards sharps container.

30. The Physician will check the drug dosage and inject the drug into the eye.

31. As the physician withdraws the needle, the nurse/injection specialist will instill two drops of antibiotic solution.

32. The Nurse/Injection Specialist or physician will remove the eye lid speculum.

33. The Nurse/Injection Specialist will check the patient for light perception in the Treated eye and notify the physician immediately of no light perception (NLP).

Procedure for Monitoring subjects after injection

1. The Nurse/Injection Specialist will monitor the intraocular pressure (IOP) with a Tonopen every 15 minutes post-injection until the IOP is under 30. If, the IOP is under 30, the patient can be discharged.

2. If the IOP is over 30, then the patient can be discharged if approved by the physician OR the IOP and vision will be monitored every 15 minutes and the patient discharged when the IOP is Under 30.

3. The IOP at discharge is recorded.

4. The Nurse/Injection Specialist will review discharge instructions and have the Patient sign the Patient Discharge Instruction form.

Risks Associated with Study Procedures

Risks associated with visual function tests include risks from eye drops. Rarely, a patient may experience redness, discomfort or an allergic reaction

to the eye drops used to dilate the pupil or anesthetize the cornea. Angle closure glaucoma may be precipitated by the dilating drops in a susceptible individual. If a patient has hypertension or cardiac disease, dilating drops can, in rare circumstances, temporarily exacerbate these conditions.

Phlebotomy has the risks of discomfort, psychological stress leading to syncopal reactions and hematomas at the site of venipuncture.

The chest X-ray performed as part of this study is not necessary for a subject's medical care and will occur only as a result of participation in this study. Risks associated with radiation exposure of the magnitude in a chest X-ray are considered to be extremely low.

Adequacy of Protection against Risks

Recruitment and Informed Consent

Many individuals and families with LHON have expressed interest and willingness to participate in a clinical trial. Additional volunteer participants will be recruited from patients [redacted] at Bascom Palmer Eye Institute, University of Miami. We expect continued inquiries from newly diagnosed LHON patients and families who are interested in the research and clinical studies due to the protocol listing on GeMCRIS and widely-available genetic testing for retinal degeneration-associated genes.

Before subjects are enrolled in this trial, an Informed Consent document will be approved by the IRB of University of Miami. An investigator will completely explain the study to potential participants. At the discretion of the potential participants, friends and/or family members will be included in these discussions. Subjects will be encouraged to ask questions prior to providing written informed consent. Participation in this clinical trial is completely voluntary and great care will be taken to ensure that individuals do not feel coerced to participate. Subjects will be able to terminate their participation at any time without prejudice.

Protection against Risk

The following procedures are likely to minimize the potential risks associated with study procedures.

The medical history and physical examination performed prior to study agent administration will identify patients with medical or ophthalmic conditions that would increase the risks associated with study procedures. Those subjects will be excluded from participation. Surgical administration of the study agent will be performed in standard fashion by experienced retinal

surgeons and with strong attention paid to the risks listed above. Administration of topical corticosteroids and antibiotics following intravitreal injection of the study agent is planned for this clinical trial to minimize the risk of local inflammation or infection. To minimize the risks of potential adverse events, following intravitreal injection, study subjects will be monitored and discharged only after their intraocular pressure (IOP) is less than 30 mm. Subjects will also be examined one day after injection and again on day 7. If ocular inflammation is noted at day 1, an additional visit will be scheduled before the day 7 visit. Subjects will be provided with contact numbers for any questions or concerns arising regarding the possible effects of the administration of scAAV2-P1ND4v2. Results of all laboratory and safety examinations will be reviewed by an investigator throughout the study. Since the risks of rAAV vector administration to pregnancies and developing fetuses are unknown, female subjects of childbearing potential will have two pregnancy tests prior to administration and pregnant and breastfeeding females will be excluded from participation. Subjects (men and women) of reproductive ability will be required to use effective contraception for 1 year following gene transfer. Subjects must also be willing to use barrier contraception following gene transfer. If subjects do not agree to use contraception, they will be excluded from the study.

A slit lamp exam will be performed to be certain no abrasions were sustained.

Phlebotomy will be performed by experienced phlebotomists, thus decreasing the likelihood of discomfort, stress and the occurrence of bruising.
Subjects will be provided with emergency contact information for [redacted] and will be encouraged to call if they feel that an adverse event is occurring. The Bascom Palmer Eye Institute maintains a 24-hour fully staffed emergency room. In the event of an adverse event, immediate medical care will be provided without cost to the subject or the subject's family.

Confidentiality of research materials obtained through this study will be maintained by coding data collection forms. Patients included as subjects in reports or publications will be referenced with numbers.

This is the first time study agent, scAAV2(Y444+500+730)-smCBA-P1ND4v2, abbreviated as **scAAV2-P1ND4v2**, will be used in Humans.

The purpose of this dose-escalation study is to assess the safety and tolerability of scAAV2-P1ND4v2 gene replacement therapy in subjects confirmed with the G11778A mutation in mtDNA responsible for Leber's Hereditary Optic Neuropathy. Ocular and systemic toxicity will be assessed following vector administration to determine if there are adverse changes that may be associated with vector administration.

This first-in-man (FIM) clinical trial will assess safety, tolerability, and potentially efficacy of a single intravitreal injection in patient groups reflecting the acute, presymptomatic and chronic stages and manifestation of the LHON disease.

The Food and Drug Administration (FDA), **IND#15941** and National Institutes of Health/National Eye Institute (NIH/NEI) grant number: **1U10EY023558-01A1,** have approved this clinical trial.

Sources of Materials

Research materials obtained from human subjects related to this trial will include history and physical assessments, results of blood, urine, and LHON specific analyses, results of retinal and ophthalmic evaluations, AE reports, chest x-rays, electrocardiogram. Copies of CRFs, original test results, subject medical records, signed informed consent forms, correspondence, and all other trial-related documents will be kept at the clinical sites in locked cabinets (McKnight Building, Room 408, 1638 NW 10TH AVE, MIAMI, FL 33136). All material collected as part of the studies will be obtained specifically for research purposes and only trained personnel directly involved in the study will be able to access study materials. Subjects will be identified by code number on all data collection forms and in any reports or publications. All information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Data management will be 21 CFR compliant.

Long-term follow-up of trial participants will be performed according to regulatory guidelines by [redacted] or his designees.

Long-term follow-up will occur for 10 years after study agent administration. All subjects will be provided with written contact information for [redacted] should they experience any SAE that they consider possibly related to study treatment or study participation. Subjects will be instructed to notify [redacted] of changes in their contact information.

Annually, subjects will be contacted by mail or by telephone and information concerning any cancer, neurological, autoimmune or hematological disorder that developed or worsened since the previous contact will be obtained. The subjects will also be asked for information concerning hospitalizations and use of medications.

At the time of death, no matter what the cause, permission for an autopsy will be requested of subjects' families. Subjects will be asked to advise their families of this request and of its scientific and medical importance.

11) **Data and Specimen Banking (for research purposes)**. Specimens of blood and anterior chamber fluid will be stored for analysis of vector DNA and anti-AAV antibodies.

12) Data Management*

Study data will be managed by the Biostatistics Center as part of a separate National Eye Institute grant U10EY024247 which has already been I.R.B. approved (protocol 20140294, approval date 9-June-2014). The following is abstracted from that protocol.

Assignment of Patient Identification (ID) Number

The Baseline Qualifying Assessment establishes whether the patient satisfies the eligibility criteria. If the patient appears to be eligible for the study, the Clinical Center (CC) completes the Qualifying Assessment Form and Pre-injection Form. Both forms are faxed to the Biostatistics Center (BC), along with a copy of the consent form. The BC reviews each of the inclusion and exclusion criteria to ensure that the patient is eligible.

Any patient who is confirmed by the BC to meet the eligibility criteria and is enrolled in the Phase 1 Safety Study is assigned a patient identification (ID) number. The patient identification number is a 3 digit, 3 letter code which is unique for each patient. The first digit refers to the Group (1-3). The second digit refers to the dose (1=low, 2=medium, 3=high, or 4=higher). The third digit is a sequential number for each patient. The 3 letters are the subjects initials. A line through will be used in the center box for those subjects with only two initials. . For example, ID number 2-3-1-AZE refers to the first patient enrolled with the high dose in group 2.

Data Management

A master log is kept of each patient in the Study. An appointment schedule is made for each patient and sent to the Clinical Center. When a data form is received at the Biostatistics Center, it is processed for filing and data entry. Each form is data entered by a data entry clerk and then verified by double entry by the BC Research Coordinator. Edit checks, such as missing data and out-of-range values, will be clarified within the Clinical Center.

BC procedures provide for

(i) CRF tracking and annotation

(ii) Audit from source document to database

(iii) Database design
(iv) Data entry
(v) Data validation
(vi) Discrepancy management
(vii) Database lock

(i) The BC maintains a stand-alone patient log recording dates of enrollment and study visits as they occur, as well as documenting receipt of CRFs from the clinical center study coordinator. The BC will accept photocopies of the original CRFs or will copy them for the study coordinator if necessary. Originals will be returned to the coordinator and the copies retained at the BC. The data entry operator inspects the forms visually for missing fields and reports these to the clinical coordinator who either supplies the missing data or provides an explanation for why the data were not collected. CRFs awaiting data entry are filed in the data entry operator's office – access to this office is by magnetic card key.

(ii) A separate dataset and entry screen is provided by the data manager for each of the study forms: Inclusion-Exclusion/Group Assignment, Adverse Event, AntiAAV Antibodies, Baseline / Follow Up Exam, Clinical Chemistry, Coagulation, Chest Xray Exam, Demographic Data, Electrocardiogram, Hematology, Injection/Treatment Day 0, Concomitant Medications, Vital Signs/Physical Exam, Serum Pregnancy, Urinalysis, NEI VFQ 25 Quality of Life Questionnaire. The family tree CRF is not data entered.

(iii) Computer data entry will be performed in two stages.

a. Initial data entry will be performed by the BC data entry operator into the 21 CRF part 11 compliant Velos eResearch system which is maintained by the University of Miami's Office of Research Information Management, which supports audit trails and controls access to the datasets.
b. These data will be exported to IBM/SPSS data collection version 7, which supports two pass verification to foster data quality. Any errors found in the verification pass will be corrected in the Velos datasets and these corrections recorded with audit trails.

As a further monthly check on database integrity, the current dataset will be compared with the previous month's backup and differences will be reconciled. The CRFs are data entered and a subsequent verification data entry pass is performed, typically, days or weeks later, after which the CRF copies are stored in locked file cabinets within the BC.

(iv) Following entry, data will be subjected to range and consistency checks. Data entered from the CRFs are merged by patient ID to create a single record for each patient and these records are subjected to range and consistency checks by the study statistician. Data entry errors uncovered in this way are corrected by the data entry operator in the Velos system and documented with its audit trail feature.

(v) Similarly, data fields containing values demonstrated to be unlikely or impossible by consistency checks are reported to the clinical coordinator for resolution. Necessary corrections are made and documented as with data entry errors. Interim versions of these data files are maintained for reference by the BC. Any updates to the paper forms (i), based on this procedure are dated and made to the paper forms in a different color ink from that originally used to fill out the form.

(vi) After a proper quality check and assurance, the final data validation is run. If there are no discrepancies, the datasets are finalized by the study statistician and data manager. BC hardware and software will be identified and available for inspection by the FDA or the representatives of the NEI or DSMC.

The BC ensures that safety events, systemic, ocular, and/or acuity loss (described below), are captured on study CRFs (Adverse Event and Follow Up Exam) and reported to the FDA, NEI, and DSMC.

Data Security

The paper data forms for the Study are kept in file cabinets in the Biostatistics facility in the Dominion Tower. The building is locked and access is by card key entry. A security guard is present during working hours. The computer files for the study are kept on computers in the same location. These rooms are kept locked when not in use, and the study computer files are password-protected as described in Resources. The data files are backed up daily and are stored at a remote facility. Computer data files used for publication are saved and stored as separate files.

Data Forms

The data forms will be designed to be self-explanatory. Their completion should not require reference to separate information manuals. The data forms contain information to be collected at a given point in time during the study. Information collected at another date is incorporated into a separate form. Data forms are faxed to the

Biostatistics Center for data entry. Forms will be reviewed periodically and revised as dictated by protocol changes.

Data Analysis

The analysis of data will be performed by the Biostatistics Center under the supervision of WJ Feuer. He is the principal investigator biostatistics Center for the Phase 1 Safety trial, descriptive statistics with 95% confidence intervals around the success rates and the adverse event rates will be calculated. To reiterate, the results of the ongoing monitoring of each patient's course by the BC and the Data and Safety Monitoring Committee (DSMC) will be evaluated to decide about continuing recruitment in this phase of the trial; statistical considerations will only provide guidelines.

Assessments of change in visual acuity will be calculated from the Baseline 2 measurement to guard against regression to the mean from the screening acuity, Baseline 1.

These patients will also be monitored for ocular and systemic adverse events which will be reviewed by the DSMC on an ongoing basis as deemed necessary by the DSMC and the NEI.

Ancillary data analysis will include assessments of the changes in acuity, visual fields, and OCT RNFL thickness as well as correlations between these measurements to the limited extent that this can be studied with such a small sample size. Assuming that the earlier the stage, the more likely the benefit of treatment, we will examine and develop outcomes that can be used in later phases and other trials that could include a wide range of these patients before the onset of permanent visual deficits.

Evaluations of Efficacy in a phase I trial.
Patients with molecularly confirmed G11778A mitochondrial DNA will be recruited into one of 3 groups.

	Low-dose ($1.18x10^9$ vg)	Medium dose ($5.81x10^9$ vg)	High dose ($2.40x10^{10}$ vg)	Higher dose ($1x10^{11}$vg)
Group I	3	3	3	
Group II	3	3		3
Group III	3	3		3

The certified technician performing the visual acuity assessments will be masked to treatment. The elimination of improvement to ≥70 letters from the efficacy endpoint increases the likelihood of a success by natural history and correspondingly decreases power. To obtain the requisite patient numbers needed to complete the efficacy

assessments, we will submit for another U10 for phase II, if phase I safety assessment supports further investigation. Our previous power estimates were based on improvement by 15 letters and acuity of 20/40 and included both best and worst eyes of patients (Aim 1 and 2). The new endpoint, which drops the latter requirement, is more common in natural history but is assessed only on the 31 worst eyes eligible for Aim 1 and Aim 2. We include the upper 95% confidence intervals along with the point estimates as recommended.

Power for detecting 15 letter improvement with N=9 in group I

			Treatment Efficacy		
Natural History Estimate			50%	75%	95%
Point:	9%	Power	35%	83%	>95%
Upper 95% CI:	28%	Power	13%	41%	92%

Power for detecting 15 letter improvement with N=9 in group 3

			Treatment Efficacy		
Natural History Estimate			50%	75%	95%
Point:	33%	Power	9%	30%	88%
Upper 95% CI:	70%	Power	NA	NA	17%

Sample size for 80% (90%) power to detect a 15 letter improvement in group I

			Treatment Efficacy		
Natural History Estimate			50%	75%	95%
Point:	9%	N	18 (22)	9 (10)	6 (6)
Upper 95% CI:	28%	N	76 (100)	18 (21)	9 (9)

Sample size for 80% (90%) power to detect a 15 letter improvement in group 3

			Treatment Efficacy		
Natural History Estimate			50%	75%	95%
Point:	33%	N	130 (175)	22 (27)	9 (11)
Upper 95% CI:	70%	N	NA	NA	34 (41)

13) **Provisions to Monitor the Data to Ensure the Safety of Subjects***

The DSMC is responsible for the ethical conduct of the study. This committee oversees the informed consent process and major changes in the

protocol. The DSMC reviews the accumulating data for evidence of adverse and beneficial treatment effects. This committee will meet as often as they deem appropriate, but at least twice each year for the duration of the study. Telephone conferences will occur as needed. The responsibilities of the DSMC are as follows:

- To review the study design and study documents before the start of the study to identify any problems that may affect patient safety
- To monitor adherence to the study protocol
- To review treatment reports prepared by the BC for evidence of adverse and beneficial treatment effects
- To terminate the study if treatment risk is so high that continuation of the trial is deemed unethical
- To collaborate with the investigators on interpretation of study data.
- To recommend changes to the study protocol based on periodic data analysis
- To review and approve all publications and presentations
- **To determine when data collected in the study should be released to study investigators, study patients, the medical community, and the public**

14) **Withdrawal of Subjects***

Subjects may withdraw or be withdrawn from the study for any of the following reasons:

- Occurrence of intolerable adverse events, including clinically significant abnormal laboratory findings which, in the opinion of the investigator, preclude further participation in the study;
- Voluntary withdrawal by participant;
- Subject non-compliance with study procedures;
- Investigator decision that withdrawal from further participation would be in the subject's best interest;
- Instruction to terminate study by regulatory, safety, institutional authorities or the DSMB.

The reason for withdrawal will be documented for each subject. Subjects who withdraw due to an adverse event will be followed until the event has stabilized

or resolved. Subjects will be considered withdrawn from the study at the time that any of the above criteria for withdrawal are met. If subjects withdraw prematurely from the study, data collection will occur throughout the protocol-defined follow-up period. If a subject withdraws consent for participation, attempts will be made to record at least survival data through the end of the follow-up period. If a subject withdraws from the study and contact is lost, three attempts will be made to contact the subject by phone after which next-of-kin will be contacted in order to obtain survival data. Any subject who withdraws prior to administration of study agent will be replaced.

15) **Risks to Subjects***

Summary of the known and potential risks and benefits to human subjects

Subjects may experience burning or pain in the eye after the anesthesia given for the injection wears off. This clinical trial is the first human study of scAAV2-P1ND4v2 so the risks associated with vector administration are not precisely known. Based on our preclinical studies of scAAV2-P1ND4v2 in normal rats and normal non-human primates there is a high likelihood that intravitreal administration of scAAV2-P1ND4v2 will be safe in humans. Safety of rAAV2 vectors in humans is also supported by previous and on-going clinical trials. Most relevant are studies performed at the University of Florida's Powell Gene Therapy Center. These trials include several Phase I and II clinical studies in individuals with cystic fibrosis with alpha-1-antitrypsin deficiency, with RPE65-associated retinal disease. Overall, to date, results indicate no significant vector-related toxicity and demonstrate the feasibility of gene transfer. Potential risks associated with scAAV2-P1ND4v2 and the study procedures are discussed below.

Specifically associated with intravitreal-injections of scAAV2-P1ND4v2, there is a risk of degeneration of lens and cataract formation in the injected eye. Detachment of the retina is also possible.

There is a potential risk of vector spread to other organs. In our safety studies, all major organs of rats and primates were essentially negative (≤ 100 copies/ug genomic DNA) for vector. Our biodistribution results after scAAV2-P1ND4v2 (intravitreal) administration to rats and non-human primates indicate only limited vector spread outside the injected eye and no evidence of a vector dose-related effect. The risks of vector spread and germline transmission (all gonad samples were negative) are therefore presumed to be low in the planned human trial.

There is a risk that subjects may develop an immune response to the vector. Our neutralizing antibody assay data from scAAV2-P1ND4v2 administration in large animals suggests that the risk of adverse immune response in humans is minimal. It is possible, but unlikely, that scAAV2-P1ND4v2 could interact with other viruses

with which the subject comes in contact, forming a new virus that could produce new side effects.

In preclinical studies, there was minimal ocular inflammation which subsided with time after administration; in one animal of the non-human primate studies. Administration of corticosteroids and antibiotics following intravitreal injection of the study agent is planned for this clinical trial to minimize the risk of local inflammation. There is potential that vision of subjects could be further reduced. Based on preclinical studies in animal models and in normal animals, the risk of further visual impairment due to scAAV2-P1ND4v2 is believed to be low.

There is a chance that the study agent could damage the DNA in the cells of a subject's retina. In the unlikely event that this occurred, it could put the subject at risk for developing retinal tumors in the future. In animal and human studies to date, cancer has not developed and therefore, the risk is considered to be low. In our preclinical work with the AAV vector, the majority of vector DNA appears to persist as episomal rather than integrated into genomic DNA, which makes it very unlikely that mutagenesis and tumor development will occur.

Intravitreal administration of scAAV2-P1ND4v2, as mentioned above, carries a risk of retinal cell loss from administration trauma. There is also a risk of cataract development that could require additional surgery. There is a risk of retinal tears or retinal detachment and there is the possibility that these could lead to worse vision. Exogenous intraocular infection (endophthalmitis) is rare but can occur. The most common pathogens can be treated but poor vision (in patients with better vision than those entering this study) can result from infection despite successful antibiotic treatment. Intraocular hemorrhage is rare in patients without bleeding diathesis. Vitreous hemorrhage is benign and usually resolves spontaneously. Choroidal or subretinal hemorrhage is usually self-limited and could affect vision (or potential vision) significantly. Temporary increases in intraocular pressure can occur following ocular/retinal injections and are treatable with topical or systemic medications to lower eye pressure. Risk of anesthesia, which is local (retrobulbar) is low. There is a small risk of penetration of the eye or optic nerve from retrobulbar injection and also the possibility of hemorrhage, which can be decompressed if necessary. In addition to these risks, there may be other longer-term risks which are not known.

16) Potential Benefits to Subjects*

Benefits: Due to the initial, safety establishing nature of this investigation, there is no anticipated benefit to the subjects in this trial.

This Phase I clinical trial will define the safety of the proposed procedures and study agent. Since this is the first study of scAAV2-P1ND4v2, the potential risks and benefits are unknown and **there are no expected direct benefits to the individual subjects**. The results of this trial will provide information on the biological response to intravitreal gene delivery in this human blinding disease and may be extended to other degenerative diseases caused by mutated mitochondrial genes.

17) Vulnerable Populations*

No special populations are to be included in this research, such as children 14 years old or younger, prisoners, or pregnant women. We **wish to exclude pregnant women**. There is no reason to unnecessarily endanger the unborn. The data obtained from this study may provide additional data to warrant extension of similar, future studies to additional populations.

18) Multi-Site Research*N/A

19) Community-Based Participatory Research* N/A

20) Sharing of Results with Subjects*

DSMC will determine when data collected in the study should be released to study investigators, study patients, the medical community, and the public

21) **Setting**

Describe the sites or locations where your research team will conduct the research.

Bascom Palmer Eye Institute

Clinics of [redacted]

See Manual Of Procedures for the methods that will be used in the examinations and testing as well as the injection procedure.

22) **Resources Available**

- [redacted] with long-standing severe vision loss of 20/200 or worse, shows that we have 44 patients who would be eligible for the Phase 1 study in Groups 1 and 2. We expect that the availability of a potential treatment will draw more patients in groups 2 and 3. We have also followed 45 asymptomatic carriers. One

of them has converted to affected. Others may also. Thus, we should have a sizable pool of patients for the Phase 1 safety study of the AAV-ND4 vector.

- The Principal Investigator is listed as 40% effort for this study. [redacted] this NIH funded grant.
  Bascom Palmer Eye Institute: Founded in 1962, Bascom Palmer Eye Institute serves as the Department of Ophthalmology for the University of Miami Miller School of Medicine. The Institute comprises clinical care, teaching, and research activities. In the past two years, Bascom Palmer Eye Institute was rated as the number one eye hospital in the nation by board-certified ophthalmologists from across the United States. It has been either first or second in the rankings since the survey began. Ophthalmology Times, an industry periodical for and by ophthalmologists consistently rates Bascom Palmer's patient care and residency programs as among the best in the nation.

- Currently there are 42 board-certified ophthalmologists on the full-time clinical faculty, almost all fellowship-trained. In addition, 10 adjunct faculty members take part in various training activities for 22 board-certified fellows and 20 residents. All ophthalmic sub-specialties are represented within this group. More than half of the Institute's clinical faculty members are listed in Castle Connolly "Top Doctors in America" including the PI.

- In 2001, Bascom Palmer began an intensive recruitment effort to build its research program. There are currently 21 research faculty members in the department. Hiring continues, including scientists and clinician-scientists for the research program, as well as clinicians to staff the expanding clinical facilities.

- The **Anne Bates Leach Eye Hospital** is Bascom Palmer's main facility for ophthalmic care. Operating within a six story building located on the medical campus (opened in 1976), the hospital serves individuals of all ages. More than 200,000 patients are seen each year and at least 10,000 surgical procedures are performed. Additionally, 24-hour emergency care and community-based ophthalmic care for indigent and low-income patients of Miami is provided. The hospital employs numerous optometrists as well as other professional staff.

- The neuro-ophthalmology clinic is run 5 days a week. 3 of these days are staffed by [redacted] one of these days staffed by [redacted] Therefore we have coverage 80% of the time of every week for our LHON patients. Bascom Palmer

mint maintains a fully staffed eye emergency room 24/7. Therefore we have coverage for any conceivable problem that could arise.

The manual of procedures delineates the protocol to be followed by all personnel in the study. [redacted] have **just completed a five-year study of patients with LHON that included all evaluations proposed here with the exception of the intravitreal injections of study drug.**

23) **Prior Approvals**

University of Miami Institutional Review Board, University of Miami Biosafety Committee and University of Miami Environmental Health and Safety Department.

Approvals from the Federal Drug Administration and National Institutes of Health/National Eye Institute have been obtained by Sponsor-Investigator, [redacted]

24) **Recruitment Methods**

Patients in this single center study will be recruited from the existing LHON patient pool [redacted], where access to records is already given outside of research, and from referrals of outside ophthalmologists. This is the same strategy used to recruit patients for the completed natural history study of Leber's Hereditary Optic Neuropathy (references below). Sufficient participants (N>40) were recruited in this way, even without the possibility of offering participants the possibility of an active treatment. We do not anticipate the need for advertisements or other recruitment materials.

Patients will be reimbursed for travel expenses as needed and in accordance with the budget in the grant application approved by the national eye Institute

25) **<u>Local</u> Number of Subjects: N/A**

Number of Study Centers: There is only a single study center at, Bascom Palmer Eye Institute, University of Miami, Miami, FL.

Many individuals and families with LHON have expressed interest and willingness to participate in a clinical trial. Phase I. A review of the LHON patients currently

being seen by [redacted] with long-standing severe vision loss of 20/200 or worse, shows that we have 23 patients who would be eligible for the Phase 1 study in Groups 1 and 2. We expect continued inquiries from newly diagnosed LHON patients and families who are interested in the research and clinical studies due to the protocol listing on GeMCRIS and widely-available genetic testing for retinal degeneration-associated genes. We expect that the availability of a potential treatment will draw more patients in groups 2 and 3. Thus, we should have a sizable pool of patients for the Phase 1 safety study of the AAV-ND4 vector.

Individuals who have been diagnosed with G11778A mutation associated Leber's hereditary Optic Neuropathy and who are eligible based on inclusion and exclusion criteria will be invited to participate in this clinical trial. A complete physical examination and clinical chemistries will be performed at a pre-treatment, screening evaluation to further determine eligibility. Subjects will be considered enrolled when they sign the Informed Consent.

26) **Confidentiality: N/A**

This is not a multicenter study.

27) **Provisions to Protect the Privacy Interests of Subjects**

Patients enrolled in the study are assigned a patient identification (ID) number. The patient identification number is a 3 digit, 3 letter code used are the subjects initials. A line through will be used for subjects without a middle initial . This ID number is used on CRFs instead of the patient name.

Access to datasets, including Personal Health Information, is restricted to Biostatistics Center (BC) personnel associated with the study and identified as such in Eprost. For a complete description of data management procedures see section 12.

Section 12 also describes the physical facilities of the BC and measures taken to ensure data security.

Direct Access To Source Data/Documents

Qualified representatives of regulatory agencies, internal auditors or monitors will have the right, both during and after this study, to conduct inspections and to audit and review medical records and/or study documents (CRFs, source data/documents, etc.) pertinent to the clinical study as permitted by the regulations (21 CFR 312.58; 312.68, ICH-GCP E6; 6.10).

Quality Control & Quality Assurance

Monitoring of the study will be provided through the University of Miami's Office of Clinical Research Operations and Regulatory Support (CRORS) or an experienced external monitoring service.

28) **Compensation for Research-Related Injury**

Provisions for Injury

In event of injury resulting from procedures associated with the clinical trial, the cost of medical treatment in excess of that covered by a third party payer will be provided to the subject but financial compensation is not available for injury.

29) **Economic Burden to Subjects (From ICF form)**

If you choose to take part in this study, will it cost you anything?

Subject nor their insurance company will be charged for any tests or procedures that are done for the purposes of the research study. Study agent will be provided without charge. **Subject or Subject's insurance company will be responsible for costs of tests or procedures that are part of your routine medical care.**

Will you receive payment for your participation in this study?

Study Subject will not receive financial compensation for participation in this study. Travel will be provided and arranged by our research staff. In the event that we are unable to provide this service, with documentation of expenses, Study Subject will be compensated for travel expenses. This reimbursement for costs will be done in accordance with the travel policies and procedures of Bascom Palmer Eye Institute and the University of Miami School of Medicine. Study Subject's name and social security number will be reported to University administrative personnel for purposes of making and recording the payment. The research staff will arrange for payment or reimbursement of procedures associated with the study and performed at non-University locations.

- *30) Consent Process* Consent process will take place in the Bascom Palmer Eye Institute.
- The informed consent form will be read to all subjects through a laptop using the Dragon Software. A hardcopy of the consent form will be provided to the patients to follow while the ICF is being read to them. If

the subject is a minor the hardcopy will be provided to the parent. A study member is present during the consent process and he/she can pause, or make adjustments to volume and reading speed to subjects satisfaction. After the consent form has been read the subject will have the opportunity to ask questions.

- The waiting period will be as long as the prospective Subject requires.
- Patient will be asked at the start of every visit whether they wish to continue participating in LHON: Gene Therapy Clinical Trial.
- An investigator will also provide complete explanation of the study to potential participants and will ensure that all questions are answered prior to decisions about participation.
- Time that will be devoted to the consent discussion will as much time as potential participant requires.
- At the discretion of the potential participants, friends and/or family members will be included in these discussions. Subjects will be encouraged to ask questions prior to providing written informed consent. Participation in this clinical trial is completely voluntary and great care will be taken to ensure that individuals do not feel coerced to participate. Subjects will be able to terminate their participation at any time without prejudice.
- To ensure Subjects understand in participating in the clinical trial, subject will be asked questions regarding ICF.
- As the PI and co investigators speak only English no non-English speaking subjects will be enrolled to ensure communications between studies subjects and investigators.
- Patients who do not speak English will not be enrolled.
- Signatures will be obtained on an IRB approved assent form for all subjects ages 15-17 years old.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception) – N/A

Subjects who are not 15 years old or older- Excluded

Cognitively Impaired Adults - Excluded

Adults Unable to Consent - Excluded

Adults Unable to Consent - Excluded

31) **Process to Document Consent in Writing**

After the patient is given verbal informed consent, he or she will be asked to sign the written informed consent form.

32) **Drugs**

Handling of Investigational Agent

The study agent will be stored according to regulatory guidelines (CFR 21, Part 312.69). The investigators will take adequate precautions, including storage of the investigational agent in a securely locked (**McKnight Building**) , substantially constructed cabinet or other securely locked (**Locked freezer**) to prevent theft or diversion of the substance into illegal channels of distribution. Only the PI has the key to this freezer.

Disposition of Study Agent

The investigators, in conjunction with the Research Pharmacy Services will maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the trial is terminated, suspended, discontinued, or completed, the investigators will provide for disposition of the unused supplies, in accordance with regulatory guidelines (CFR 21, Part 312.62).

All requirements completed per FDA regulations

	Applicable to:
FDA Regulation	**IND Studies**
21 CFR 11	X
21 CFR 54	X
21 CFR 210	X
21 CFR 211	X
21 CFR 312	X
21 CFR 812	
21 CFR 820	

The FDA has given us approval for the low and medium dose injections. We will be supplying them pre-clinical data for the high dose injections before administering that dose to our patients with LHON. They have approved the injection procedure and our manual of procedures which we will follow according to all FDA regulations. In addition the Data Safety and Monitoring Committee constituted by the NIH will be reviewing our forms and data every 6 months.

33) Record Keeping

Accurate and complete study records will be maintained and kept in the McKnight Bldg

34) Publication and Presentation Policy

A Study publication is one which contains details of the design, methods, or results of the Study, and is written by investigators. Any paper classified as a Study publication must be approved by the Data and Safety Monitoring Committee prior to submission for publication. Similarly, any presentation made on behalf of the Study must be approved by the Data Safety and Monitoring Committee.