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Gene Therapy for Leber Hereditary Optic Neuropathy:

Initial Results

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Abstract

Purpose—Leber hereditary optic neuropathy (LHON) is a disorder characterized by severe and rapidly progressive visual loss when caused by a mutation in the mitochondrial gene encoding NADH:ubiquinone oxidoreductase subunit 4 (ND4). We have initiated a gene therapy trial to determine the safety and tolerability of escalated doses of an adeno-associated virus vector (AAV) expressing a normal ND4 complementary DNA in patients with a G to A mutation at nucleotide 11778 of the mitochondrial genome.

Design—In this prospective open-label trial (NCT02161380), the study drug (self-complementary AAV [scAAV]2(Y444,500,730F)-*PIND4v2*) was intravitreally injected unilaterally into the eyes of 5 blind participants with G11778A LHON. Four participants with visual loss for more than 12 months were treated. The fifth participant had visual loss for less than 12 months. The first 3 participants were treated at the low dose of vector (5×10^9 vg), and the fourth participant was treated at the medium dose (2.46×10^{10} vg). The fifth participant with visual loss for less than 12 months received the low dose. Treated participants were followed for 90 to 180 days and underwent ocular and systemic safety assessments along with visual structure and function examinations.

Participants—Five legally blind patients with G11778A LHON.

Main Outcome Measures—Loss of visual acuity.

Results—Visual acuity as measured by the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart remained unchanged from baseline to 3 months in the first 3 participants. For 2 participants with 90-day follow-up, acuity increased from hand movements to 7 letters in 1 and by

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“Reducing cellular dysfunction caused by mitochondrial genes”), and it is possible that he may receive royalties in the future should the product ever become commercialized.

15 letters in 1, representing an improvement equivalent to 3 lines. No one lost vision, and no serious adverse events were observed. Minor adverse events included a transient increase of intraocular pressure (IOP), exposure keratitis, subconjunctival hemorrhage, a sore throat, and a transient increase in neutralizing antibodies (NABs) against AAV2 in 1 participant. All blood samples were negative for vector DNA.

Conclusions—No serious safety problems were observed in the first 5 participants enrolled in this phase I trial of virus-based gene transfer in this mitochondrial disorder. Additional study follow-up of these and additional participants planned for the next 4 years is needed to confirm these preliminary observations.

Mitochondria are thought to have evolved from free-living aerobic bacteria. They have their own genomic DNA (mitochondrial DNA [mtDNA]) encoding 37 genes (2 for ribosomal RNAs, 22 for transfer RNAs, and 13 for proteins) that are essential for cell function and viability. In 1988, a G to A transition at nt-11778 in the ND4 subunit gene of complex I of mtDNA was first linked to the blindness of Leber hereditary optic neuropathy (LHON).¹ The clinical features of LHON were described a century earlier.² Visual loss is usually severe and bilateral. Unilateral visual loss is typically followed by involvement of the fellow eye within months. Spontaneous recovery of 3 lines is 18%.³ Two other primary mutations, G3460A and T14484C, have been identified in mtDNA in patients with LHON.^{4,5} Each mutation affects a different subunit of the NADH:ubiquinone oxidoreductase (complex I) in the oxidative phosphorylation pathway where electrons first enter the electron transport chain. This large enzyme consists of 7 subunits (ND1, 2, 3, 4, 4L, 5, and 6) encoded by mtDNA; the remaining 35 subunits are nuclear encoded. The G11778A mutation that accounts for most LHON cases affects the ND4 subunit. This mutation has the least propensity for spontaneous recovery, making it a good candidate to test potential remedies. Unlike most mitochondrial diseases that have a mix of wild-type and mutant mtDNAs (heteroplasmy), LHON is typically homoplasmic, having 100% mutated mtDNA. It is unclear whether the presence of the wild-type allele is protective against visual loss.^{6,7} Approximately half of the men and 10% of the women with the mutant ND4 allele develop the disease phenotype.^{8,9} There is no predictive test to determine who is at risk for vision loss.¹⁰

Therapies for LHON in common with most mitochondrial diseases are inadequate.^{11–14} Many experimental approaches have been proposed.^{15–17} However, only the allotopic expression approach^{18–27} has reached human testing in China (NCT01267422) and France (NCT02064569). We describe the preliminary findings from the first 5 participants enrolled in this trial based in the United States (NCT02161380). Our Investigational New Drug (#15941) was approved by the Food and Drug Administration in March 2014, the Research Advisory Council of the National Institutes of Health in June 2014 (protocol #1404–1306), and the institutional review board (#20140248) in July 2014.

Methods

Approximately 1500 mitochondrial proteins are encoded by nuclear genes and imported into the organelle,²⁸ but in the current study a full-length mitochondrial encoded gene (the ND4 subunit) was recoded in the “universal” genetic code and imported into the mitochondrion

from the cytoplasm by adding a targeting sequence derived from the P1 isoform of subunit c of ATP synthase. We refer to the nuclear encoded ND4 with the appended subunit c of ATP synthase targeting sequences as *PIND4v2*. It was inserted into a self-complementary adeno-associated vector (scAAV2)(Y444,500,730F). The use of this vector was followed by ND4 expression in almost all retinal ganglion cells by 1 week after intravitreal injection in the mouse.²⁹ We first determined its safety and tolerability in rodents and nonhuman primates,³⁰ and then in patients with LHON. This open-label, phase I, dose-escalation study aims to assess the safety of 3 vector doses (5×10^9 vg, 2.46×10^{10} vg, and 1×10^{11} vg) of scAAV2(Y444,500,730F)-*PIND4v2* in 3 groups of patients with LHON with molecularly confirmed G11778A DNA as follows.

Group 1

The bilateral chronic group includes participants with ≥ 12 months since the onset of visual loss in 1 eye and at least 6 months since the onset in the more recently affected eye. For inclusion in the study, both eyes must have acuity reduced to ≥ 35 letters (Snellen = 20/200). If both eyes have ≥ 12 months since the onset, the eye with worse visual acuity is injected. If both eyes have the same acuity, the eye with the longest duration of vision loss is injected. If 1 eye has <12 months and >6 months since onset and the difference in acuity between the eyes is ≥ 10 letters, the eye with ≥ 12 months is injected. If eyes have an acuity difference >10 letters, the eye with worse acuity is injected.

Group 2

Group 2 includes those with acute and bilateral loss of visual acuity to <35 Early Treatment Diabetic Retinopathy Study (ETDRS) letters in both eyes for less than 12 months. The worse eye is injected.

Group 3

Group 3 includes those with acute unilateral loss of acuity to <35 ETDRS letters for less than 12 months in 1 eye, but with mildly impaired but good acuity ≥ 70 letters (Snellen = 20/40) in the contralateral eye. The better eye will be injected.

If none of the 3 subjects at a given dose level develops visual loss or a systemic toxicity, the next cohort of participants will receive the next higher dose. We wait a minimum of 6 weeks between injections of the same dose and 3 months before moving to the next higher dose. We test low dose first in 3 participants of group 1 before moving to testing of this low dose in group 2, then last in group 3. Because group 3 is to receive an injection into an eye with good vision before it loses vision like the fellow eye, we wait longer to ensure that there is no toxicity in the blind eyes of groups 1 and 2 with $\geq 20/200$ before injecting group 3.

The primary outcome measure is a loss of visual acuity to no light perception. Entry into the study included 2 baseline visits, followed by the injection, clinic visits on postinjection day 1, 7, 30, 60, 90, 180, 270, and 365, and then visits every 180 days for up to 3 years. Serial testing included best-corrected visual acuity using the ETDRS chart performed by a certified ophthalmic technologist masked to the identity of the treated eye. Qualifying visual acuity was assessed at a baseline 1 visit, and change in acuity during follow-up was determined

from a preinjection baseline 2 measurement to guard against regression to the mean. Humphrey 30-2 white-on-white standard visual field, pattern electroretinogram (PERG), and spectral-domain optical coherence tomography (SD OCT) were also performed. Neuro-ophthalmic examination included assessments of the pupils, anterior segment, and ophthalmoscopy of the optic nerve head and retina.

Molecular Analysis

A polymerase chain reaction (PCR)-based test using the amplification refractory mutation system was used to detect the presence or absence of 3 nucleotide substitutions known to cause LHON (3460 G>A, 11778 G>A, or 14484 T>C). The affected patients had molecular genetic analysis performed by the Carver laboratory (University of Iowa), Athena Laboratory, or Emory University Genetics Laboratory.

Optical Coherence Tomography

Peripapillary retinal nerve fiber layer (RNFL) thickness was measured by an Optic Disk Cube 200 × 200 scan of the spectral-domain Cirrus HD OCT (software version 3.0; Carl Zeiss Meditec, Dublin, CA). Cirrus SD OCT uses SD OCT technology to acquire OCT data with better resolution (5 μm) and has a scanning rate of 27 000 A-scans per second. The Optic Disk Cube 200 × 200 protocol was used for acquisition and analysis. This protocol generates a cube of data through a 6-mm square grid by acquiring a series of 200 horizontal scan lines each composed of 200 A-scans; the total scan time was 1.48 seconds. For analysis, Cirrus algorithms identify the center of the optic disc and automatically place a calculation circle of 3.46 mm diameter around it. The anterior and posterior margins of the RNFL are delineated, and after extracting from the data cube 256 A-scan samples along the path of the calculation circle, the system calculates the RNFL thickness at each point on the circle. Three consecutive Optic Disk Cube scans were acquired and analyzed for each eye.

Pattern Electroretinogram

The PERG was simultaneously recorded from both eyes according to a paradigm optimized for glaucoma detection. The method is highly reproducible, and normative data are available. Pattern electroretinogram waveforms were automatically analyzed in the frequency domain by discrete Fourier transform to isolate the frequency component at the contrast-reversal rate (16.28 Hz) and compute its amplitude in μV and phase in π rad.

Dose Preparation

To adjust the study drug to 300 mOsm, 199.5 μl of water for injection was added to 150 μl of freshly thawed scAA-V2(Y444,500,730F)-*PIND4v2*. This gave a titer of 1.678×10^{11} vg/ml in a total volume of 349.5 μl of a solution that is iso-osmotic relative to the vitreous. To prepare the low dose (5×10^9 vg), 1996.5 μl of balanced salt solution (Alcon, Fort Worth, TX) was added to the iso-osmotically adjusted solution. To prepare the medium dose (2.46×10^{10} vg), 127.3 μl of balanced salt solution was added to the iso-osmotically adjusted solution. The volume to be administered, 200 μl, was loaded into a 1-ml tuberculin syringe.

Intravitreal Injection Procedure

Participants received injections in a clinic room in the outpatient clinic. One drop of proparacaine 0.5% followed by 1 drop of lidocaine 4% and 1 drop of Betadine 5% (Alcon) was instilled into the study eye; this was repeated twice. The eyelashes and lids of the study eye were swabbed with a Betadine swab stick, and a sterile drape was put in place. Personnel and participant were masked, and sterile gloves were used to handle drapes and instruments. A sterile eyelid speculum was placed in the study eye to keep the eyelids open. An anterior chamber paracentesis was performed using a 3-ml syringe mounted with a 32-gauge needle, and 0.15 to 0.2 ml of aqueous was removed to reduce risk of reflux after injection of study drug and prevent a spike in intraocular pressure (IOP). Viral vector was in a volume of 0.2 ml in a 1-ml BD Luer-Lok syringe (Becton Dickinson, Franklin Lakes, NJ) mounted with a 32-gauge stainless-steel needle (TSK Laboratory, Tochigi, Japan). Study drug was injected into the vitreous over approximately 3 to 4 seconds at a position 3.5 to 4 mm posterior to the limbus, inserting the needle to the hub and rolling a sterile swab over the site after needle removal to reduce reflux. The eyelid speculum and drapes were then removed. The used injection syringe and disposables were discarded in approved biohazard waste containers. The eye was rinsed with sterile saline, and the lids were wiped with a sterile pad. Two drops of polymyxin-trimethoprim ophthalmic solution were placed into the study eye. The IOPs was measured with a Tono-Pen (Reichert Inc., Depew, NY) beginning 5 minutes after the injection and repeated every 15 minutes. If the IOP was less than 30 mmHg at 15 minutes after injection, the participant was discharged to return the following day. If the IOP was more than 30 mmHg, this procedure was repeated until the pressure was less than 30 mmHg.

Neutralizing Antibody Assay

Cells (RGC-5) were grown in T-75 flasks in Dulbecco's modified Eagle's medium (DMEM) containing high levels of glucose (4.5 mg/ml), 2 mmol/l L-glutamine, and 110 mg/l sodium pyruvate, supplemented with 10% fetal bovine serum. Cells were incubated at 37°C in 5% CO₂. The cells were harvested by trypsinization followed by a phosphate-buffered saline wash. Serum samples from 5 subjects with LHON obtained before and after intraocular injections and anterior chamber fluid (ACF) obtained at the time of intraocular injections were heat inactivated at 56°C for 35 minutes. Self-complementary AAV2 (Y444,500,730F)-smCBA-*mCherry* vector (5×10^3 genomic copies per cell)³¹ was added to serum-free DMEM medium and added to the heat-inactivated serum or ACF samples in DMEM at serial dilutions ranging from 1:5 to 1:20 480. The serum or ACF-vector mixtures were then used to infect RGC-5 cells and seeded in 96 wells at a density of 1×10^4 cells/well for 3 hours. After a 3-hour incubation, an equal volume of 10% fetal bovine serum DMEM was added to each well and incubated for 48 hours at 37° C in 5% CO₂. Two days postinfection, cells were observed under a fluorescent microscope (Olympus [Pittsburgh, PA] IX 50 Inverted Fluorescent Microscope). Each sample dilution was seeded in triplicate. Cells were then harvested by trypsinization, and 10 000 cells per sample were analyzed using a LSR-Fortessa-HTS flow cytometer equipped with DiVa-6 software (BD Biosciences, San Jose, CA). Fluorescence of *mCherry* was quantified with a PE-Texas-Red-A filter at an excitation wavelength of 532 nm and an emission band pass of 600 to 620 nm. The neutralizing antibody (NAb) titer was reported as the highest serum or ACF sample dilution that inhibited

scAAV2(Y444,500,730F)-smCBA-*mCherry* transduction by 50%, compared with no serum control.

Quantitative SYBR-Green Quantitative Polymerase Chain Reaction

Genomic DNA was extracted from participants' whole blood at baseline and postinjection days 1 and 7 using a kit (Qiagen [Venlo, The Netherlands], Cat. 69506) according to the manufacturer's instruction. The DNA concentration was measured spectrophotometrically (BioTek, Winooski, VT). Each sample was analyzed twice in triplicate using 0.5 µg of DNA in each reaction using a Bio-Rad (Hercules, CA) real-time PCR system (CFX Connect Real time SYSTEM). In addition, as a spike-in control, 10 pg of *PIND4v2* DNA plasmid was added to the participant's blood DNA. Quantitative SYBR-Green (Thermo Fisher Scientific, Waltham, MA) PCR was performed using the bovine growth hormone poly A primers (the bovine growth hormone polyadenylation): F1-5'-AGCCTCGACTGTGCCTTCTAGTT-3' and R1-5'-GGGTTCTGCTATTGTCTTCCCAA with SsoAdvanced (Bio-Rad) universal SYBR Green Supermix (Cat. 172-5271) according to a standard protocol as follows: 1 cycle at 95°C for 3 minutes and 40 cycles at 95°C for 30 seconds, annealing at 60 °C for 30 seconds and holding at 4°C. DNA plasmid was serially diluted into 10⁰, 10¹, 10², 10³, and 10⁴ pg; each dilution was in duplicate. A standard curve was constructed according to 10⁰ pg of 5136 base pairs DNA plasmid = 1.74 × 10⁵ copy number, 10¹ pg = 1.74 × 10⁶, 10² pg = 1.74 × 10⁷, 10³ pg = 1.74 × 10⁸, 10⁴ pg = 1.74 × 10⁹ copy number. The copy numbers were calculated by Bio-Rad Real-Time PCR system (CFX) software according to Ct value.

Results

Study Entry and Dosing

After obtaining informed consent, 5 legally blind participants with LHON with the G11778A mutation were entered in year 1 of the study (Table 1). Participants 1 to 4 were men, and participant 5 was a woman. All patients were white. Each received a single intravitreal injection of scAAV2(Y444,500,730F)-*PIND4v2* and returned for 2 or more postinjection visits (Table 2). Four participants had chronic visual loss for more than 1 year (group 1). The first 3 participants received the low dose (5 × 10⁹ vg), and the fourth participant received the medium dose (2.46 × 10¹⁰ vg). The fifth participant with bilateral visual loss for less than 12 months (group 2) received the low dose. None of the participants were taking idebenone, which must be stopped 1 month before injections and for the entire duration of the study.

Adverse Events

There was no loss of vision and no serious ocular or systemic adverse events. Minor adverse events included transient post-injection spike of IOP (participant 5), painful toxic/exposure keratitis (participant 5), subconjunctival hemorrhage (participant 5), and sore throat (participant 4) that resolved spontaneously. No haze, cells, or inflammation was detected in the vitreous or anterior chamber, and retinal examinations remained unchanged from baseline examinations.

Visual Function

Three participants (group 1, low dose) experienced no change in ETDRS visual acuity (Fig 1A–C), and 2 participants (group 1, medium dose [participant 4] and group 2, low dose [participant 5]) had an increase equivalent to 3 lines of vision. Visual acuity of participant 1 was hand movements before injection and remained unchanged 6 months after injection (Table 3). Participant 2 had vision at the level of finger counting before vector injection and 6 months after injection (Table 4). Participant 3 identified 10 to 14 ETDRS letters on baseline examinations and remained at 14 letters at day 60 (Table 5).

Three months after injection in participant 3, we enrolled a participant of group 1 to receive the medium dose. Participant 4 had visual loss in the right eye for more than 12 months, but visual loss in the left eye for less than 12 months. At baseline, he demonstrated hand movements and had dense central scotomas in both eyes (Fig 2F). On postinjection day 7, the participant identified a single ETDRS letter (Fig 1D) (Table 6). At follow-up visit day 30, the participant read 2 letters, an increase equivalent to 3 lines.³² The uninjected eye varied between hand movements and finger counting during this time. By day 90, he read 7 letters in the injected right eye and 9 letters in the uninjected eye.

Three months after injection in participant 3, we enrolled a participant from group 2. This fifth participant had visual loss for less than 12 months with 15 letters at baseline and central scotomas (Fig 2G). After receiving the low dose, the participant identified 30 letters in the injected eye on day 30, an increase of 3 lines that persisted to day 90 (Fig 1E) (Table 7). The uninjected eye went from 20 to 22 letters during the same interval (day 90), thus a smaller change of only 2 letters.

Spectral-Domain Optical Coherence Tomography and Pattern Electretinogram

In all 5 participants, we found no changes in RNFL thickness as measured by SD OCT (Fig 3). Participants 1 to 4 with chronic visual loss had loss of the RNFL and optic atrophy before (shown in Fig 3F for participant 4) that remained unchanged after injections. Participant 5 had normal average RNFL values and optic nerve heads that were normal by ophthalmoscopy (Fig 3G). In all participants, PERG amplitudes did not change and were at or near noise levels before and after injections (Fig 4).

Neutralizing Antibodies and Quantitative Polymerase Chain Reaction

Neutralizing antibodies were detected in all participants before injection (Table 8). A single participant had a transient postinjection increase in NAb titers in serum. Preinjection serum samples screened for the existence of NAb before injection of study drug found that 3 participants (participants 1, 2, and 5) exhibited strong inhibition to *mCherry* expression due to NAb against AAV2 (NAb = 20 480) at baseline, 1 day, 7 days, 3 months, and 6 months postinjection (Table 8). Participant 3 had a baseline NAb of 5, indicating low levels of NAb to AAV2 before and 1 day after injection that increased at day 7 to 20 then decreased to baseline levels at 3 months postinjection. The fourth participant had a NAb titer of 5120 at baseline, 1 day and 7 days postinjection. NAb in the ACF of all participants were lower than the serum levels. Preinjection and postinjection blood samples were negative for vector

DNA, indicating there was no significant leakage of vector into the systemic circulation (Table 9).

Discussion

None of the 5 participants in this phase I trial experienced any serious ocular or systemic adverse effects associated with this approach to gene therapy for LHON during the follow-up period. Ocular adverse events were transient and seen in a single participant (Table 10 shows the scaling of potential ocular toxicity). They consisted of elevation of IOP, painful exposure keratitis, and subconjunctival hemorrhage in 1 participant. Systemic adverse events consisted of transient sore throat in 1 participant who received the highest dose (the medium dose) of vector of the 5 participants treated.

Although the low dose of study drug had no effect on visual acuity in those with chronic visual loss (12 months, participants 1–3) and marked loss of RNFL, we observed an increase in acuity equivalent to 3 lines on the ETDRS chart when given at the medium dose to participant 4. This was seen even in the face of marked loss of the RNFL. In our natural history study,³ only 3 patients recovered vision of 15 letters recruited into that study 12 months after the onset of their visual loss (i.e., 7%). Participant 4 showed later improvements in the uninjected eye that had visual loss for less than 12 months. We also observed increases in acuity of 3 lines on the ETDRS chart with injection of the low dose to the only participant (#5) treated with acute visual loss who also had normal RNFL values, at a time before the optic nerve had undergone the characteristic neurodegeneration. In our natural history study, 10 of the 13 patients who recovered vision of 3 lines had experienced visual loss for <12 months. The improvements in vision of both participants in this trial were seen quickly within 7 to 30 days postinjection. Although these increases are within the range of spontaneous improvements sometimes seen in the natural history of G11778A LHON, similar changes were not observed on the 3 examinations before the injection (baseline 1 and 2 and the injection day). Rapid expression of the vector seen in our animal studies may have played some role.²⁹ More important, we want to point out that none of the 5 patients in this study lost vision, whereas in our natural history study, vision worsened in 6 patients (14%), although eligibility criteria were different in the 2 studies. Therefore, at this point, allotopic ND4 gene therapy does not seem to be harmful.

Despite the absence of vector DNA detection in our participant blood samples, NABs to AAV2 that could reduce the expression of the delivered normal ND4 gene were elevated in all participants before injection and increased transiently in 1 participant. However, the baseline NAB levels detected from intraocular fluid removed at the time of injection were lower. The 2 participants with improved vision had the lowest levels of NABs detected in ocular fluid, but high levels in serum. These findings suggest that high serum levels of NABs may not be a barrier to successful ocular gene therapy. Additional enrollment and follow-up planned over the next 4 years are needed to confirm these preliminary observations.

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Abbreviations and Acronyms

AAV	adeno-associated virus vector
ACF	anterior chamber fluid
DMEM	Dulbecco's modified Eagle's medium
ETDRS	Early Treatment Diabetic Retinopathy Study
IOP	intraocular pressure
LHON	Leber hereditary optic neuropathy
mtDNA	mitochondrial DNA
NAb	neutralizing antibody
PCR	polymerase chain reaction
PERG	pattern electroretinogram
RNFL	retinal nerve fiber layer
scAAV	self-complementary adeno-associated vector

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Figure 1.

Plot of visual acuity (logarithm of the minimum angle of resolution) improvement of injected and uninjected fellow eyes of participants 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). BL1 = baseline 1 examination; BL2 = baseline 2 examination; D01 = postinjection day 1; D07 = postinjection day 7; D30 = postinjection day 30; Inj = intravitreal injection day; logMAR = logarithm of the minimum angle of resolution; M03 = postinjection month 3; M06 = postinjection month 6.

Figure 2.

Plot of automated visual field mean defect improvement of injected and uninjected fellow eyes of participants 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). The baseline visual fields are shown for participants 4 (F) and 5 (G). BL2 = baseline 2 examination; dB = decibels; D01 = postinjection day 1; D07 = postinjection day 7; D30 = postinjection day 30; HVF = Humphrey visual field; Inj = intravitreal injection day; MD = mean defect; M03 = postinjection month 3; M06 = postinjection month 6; OD = right eye; OS = left eye.

Figure 3.

Plot of spectral-domain optical coherence tomography (SD OCT) retinal nerve fiber layer (RNFL) improvement of injected and uninjected fellow eyes of participant 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). The baseline fundus photographs of the optic nerve head are shown for participants 4 (F) and 5 (G). BL2 = baseline 2 examination; D01 = postinjection day 1; D07 = postinjection day 7; D30 = postinjection day 30; Inj = intravitreal injection day; M03 = postinjection month 3; M06 = postinjection month 6; OD = right eye; OS = left eye.

Figure 4.

Plot of pattern electroretinogram (PERG) amplitude improvement of injected and uninjected fellow eyes of participants 1 (A), 2 (B), 3 (C), 4 (D), and 5 (E). BL2 = baseline 2 examination; D01 = postinjection day 1; D07 = postinjection day 7; D30 = postinjection day 30; Inj = intravitreal injection day; M03 = postinjection month 3; M06 = postinjection month 6.

Table 1

Participation Demographics

Subject ID	Gender	Age, yrs	LHON Diagnosis Since (Age, yrs)
01	Male	45	41
02	Male	55	35
03	Male	37	34
04	Male	35	32
05	Female	49	49

LHON = Leber hereditary optic neuropathy.

Table 2

Length of Follow-Up Data

Subject ID	Injection (Day 0)	Day 1	Day 7	Day 30	Day 60	Day 90	Day 180	Day 270	Month 12
01	✓	✓	✓	✓	✓	✓	✓		
02	✓	✓	✓	✓	✓	✓	✓		
03	✓	✓	✓	✓	✓	✓			
04	✓	✓	✓	✓	✓	✓			
05	✓	✓	✓	✓	✓	✓			

Table 3

Visual Data of Participant 1

	Injected Eye (OS)				Uninjected Eye (OD)			
	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp uV</i>	<i>RNFL μm</i>	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp uV</i>	<i>RNFL μm</i>
BL1	HM	-32.76	0.17	54	HM	-32.76	0.30	57
BL2	HM	-32.76	0.18	54	HM	-32.76	0.24	58
Before injection	HM	-	-	-	HM	-	-	-
Day 1	HM	-32.76	0.27	-	HM	-32.76	0.25	-
Day 7	HM	-32.76	0.05	56	HM	-32.76	0.11	58
Month 1	HM	-32.76	0.27	-	CF	-32.76	0.37	-
Month 2	HM	-32.76	0.26	63	HM	-32.76	0.31	57
Month 3	HM	-32.71	*	-	HM	-32.71	*	-
Month 6	HM	-32.71	0.24	-	HM	-32.71	0.20	-

BL = baseline; CF = count fingers; dB = decibels; HM = hand movements; HVF = Humphrey visual field; OD = right eye; OS = left eye; PERG = pattern electroretinogram amplitude; RNFL = retinal nerve fiber layer; - = test is not required for this visit.

No changes in acuity, HVF, PERG amplitude, or RNFL thickness were detected up to 6 months postinjection, the longest time period studied thus far. The dynamic range of the PERG amplitude 1 to 0.25 uV. Normal RNFL is 80 to 100 μ m.

* PERG technician was unavailable for month 3 examination.

Table 4

Visual Data of Participant 2

	Injected Eye (OD)				Uninjected Eye (OS)			
	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RR RNFL</i>	<i>Acuity</i>	<i>H HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>
BL1	CF	-30.99	0.45	54	CF	-24.69	0.35	53
BL2	CF	-31.57	0.48	51	CF	-30.47	0.42	52
Before Injection	CF	-	-	-	CF	-	-	-
Day 1	CF	-31.43	0.40	-	CF	-28.97	0.46	-
Day 7	CF	-31.81	0.57	55	CF	-30.96	0.40	54
Month 1	CF	-30.38	0.46	-	CF	-26.20	0.49	-
Month 2	CF	-27.80	0.30	51	CF	-29.52	0.31	51
Month 3	CF	-26.20	0.3	-	CF	-27.34	0.33	-
Month 6	CF	-20.14	0.33	-	CF	-22.23	0.33	-

BL = baseline; CF = count fingers; HVF = Humphrey visual field; OD = right eye; OS = left eye; PERG = pattern electroretinogram amplitude; RNFL = retinal nerve fiber layer; - = test is not required for this visit.

Visual acuity shows no changes in participant 2.

Table 5

Visual Data of Participant 3

	Injected Eye (OS)				Uninjected Eye (OD)			
	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>
BL1	10	-28.10	0.16	59	13	-27.15	0.38	59
BL2	14	-25.40	0.45	56	18	-20.42	0.32	61
Before injection	14	-	-	-	18	-	-	-
Day 1	9	-22.60	0.25	-	10	-22.50	0.44	-
Day 7	11	-19.87	0.24	56	17	-19.72	0.27	58
Day 30	10	-26.71	0.33	-	15	-23.74	0.42	-
Month 2	14	-26.04	0.23	61	15	-22.79	0.34	64
Month 3	14	-26.60	0.35	-	20	-23.48	0.33	-

BL = baseline; dB = decibels; OD = right eye; OS = left eye; PERG = pattern electroretinogram; RNFL = retinal nerve fiber layer.

No changes in visual acuity, visual fields, PERG, or RNFL were detected by month 3 postinjection.

Table 6

Visual Data of Participant 4

	Injected Eye (OD)				Uninjected Eye (OS)			
	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>
BL1	HM	-32.99	0.25	73	HM	-33.26	0.13	78
BL2	HM	-33.22	0.1	62	HM	-33.32	0.16	80
Before injection	HM	-		-	HM			-
Day 1	HM	-32.74	0.08	-	CF	-32.45	0.08	-
Day 7	1 letter	-32.92	0.13	58	HM	-32.66	0.14	72
Day 30	2 letters	-32.80	0.09		CF	-31.31	0.14	

BL = baseline; CF = count fingers; dB = decibels; HM = hand movements; HVF = Humphrey visual field; OD = right eye; OS = left eye; PERG = pattern electroretinogram; RNFL = retinal nerve fiber layer.

By day 7, the patient read 1 letter and was able to count fingers that persisted at 1 month. As Scott et al³⁰ used the convention that count fingers = 1/200 or logarithm of the minimum angle of resolution (logMAR) 2.30 and HM = 0.5/200 or logMAR -2.60, in patient 4 an improvement from 2.3 to 2.6 is 0.30 corresponds to 15 letters. Therefore, it is reasonable to consider this patient's improvement is approximately 3 lines or 15 letters.

Table 7

Visual Data of Participant 5

	Injected Eye (OD)				Uninjected Eye (OS)			
	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>	<i>Acuity</i>	<i>HVF (dB)</i>	<i>PERG Amp</i>	<i>RNFL</i>
BL1	13	-12.24	0.19	98	15	-14.64	0.23	100
BL2	15	-16.33	0.23	103	20	-9.67	0.16	104
Before injection	19	-		-	21	-		-
Day 1	20	-10.05	0.06	-	18	-16.35	0.31	-
Day7	26	-10.59	0.14	97	21	-8.23	0.22	110
Day 30	30	-7.46	0.12	-	24	-9.62	0.25	-
Day 60	29	-7.87	0.16	99	22	-7.95	0.15	104
Day 90	30	-8.43	0.22	-	22	-13.83	0.17	-

BL = baseline; dB = decibels; HVF = Humphrey visual field; PERG = pattern electroretinogram; OD = right eye; OS = left eye; RNFL = retinal nerve fiber layer.

The injected eye had a 3-line or 15-letter increase by day 30 that persisted at day 90. The uninjected eye saw only 2 more letters by day 90 relative to baseline 2.

Table 8

Neutralizing Antibody Titers

Sample	Time Points	LHON Subject ID				
		01	02	03	04	05
Serum	BL1	20 480	20 480	5	5120	20 480
	Postinjection day 1	20 480	20 480	5	5120	20 480
	Postinjection day 7	20 480	20 480	20	5120	20 480
	Postinjection day 90	20 480	20 480	5	-	-
	Postinjection day 180	20 480	-	-	-	-
ACF	DOI	118	10	5	5	5

ACF = anterior chamber fluid; BL1 = baseline; DOI = day of injection; LHON = Leber hereditary optic neuropathy.

Neutralizing antibodies were detected in the serum of all participants before injections. Only patient 3 had a transient postinjection increase in NAb titers. Neutralizing antibodies in ACF of the eye were lower than serum values.

Table 9

Blood Quantitative Polymerase Chain Reaction Detection of Vector DNA

Subject ID	Samples	qPCR	Spike -DNA	Copy Number of AAV2P1ND4v2 in 0.5 µg Blood DNA				Score
				Replicate 1	Replicate 2	Replicate 3	Average	
01	BL	Run 1	1.79e+06	44	78	0	61	0
		Run 2	3.42e+05	0	0	0	0	0
	PI Day 1	Run 1	1.54e+06	20	14	0	17	0
		Run 2	6.72e+05	0	0	0	0	0
	PI Day 7	Run 1	2.01e+05	0	0	0	0	0
		Run 2	5.94e+05	0	0	0	0	0
02	BL	Run 1	1.96e+06	0	0	0	0	0
		Run 2	4.98e+05	0	0	0	0	0
	PI Day 1	Run 1	2.27e+05	0	0	0	0	0
		Run 2	4.85e+05	0	0	0	0	0
	PI Day 7	Run 1	9.47e+05	0	0	0	0	0
		Run 2	3.28e+05	0	0	0	0	0
03	BL	Run 1	9.20e+05	32	28	0	20	0
		Run 2	8.29e+05	0	0	0	0	0
	PI Day 1	Run 1	4.10e+05	50	33	0	21	0
		Run 2	9.90e+05	0	0	0	0	0
	PI Day 7	Run 1	8.30e+05	161	351	0	171	1
		Run 2	1.44e+06	0	0	0	0	0
04	BL	Run 1	9.95e+05	0	0	0	0	0
		Run 2	3.86e+05	16	0	48	21	0
	PI Day 1	Run 1	4.40e+05	0	0	0	0	0
		Run 2	2.26e+05	8	0	259	89	0
	PI Day 7	Run 1	1.21e+06	0	0	0	0	0
		Run 2	1.26e+06	9	10	273	97	0
05	BL	Run 1	2.33e+06	0	55	0	18	0
		Run 2	2.46e+06	92	63	55	70	0
	PI Day 1	Run 1	4.86e+05	0	0	0	0	0
		Run 2	5.91e+05	8	0	170	59	0
	PI Day 7	Run 1	3.68e+05	0	0	6	2	0
		Run 2	4.25e+05	12	9	0	7	0

BL = baseline; PI = postinjection; qPCR = quantitative polymerase chain reaction.

Blood samples were negative for vector DNA. Scoring 0 = 0–99, +1 = 100–500, +2 = 501–5000, +3 = 5001–50,000, +4 = >50,000 copies/0.5 µg DNA. No template control 500, copy number 500 as negative.

Table 10**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	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
Retinal detachment	Localized and intervention not indicated	Not resolving but intervention not yet indicated	Operative intervention indicated	Operative failure threatening ocular integrity

EIOP = elevated intraocular pressure.