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Efficacy and Safety of Intravitreal Gene Therapy for Leber Hereditary Optic Neuropathy Treated within 6 Months of Disease Onset

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Purpose: To evaluate the efficacy of a single intravitreal injection of rAAV2/2-*ND4* in subjects with visual loss from Leber hereditary optic neuropathy (LHON).

Design: RESCUE is a multicenter, randomized, double-masked, sham-controlled, phase 3 clinical trial.

Participants: Subjects with the m.11778G>A mitochondrial DNA mutation and vision loss ≤6 months from onset in 1 or both eyes were included.

Methods: Each subject's right eye was randomly assigned (1:1) to treatment with rAAV2/2-*ND4* (single injection of 9×10^{10} viral genomes in 90 μl) or to sham injection. The left eye received the treatment not allocated to the right eye.

Main Outcome Measures: The primary end point was the difference of the change from baseline in best-corrected visual acuity (BCVA) between rAAV2/2-*ND4*–treated and sham-treated eyes at week 48. Other outcome measures included contrast sensitivity, Humphrey visual field perimetry, retinal anatomic measures, and quality of life. Follow-up extended to week 96.

Results: Efficacy analysis included 38 subjects. Mean age was 36.8 years, and 82% were male. Mean duration of vision loss at time of treatment was 3.6 months and 3.9 months in the rAAV2/2-*ND4*–treated eyes and sham-treated eyes, respectively. Mean baseline logarithm of the minimum angle of resolution (logMAR) BCVA (standard deviation) was 1.31 (0.52) in rAAV2/2-*ND4*–treated eyes and 1.26 (0.62) in sham-treated eyes, with a range from −0.20 to 2.51. At week 48, the difference of the change in BCVA from baseline between rAAV2/2-*ND4*–treated and sham-treated eyes was −0.01 logMAR ($P = 0.89$); the primary end point of a −0.3 logMAR (15-letter) difference was not met. The mean BCVA for both groups deteriorated over the initial weeks, reaching the worst levels at week 24, followed by a plateau phase until week 48, and then an improvement of +10 and +9 Early Treatment Diabetic Retinopathy Study letters equivalent from the plateau level in the rAAV2/2-*ND4*–treated and sham-treated eyes, respectively.

Conclusions: At 96 weeks after unilateral injection of rAAV2/2-*ND4,* LHON subjects carrying the m.11778G>A mutation treated within 6 months after vision loss achieved comparable visual outcomes in the injected and uninjected eyes. *Ophthalmology 2021;128:649-660 © 2020 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

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See Commentary on page 661.

Leber hereditary optic neuropathy (LHON) is a maternally inherited, blinding, bilateral optic neuropathy[1] that affects approximately 1 in 30 000 to 1 in 50 000 people, particularly young adult men.[2] It is the most common primary mitochondrial DNA (mtDNA) disorder, with 3 mtDNA point mutations accounting for approximately 90% of all LHON cases, namely, m.3460G>A (*MT-ND1*), m.11778G>A (*MT-ND4*), and m.14484T>C (*MT-ND6*), with m.11778G>A being the most common mutation worldwide.[1,3] These mutations affect complex I subunits of the mitochondrial respiratory chain, impairing mitochondrial function and increasing the levels of reactive oxygen species. The pathophysiology of LHON is characterized by selective loss of retinal ganglion cells (RGCs) and their axons, which may lead, after a prolonged asymptomatic period, to subacute, rapidly

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progressive bilateral vision loss.[2] Retinal ganglion cells are particularly vulnerable to mitochondrial dysfunction, which leads to axonal swelling and apoptotic cell death, and results in optic nerve degeneration and the development of optic atrophy.[3]

The m.11778G>A mutation is associated with a poor visual outcome as most patients progress to vision worse than 20/200 within the first year after disease onset.[4] Spontaneous recovery occurs in a minority of patients (14.4% in a recent large meta-analysis),[5] and most patients remain severely visually impaired.[6] This rapid, dramatic, irreversible loss of vision is life changing, with a major emotional and socioeconomic impact on affected patients and their families.[7] Current treatments for LHON remain inadequate.[8] Idebenone (Raxone, Santhera GmbH) was granted market authorization by the European Medicines Agency (EMA) for the treatment of LHON under exceptional circumstances,[9,10] but its benefits are limited. Leber hereditary optic neuropathy remains a seriously debilitating disease with a continued unmet medical need for efficacious therapies.

Over the past decade, substantial progress has been made in the application of gene therapy to monogenic blinding diseases, with the first treatment approved by both the U.S. Food and Drug Administration and the EMA for an inherited retinal degenerative disorder, Leber congenital amaurosis caused by recessive *RPE65* mutations.[11] Gene therapy for mitochondrial disorders, however, is more challenging because of the multicopy nature of mtDNA and the current lack of efficient means to directly deliver nucleic acids to the mitochondrial matrix compartment (which requires efficient transfer across both the outer and inner mitochondrial membranes). However, by using an allotopic expression strategy, LHON may be amenable to gene therapy wherein the wild-type recoded replacement gene is delivered and expressed into the cell's nucleus, then the cytoplasm-translated protein is targeted for mitochondrial import by a specific mitochondrial targeting sequence (MTS), similar to what normally occurs with those mitochondrial proteins naturally encoded by nuclear DNA.[12] The rAAV2/2-*ND4* (GS010) product is a recombinant replication-defective adeno-associated virus, serotype 2, carrying a modified complementary DNA (cDNA) encoding the human wild-type mitochondrial ND4 protein and a specific MTS. Preclinical studies showed that rAAV2/2-*ND4* efficiently rescued complex I dysfunction in cells carrying the m.11778G>A mutation and in an induced rat model of LHON.[12-15] Based on these preclinical studies, a first-in-human trial (GS-LHON-CLIN-01) showed that a single intravitreal administration of increasing doses of rAAV2/2-*ND4* was safe and well tolerated in LHON subjects.[16,17] Two phase 3 clinical trials with a follow-up of 96 weeks postadministration of the gene therapy vector and assessing the efficacy of intravitreal injection of rAAV2/2-*ND4* in LHON subjects with the m.11778G>A mutation are now completed (RESCUE NCT02652767 and REVERSE NCT02652780). An additional phase 3 clinical study with bilateral intravitreal injections is ongoing (REFLECT NCT03293524).

In the REVERSE trial, LHON subjects had loss of vision in both eyes of 6 months to 1 year at study enrollment, and a bilateral visual improvement was observed over the 96 weeks after treatment administration.[18] The RESCUE trial was designed concurrently with the REVERSE trial with similar assessment protocols and outcome measures, but subjects had to have vision loss within 6 months in at least 1 eye and vision loss of no longer than 6 months in both eyes, enabling a study of earlier treatment in the subacute phase of LHON. We present the results of the RESCUE trial at 96 weeks after unilateral intravitreal injection of rAAV2/2-*ND4*, with a comparative analysis to the results of the REVERSE trial.

Methods

Study Design

RESCUE (NCT02652767) was a randomized, double-masked, sham-controlled, phase 3 clinical trial to evaluate the efficacy of a single intravitreal injection of rAAV2/2-*ND4* in LHON subjects with the m.11778G>A mutation and vision loss within 6 months in at least 1 eye and vision loss of no longer than 6 months in both eyes. Subjects were enrolled in 7 centers in France, Germany, Italy, the United Kingdom, and the United States. The objective was to evaluate the efficacy of rAAV2/2-*ND4* compared with a sham injection at weeks 48 and 96, using the difference in change in logarithm of the minimal angle of resolution (logMAR) visual acuity from baseline, with the primary end point being at week 48. An interim analysis was planned at week 72 to gain additional insights on the results at week 48. We report these data and the follow-up data at 96 weeks.

The right eye of each subject was randomly allocated to receive treatment with rAAV2/2-*ND4* via intravitreal injection (9×10^{10} viral genomes in 90 μl per eye) or a sham injection irrespective of which eye was first affected. The fellow (left) eye received the treatment not allocated to the right eye in a 1:1 ratio. Treatment with prophylactic oral or topical steroids was not provided. Both pupils were dilated. Both eyes received standard antiseptic preparation, followed by the administration of a topical ocular anesthetic agent and a topical intraocular pressure−lowering agent. The viral vector rAAV2/2-*ND4* was administered in a single intravitreal injection. For the sham-treated eye, the blunt end of a syringe was applied on the eye at a typical injection site. Only the pharmacy team, the injecting physician, and the medical team assisting in treatment were unmasked to treatment allocation. The unmasked study team performed the assessment on the first day after treatment, whereas a separate medical team masked to treatment allocation performed all the subsequent ocular examinations.

The protocol was reviewed and approved by independent ethics committees at all recruitment sites, and informed consent was obtained from all participants. The study was conducted in accordance with the principles and requirements of the International Conference on Harmonization Good Clinical Practice and adhered to the ethical principles outlined in the Declaration of Helsinki. An independent Data Safety Monitoring Board periodically reviewed study data to ensure the continued safe conduct of the trial and protection of subjects.

To be enrolled, LHON subjects had to be age 15 years or older. Vision loss could be in 1 or both eyes, but with duration of no longer than 6 months in each eye, and with visual acuity of counting fingers or better in each eye. Documented genotyping was required to confirm the presence of the m.11778G>A

mutation in the *MT-ND4* gene and the absence of other primary LHON-associated mutations (m.3460G>A in *MT-ND1* or m.14484T>C in *MT-ND6)*. Whole mitochondrial genome sequencing was not performed. The subjects recruited into RESCUE had classic LHON phenotypes and nuclear genome sequencing was not specifically requested to exclude other optic atrophy genes, although the exclusion criteria also included any previously known inherited retinal or optic nerve conditions. Additional exclusion criteria were previous treatment with an ocular gene therapy product, glaucoma, optic neuropathy other than LHON, history of amblyopia, previous vitrectomy in either eye, or ocular surgery of clinical relevance within 90 days. Prior use of idebenone was required to have ceased at least 7 days before enrollment. This was thought to be a sufficient length of time because idebenone is rapidly absorbed with an average plasma half-life of approximately 15 hours.[19]

Outcome Measures

Ophthalmic evaluations included assessment of best-corrected visual acuity (BCVA) using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart at 1 or 4 m, assessment of contrast sensitivity using the Pelli-Robson chart, Humphrey visual field (HVF) perimetry, Farnsworth-Munsell 100-Hue Color Vision testing, slit-lamp biomicroscopy, Goldmann applanation tonometry, funduscopy, spectral-domain OCT, and color fundus photography. A quality of life assessment was performed before and after treatment at 96 weeks.

When subjects could not read any letters on the ETDRS chart, they were asked to count the assessor's fingers or to detect hand motion. An off-chart Snellen equivalent was derived using both the distance at which the assessment was made and the size of the assessor's fingers.[20] The method was also adapted to hand motion visual acuity to provide conversion into a logMAR value. Light perception and no light perception visual acuities were assigned a value of 4.0 and 4.5 logMAR, respectively.

The contrast sensitivity (CS), which is the reciprocal of contrast threshold, was measured using the Pelli-Robson chart at 1 m according to test instructions and expressed as a logarithm (LogCS).[21] Subjects who could not read any letters on the Pelli-Robson chart were assigned the worst possible score (0 LogCS).

Intraocular inflammation was assessed and graded according to the Standardization of Uveitis Nomenclature[22] and the National Institutes of Health Grading Scale for Vitreous Haze.[23]

Spectral domain-OCT was performed with the Spectralis OCT (Heidelberg Engineering). Parameters were measured for the optic nerve and posterior pole (peripapillary retinal nerve fiber layer [RNFL], macular ganglion cell layer, and RNFL of the papillomacular bundle), as per standard protocols included in the Spectralis software. At prespecified visits, certified technicians performed 1 "optic nerve head − radial scan and concentric circle scan" and 1 "posterior pole N scan" for each eye, after maximal dilation. A reading center masked to treatment allocation—the William H. Annesley EyeBrain Center at Thomas Jefferson University partnered with Wills Eye Hospital—performed quality control, analysis, manual segmentation when necessary, and interpretation of all spectral-domain OCT data.

The standardized automated HVF 30-2, central threshold, SITA-FAST procedure was performed with the HVF Analyzer II and a white size III stimulus. The reliability of the HVF test results was quality controlled by the EyeBrain reading center, and the HVF test was repeated if considered unreliable (i.e., fixation losses ≥15%, false-positive errors ≥20%, or false-negative errors ≥33%).

Quality of life was assessed at enrollment and at week 96 using the National Eye Institute Visual Function Questionnaire.[24] The National Eye Institute Visual Function Questionnaire-25 consists of 25 vision-targeted questions representing 11 vision-related constructs and a General Health rating question. All items were scored on a 0 to 100 scale with a high score representing better functioning. An overall Composite Score was calculated as the average of the vision-targeted subscale scores, excluding the General Health rating question. For each subscale, the change from baseline was calculated in terms of the average score increase/decrease, and as the average of percentage changes in scores. Clinically relevant change was determined as described previously.[25]

Statistical Analyses

The primary end point for the RESCUE study was the between-eye change of logMAR BCVA from baseline to week 48 after treatment. A difference of −0.3 logMAR (15 ETDRS letters equivalent) between the change from baseline in the rAAV2/2-*ND4*−treated eyes and the sham-treated eyes was considered clinically significant based on Food and Drug Administration recommendations. The sample size calculation was based on the primary end point and a paired comparison of rAAV2/2-*ND4*−treated and sham-treated eyes. Based on available data,[16] the standard deviation of the change from baseline for the within-subject difference between rAAV2/2-*ND4*−treated eyes and the sham-treated eyes was estimated to be 0.594 logMAR. The treatment effect was estimated to be 0.3 logMAR. By using these estimates, a sample size of 36 subjects would provide a power of 85%.

The baseline value of functional end points for BCVA, CS, and HVF perimetry was defined as the last reported value before treatment administration, considering the subacute progression of visual signs. For OCT parameters, baseline was defined as the average value of screening and inclusion visits assessments. Measurements of BCVA were performed at the following time points after treatment: 1 day, 2 weeks (±2 days), 4 weeks (±3 days), 8 weeks (±6 days), 12 weeks (±9 days), 24 weeks (±17 days), 48 weeks (±30 days), 72 weeks (±30 days), and 96 weeks (±30 days). We defined the "nadir" for each eye of each subject as the worst BCVA measured from baseline to week 96 (including baseline and week 96 values). A statistical analysis plan was prepared after the study protocol was approved and before the database lock.

The efficacy analyses were run using the intention-to-treat (ITT) population including all subjects who were randomized and received the actual study treatment (rAAV2/2-*ND4*). The safety analyses were run using the safety population including all subjects who received the study treatment (rAAV2/2-*ND4*).

The change of logMAR BCVA from baseline to week 96 in rAAV2/2-*ND4*−treated eyes was compared with that in sham-treated eyes (intra-subject comparison) using a mixed-effects analysis of covariance model with subject and eyes of the subject as random factors, treatment as a fixed effect, and baseline logMAR BCVA as a covariate. The difference in the mean change from baseline between the 2 treatment groups and associated 95% confidence interval were reported. Statistical significance was assessed using a 2-sided alpha of 0.05.

A clinically relevant recovery (CRR) at week 96 was defined from baseline and from nadir as either an eye that was on-chart (i.e., able to see letters on the chart) at baseline or nadir and showed an improvement of at least 10 ETDRS letters or an eye that was off-chart (i.e., not able to see letters on the chart) at baseline or nadir and became able to read 5 ETDRS letters on-chart at 1 m.[9,10,26,27] A subject responder was defined as having this response in at least 1 eye at week 96. The CRR was considered by the EMA in its assessment of the efficacy of idebenone (Raxone) in LHON.[9] A group of experts also endorsed the use

of CRR as a valid outcome measure when assessing the effect of treatment with idebenone in LHON.[1]

Results

Subject Disposition

A total of 39 subjects with LHON due to the m.11778G>A mutation and vision loss for ≤6 months were enrolled in the RESCUE trial between February 2016 and July 2017. One subject received a lower dose than planned in the protocol, and this case was not included in the ITT analysis (n = 38). One subject died and was discontinued from the study before week 48. Three subjects were discontinued after week 48 and before week 96, 1 of whom died. The 2 deaths were deemed unrelated to study treatment or procedure by the investigators, and the last follow-up data were obtained at week 8 and week 48. One subject withdrew consent, and 1 subject was lost to follow-up, with their last visit performed at week 48 (Table 1).

Demographics and Baseline Characteristics of the Study Population

The mean age of participants was 36.8 years (standard deviation [SD], 15.4 years) and 82% were male. The duration of vision loss at enrollment was similar in the rAAV2/2-*ND4*–treated eyes and sham-treated eyes, on average 3.6 months (109 days) and 3.9 months (116 days), respectively (Table 2). Ten subjects (25.6%) had been treated with idebenone before enrollment in the RESCUE trial; they all had discontinued this medication at least 7 days before enrollment.

At baseline, the mean (SD) logMAR BCVA was 1.31 (0.52) and 1.26 (0.62) in rAAV2/2-*ND4*–treated and sham-treated eyes, respectively (Table 2), with 64 of 76 eyes being on-chart. At baseline, no eyes were off-chart for the Pelli-Robson test, with a mean (SD) baseline CS score of 0.64 (0.53) and 0.63 (0.53) LogCS in rAAV2/2-*ND4*–treated and sham-treated eyes, respectively. The mean (SD) of the mean deviation (MD) on HVF perimetry at baseline was −17.1 (12.3) decibels (dB) and −18.3 (12.4) dB for rAAV2/2-*ND4*–treated and sham-treated eyes, respectively. Four eyes in 4 subjects were not affected by vision loss at baseline, with BCVA ranging from 20/13 (logMAR −0.2) to 20/16 (logMAR −0.1), LogCS from 1.35 to 1.65, HVF MD from −0.57

Table 1. Subject Screening and Follow-Up

Screened subjects	N	49
Randomized subjects	N	39
Treated subjects*	N	39
ITT population†	N	38
Subjects who withdrew before week 48	N	1
Subjects who completed week 48	n	38
Subjects who withdrew between week 48 and week 96	n	3
Subjects who completed the study (week 96)	n	35
Primary reason for withdrawal at any time:		
Adverse event	n	0
Death	n	2
Lost to follow-up	n	1
Physician's decision	n	0
Pregnancy	n	0
Protocol violation	n	0
Subject's decision	n	1
Other	n	0

ITT = intention-to-treat.
*All subjects who were administered the study treatment were included in the Safety population. The Safety population was used for all safety analyses.
†The ITT population consisted of subjects who received the study treatment as per protocol. One subject who received an incomplete dose of rAAV2/2-*ND4* was removed from this population. The ITT population was used for the efficacy analyses.

Table 2. Baseline Characteristics of Subjects in the RESCUE Study

Age (yrs)			
	N	38*	
	Mean (SD)	36.8 (15.4)	
	Min; Max	15; 69	
Gender			
	N	38*	$P = 0.00$
Female	n (%)	7 (18.4%)	
Male	n (%)	31 (81.6%)	
Duration of Vision Loss (Days)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	108.5 (43.0)	$P = 0.33$
	Min; Max	40; 179	
Sham-treated eyes	Mean (SD)	115.8 (42.9)	
	Min; Max	24; 179	
logMAR			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	1.31 (0.52)	$P = 0.65$
	Min; Max	−0.10; 2.51	
Sham-treated eyes	Mean (SD)	1.26 (0.62)	
	Min; Max	−0.20; 2.35	
Contrast Sensitivity (LogCS)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	0.64 (0.53)	$P = 0.96$
	Min; Max	0.00; 1.65	
Sham-treated eyes	Mean (SD)	0.63 (0.53)	
	Min; Max	0.00; 1.65	
HVF Mean Deviation (dB)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	−16.26 (10.59)	$P = 0.61$
	Min; Max	−33.93; −0.57	
Sham-treated eyes	Mean (SD)	−16.73 (11.48)	
	Min; Max	−34.71; −1.20	
Temporal RNFL Thickness (μm)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	49.5 (18.6)	$P = 0.74$
	Min; Max	28.5; 111.0	
Sham-treated eyes	Mean (SD)	50.3 (23.6)	
	Min; Max	24.5; 147.5	
PMB RNFL Thickness (μm)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	34.1 (10.5)	$P = 0.51$
	Min; Max	24.0; 67.0	
Sham-treated eyes	Mean (SD)	35.7 (17.8)	
	Min; Max	21.0; 123.0	
GCL Macular Volume (mm^3)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	0.741 (0.159)	$P = 0.57$
	Min; Max	0.550; 1.255	
Sham-treated eyes	Mean (SD)	0.730 (0.183)	
	Min; Max	0.495; 1.275	
ETDRS Total Macular Volume (mm^3)			
rAAV2/2-*ND4*–treated eyes	Mean (SD)	8.418 (0.460)	$P = 0.35$
	Min; Max	7.665; 9.580	
Sham-treated eyes	Mean (SD)	8.388 (0.499)	
	Min; Max	7.535; 9.580	

BCVA = best-corrected visual acuity; dB = decibels; ETDRS = Early Treatment Diabetic Retinopathy Study; GCL = ganglion cell layer; HVF = Humphrey visual field; PMB = papillomacular bundle; RNFL = retinal nerve fiber layer; SD = standard deviation.
*The ITT population of 38 patients.

to −2.4 dB, and OCT parameters all within normal limits. Among these 4 eyes, only 1 eye was treated, the 3 others being sham injected.

Efficacy Data

At week 48, a comparable deterioration in visual acuity was observed in the rAAV2/2-*ND4*−treated eyes and the sham-treated eyes, with a least squares (LS) mean change from baseline to week 48 of +0.38 (−19 ETDRS letters equivalent) and +0.39 logMAR (−20 ETDRS letters equivalent), respectively. The difference of the change in BCVA from baseline between rAAV2/2-*ND4*−treated and sham-treated eyes was −0.01 logMAR (+1 ETDRS letters equivalent). The primary end point, that is, a clinically significant difference of −0.3 logMAR between rAAV2/2-*ND4*−treated and sham-treated eyes, was not met. When the analysis was repeated, including the 1 underdosed subject who had been removed from the ITT, the *P* value for the difference between rAAV2/2-*ND4*−treated and sham-treated eyes differed only at the third decimal place, with the lack of significance maintained.

At week 96, the LS mean change from baseline was +0.18 logMAR (−9 ETDRS letters equivalent) and +0.21 logMAR (−10 ETDRS letters equivalent) in the rAAV2/2-*ND4*−treated and sham-treated eyes, respectively. The difference of the change in BCVA from baseline between rAAV2/2 *ND4*−treated and sham-treated eyes at week 96 was −0.03 logMAR (1.5 EDTRS letters equivalent) (Table 3). The week 96 analysis repeated to include the 1 underdosed subject removed from the ITT population resulted in insignificant change.

The change in mean BCVA showed a parallel evolution for rAAV2/2-*ND4*−treated and sham-treated eyes (Fig 1). The mean BCVA for both groups deteriorated over the initial weeks reaching the worst levels at week 24, followed by a plateau phase until week 48, and then improved by week 96 by +10 and +9 ETDRS letters equivalent over the plateau level in the rAAV2/2-*ND4*−treated and sham-treated eyes, respectively. Of the eyes that were off-chart at week 24, 6 of 14 (43%) rAAV2/2-*ND4*−treated eyes and 5 of 12 (42%) sham-treated eyes moved to on-chart vision at week 96.

For each eye, a nadir BCVA value was identified between baseline and week 96. The average nadir BCVA was 1.9 logMAR (20/1600) in both eyes (Table 4). The mean (SD) time to nadir was 153.5 (171.8) days and 164.1 (181.2) days for rAAV2/2-*ND4*−treated and sham-treated eyes, respectively (Table 4). The LS mean change from the nadir to week 96 was −0.53 logMAR (+26 ETDRS letters equivalent) and −0.46 logMAR (+23 ETDRS letters equivalent) in the rAAV2/2-*ND4*−treated and sham-treated eyes, respectively. The improvement from the nadir to week 96 was statistically significant in both eye groups ($P < 0.0001$). The magnitude of improvement was similar in both eye groups with a nonstatistically significant difference of −0.07 logMAR (+3 ETDRS letters equivalent, $P = 0.40$). When assessing individual changes for each subject, the change from the nadir to week 96 in the rAAV2/2-*ND4*−treated eyes was overall comparable to that of the sham-treated eyes ($R = 0.70$) (Fig 2).

The proportion of eyes with an improvement of at least −0.3 logMAR from the nadir to week 96 was 63% for rAAV2/2-*ND4*−treated eyes and 55% for sham-treated eyes ($P = 0.24$) (Table 5). In a second responder analysis based on the CRR from the nadir to week 96, the eye responder rate was 61% for rAAV2/2-*ND4*−treated eyes and 53% for sham-treated eyes ($P = 0.40$). At week 96, 71% of subjects (27/38) had an improvement of at least −0.3 logMAR (15 ETDRS letters equivalent) from the nadir in at least 1 eye, and 71% of subjects (27/38) had a CRR from nadir in at least 1 eye (Table 5 and Fig S1, available at www.aaojournal.org). A subset of 5 RESCUE subjects showed an even more marked bilateral improvement in vision (Fig 2 and Table S1, available at www.aaojournal.org).

The changes in mean BCVA for the RESCUE trial were compared with those of the REVERSE trial based on the time from onset of vision loss (Fig 3 and Fig S2, available at www.aaojournal.org). RESCUE subjects were treated on average 16 weeks after onset of vision loss, whereas REVERSE subjects were treated on average 39 weeks after onset of vision loss. On average, recovery of BCVA was observed 24 weeks after treatment in the RESCUE trial and 12 weeks after treatment in the REVERSE trial. Subsequently, the mean BCVA curves for both studies showed a consistent improvement up to week 96. There was a statistically significant difference in mean logMAR BCVA between the rAAV2/2-*ND4*−treated eyes in RESCUE and REVERSE at approximately week 63.5 ($P = 0.04$), week 87.5 ($P = 0.04$), and week 111.5 ($P = 0.02$) from onset of vision loss.

Other Functional and Structural Outcome Measures

Contrast sensitivity worsened from baseline to week 48 and then improved from week 48 to week 96 in rAAV2/2-*ND4*−treated and

Table 3. Change in BCVA from Baseline to Week 96

BCVA (logMAR)		rAAV2/2-*ND4*−Treated Eyes	Sham-Treated Eyes
At Baseline	N	38	38
	Mean (SD)	1.31 (0.52)	1.26 (0.62)
	Min, Max	−0.1, 2.51	−0.2, 2.35
At Week 96	Mean (SD)	1.47 (0.77)	1.49 (0.74)
	Min, Max	−0.2, 4.0	−0.2, 4.0
Change from Baseline	LS Mean (SE)	0.18 (0.12)	0.21 (0.12)
	95% CI	−0.06, 0.42	−0.03, 0.45
Between-Eye Difference in Change from Baseline*	N	38	
	LS Mean	−0.03	
	95% CI	−0.20, 0.14	
	P value	0.74	

BCVA = best-corrected visual acuity; CI = confidence interval; logMAR = logarithm of the minimal angle resolution; LS = least square; SD = standard deviation; SE = standard error.
*A mixed-effects analysis of covariance model was used with change from baseline at as the response, and subject and eyes of the subject as random factors, treatment as a fixed effect, and the baseline logMAR value as covariate. *P* value is used to assess the significance of the difference between rAAV2/2-*ND4* and sham-treated eyes with respect to change from baseline.

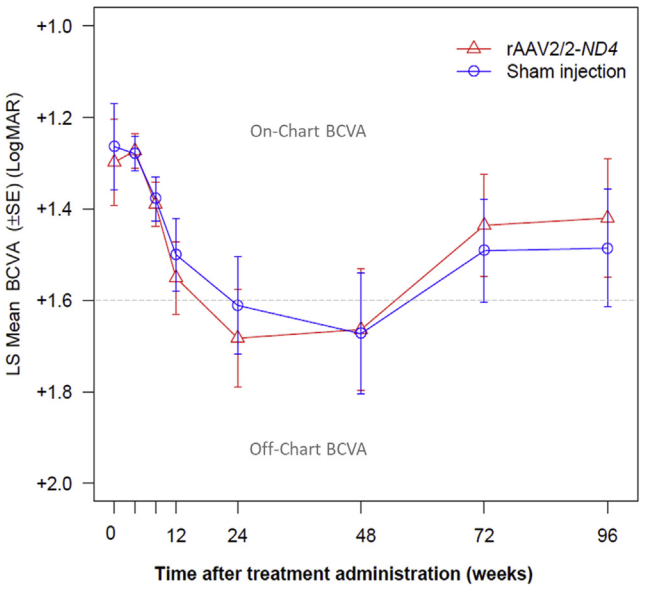

Figure 1. Mean change from baseline in logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) up to 96 weeks post-administration of rAAV2/2-*ND4* gene therapy. Error bars ±1 standard error (SE). The Y-axis was inverted to represent BCVA improving with the line moving upward. Red line: rAAV2/2-*ND4*; Blue line: Sham injection.

sham-treated eyes, leading to a mean (SD) decrease from baseline of −0.27 (0.07) and −0.25 (0.07) LogCS, respectively (Table S2, available at www.aaojournal.org). Despite HVF perimetry being repeated when needed, 55% of HVFs were assessed as unreliable by the reading center. The mean MD worsened from baseline to week 48 and then stabilized from week 48 to week 96 in both rAAV2/2-*ND4*−treated and sham-treated eyes (Table S2, available at www.aaojournal.org). From baseline to week 96, the LS mean of the ganglion cell layer volume decreased by −0.207 and −0.218 mm^3 in rAAV2/2-*ND4*−treated and sham-treated eyes, respectively (Table S2, available at www.aaojournal.org).

Quality of Life

Patient-reported outcome measures were evaluated using the National Eye Institute Visual Function Questionnaire 25.[24] The change in quality of life was assessed and compared with the baseline values (i.e., before treatment). At week 96, the score increase from baseline was clinically relevant in the following subscales: mental health (+15.8 points), dependency (+7.8 points), and role difficulties (+7.7 points).[25] No significant changes from baseline were observed for the mean Composite Score, near activities, and social functioning (Table 6). The scores for distance activities and peripheral vision worsened. The General Health scale could not be adequately assessed because of numerous missing data points. There was a correlation between quality of life scores and visual function in that for every line (0.1 logMAR) better or worse than baseline at week 96, Visual Function Questionnaire Composite scores were increased or decreased from baseline by 1.387 points ($P = 0.0008$), respectively.

Safety Data at Week 96

The safety population included the 39 subjects who were treated in the study. The treatment with the viral vector was well tolerated with no occurrences of study discontinuation related to ocular adverse events. One subject withdrew from the study, and 1 subject was lost to follow-up, with their last visit performed at week 48. Two subjects died after serious adverse events related to alcohol use; these deaths were not considered related to the viral vector or study interventions, and the last follow-up data were obtained at week 8 and week 48. No prophylactic oral or topical steroids were provided before or immediately after the intravitreal injection. In rAAV2/2-*ND4*−treated eyes, the most frequent ocular adverse event was intraocular inflammation, which was documented in 29 eyes (74%) and assessed as mild in 22 eyes and moderate in 7 eyes. No intraocular inflammation was graded

Table 4. Change in BCVA from Nadir to Week 96

		rAAV2/2-*ND4*−Treated Eyes	Sham-Treated Eyes
Time to Nadir	N	38	38
(days)	Mean (SD)	153.5 (171.8)	164.1 (181.2)
	Min, Max	−1, 679	−1, 679
BCVA (logMAR)			
At Nadir*	N	38	38
	Mean (SD)	1.95 (0.83)	1.92 (0.78)
	Min, Max	0.6, 4.5	0.5, 4.0
Change from Nadir to Week 96†	N	34	34
	LS Mean (SE)	−0.53 (0.08)	−0.46 (0.08)
	95% CI	−0.68, −0.37	−0.61, −0.30
Between-Eye Difference in Change from Nadir‡	N	34	
	LS Mean	−0.07	
	95% CI	−0.18, 0.04	
	P value	0.40	

BCVA = best-corrected visual acuity; CI = confidence interval; logMAR = logarithm of the minimal angle resolution; LS = least square; SD = standard deviation; SE = standard error.
*Nadir was defined as the worst BCVA value observed from baseline to week 96.
†Change from nadir to week 96 was calculated with no imputation (n=34).
‡A mixed-effects analysis of covariance model was used with change from nadir at as the response, and subject and eyes of the subject as random factors, treatment as a fixed effect, and the nadir logMAR value as covariate. *P* value is used to assess the significance of the difference between rAAV2/2-*ND4* and sham-treated eyes with respect to change from nadir.

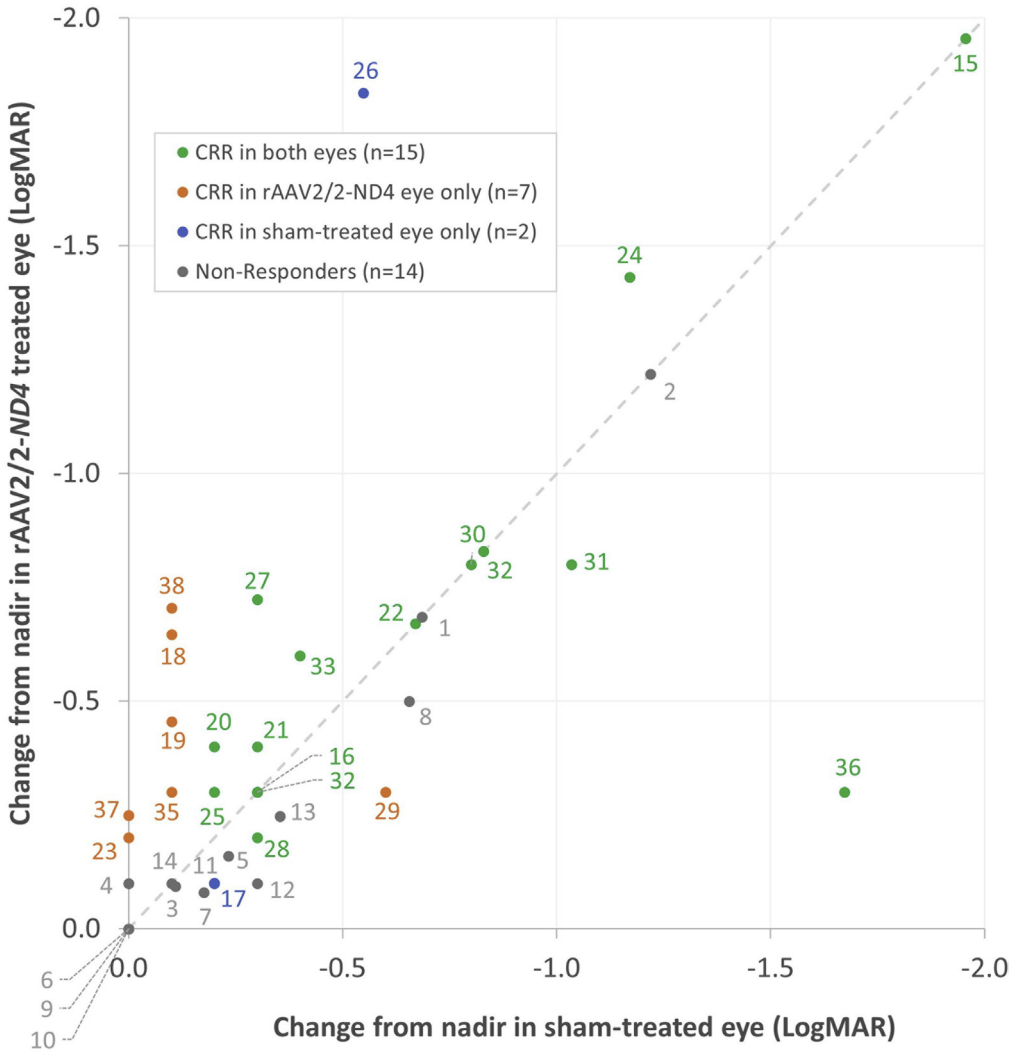

Figure 2. Individual changes in logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) from nadir to week 96. CRR = clinically relevant response from nadir at week 96. Subjects were considered responders at week 96 if they had a CRR from nadir in at least one eye. CRR was defined as either an eye on-chart at nadir that gained at least -0.2 logMAR (i.e., 10 Early Treatment Diabetic Retinopathy Study [ETDRS] letters equivalent) at week 96, or an eye off-chart at nadir that became on-chart with at least 5 ETDRS letters read at week 96. Data labels represent subject ID numbers. The X and Y-axes were inverted to represent BCVA improving going right for sham-treated eyes and upward for rAAV2/2-*ND4*-treated eyes. Diagonal bisector indicates an equal change in BCVA for both eyes of a subject. A subset of 5 RESCUE subjects (nos. 15, 24, 26, 31, and 36) showed a marked improvement in vision and is summarized in Table S1. Correspondence for points in green, orange, blue, and grey.

as severe. Anterior and intermediate uveitis was reported in 22 eyes (56%) and 25 eyes (64%), respectively, with no posterior uveitis affecting the retina or optic nerve reported. The anterior uveitis was graded as mild in 17 eyes and moderate in 5 eyes. The intermediate uveitis was graded as mild in 19 eyes and moderate in 6 eyes. Intraocular inflammation resolved without sequelae after standard therapy in all patients. Of the 29 eyes that developed intraocular inflammation, 27 (93%) received topical steroids. A course of oral steroids was prescribed for 5 subjects (13%) based on the examining clinician's judgment. Two (5%) sham-treated eyes developed mild anterior chamber inflammation that resolved fully after a course of topical steroids. An increase in intraocular pressure was reported in 13 (33%) of rAAV2/2-*ND4*–treated eyes and this was mostly mild, resolving with standard topical therapy. No subject developed persistently elevated intraocular pressures during follow-up.

Viral vector biodissemination was assessed for all subjects at 2 weeks posttreatment. The tested blood samples showed negative quantitative polymerase chain reaction results, except for 2 subjects who tested positive for DNA presence (674 copies/µg of DNA in 1 subject and 350 copies/µg of DNA in the other subject).

Discussion

Among the 3 phase 3 clinical trials of rAAV2/2-*ND4* (GS010) treatment in LHON subjects carrying the m.11778G>A mtDNA mutation (RESCUE NCT02652767, REVERSE NCT02652780, REFLECT NCT03293524), RESCUE is the second phase 3 trial for which results are now reported. This study represents the largest cohort of

Table 5. Visual Responders at Week 96

	Eye Responders		
	rAAV2/2-ND4—Treated	*Sham-Treated*	**Subject Responders***
Improvement of at least −0.3 logMAR from nadir			
Responder	24 (63%)	21 (55%)	27 (71%)
Nonresponder	14 (37%)	17 (45%)	11 (29%)
	$P = 0.24$†		
CRR from nadir‡			
Responder	23 (61%)	20 (53%)	27 (71%)
Nonresponder	15 (39%)	18 (47%)	11 (29%)
	$P = 0.40$†		

CRR = clinically relevant recovery; logMAR = logarithm of the minimum angle of resolution.
*A subject responder was defined as having a response in at least 1 eye at week 96.
†*P* value from McNemar test compares the rates of eye responders between treatment groups.
‡Response definition: either an eye that is on-chart at the nadir with an improvement at week 96 of at least 10 ETDRS letters or an eye that is off-chart at the nadir that became on-chart with at least 5 letters read at week 96.

patients treated with unilateral intravitreal injection (IVT) of rAAV2/2-*ND4*, with 39 patients in the safety population, 38 patients in the efficacy population (ITT population), and 35 patients who completed follow-up to the week 96 visit post-treatment administration. Baseline demographics were consistent with previous descriptions, with male predominance (82%) and young age at disease onset (36.8 years on average), recognizing that subjects had to be at least age 15 years when enrolled in the study.[1-4] As per the study design with enrollment within 6 months of vision loss, RESCUE subjects were representative of the LHON population in the early stages after disease onset, with relative preservation of visual function and retinal anatomy at baseline. Indeed, a greater baseline RNFL thickness among RESCUE patients compared with REVERSE patients (mean RNFL thickness 99.1 μm vs. 69.7 μm, respectively) likely reflects a combination of less nerve fiber loss and more axonal swelling in the earlier stages of LHON.

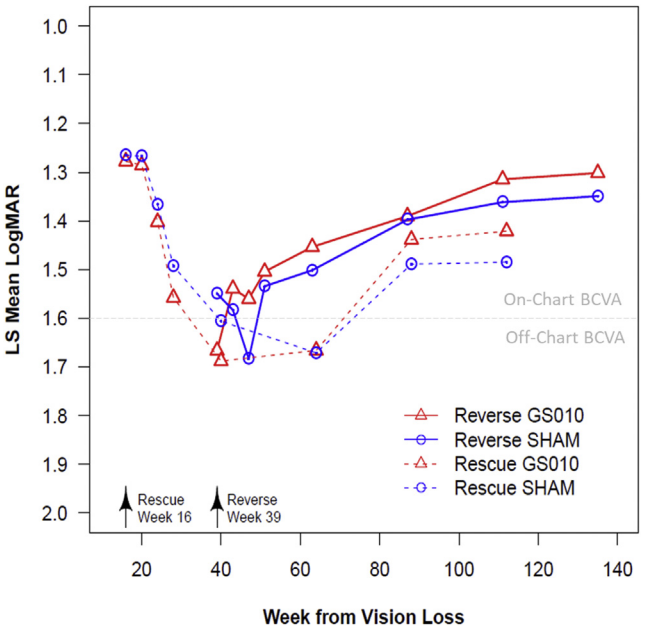

Figure 3. Evolution of logarithm of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) in the RESCUE and REVERSE trials. RESCUE subjects (n = 38) were treated on average 16 weeks after onset of vision loss whereas REVERSE subjects (n = 37) were treated on average 39 weeks after onset of vision loss. The Y-axis was inverted to represent BCVA improving with the lines moving upward. A more detailed statistical comparison is provided in Figure S2. Red solid line: Reverse GS010; Blue solid line: Reverse SHAM; Red dotted line: Rescue GS010; Blue dotted line: Rescue SHAM.

In the REVERSE trial, the mean baseline BCVA was 1.61 logMAR (Snellen 20/800), which is worse than in RESCUE (1.29 logMAR; Snellen 20/400), suggesting that most patients had reached their nadir when treated. In the REVERSE study, at week 96, rAAV2/2-*ND4*—treated eyes showed a LS mean improvement from baseline in BCVA of −0.308 logMAR (+15 ETDRS letters equivalent), and sham-treated eyes showed an LS mean improvement from baseline of −0.259 logMAR (+13 ETDRS letters equivalent).[18] A total of 78% of patients experienced some bilateral improvement of BCVA from baseline; 81% experienced a CRR from nadir in at least 1 eye, and 76% had an improvement of at least −0.3 logMAR (15 ETDRS letters equivalent) from nadir in at least 1 eye. Among RESCUE subjects, BCVA evolution was comparable in both rAAV2/2-*ND4*—treated and sham-treated eyes, similar to the bilateral changes seen among subjects in the REVERSE study.[18] The coherence of BCVA curves over time between RESCUE and REVERSE studies is noteworthy, with bilateral recovery occurring on average 24 weeks and 12 weeks post-treatment in the RESCUE and REVERSE studies, respectively (Fig 3). In RESCUE patients, this bilateral improvement of BCVA was most pronounced between 48 and 96 weeks, with a gain of +10 and +9 ETDRS letters equivalent in rAAV2/2-*ND4*—treated and sham-treated eyes from week 48, and a gain from nadir of +26 and +23 ETDRS letters equivalent in the rAAV2/2-*ND4*treated and sham-treated eyes, respectively.

The rAAV2/2-*ND4*—treated eyes in REVERSE seemed to achieve a slightly better visual outcome compared with those in RESCUE at comparable times after the onset of

Table 6. Vision-Related Quality of Life at Week 96

Visual Function Questionnaire 25 Subscales*	Baseline Score	Change from Baseline	
	Mean (SD)	*Mean (SD)*	*Mean %*†
Mental Health	29.5 (20.8)	15.8 (24.0)	101.2%
Dependency	34.0 (27.5)	7.8 (35.6)	91.7%
Role Difficulties	32.2 (28.3)	7.7 (33.4)	14.1%
General Vision	39.5 (19.1)	2.4 (24.4)	20.9%
Near Activities	31.8 (24.9)	−3.4 (29.5)	19.1%
Social Functioning	47.4 (26.8)	−3.7 (35.3)	11.9%
Ocular Pain	89.1 (18.2)	−4.8 (17.7)	−1.0%
Distance Activities	44.7 (24.7)	−5.9 (26.9)	−3.3%
Peripheral Vision	66.4 (25.5)	−11.8 (37.0)	−6.3%
Color Vision	76.3 (27.8)	−18.4 (37.6)	−20.5%
Composite Score‡	46.4 (18.4)	−2.2 (23.2)	7.7%

SD = standard deviation.
*Subscales not reported in this table: General Health (missing values), Driving (not applicable to LHON).
†The mean percent change from baseline was calculated from individual percent changes from baseline.
‡The Composite Score is the average of all vision-targeted subscale scores, excluding the General Health rating question.

vision loss (Fig S2, available at www.aaojournal.org). Indeed, the difference in mean BCVA between the 2 trials was statistically significant from week 63.5 after disease onset. Counterintuitively, despite earlier treatment in RESCUE (within 6 months from onset), visual outcomes at 96 weeks were actually inferior to those seen in subjects treated at later disease stages in REVERSE (6−12 months after onset). Among the 4 eyes with preserved BCVA at baseline, only 1 eye was treated with the active product. Early unilateral rAAV2/2-*ND4* treatment did not spare the second eye from vision loss, with a gradual decrease of BCVA up to week 72 (1.60 logMAR; Snellen 20/800), although there was improvement in this eye from week 72, with a BCVA of 1.30 logMAR (Snellen 20/400) at week 96. Further work is needed to confirm and explain these observations. Although speculative, it is possible that RNFL thickness, which is greater in the initial stages of the disease due to axonal swelling, may play a role by imposing a physical barrier to the diffusion of the rAAV2/2-*ND4* to RGCs and potentially impeding the distribution of the viral vector throughout the RNFL after viral transduction.

Given that both treated and untreated eyes may have benefitted from the administration of gene therapy in 1 eye, the proposed treatment effect observed in RESCUE and REVERSE implies a degree of RGC preservation and reactivation to function, which must then be compared with untreated LHON patients outside of the study. Natural history studies of LHON provide some insight on reported final visual acuities and on spontaneous improvement of vision after initial decline. In a recent meta-analysis of English-language peer-reviewed publications with study cohorts of at least 5 LHON patients confirmed to carry the m.11778G>A mutation, 12 retrospective and 3 prospective studies were identified providing visual function information on 695 subjects, among whom 100 (14.4%) were reported to have recovered some vision, although definitions of recovery varied among studies.[5] Specifically focusing on those patients who were at least 15 years old at the time of visual loss, spontaneous meaningful visual recovery occurred in 23 of 204 (11.3%) cases. Unfortunately, adequate prospective natural history studies with sufficient sample sizes of LHON patients stratified by mutation and age, and followed regularly with standardized measures of visual function at consistent intervals from the time of onset of visual loss until 96 weeks or longer are lacking, making direct comparison to our study challenging. Perhaps the closest such study is the subgroup analysis of the 61 m.11778G>A LHON patients in the retrospective study of Silva et al,[26,27] which reported a spontaneous CRR in at least 1 eye at the last follow-up of 15% and 28% from baseline and nadir, respectively. In comparison, 32% and 71% of RESCUE subjects had a CRR in at least 1 eye at week 96, respectively, from baseline and nadir.

RESCUE and REVERSE[18] are not the only studies to report bilateral BCVA improvement after unilateral injection of gene therapy. Indeed, similar improvement in visual function of the untreated eye after unilateral intravitreal administration of a viral vector containing a cDNA encoding the human wild-type mitochondrial ND4 protein was observed in clinical studies from 2 other gene therapy programs for LHON caused by the m.11778G>A mutation, 1 in China and 1 in the United States. The gene therapy used by the Chinese group consists of a recombinant AAV2-*ND4* construct with a targeted MTS to allow for allotopic rescue, similar to our approach.[28-32] In their pilot study of 8 LHON subjects who received a unilateral IVT of rAAV2-*ND4*, a sustained and prolonged bilateral improvement in BCVA was demonstrated in 5 patients (all of whom were aged less than 15 years at onset of visual loss) over 75 to 90 months of follow-up.[28,30] The same group subsequently reported on 149 patients (mean age of 19 years) who received a unilateral IVT of rAAV2-*ND4*, among whom 54 (36%) showed an early BCVA improvement, which was bilateral for 32% (n = 17), in only the treated eye for 48% (n=26) and only in the contralateral eye for 20% (n = 11).[31] At the American Academy of Ophthalmology 2019 Annual Meeting, Yuan et al[33] reported further results of this prospective open-label study, including 159 patients unilaterally injected

with rAAV2-*ND4* (aged 7−45 years), with a follow-up duration of at least 12 months for 106 patients. For the subgroup of 106 patients with 12-month follow-up data, BCVA improved (≥0.3 logMAR) in 67 patients (63%), with an average improvement of 0.6 logMAR in the treated eyes and an average improvement of 0.9 logMAR in the untreated eyes.[34] However, this cohort included an unspecified percentage of subjects with childhood-onset LHON, for whom spontaneous visual recovery, even years after onset, is more frequent than among patients with adult onset.[5] Guy et al,[35,36] at the University of Miami, have also developed a self-complementary adeno-associated vector with a nuclear-encoded *ND4* and an appended MTS for allotopic rescue of the m.11778G> mutation. They conducted a prospective, dose-escalation, open-label trial, including 14 LHON subjects who were 18 years or older and unilaterally injected with self-complementary adeno-associated vector 2-*ND4*. The data and BCVA curves provided showed a bilateral improvement in BCVA in 7 patients (50%), with a follow-up from 3 to 18 months.[35,36]

A weakness of all the gene therapy studies conducted to date is the lack of a true placebo arm assessed using the same comprehensive protocol to fully confirm that the observed visual improvement is not due, at least in part, to the natural history of the disease. Although the body of published natural history data on visual outcomes among LHON subjects with the m.11778G>A mutation reports worse improvement rates and outcomes than among the RESCUE and REVERSE cohorts, it is important to acknowledge that under the rigorous conditions of a clinical trial, vision is more carefully obtained and prospectively measured more often, allowing for either an erroneous vision measurement to become the "nadir" with later regression to the mean or the demonstration of a true nadir point in study patients, the equivalent of which may have been missed during the course of visual loss in LHON patients assessed in retrospective natural history studies. It is also not possible to fully exclude that some of the visual gains are due to better use of eccentric fixation as has been documented in patients with central scotomas from macular disease.[37]

Allowing for the possibility that gene therapy delivered in 1 eye caused some ipsilateral and contralateral vision improvement among LHON patients in RESCUE, REVERSE, and other independent studies, a mechanistic explanation is required. In a recent nonhuman primate study, the presence of rAAV2/2-*ND4* vector DNA was demonstrated in the contralateral eye's anterior segment, retina, and optic nerve (mean quantity of rAAV2/2-*ND4* vector DNA ranged from 3.39×10^3 to 1.00×10^4 vg/μg) 3 months after unilateral intravitreal injection in Cynomolgus monkeys, a primate species with comparable intracranial visual pathways to that of humans.[18] The findings from this preliminary nonhuman primate study suggest that genetic material is being transferred from 1 optic nerve to the other, with the routes involved requiring further investigation. Additional research is needed to better understand mechanisms to account for the possibility of bilateral visual improvement after unilateral treatment and how the timing of treatment could potentially influence RGC rescue and the extent of visual recovery.

In conclusion, the RESCUE study did not meet its primary end point of a −0.3 logMAR (15-letter) difference between treated and untreated eyes at week 48 in LHON subjects with recent visual loss treated with a unilateral intravitreal injection of rAAV2/2-*ND4*. The RESCUE study did demonstrate bilateral improvement of visual acuity from nadir to week 96, consistent with results from the REVERSE study and 2 other independent groups, but not aligned with the reported natural history of visual outcomes in LHON patients. These findings have major implications for the design of future neuro-ophthalmologic gene therapy clinical trials, in which the appropriate choice of outcome measures and controls will be crucial.

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No animal subjects were used in this study.

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Abbreviations and Acronyms:

BCVA = best-corrected visual acuity; **cDNA** = complementary DNA; **CRR** = clinically relevant recovery; **CS** = contrast sensitivity; **dB** = decibels; **EMA** = European Medicines Agency; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **HVF** = Humphrey visual field; **ITT** = intention-to-treat; **IVT** = intravitreal injection; **LHON** = Leber hereditary optic neuropathy; **LogCS** = logarithm of contrast sensitivity; **logMAR** = logarithm of the minimal angle of resolution; **mtDNA** = mitochondrial DNA; **MTS** = mitochondrial targeting sequence; **RGC** = retinal ganglion cell; **RNFL** = retinal nerve fiber layer.

Keywords:

Leber Hereditary Optic Neuropathy, intravitreal gene therapy, efficacy, safety, bilateral visual improvement, best-corrected visual acuity, Phase 3 randomized double-masked clinical trial, contrast sensitivity, Humphrey visual field perimetry, retinal anatomic measures, Quality of life.

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