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ORIGINAL RESEARCH

Indirect Comparison of Lenadogene Nolparvovec Gene Therapy Versus Natural History in Patients with Leber Hereditary Optic Neuropathy Carrying the m.11778G>A *MT-ND4* Mutation

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ABSTRACT

Introduction: Lenadogene nolparvovec is a promising novel gene therapy for patients with Leber hereditary optic neuropathy (LHON) carrying the m.11778G>A *ND4* mutation (*MT-ND4*). A previous pooled analysis of phase 3 studies showed an improvement in visual acuity of patients injected with lenadogene nolparvovec compared to natural history. Here, we report updated results by incorporating data from the latest phase 3 trial REFLECT in the pool, increasing the number of treated patients from 76 to 174.

Methods: The visual acuity of 174 *MT-ND4*-carrying patients with LHON injected in one or both eyes with lenadogene nolparvovec from

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four pooled phase 3 studies (REVERSE, RESCUE and their long-term extension trial RESTORE; and REFLECT trial) was compared to the spontaneous evolution of an external control group of 208 matched patients from 11 natural history studies.

Results: Treated patients showed a clinically relevant and sustained improvement in their visual acuity when compared to natural history. Mean improvement versus natural history was − 0.30 logMAR (+ 15 ETDRS letters equivalent) at last observation ($P < 0.01$) with a maximal follow-up of 3.9 years after injection. Most treated eyes were on-chart as compared to less than half of natural history eyes at 48 months after vision loss (89.6% versus 48.1%; $P < 0.01$) and at last observation (76.1% versus 44.4%; $P < 0.01$). When we adjusted for covariates of interest (gender, age of onset, ethnicity, and duration of follow-up), the estimated mean gain was − 0.43 logMAR (+ 21.5 ETDRS letters equivalent) versus natural history at last observation ($P < 0.0001$). Treatment effect was consistent across all phase 3 clinical trials. Analyses from REFLECT suggest a larger treatment effect in patients receiving bilateral injection compared to unilateral injection.

Conclusion: The efficacy of lenadogene nolparvovec in improving visual acuity in *MT-ND4* LHON was confirmed in a large cohort of patients, compared to the spontaneous natural history decline. Bilateral injection of gene therapy may offer added benefits over unilateral injection.

Trial Registration Numbers: NCT02652780 (REVERSE); NCT02652767 (RESCUE); NCT03406104 (RESTORE); NCT03293524 (REFLECT); NCT03295071 (REALITY).

Keywords: Gene therapy; Leber hereditary optic neuropathy; LHON; *MT-ND4*; Natural history; Visual acuity

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Key Summary Points

Lenadogene nolparvovec is an investigational gene therapy for *MT-ND4* Leber hereditary optic neuropathy (LHON), a rare disease that causes severe vision loss and for which treatment options are currently limited.

Lenadogene nolparvovec previously demonstrated visual acuity benefits in a pooled analysis of three phase 3 trials (REVERSE, RESCUE and their long-term extension trial RESTORE) when compared to an external control group of natural history patients.

Here, we present updated efficacy results with the inclusion of data from the latest phase 3 trial REFLECT in the pool, increasing the number of treated patients from 76 to 174. The same pool of 208 natural history patients was used as an external control group.

The clinically significant and sustained improvement in visual acuity induced by lenadogene nolparvovec was confirmed, with a mean gain of − 0.30 logMAR versus natural history up to 3.9 years after treatment. The treatment effect remained clinically significant when controlling for potential confounding factors.

Bilateral treatment with lenadogene nolparvovec may induce larger visual acuity benefit than unilateral treatment.

Lenadogene nolparvovec is a promising novel therapy for the treatment of *MT-ND4* LHON.

INTRODUCTION

Leber hereditary optic neuropathy (LHON), is a rare, maternally inherited genetic disorder that manifests as severe bilateral central vision loss,

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leading to a dramatic impact on the quality of life of patients [1, 2]. Most of the LHON causative mutations affect mitochondrial DNA (mtDNA) genes encoding for NADH dehydrogenase (ND) subunits of the respiratory chain complex I, leading to a subacute and catastrophic degeneration of retinal ganglion cells (RGCs) (with the final outcome of optic nerve atrophy) [1]. Mutation carriers are asymptomatic before expression of the disease, and the onset of LHON manifests as a rapidly evolving subacute bilateral decline in visual acuity, frequently characterized by the asymmetric rapid deterioration in one eye followed by the second eye a few weeks later [3–6]. While onset of vision loss classically affects young men in their teens and twenties, LHON has been reported in both men and women of all ages and may occur in younger or older individuals [6–9]. In most patients, the final Snellen visual acuity a few months after onset is worse than 20/200 [3, 6]. The majority of LHON cases are caused by one of the three mtDNA missense point mutations at positions m.3460G>A/*MT-ND1*, m.11778G>A/*MT-ND4*, and m.14484T>C/*MT-ND6*, with several other rare mtDNA point mutations accounting for a few cases worldwide, and an additional subset of cases recently described as due to recessive mutations in the nuclear DNA, all involving complex I as in the case of the *DNAJC30* gene [10, 11]. The *MT-ND4* m.11778G>A mutation remains, however, the most prevalent with severe visual prognosis and infrequent spontaneous visual recovery [12–14]. A recent meta-analysis conducted on 204 *MT-ND4* mutation carriers aged 15 years or older at onset of vision loss demonstrated recovery in only 11% of patients [13]. On the opposite end of the spectrum, the *MT-ND6* m.14484T>C mutation is associated with the most favourable prognosis, with up to 70% of cases experiencing a spontaneous recovery of visual acuity, in particular when age at onset is before age 15 [15–17].

The only approved therapeutic option for patients with LHON is limited to the quinone analogue idebenone, which is approved in Europe by the European Medicines Agency (EMA) for the treatment of LHON, but not in the USA [18]. Idebenone has been shown to improve the visual outcomes of patients with LHON in randomised and real-word settings [19–21]. However the therapeutic benefit varies greatly depending on the causal LHON mutation, with a reported clinically relevant recovery of 39% in *MT-ND4*-carrying patients treated with long-term idebenone versus 75% in *MT-ND6*-carrying patients [20]. There remains a definite unmet therapeutic need in LHON, especially for patients carrying the *MT-ND4* mutation, representing the majority of the LHON patient population.

Lenadogene nolparvovec (rAAV2/2-*ND4*) is a modified adeno-associated virus gene therapy product in clinical development for LHON, which was specifically designed to complement the defective *ND4* gene by the allotopic expression of the wild-type ND4 subunit from the nucleus followed by mitochondrial import of the protein product [22–24]. A phase 1/2 study conducted in *MT-ND4*-carrying patients with LHON showed that intravitreal injection (IVT) of lenadogene nolparvovec was associated with clinical benefits and was well tolerated [25, 26]. These encouraging results were later confirmed in the phase 3 randomised, double-masked pivotal clinical studies REVERSE, RESCUE and REFLECT where lenadogene nolparvovec showed a clinically significant improvement in best-corrected visual acuity (BCVA) of patients with LHON affected with the *ND4* m.11778G>A mutation and enrolled in the studies within the first year after onset of vision loss [27–30]. In REVERSE and RESCUE studies, lenadogene nolparvovec was administered as a single IVT in one eye while the other eye received a sham IVT. At 96 weeks after treatment, in REVERSE and RESCUE respectively, the mean gain in ETDRS letters from nadir (worst vision point) was respectively + 28 and + 26 in eyes injected with the gene therapy, and + 24 and + 23 in eyes that received the sham IVT. The unexpected positive effect observed in sham-injected eyes, which mirrored the treatment effect observed in lenadogene nolparvovec-injected eyes, was consistent with nonhuman primate data that demonstrated viral vector DNA in both eyes, suggesting transfer from one eye to the other, possibly through the optic chiasm [27, 31]. The visual

improvement at 96 weeks after treatment observed in REVERSE and RESCUE trials was maintained at 3 years, as shown by the interim results of the joint long-term follow-up RESTORE study, in which patients are still being followed for a total duration of 5 years [32]. In the REFLECT study, *MT-ND4*-carrying patients with LHON were randomised to receive the treatment as a unilateral IVT (with the other eye receiving a placebo IVT) or as a bilateral IVT. At 1.5 years after treatment, all eyes (including those injected with placebo) showed a clinically significant improvement in BCVA from nadir [29, 30].

As a result of the ethical considerations concerning the rapid and irreversible nature of LHON, clinical studies with lenadogene nolparvovec to date have not included a control group of untreated patients, thus preventing any formal estimation of the treatment effect size. To overcome this limitation, we adopted an indirect approach for estimating the treatment effect by comparing the visual acuity of patients treated with lenadogene nolparvovec in phase 3 studies to the spontaneous evolution of visual acuity of an external pooled control group of natural history patients with LHON matched for age and *MT-ND4* genotype [33]. The natural history patients were from a LHON registry (REALITY) and from 10 natural history published reports [33, 34]. We previously reported the results of this analysis using all BCVA data of treated patients available at the time, which included patients from REVERSE, RESCUE and RESTORE studies [33]. The results showed a statistically and clinically relevant difference in visual acuity of − 0.33 logarithm of the minimal angle of resolution (logMAR) in lenadogene nolparvovec eyes versus natural history eyes ($P < 0.01$) at 48 months after vision loss. Here, we present an update of this analysis by inclusion of the latest available BCVA data from the REFLECT study. The same pool of external natural history patients was used as a control group.

METHODS

Patients Treated with Gene Therapy

We analysed the evolution of BCVA in a pooled data set of 174 *MT-ND4*-carrying patients with LHON who received lenadogene nolparvovec (rAAV2/2-*ND4*) as a single unilateral or bilateral IVT at a dose of 9×10^{10} viral genomes/eye. The BCVA data were collected from study inclusion to week 96 after treatment in REVERSE (NCT02652780) [27] and RESCUE (NCT02652767) [28], from study inclusion to year 1.5 after treatment in REFLECT (NCT03293524) [29, 30] and from week 96 after treatment to the last available observation in the ongoing long-term follow-up RESTORE study of REVERSE and RESCUE (NCT03406104) [32] (Table 1).

The study design and results of REVERSE, RESCUE and RESTORE have been previously reported [27, 28, 32]. Briefly, REVERSE and RESCUE were randomised, double-masked, sham-controlled phase 3 studies. Both studies enrolled symptomatic patients with LHON aged 15 years or older with a confirmed m.11778G>A *ND4* mutation and only differed in the timing of onset of vision loss: from 181 to 365 days in both eyes in REVERSE and at most 180 days in the first-affected eye in RESCUE. All REVERSE ($N = 37$) and RESCUE ($N = 39$) patients received an IVT of lenadogene nolparvovec in one eye and a sham injection in the other eye according to a 1:1 allocation ratio. A total of 62 patients who completed REVERSE and RESCUE were enrolled in the RESTORE extension study for a follow-up of 5 years after treatment. The RESTORE study is ongoing at the time of this report and interim data up to 3 years after treatment was available for use in this report.

The study design and results of REFLECT have been previously reported [29, 30]. Briefly, REFLECT is a randomised, double-masked, placebo-controlled phase 3 study. The study enrolled symptomatic patients with LHON aged 15 years or older with a confirmed m.11778G>A *ND4* mutation and an onset of vision loss within at most 1 year. A total of 98 patients received lenadogene nolparvovec as a bilateral

Table 1 Studies and patients included in the analysis

Study ID	Type of visual acuity data[a]	Number of patients with the m.11778G>A *MT-ND4* mutation	Number of patients included in the analysis[b]
Patients treated with lenadogene nolparvovec (rAAV2/2-*ND4*)			
REVERSE[c]	Longitudinal	37	37
RESCUE[c]	Longitudinal	39	39
RESTORE[c]	Longitudinal	62	62
REFLECT[d]	Longitudinal	98	98
Total		174	174
Natural history patients[e]			
REALITY	Longitudinal	27	23
Hotta et al. (1995) [35]	Cross-sectional	89	32
Lam et al. (2014) [36]	Longitudinal	44	36
Nakamura et al. (1993) [37]	Cross-sectional	9	9
Newman et al. (1991) [4]	Cross-sectional	56	40
Qu et al. (2007) [38]	Cross-sectional	10	7
Qu et al. (2009) [39]	Cross-sectional	14	12
Romero et al. (2014) [40]	Cross-sectional	21	15
Sadun et al. (2004) [41]	Cross-sectional	20	14
Yang et al. (2016) [42]	Longitudinal	16	5
Zhou et al. (2010) [43]	Cross-sectional	25	15
Total		331	208

IVT intravitreal injection, *LHON* Leber hereditary optic neuropathy, *ND4* gene coding for NADH dehydrogenase 4

[a]Visual acuity data were either longitudinal (several measurements over time per patient) or cross-sectional (measurement at a single time point per patient)

[b]Patients with LHON with the m.11778G>A *ND4* mutation who were 15 years or older at onset of vision loss and who had at least one visual acuity value with reported time of measurement since vision loss

[c]REVERSE and RESCUE patients received a single IVT lenadogene nolparvovec in one eye and were followed up in the joint long-term extension study RESTORE; no lenadogene nolparvovec was administered as part of RESTORE

[d]REFLECT patients received an IVT of lenadogene nolparvovec in one eye (50 patients) or in both eyes (48 patients)

[e]Natural history patients did not receive lenadogene nolparvovec or any other gene therapy treatment but may have received idebenone

treatment or as a unilateral treatment according to a 1:1 allocation ratio. Patients randomised in the bilateral treatment arm ($N = 48$) received an IVT of lenadogene nolparvovec in both eyes. Patients randomised in the unilateral treatment arm ($N = 50$) received an IVT of lenadogene nolparvovec in their first-affected eyes and an IVT of placebo in their second-affected eyes. The available REFLECT data used in this report are from the double-masked period of 1.5 years after treatment. The REFLECT study is ongoing at the time of this report with patients being followed in the long-term unmasked follow-up period from 1.5 to 5 years after treatment.

On the basis of BCVA data from phase 3 studies and biodistribution data in non-human primates [27, 31], both treated eyes and untreated eyes (i.e. eyes receiving placebo IVT or sham IVT) were considered exposed to the gene therapy across all studies and were pooled in the treated group in this analysis.

The protocols of REVERSE, RESCUE, RESTORE and REFLECT were approved by local independent ethics committees, and informed consent was obtained from all participants. All studies were performed in compliance with Good Clinical Practice and adhered to the ethical principles outlined in the Declaration of Helsinki.

External Control Group of Natural History Patients

Natural history patients (those not treated with lenadogene nolparvovec, although they could have received idebenone treatment), with age and LHON genotype adjusted to those of treated patients, were used as an external control for the analysis. To this end, we created a large database containing visual acuity data from 11 studies originating from two main sources: (i) the REALITY LHON registry (NCT03295071) sponsored by GenSight Biologics [34] and (ii) 10 published studies on LHON identified after a systematic review of the literature [4, 35–43]. Studies were included in the database only if they reported individual (patient- and eye-level) visual acuity values, along with documentation of the time after vision loss, in cohorts of at least five patients with LHON carrying the *MT-ND4* mutation. For relevant comparison with treated patients, we included only patients from the pooled database who matched the inclusion criteria of REVERSE, RESCUE and REFLECT as regards age and LHON genotype (i.e. symptomatic patients with LHON carrying the m.11778G>A ND4 mutation who were 15 years or older at the onset of vision loss). Further details on the REALITY registry study and on the systematic literature review can be found in our previous report [33].

Handling of Data

Handling of visual acuity data was conducted as previously described [33]. Briefly, visual acuity values were converted to logMAR using the same methodology for treated patients and for natural history patients as previously described [33]. Specifically, conversions of off-chart visual acuities to logMAR values in natural history literature studies were aligned with the conventions used in lenadogene nolparvovec clinical trials and in the REALITY registry as follows: count fingers, logMAR + 2.0; hand motion, logMAR + 2.3; light perception, logMAR + 4.0; no light perception, logMAR + 4.5 [30, 33, 34, 44]. All treated and natural history eyes were assigned a logMAR value of 0 at 1 month before the onset of vision loss, in line with the normal visual acuity of LHON mutation carriers before expression of the disease as described in the literature [45, 46] and consistent with pre-symptomatic data of lenadogene nolparvovec studies (logMAR ranging from − 0.3 to 0 for unaffected eyes in the RESCUE and REFLECT studies), the REALITY registry and early access programs. All extracted data and conversions of visual acuity values to logMAR underwent a quality control process and review to ensure the accuracy of reported values.

Statistical Methods

All data from treated and natural history patients were imported into a pooled database and analysed at the eye level. Statistical analyses

Table 2 Description of the treated and natural history population

	Treated ($N = 174$)	Natural history ($N = 208$)	Total ($N = 382$)	*P* value
Number of eyes with visual acuity values	348	408[a]	756	
Gender				0.52 (C)
Male (%)	139 (79.9%)	142 (82.6%)	281 (81.2%)	
Missing data	0	36[b]	36	
Ethnicity[c]				< 0.01 (C)
Asian (%)	15 (8.6%)	80 (38.5%)	95 (24.9%)	
Age at onset of vision loss (years)				< 0.01 (KW)
Mean (SD)	33.4 (14.5)	27.6 (12.4)	30.2 (13.7)	
Median	29.0	23.5	26.0	
Range	15.0–74.0	15.0–71.0	15.0–74.0	
Number of visual acuity assessments per patient				< 0.01 (KW)
Mean (SD)	24.8 (4.3)	4.1 (4.0)	13.5 (11.1)	
Median	24.0	2.0	12.0	
Range	12.0–44.0	1.0–22.0	1.0–44.0	
Follow-up since vision loss (months)[d]				0.04 (KW)
Mean (SD)	33.5 (8.1)	84.8 (132.3)	61.4 (101.0)	
Median	32.3	25.3	31.7	
Range	8.1–51.5	0.0–768.0	0.0–768.0	
Number of patients with follow-up > 36 months	65 (37.4%)	79 (38.0%)	144 (37.7%)	0.90 (C)
Time from vision loss to treatment (months)				
Mean (SD)	7.9 (3.3)	NA	NA	
Median	8.3	NA	NA	
Range	1.7–12.8	NA	NA	

C chi-squared test, *KW* Kruskal–Wallis test, *NA* not applicable, *SD* standard deviation

[a]Eight natural history patients had visual acuity values in one eye only, leading to a sample size of 408 eyes

[b]All missing gender data were from the natural history study of Lam et al. [36]

[c]Patient ethnicity data were not collected in any studies. Patient ethnicity was assigned as Asian/non-Asian solely on the basis of geographic location of their study site

[d]Defined as the time from vision loss to the last available visual acuity value, regardless of the eye

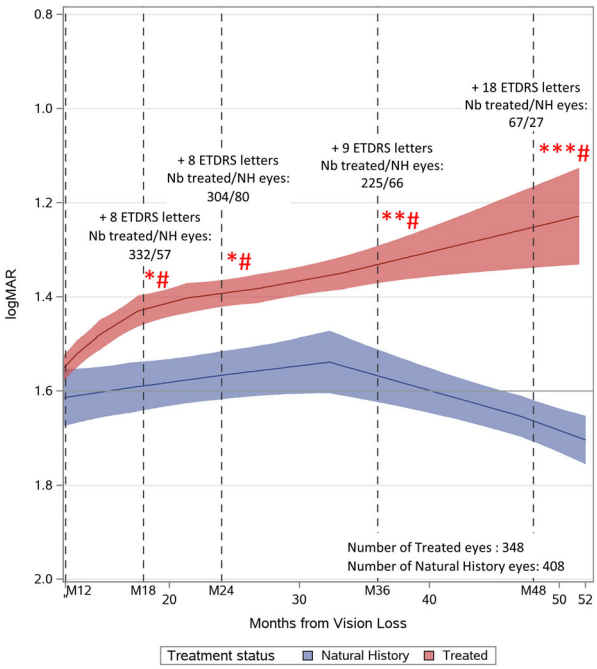

Fig. 1 Evolution of visual acuity of treated eyes versus natural history eyes. The evolution of visual acuities over time for treated eyes (n = 348) and natural history eyes (n = 408) was estimated by LOESS regression (solid line) with 95% CI around the fitted curve (shaded area). Visual acuity values > 52 months were assigned to the 52-month time point using the next observation carried backward method. Smoothing parameter: 0.315 for treated eyes and 0.408 for natural history eyes. The statistically significant difference between treated and natural history eyes is illustrated by the non-overlapping CIs of LOESS curves. Mean differences at month 18 [15; 21], month 24 [21; 30], month 36 [30; 42] and month 48 [42; 54] were estimated by a mixed-model ANCOVA with repeated measures: $^{*}P$ = 0.03, $^{**}P$ = 0.02 and $^{***}P < 0.01$ versus natural history; and with Kruskal Wallis test: $^{\#}P < 0.01$ versus natural history (details in Table 3).

were carried out with SAS® software version 9.4. Statistical significance was set at $P < 0.05$.

In a first step, we explored graphically the evolution of visual acuity in treated and natural history eyes from 12 months after vision loss, when all REVERSE, RESCUE and REFLECT patients would have been treated with lenadogene nolparvovec, using a locally estimated scatterplot smoothing (LOESS), non-parametric, local regression model in which each patient's eyes were considered independently. Smoothing parameters were based on the corrected Akaike Information Criterion (SAS default method with values from 0.3 to 0.6). LOESS curves with 95% confidence interval (CI) were presented from 12 months up to 52 months after vision loss, corresponding to the maximal duration of follow-up for treated eyes in the extension study RESTORE. All subsequent visual acuity values of natural history eyes were assigned to the 52-month time point using the next observation carried backward method, allowing the regression curves of treated eyes and natural history eyes to be plotted on the same figure.

In a second step, we compared the visual outcomes between treated eyes and natural history eyes at 12, 18, 24, 36, and 48 months after vision loss (when all treated eyes were on treatment) and at the last available visual acuity value. For the 12- to 48-month analysis, only the closest value to the nominal time point was selected for each eye on the basis of prespecified time windows (month 12, [9; 15] months; month 18, [15; 21] months; month 24, [21; 30] months; month 36, [30; 42] months; month 48, [42; 54] months). For the analysis at the last available visual acuity value, final visual acuity values from all eyes were considered in the analysis. The following visual outcomes were analysed: visual acuity values in logMAR and eye response rates using a threshold of logMAR $\leq$ 1.6 (on-chart values on the ETDRS scale) and logMAR $\leq$ 1.3 (cut-off for blindness according to World Health Organization, WHO). Comparisons of visual outcomes between treated eyes and natural history eyes were performed by a non-parametric test (Kruskal–Wallis for visual acuity values and chi-squared test for eye response rates). In addition, a parametric model with repeated measures on patients was also used in order to take into account the inter-eye correlation of each patient (mixed-model analysis of covariance [ANCOVA] for visual acuity values and generalized linear mixed model for eye response rates).

In order to control for potential confounding covariates, we conducted sensitivity analyses to estimate the treatment effect on visual acuity values at last available observation taking into account the following set of covariates of

Table 3 Visual acuity of treated eyes versus natural history eyes at each time point

Time from vision loss with time intervals[a]	Treated (N = 348 eyes)	Natural history (N = 408 eyes)
12 [9; 15] months		
Number of eyes	346	76
Visual acuity (logMAR)		
Median	1.50	1.70
Mean (SD)	1.56 (0.53)	1.69 (0.67)
95% CI (mean)	[1.50; 1.61]	[1.54; 1.84]
P values (KW/ANCOVA[b])	0.02/0.06	
Mean difference [95% CI][b]	− 0.133 [− 0.272; 0.006]	
18 [15; 21] months		
Number of eyes	332	57
Visual acuity (logMAR)		
Median	1.40	1.60
Mean (SD)	1.44 (0.54)	1.60 (0.54)
95% CI (mean)	[1.38; 1.49]	[1.46; 1.75]
P values (KW/ANCOVA[b])	< 0.01/0.03	
Mean difference [95% CI][b]	− 0.165 [− 0.317; − 0.013]	
24 [21; 30] months		
Number of eyes	304	80
Visual acuity (logMAR)		
Median	1.40	1.52
Mean (SD)	1.38 (0.58)	1.54 (0.52)
95% CI (mean)	[1.32; 1.45]	[1.42; 1.65]
P values (KW/ANCOVA[b])	< 0.01/0.03	
Mean difference [95% CI][b]	− 0.157 [− 0.297; − 0.017]	
36 [30; 42] months		
Number of eyes	225	66
Visual acuity (logMAR)		
Median	1.30	1.55
Mean (SD)	1.34 (0.54)	1.52 (0.47)
95% CI (mean)	[1.27; 1.41]	[1.40; 1.63]
P values (KW/ANCOVA[b])	< 0.01/0.02	
Mean difference [95% CI][b]	− 0.172 [− 0.317; − 0.027]	

Table 3 continued

Time from vision loss with time intervals[a]	Treated (*N* = 348 eyes)	Natural history (*N* = 408 eyes)
48 [42; 54] months		
Number of eyes	67	27
Visual acuity (logMAR)		
Median	1.30	1.62
Mean (SD)	1.23 (0.44)	1.59 (0.44)
95% CI (mean)	[1.13; 1.34]	[1.41; 1.76]
P values (KW/ANCOVA[b])	< 0.01/< 0.01	
Mean difference [95% CI][b]	− 0.352 [− 0.554; − 0.149]	

ANCOVA analysis of covariance, *CI* confidence interval, *KW* Kruskal–Wallis, *logMAR* logarithm of the minimal angle of resolution, *NH* natural history, *SD* standard deviation

[a]For each eye, only the closest value to the nominal time point was selected on the basis of the time windows indicated in brackets

[b]Mixed-model ANCOVA with repeated measures taking into account the inter-eye correlation of each patient

clinical interest: age at onset of vision loss, gender, ethnicity (Asian vs. non-Asian) and duration of follow-up post vision loss. No individual ethnic data were available for natural history and treated patients, and patient ethnicity was assigned as Asian/non-Asian solely on the basis of geographic location of their study site. We used three different approaches for covariate adjustment: multivariate analyses, propensity score weighting and propensity score matching. The estimated treatment effect with 95% CI versus natural history eyes and its associated *p* value was presented for each approach, taking into account the inter-eye correlation of each patient (repeated measures).

In order to explore the effect of bilateral treatment versus unilateral treatment on visual acuities and response rates, we conducted the following additional comparisons at last available observation: all eyes of REFLECT patients treated bilaterally versus natural history eyes, and all eyes of REFLECT patients treated unilaterally versus natural history eyes.

Finally, the following factors possibly impacting the response to treatment were explored by univariate and multivariate analysis in the treated patients cohort: age at onset of vision loss, gender, ethnicity (Asian vs. non-Asian) and time from vision loss to treatment.

RESULTS

Characteristics of Natural History Patients and Treated Patients at Onset of Vision Loss

A total of 208 *MT-ND4*-carrying patients with LHON aged 15 years or older (185 patients from 10 published studies and 23 patients from the REALITY registry) were used as the external control cohort for the comparison with treated patients. Approximately 30% of natural history patients had longitudinal data for each eye (i.e. at least two visual acuity values per eye) (Table 1).

These 208 natural history patients (408 eyes) were compared to the 174 treated patients (348 eyes) in the pooled lenadogene nolparvovec studies. The characteristics of each patient cohort are described in Table 2. Both cohorts were predominantly male patients (81.2%) with a median age at onset of vision loss in their twenties (26.0 years). Natural history patients were slighter younger than treated patients (median age at onset of 23.5 years versus 29.0 years, $P < 0.01$). Five percent of patients were 60 years or older at onset of vision loss (6.9% of treated patients and 3.4% of natural history patients), consistent with the reported

Table 4 Visual acuity at last observation

	Treated eyes				Natural history
	All Treated	REVERSE/ RESTORE[a]	RESCUE/ RESTORE[a]	REFLECT	
Number of eyes	348	74	78	196	408
Visual acuity (logMAR)					
Median	1.40	1.30	1.40	1.40	1.70
Mean (SD)	1.38 (0.59)	1.29 (0.47)	1.43 (0.71)	1.39 (0.57)	1.68 (0.61)
95% CI (mean)	[1.31;1.44]	[1.18; 1.40]	[1.27; 1.59]	[1.31;1.47]	[1.62; 1.74]
P values versus NH (KW/ANCOVA[b])	< 0.01/< 0.01	< 0.01/< 0.01	< 0.01/< 0.01	< 0.01/< 0.01	–
Mean difference versus NH [95% CI][b]	− 0.301 [− 0.387; − 0.215]	− 0.384 [− 0.531; − 0.238]	− 0.248 [− 0.400; − 0.096]	− 0.290 [− 0.392; − 0.188]	–
Time from vision loss to last observation (months)					
Median (range)	32.3 (8.1–51.5)	43.7 (29.0–49.2)	35.6 (8.1–51.5)	30.0 (13.4–48.1)	25.3 (0.0–768.0)
Mean (SD)	33.5 (8.1)	42.6 (5.4)	33.5 (8.4)	30.3 (5.9)	84.8 (132.3)
Time from treatment to last observation (months)					
Median (range)	24.8 (1.8–46.7)	34.7 (21.1–40.4)	30.5 (1.8–46.7)	24.0 (2.9–36.4)	–
Mean (SD)	26.5 (7.3)	33.1 (4.9)	29.5 (8.5)	22.9 (5.0)	–

ANCOVA analysis of covariance, *CI* confidence interval, *KW* Kruskal–Wallis, *logMAR* logarithm of the minimal angle of resolution, *NH* natural history, *SD* standard deviation

[a]Including follow-up data from the RESTORE study

[b]Mixed-model ANCOVA with repeated measures taking into account the inter-eye correlation of each patient

demographics of LHON, which show that some individuals may manifest the disease at a later age [12]. The proportion of Asian patients was significantly higher in the natural history cohort (38.5%) as compared to the treated cohort (8.6%) ($P < 0.01$). In the treated group, all Asian patients were from a single Taiwan centre of REFLECT, and in the natural history group, Asian patients were from either China or Japan [35, 37–39, 42, 43].

The mean number of visual acuity assessments per patient was larger in the treated group (24.8) as compared with the natural history group (4.1). Median follow-up after vision loss was slightly longer in treated patients (32.3 months) than in natural history patients (25.3 months; $P = 0.04$). Conversely, follow-up was distributed over a narrower range for treated patients (8.1–51.5 months) compared to natural history patients (0 to 768 months). About 38% of patients of each cohort were followed up for at least 36 months.

The treated patients received lenadogene nolparvovec as a unilateral (126 patients, 72.4%) or as a bilateral (48 patients, 27.6%) IVT between 1.7 and 12.8 months after vision loss (median, 8.3 months). Half of the eyes (53.4%) had received treatment at month 6 ([3; 9] months) after vision loss and nearly all eyes (96.8%) at month 12 ([9; 15] months) after

Table 5 Visual acuity at last observation with adjustment for covariates

	Treated (N = 348 eyes)	Natural history (N = 408 eyes)
Multivariate analysis		
Number of eyes	348	336[a]
Visual acuity (logMAR)		
LS mean [95% CI]	1.29 [1.20; 1.38]	1.72 [1.64; 1.80]
Effect estimate with 95% CI; P value[b]	− 0.43 [− 0.53; − 0.33]; $P < 0.0001$	
Propensity score weighting		
Number of eyes	348	336[a]
Visual acuity (logMAR)		
LS mean [95% CI]	1.35 [1.29; 1.42]	1.76 [1.7; 1.83]
Effect estimate with 95% CI; P value[b]	− 0.41 [− 0.50; − 0.32]; $P < 0.0001$	
Propensity score matching		
Number of eyes	186	186
Visual acuity (logMAR)		
LS mean [95% CI]	1.32 [1.2; 1.44]	1.65 [1.52; 1.78]
Effect estimate with 95% CI; P value[b]	− 0.33 [− 0.47; − 0.19]; $P < 0.0001$	

The four clinical covariates of interest included in the analyses were gender, ethnicity (Asian vs. non-Asian), age at onset of vision loss and duration of follow-up

CI confidence interval, *logMAR* logarithm of the minimal angle of resolution, *LS* least squares

[a]72 natural history eyes were excluded from the analysis because of missing gender data

[b]Repeated measure analyses taking into account the inter-eye correlation of each patient

vision loss. In this analysis we report visual acuity data starting from month 12 after vision loss, which is the time when nearly all eyes had received the treatment with lenadogene nolparvovec.

Global Evolution of Visual Acuity Over Time

The LOESS regression curves of visual acuity data are shown in Fig. 1 between 12 and 52 months since vision loss, which is the maximal follow-up duration for treated eyes. For natural history eyes, the 52-month time point also takes into account visual acuity values post month 52 using the next observation carried backward method. The LOESS curves of visual acuity up to 300 months, which allows for a better visualization of the evolution of natural history eyes at later time points, is shown in Supplementary Material Fig. 1.

The evolution of natural history eyes (shown in blue in Fig. 1) showed an absence of recovery throughout the entire follow-up period, with visual acuity values plateauing around 1.6 logMAR up to 36 months after vision loss followed by a slow decline to off-chart values from 36 months [33]. In contrast, the eyes of patients treated with lenadogene nolparvovec (in red) showed a progressive, continuous and sustained improvement between 12 and 52 months after vision loss, with the lowest point of the LOESS regression curve (worst BCVA) remaining on-chart with BCVA values not worse than 1.6 logMAR. The improvement

Table 6 Multivariate analysis of visual acuity at last observation: influence of covariates

Effect	Effect estimate [95% CI] (logMAR)	*P* value[a]
Treatment (reference = natural history)	− 0.43 [− 0.53; − 0.33]	< 0.0001
Covariates		
Gender (reference = male)	− 0.0305 [− 0.1459; 0.0848]	0.6029
Ethnicity (reference = non-Asian)	− 0.4866 [− 0.7571; − 0.2161]	0.0005
Age at onset of vision loss	0.0012 [− 0.0025; 0.0048]	0.5305
Duration of follow-up	0.0007 [0.0002; 0.0012]	0.0028
Interaction Age × Ethnicity	0.0084 [− 0.0014; 0.0182]	0.0913

CI confidence interval, *logMAR* logarithm of the minimal angle of resolution
[a]Repeated measure analyses taking into account the inter-eye correlation of each patient

was statistically significant as evidenced by the absence of overlap in the 95% CI of the regression curves, with consistently better visual acuity of treated eyes versus natural history eyes between month 12 and month 52. Similarly, patients recruited into the REFLECT trial showed consistently better visual acuity versus natural history eyes as illustrated in Supplementary Material Fig. 2.

Visual Acuities at Each Time Point

Visual acuity values between 12 and 48 months after vision loss are shown in Table 3. In agreement with LOESS regression analyses, eyes treated with lenadogene nolparvovec had better visual acuity at all time points when compared to natural history eyes. The difference in visual acuity between treated and natural history eyes was statistically significant at all evaluated time points between 18 and 48 months (Kruskal–Wallis test and mixed-model ANCOVA with repeated measures). The mean visual acuities [95% CI] for treated eyes and natural history eyes were, respectively, 1.23 [1.13; 1.34] and 1.59 [1.41; 1.76] logMAR at month 48, with a clinically relevant mean difference of − 0.352 [− 0.554; − 0.149] logMAR in favour of treated eyes ($p < 0.01$ with no overlap of CIs).

Visual Acuities at Last Available Observation

Visual Acuities Without Covariate Adjustment

Analysis conducted at last available observation in Table 4 showed a statistically significant and clinically relevant better visual acuity of treated eyes when compared to natural history eyes, overall and for each study, using both parametric and non-parametric statistical approaches.

The mean (unadjusted) difference of treated eyes versus natural history eyes was − 0.301 [− 0.387; − 0.215] logMAR in favour of treated eyes ($p < 0.01$ for both Kruskal–Wallis and mixed-model ANCOVA with repeated measures, and no overlap of CIs). The mean time from treatment to last available visual acuity was 26.5 months (2.2 years), with a maximum follow-up of up to 46.7 months (3.9 years).

Visual Acuities with Covariate Adjustment

For sensitivity analyses, the following clinical covariates of interest were taken into account when evaluating the treatment effect at last observation: gender, ethnicity (Asian vs. non-Asian), age at onset of vision loss and duration of follow-up (Table 5). Adjustment by multivariate analysis (Tables 5 and 6) confirmed the statistically significant and clinically relevant effect of treatment on last observed visual

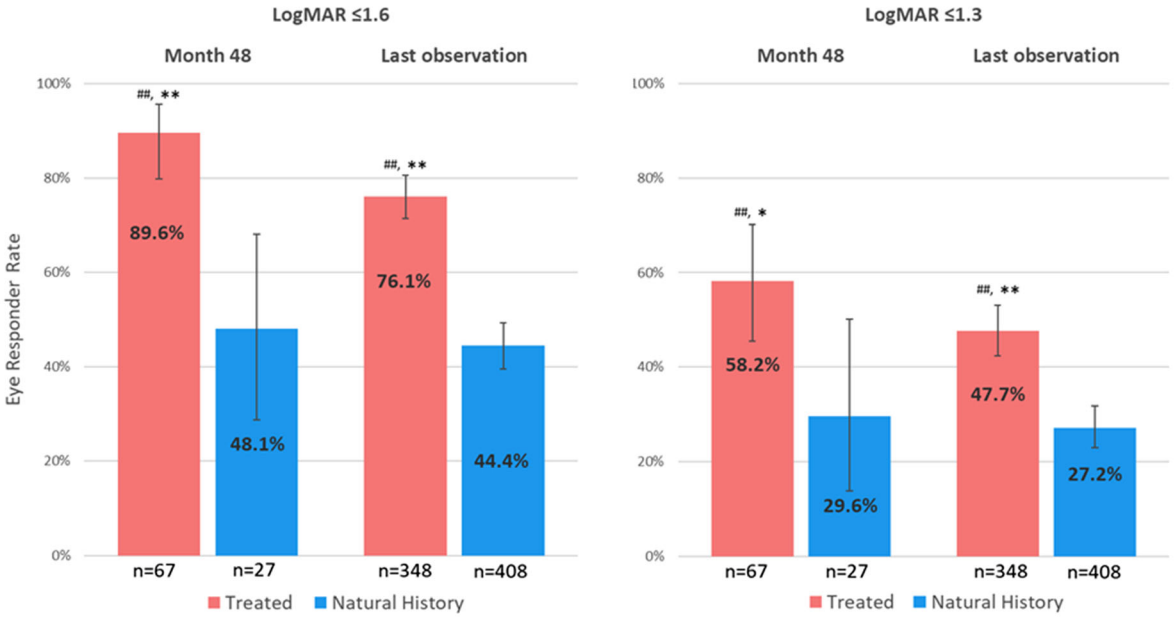

Fig. 2 Eye responder rate at month 48 since vision loss and at last observation. *logMAR* logarithm of the minimal angle of resolution, *n* number of eyes. Response rates (%) are defined as the proportion of eyes with visual acuity values ≤ 1.6 logMAR (left panel) or ≤ 1.3 logMAR (right panel). Error bars represent 95% confidence interval. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$: statistically significant difference vs. natural history eyes using chi-squared test. $^{*}P < 0.05$, $^{**}P < 0.01$: statistically significant difference vs. natural history eyes using a generalized linear mixed model with repeated measures taking into account the inter-eye correlation of each patient

acuity, with a LS mean difference of − 0.43 [− 0.53; − 0.33] logMAR in favour of treated eyes ($P < 0.0001$ by mixed-model ANCOVA with repeated measures, and no overlap of CIs). Both duration of follow-up ($P = 0.0028$) and ethnicity ($P = 0.0005$) showed a statistically significant effect on visual acuity (i.e. patients with a shorter follow-up and Asian patients had better visual acuity independent of treatment). In contrast, gender ($P = 0.6029$) and age of onset (0.5305) had no significant effect on visual acuity. There was no statistically significant interaction between age and ethnicity ($P = 0.0913$).

Results of covariate adjustment using propensity score methods were consistent with results of the multivariate analysis, with a statistically significant and clinically relevant LS mean difference of − 0.41 logMAR using propensity score weighting and of − 0.33 logMAR using propensity score matching (for both approaches $P < 0.0001$ by mixed-model ANCOVA with repeated measures, and no overlap of CIs; Table 5).

Eye Response Rates

At month 48 after vision loss, most (60/67) treated eyes (89.6%, 95% CI [79.7; 95.7]) were on-chart (logMAR ≤ 1.6) as compared to less than half of the eyes (13/27) in the natural history group (48.1%, 95% CI [28.7; 68.1]) ($P < 0.01$ with chi-squared test and generalized linear mixed model with repeated measures) (Fig. 2, left panel). Comparable statistically significant results were observed for the response rates using the logMAR threshold of 1.3, which is the cut-off for blindness according to WHO criteria (Fig. 2, right panel).

At last observation, 265 of the 348 treated eyes (76.1%; 95% CI [71.3, 80.5]) were on-chart (logMAR ≤ 1.6), as compared to 181 of the 408 natural history eyes (44.4%; 95% CI [39.5, 49.3]), with a statistically significant

Table 7 Visual acuity at last observation in REFLECT

	Treated (REFLECT)		Natural history (*N* = 408 eyes)
	Bilaterally treated (*N* = 96 eyes)	Unilaterally treated (*N* = 100 eyes)	
Visual acuity (logMAR)			
Median	1.40	1.40	1.70
Mean (SD)	1.32 (0.54)	1.45 (0.60)	1.68 (0.61)
95% CI (mean)	[1.21; 1.43]	[1.33; 1.57]	[1.62; 1.74]
P values versus NH (KW/ ANCOVA[a])	< 0.01/< 0.01	< 0.01/< 0.01	–
Mean difference versus NH [95% CI][a]	− 0.355 [− 0.488; − 0.222]	− 0.228 [− 0.361; − 0.095]	–
Response rate (logMAR ≤ 1.6)			
n (%) [95% CI]	76 (79.2%) [69.7; 86.8]	67 (67.0%) [56.9; 76.1]	181 (44.4%) [39.5; 49.3]
P values versus NH (C/ GLMM[b])	< 0.01/< 0.01	< 0.01/< 0.01	–

ANCOVA analysis of covariance, *C* chi-squared test, *CI* confidence interval, *GLMM* generalized linear mixed model, *KW* Kruskal–Wallis, *logMAR* logarithm of the minimal angle of resolution, *LS* least squares, *NH* natural history, *SD* standard deviation

[a]Mixed-model ANCOVA with repeated measures taking into account the inter-eye correlation of each patient

[b]GLMM with repeated measures taking into account the inter-eye correlation of each patient

difference with both statistical tests ($P < 0.01$) (Fig. 2, left panel).

When using the 1.3-logMAR response threshold at last observation, comparable statistically significant results were observed (Fig. 2, right panel).

Impact of Bilateral Versus Unilateral Injection in REFLECT

Analyses showed a larger treatment effect at last observation (23 months from treatment on average) in patients who received treatment as a bilateral IVT versus patients who received the treatment as a unilateral IVT (Tables 7 and 8).

The mean (unadjusted) differences versus natural history eyes were − 0.355 [− 0.488; − 0.222] logMAR for bilaterally treated patients and − 0.228 [− 0.361; − 0.095] for unilaterally treated patients ($P < 0.01$ for each treated group versus natural history by both parametric and non-parametric tests, and no overlap of the CIs) (Table 7). When we adjusted for covariates (Table 8), the LS mean differences were − 0.45 [− 0.59; − 0.32] and − 0.35 [− 0.49; − 0.22] for bilaterally treated patients and unilaterally treated patients, respectively ($P < 0.0001$ with no overlap of CIs). Both duration of follow-up and ethnicity showed a statistically significant effect on visual acuity (i.e. patients with a shorter follow-up and Asian patients had better visual acuity independent of treatment). In contrast, gender and age of onset had no significant effect on visual acuity. Results of covariate adjustment using propensity score weighting and matching were consistent with results of the multivariate analysis.

Similarly, a larger response rate for on-chart eyes was observed with bilateral IVT as compared to unilateral IVT: at last observation, the proportion of responder eyes (logMAR

Table 8 Multivariate analysis of visual acuity at last observation in REFLECT

	Number of eyes	LS mean [95% CI] (logMAR)	Effect estimate [95% CI]	*P* value[b]
Bilaterally treated versus natural history				
Treatment status			− 0.45 [− 0.59; − 0.32]	< 0.0001
Treated	96	1.25 [1.12; 1.38]		
Natural history (reference)	336[a]	1.71 [1.62; 1.79]		
Covariates				
Gender (reference = male)			− 0.0986 [− 0.2402; 0.043]	0.1713
Ethnicity (reference = non-Asian)			− 0.5666 [− 0.8636; − 0.2695]	0.0002
Age at onset of vision loss			0.0019 [− 0.0031; 0.0069]	0.4539
Duration of follow-up			0.0008 [0.0003; 0.0012]	0.0010
Interaction Age × Ethnicity			0.0124 [0.0017; 0.023]	0.0231
Unilaterally treated versus natural history				
Treatment status			− 0.35 [− 0.49; − 0.22]	< 0.0001
Treated	100	1.35 [1.21; 1.49]		
Natural history (reference)	336[a]	1.70 [1.62; 1.79]		
Covariates				
Gender (reference = male)			− 0.081 [− 0.2316; 0.0696]	0.2901
Ethnicity (reference = non-Asian)			− 0.6206 [− 0.9261; − 0.3152]	< 0.0001
Age at onset of vision loss			− 0.0002 [− 0.0055; 0.0052]	0.9553
Duration of follow-up			0.0008 [0.0003; 0.0012]	0.0014
Interaction Age × Ethnicity			0.0113 [0.0003; 0.0223]	0.0440

CI confidence interval, *logMAR* logarithm of the minimal angle of resolution, *LS* least squares
[a]72 natural history eyes were excluded from the analysis because of missing gender data
[b]Repeated measure analyses taking into account the inter-eye correlation of each patient

≤ 1.6) was 79.2% for bilaterally treated patients and 67.0% for unilaterally treated patients, as compared to 44.4% for natural history patients ($P < 0.01$ for both comparisons by parametric and non-parametric tests).

Factors Influencing Response to Treatment in the Treated Cohort

We explored the following covariates that could have influenced the results of the BCVA value at the last observation (response to treatment) in the treated cohort: age at onset of vision loss,

Table 9 Analysis of visual acuity at last observation in the treated population: assessment of predictive factors for response to treatment

Covariates	Number of eyes (N = 348)	Univariate analysis			Multivariate analysis		
		LS mean [95% CI] (logMAR)	Effect estimate [95% CI]	P value[a]	LS mean [95% CI] (logMAR)	Effect estimate [95% CI]	P value[a]
Gender (reference = male)			0.0345 [− 0.1202; 0.1892]	0.6603		0.0189 [− 0.1394; 0.1772]	0.8137
Female	70	1.40 [1.26; 1.54]			1.33 [1.16; 1.50]		
Male	278	1.37 [1.30; 1.44]			1.31 [1.19; 1.42]		
Ethnicity (reference = non-Asian)			− 0.1517 [− 0.3721; 0.0687]	0.1761		− 0.1516 [− 0.3737; 0.0705]	0.1797
Asian centre	30	1.24 [1.03; 1.45]			1.24 [1.02; 1.46]		
Non-Asian centre	318	1.39 [1.32; 1.45]			1.39 [1.31; 1.47]		
Age at onset of vision loss	348		0.0011 [− 0.0031; 0.0054]	0.6010		0.0004 [− 0.0040; 0.0048]	0.8591
Time from vision loss to treatment (months)	348		− 0.0216 [− 0.0398; − 0.0034]	0.0201		− 0.0217 [− 0.0400; − 0.0035]	0.0200

CI confidence interval, *logMAR* logarithm of the minimal angle of resolution, *LS* least squares
[a]Repeated measure analyses taking into account the inter-eye correlation of each patient

gender, ethnicity (Asian vs. non-Asian) and time from vision loss to treatment (Table 9). In the univariate analysis, a later treatment administration (within 1 year after vision loss) was associated with a better response to treatment compared to an earlier treatment, with an effect estimate of − 0.0216 (95% CI [− 0.0398; − 0.0034]) logMAR (P = 0.0201) for each month of delayed treatment, while the other factors had no statistically significant impact. In the multivariate analysis, similar results were seen, with a better response for a delayed treatment: effect estimate of − 0.0217 (95% CI [− 0.0400; − 0.0035] logMAR (P = 0.0200) for each month of delayed treatment. The other patients' characteristics were not predictive of response to treatment.

DISCUSSION

We used an indirect comparison approach to assess the efficacy of lenadogene nolparvovec by comparing the improvement of BCVA in treated patients to the spontaneous evolution of visual acuity in an external control group of *MT-ND4*–carrying patients with LHON who were not treated with the gene therapy. Both cohorts,

treated and natural history patients, were typical of the *MT-ND4* LHON population, with a predominance of male patients and a median age at onset of vision loss in their twenties.

The use of natural history data as an external control is acknowledged in European and American drug development guidelines as a valid approach in special clinical circumstances, as is often the case with rare diseases [47–49]. In recent years, studies including natural history external controls have been increasingly used to support the registration of medicinal products in special conditions such as rare metabolic diseases or severe haematologic cancers, with a high overall regulatory approval rate [50].

As previously reported, the external control cohort used in this analysis was built on the basis of a strict and robust methodology, and set up by selecting all available published visual acuity data on *MT-ND4*-carrying patients with LHON identified after a systematic review of the literature, with no restriction on study designs, including both longitudinal and cross-sectional natural history studies [33]. This systematic predefined approach enabled avoidance of bias in selecting studies, facilitating a representative sampling of the natural course of the disease. Studies were excluded when they only reported aggregated data and not individual-patient level data, preventing meaningful statistical analyses for an indirect comparison. Individual case reports were also excluded according to prespecified criteria because such reports are generally biased towards patients showing unusual disease clinical characteristics. These published natural history data were supplemented with data from our natural history registry study REALITY [34], creating the largest post-molecular diagnosis natural history database published to date on LHON. The individual visual acuity data from the included 208 patients with the *MT-ND4* genotype are representative of the spontaneous evolution of visual function of *MT-ND4*-carrying patients and comparable to the treated group as regards LHON genotype and age of onset, enabling robust comparison analyses with patients treated in interventional trials. One limitation of using such an external natural history control group is related to the retrospective design of most of those studies, as opposed to prospective collection of data. Another limitation is the unavoidably heterogeneous methods and timing of visual acuity assessments among the natural history studies.

Another research group used a comparable approach for determining the efficacy of a gene therapy product in LHON; however, their external control group was based on a single cohort study with a limited sample size [36, 51, 52]. The LEROS study group adopted a similar approach by comparing patients with LHON treated with idebenone in an open-label phase 4 interventional study to an external, natural history comparator cohort which was created using retrospective data from two LHON case record surveys [53, meeting abstract].

We previously reported that lenadogene nolparvovec improved visual acuity compared to natural history in a pool of 76 treated patients and in each separate study of the pool [33]. Here, we extend those results to 174 patients treated with the gene therapy. We show that lenadogene nolparvovec is able to induce a clinically meaningful and sustained improvement in BCVA in a large population of *MT-ND4*-carrying patients with LHON aged 15 years or older at onset of vision loss when compared to the spontaneous evolution of visual acuity of a large group of matched natural history patients. Improvement was noticeable from 12 months after vision loss, which is consistent with the proportion of patients treated with lenadogene nolparvovec over time, with half of eyes injected at month 6 and nearly all eyes at month 12. Furthermore, the absence of overlap in 95% CI between the treated and natural history LOESS regression curves, at all time points from month 12 to month 52, provides further evidence of the significance of the difference and the consistency of the better visual acuity achieved by *MT-ND4*-carrying patients in the treated pool. The mean improvement at last available observation was − 0.30 logMAR in treated eyes when compared to natural history eyes, which is consistent with the previously reported mean difference of − 0.33 logMAR in our earlier indirect analysis [33]. These differences meet the thresholds of clinical relevance defined by regulators. The conservative threshold of improvement of at

least 15 letters (− 0.3 logMAR) has been used by the US Food and Drug Administration (FDA) for drug approval, but even mean changes less than 15 letters have been considered clinically relevant in some settings, dependent on the benefit–risk balance of the treatment, a position also shared by the European agency [54]. As an example, idebenone was approved in Europe for the treatment of LHON on the basis of an improvement of 10 letters (− 0.2 logMAR) [18].

The proportion of treated eyes on-chart at last observation was 76%, close to the 80% reported in our previous indirect analysis [33]; this recovery rate is significantly higher than the 44% of on-chart eyes observed at last observation in the natural history cohort. Hence, this analysis confirms our previous findings in an extended cohort of 174 patients (instead of the previous 76 patients) by including the most recent results from the REFLECT study.

The mean time from lenadogene nolparvovec treatment to last available visual acuity was 2.2 years on average, with a maximum follow-up of up to 46.7 months (3.9 years), indicating the long-lasting effect of lenadogene nolparvovec on visual acuity. Importantly, the persistence of lenadogene nolparvovec efficacy has already been demonstrated in the RESTORE study after 3 years post-treatment [32].

Phase 3 trials with lenadogene nolparvovec demonstrated visual benefits in both eyes of patients with LHON who were treated unilaterally [27, 28, 30]; this was unanticipated and the subject of other reports. As a result, in this report, all eyes of phase 3 patients were pooled and analysed as "treated", whether or not they were injected with the therapy. This approach is supported by non-human primate data showing a bilateral biodistribution of viral vector DNA after unilateral IVT of lenadogene nolparvovec, although alternative mechanisms must also be considered in the observed contralateral therapeutic effect [31]. Consistent with results observed with lenadogene nolparvovec, bilateral improvement has also been reported with unilateral treatment in other gene therapy clinical trials in *MT-ND4* LHON [51, 55].

In the REFLECT trial, patients received the study product either as a unilateral or a bilateral IVT [29, 30]. In agreement with the contralateral therapeutic effect observed in REVERSE and RESCUE, all REFLECT eye groups, including those unilaterally injected, showed better visual outcomes at 1.5 years after treatment, with a mean gain in ETDRS letters from nadir ranging from − 0.26 logMAR (+ 13 ETDRS letters equivalent for placebo injected eyes) to − 0.38 logMAR (+ 19 ETDRS letters equivalent for eyes injected with the therapy). When compared to natural history eyes, the size of treatment effect was larger in REFLECT patients who received bilateral treatment than in patients who received unilateral treatment: at last observation (on average, 2 years after treatment), the mean difference in visual acuity versus natural history eyes was − 0.35 logMAR in bilaterally treated patients and − 0.23 logMAR in unilaterally treated patients. Similarly, the proportion of on-chart eyes at last observation was larger in bilaterally treated patients (79%) than in unilaterally treated patients (67%), and was only 44% in natural history patients. These results suggest that bilateral IVT of lenadogene nolparvovec could provide further visual benefit to patients with LHON as compared to unilateral IVT. This would also be compatible with the expected lower amount of viral vector transfer to the uninjected eye, and consequently reduced therapeutic efficiency. Of note, bilateral treatment was safe and well tolerated in the REFLECT study, with no difference between bilaterally and unilaterally treated subjects, with comparable safety findings for treated eyes [30]. The results of the ongoing long-term follow-up of REFLECT study will provide further information on the potential added benefit of bilateral treatment versus unilateral treatment.

One important limitation of our analysis relates to a possible imbalance in confounding factors between the treated group and the external control group, which may have biased the estimation of the treatment effect. While both groups showed typical features of the LHON population (predominance of male patients and a young age at onset), baseline characteristics were not all statistically comparable between the natural history and the treated group. The main differences consisted of a

slightly younger age at onset, a higher proportion of Asian patients and a shorter median follow-up in the natural history population compared to the treated patients. While it is well established that *MT-ND4*-carrying patients with LHON have a better outcome when the disease onset is in childhood, there is no documented difference in outcomes between young adults and late onset *MT-ND4* LHON. Newman et al. provided an exhaustive review of the current literature specifically related to visual function of patients with LHON carrying the m.11778G>A mutation, including 12 retrospective and three prospective studies on 695 *MT-ND4*-carrying patients with LHON and visual function documentation. They highlighted that from a clinical standpoint, all patients aged 15 years and older are considered to have a comparable disease evolution while younger patients have better visual outcomes [13]. This is in line with the results of our multivariate analysis which showed no statistically significant impact of age of onset on final visual acuity in patients aged 15 years and older. Even if there were a difference in visual outcomes in favour of younger adults, this would bias the results in favour of the natural history cohort in our study. Regarding ethnicity, a few reports have suggested that the visual outcome of *MT-ND4*-carrying Asian patients with LHON may be more favourable than in Caucasian populations [35, 56, 57]. A study examined the clinical features of LHON in 19 Thai pedigree families and compared them to patients in the USA, Europe and other Asian countries [56]. The authors noted that Thai patients with the *MT-ND4* mutation had a higher likelihood of favourable outcomes regarding visual prognosis when compared to *MT-ND4*-carrying patients with LHON in the USA. In one study conducted in 89 Japanese *MT-ND4*-carrying patients with LHON, visual outcomes were better than those of a landmark American *MT-ND4* LHON cohort and were attributed to a slower progression of vision loss after onset [4, 35]. Similarly, another study of Japanese *MT-ND4*-carrying patients showed that 15 of the 61 patients (24.6%) had a final visual acuity of at least 0.2, compared to 1.8% of *MT-ND4*-carrying patients with LHON in the same USA cohort [4, 57]. Hence, the higher proportion of Asian patients in our natural history cohort would also potentially bias our study in favour of the natural history patients. Lastly, follow-up duration can be a confounding factor. The follow-up duration across natural history LHON studies is heterogeneous, varying from a few months to many years. While spontaneous recovery is a rare event in *MT-ND4* mutation-carrying patients, the timing of this recovery may vary greatly [13]. For example, Lam et al. reported that the time to recovery after onset of vision loss ranged from 8.3 to 71.5 months, with the caveat that their study included several younger-onset patients [36].

To overcome these limitations related to the imbalance of confounding factors between the treated and external control groups, we conducted sensitivity analyses to control for four clinical covariates that could have impacted visual acuity of patients with LHON, namely gender, ethnicity, age at onset of vision loss and duration of follow-up. Using three different statistical approaches, multivariate analysis, propensity score weighting and propensity score matching, sensitivity analyses confirmed the clinically relevant treatment effect of lenadogene nolparvovec-treated eyes versus natural history eyes when accounting for these clinical covariates of interest. At last observation, up to 3.9 years post-treatment, the mean effect estimate adjusted for covariates was − 0.43 logMAR using multivariate analysis ($P < 0.0001$ versus natural history), thus larger than the treatment effect of − 0.30 logMAR without covariates adjustment. Interestingly, among the four covariates of interest considered, only ethnicity and follow-up duration had a statistically significant impact on visual outcomes independent of treatment. A shorter follow-up was associated with a better visual acuity, consistent with the progressive degenerative nature of the disease. As regards ethnicity, Asian patients tended to have more favourable visual outcomes as compared to non-Asian patients, consistent with published observation from small Thai and Japanese LHON cohorts [35, 56, 57]. Therefore, given the higher proportion of Asian patients and the shorter follow-up compared to the treated

group, the natural history group was likely biased towards better visual outcomes resulting in an underestimation of the treatment effect in our unadjusted analysis. When the same adjustment for covariates was used, bilateral IVT with lenadogene nolparvovec in REFLECT showed a clinically relevant treatment effect of − 0.45 logMAR versus natural history eyes at last observation. This was larger than the treatment effect observed without covariates adjustment, and largely met the threshold of clinical relevance.

One puzzling observation emerging from the pooled results is the unexpected better visual outcome of patients who were treated later rather than earlier within the window of 1 year from disease onset, with an improvement of approximately 1 letter for each month of delayed treatment. This was first noted in the comparison of the results of the RESCUE and REVERSE trials, and further confirmed with the addition of the REFLECT data. The intuitive expectation was that the earlier the therapy is initiated, the better the visual outcome would be, but this does not seem to be the case. It has been suggested that acutely swollen nerve fibres may act as a barrier to the delivery of the viral vector to the underlying RGCs [28]. This remains speculative, reflecting our incomplete understanding of LHON, in particular, the triggers that precipitate disease conversion, the factors that influence the pattern of vision loss and eventually the final outcome, and the mechanisms that underpin spontaneous recovery of visual function in LHON despite the catastrophic loss of RGCs and the development of optic atrophy [58, 59].

CONCLUSION

This pooled analysis confirmed a clinically relevant and sustained improvement in the visual acuity of 174 *MT-ND4*-carrying patients with LHON treated with lenadogene nolparvovec, when compared to the spontaneous evolution of vision in a large group of 208 matched natural history *MT-ND4*-carrying patients with LHON used as an external control. The mean improvement at last available observation was + 15 letters versus natural history and lasted up to 3.9 years after lenadogene nolparvovec injection. The majority of lenadogene nolparvovec-treated eyes were on-chart as compared to less than half of the natural history eyes at 48 months after vision loss and at last available observation. Importantly, all sensitivity analyses controlling for potential confounding factors of clinical interest (gender, age of onset, ethnicity and duration of follow-up) were consistent with the unadjusted analyses. When we adjusted for these covariates, the estimated mean improvement in visual acuity versus natural history at last observation was + 21.5 letters. These results mirror our previous report on 76 *MT-ND4*-carrying patients with LHON treated with lenadogene nolparvovec [33], indicating that the treatment effect of lenadogene nolparvovec is consistent across the four phase 3 clinical trials, REVERSE, RESCUE, RESTORE and REFLECT. Analyses of REFLECT data suggest that the treatment effect of lenadogene nolparvovec is larger in patients who received bilateral injections as compared to those who received unilateral injection. The best timing of treatment within the first year following disease onset remains unclear.

These results confirm a clinically relevant improvement of visual acuity in *MT-ND4*-carrying patients with LHON treated with lenadogene nolparvovec to a degree not demonstrated in natural history studies. The treatment effect of lenadogene nolparvovec was long-lasting up to the last visual acuity value currently documented. *MT-ND4* LHON disease remains an area of acute unmet medical need. Lenadogene nolparvovec provides a substantial benefit to *MT-ND4*-carrying patients with LHON, who face a blinding disease with limited treatment options at this time.

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Data Availability. The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Yu-Wai-Man P, Votruba M, Burté F, La Morgia C, Barboni P, Carelli V. A neurodegenerative perspective on mitochondrial optic neuropathies. Acta Neuropathol. 2016;132:789–806.

2. Chen BS, Holzinger E, Taiel M, Yu-Wai-Man P. The impact of Leber hereditary optic neuropathy on the quality of life of patients and their relatives: a qualitative study. J Neuro-Ophthalmol. 2022;42:316–22.

3. Riordan-Eva P, Sanders MD, Govan GG, Sweeney MG, Da CJ, Harding AE. The clinical features of Leber's hereditary optic neuropathy defined by the presence of a pathogenic mitochondrial DNA mutation. Brain. 1995;118:319–37.

4. Newman NJ, Lott MT, Wallace DC. The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. Am J Ophthalmol. 1991;111:750–62.

5. Yu-Wai-Man P, Turnbull DM, Chinnery PF. Leber hereditary optic neuropathy. J Med Genet. 2002;39:162–9.

6. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. Am J Ophthalmol. 2005;140:517–23.

7. Barboni P, Savini G, Valentino ML, et al. Leber's hereditary optic neuropathy with childhood onset. Invest Ophthalmol Vis Sci. 2006;47:5303–9.

8. Yu-Wai-Man P, Griffiths PG, Hudson G, Chinnery PF. Inherited mitochondrial optic neuropathies. J Med Genet. 2009;46:145–58.

9. Dimitriadis K, Leonhardt M, Yu-Wai-Man P, et al. Leber's hereditary optic neuropathy with late disease onset: clinical and molecular characteristics of 20 patients. Orphanet J Rare Dis. 2014;9:158.

10. Stenton SL, Sheremet NL, Catarino CB, et al. Impaired complex I repair causes recessive Leber's hereditary optic neuropathy. J Clin Invest. 2021;131:e138267. https://doi.org/10.1172/JCI138267.

11. Stenton SL, Tesarova M, Sheremet NL, et al. DNAJC30 defect: a frequent cause of recessive Leber hereditary optic neuropathy and Leigh syndrome. Brain. 2022;145:1624–31.

12. Poincenot L, Pearson AL, Karanjia R. Demographics of a large international population of patients affected by Leber's hereditary optic neuropathy. Ophthalmology. 2020;127:679–88.

13. Newman NJ, Carelli V, Taiel M, Yu-Wai-Man P. Visual outcomes in Leber hereditary optic neuropathy patients with the m11778G>A (MTND4) mitochondrial DNA mutation. J Neuro-Ophthalmol. 2020;40:547–57.

14. Koilkonda RD, Guy J. Leber's hereditary optic neuropathy-gene therapy: from benchtop to bedside. J Ophthalmol. 2011;2011: 179412.

15. Carelli V, Ghelli A, Bucchi L, et al. Biochemical features of mtDNA 14484 (ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. Ann Neurol. 1999;45:320–8.

16. Macmillan C, Kirkham T, Fu K, et al. Pedigree analysis of French Canadian families with T14484C Leber's hereditary optic neuropathy. Neurology. 1998;50:417–22.

17. Chinnery PF, Brown DT, Andrews RM, et al. The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy. Brain. 2001;124:209–18.

18. European Medicines Agency (EMA). European Public Assessment Report: Raxone (idebenone). https://www.ema.europa.eu/en/medicines/human/EPAR/raxone. Accessed Jul 22, 2022.

19. Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. Brain. 2011;134:2677–86.

20. Catarino CB, von Livonius B, Priglinger C, et al. Real-world clinical experience with idebenone in the treatment of Leber hereditary optic neuropathy. J Neuro-Ophthalmology. 2020;40:558–65.

21. Carelli V, Carbonelli M, de Coo IF, et al. International consensus statement on the clinical and therapeutic management of Leber hereditary optic neuropathy. J Neuroophthalmol. 2017;37:371–81.

22. Guy J, Qi X, Pallotti F, et al. Rescue of a mitochondrial deficiency causing Leber hereditary optic neuropathy. Ann Neurol. 2002;52:534–42.

23. Bonnet C, Augustin S, Ellouze S, et al. The optimized allotopic expression of ND1 or ND4 genes restores respiratory chain complex I activity in fibroblasts harboring mutations in these genes. Biochim Biophys Acta Mol Cell Res. 2008;1783:1707–17.

24. Ellouze S, Augustin S, Bouaita A, et al. Optimized allotopic expression of the human mitochondrial ND4 prevents blindness in a rat model of mitochondrial dysfunction. Am J Hum Genet. 2008;83:373–87.

25. Vignal C, Uretsky S, Fitoussi S, et al. Safety of rAAV2/2-ND4 gene therapy for Leber hereditary optic neuropathy. Ophthalmology. 2018;125:945–7.

26. Vignal-Clermont C, Girmens J-F, Audo I, et al. Safety of intravitreal gene therapy for treatment of subjects with Leber hereditary optic neuropathy due to mutations in the mitochondrial nd4 gene: the REVEAL study. BioDrugs. 2021;35:201–14.

27. Yu-Wai-Man P, Newman NJ, Carelli V, et al. Bilateral visual improvement with unilateral gene therapy injection for Leber hereditary optic neuropathy. Sci Transl Med. 2020;12:eaaz7423.

28. Newman NJ, Yu-Wai-Man P, Carelli V, et al. Efficacy and safety of intravitreal gene therapy for Leber hereditary optic neuropathy treated within 6 months of disease onset. Ophthalmology. 2021;128:649–60.

29. Subramanian PS, Newman NJ, Moster ML, et al. Study design and baseline characteristics for the REFLECT gene therapy trial of m.11778G>A/ND4-LHON. BMJ Open Ophthalmol. 2022. https://doi.org/10.1136/bmjophth-2022-001158.

30. Newman NJ, Yu-Wai-Man P, Subramanian PS, et al. Randomised trial of bilateral injection of lenadogene nolparvovec for m.11778G>A MT-ND4 Leber hereditary optic neuropathy. Brain. 2022. https://doi.org/10.1093/brain/awac421.

31. Calkins DJ, Yu-Wai-Man P, Newman NJ, et al. Biodistribution of intravitreal lenadogene nolparvovec gene therapy in nonhuman primates. Mol Ther Methods Clin Dev. 2021;23:307–18.

32. Biousse V, Newman NJ, Yu-Wai-Man P, et al. Long-term follow-up after unilateral intravitreal gene therapy for Leber hereditary optic neuropathy: the RESTORE study. J Neuro-Ophthalmology. 2021;41:309–15.

33. Newman NJ, Yu-Wai-Man P, Carelli V, et al. Intravitreal gene therapy vs. natural history in patients with Leber hereditary optic neuropathy carrying the m.11778G>A ND4 mutation: systematic review and indirect comparison. Front Neurol. 2021;12:662838.

34. Yu-Wai-Man P, Newman NJ, Carelli V, et al. Natural history of patients with Leber hereditary optic neuropathy—results from the REALITY study. Eye. 2022;36:818–26.

35. Hotta Y, Fujiki K, Hayakawa M, et al. Clinical features of Japanese Leber's hereditary optic neuropathy with 11778 mutation of mitochondrial DNA. Jpn J Ophthalmol. 1995;39:96–108.

36. Lam BL, Feuer WJ, Schiffman JC, et al. Trial end points and natural history in patients with G11778A leber hereditary optic neuropathy: preparation for gene therapy clinical trial. JAMA Ophthalmol. 2014;132:428–36.

37. Nakamura M, Fujiwara Y, Yamamoto M. Homoplasmic and exclusive ND4 gene mutation in Japanese pedigrees with Leber's disease. Investig Ophthalmol Vis Sci. 1993;34:488–95.

38. Qu J, Li R, Zhou X, et al. Cosegregation of the ND4 G11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. Mitochondrion. 2007;7:140–6.

39. Qu J, Zhou X, Zhang J, et al. Extremely low penetrance of Leber's hereditary optic neuropathy in 8 Han Chinese families carrying the ND4 G11778A mutation. Ophthalmology. 2009;116:558–564.e3.

40. Romero P, Fernández V, Slabaugh M, et al. Pan-American mDNA haplogroups in Chilean patients with Leber's hereditary optic neuropathy. Mol Vis. 2014;20:334–40.

41. Sadun F, De Negri AM, Carelli V, et al. Ophthalmologic findings in a large pedigree of 11778/Haplogroup J Leber hereditary optic neuropathy. Am J Ophthalmol. 2004;137:271–7.

42. Yang S, Yang H, Ma S, et al. Evaluation of Leber's hereditary optic neuropathy patients prior to a gene therapy clinical trial. Medicine (Baltimore). 2016;95:e5110.

43. Zhou X, Zhang H, Zhao F, et al. Very high penetrance and occurrence of Leber's hereditary optic neuropathy in a large Han Chinese pedigree carrying the ND4 G11778A mutation. Mol Genet Metab. 2010;100:379–84.

44. Lange C, Feltgen N, Junker B, Schulze-Bonsel K, Bach M. Resolving the clinical acuity categories 'hand motion' and 'counting fingers' using the

Freiburg Visual Acuity Test (FrACT). Graefes Arch Clin Exp Ophthalmol. 2009;247:137–42.

45. Guy J, Feuer WJ, Porciatti V, et al. Retinal ganglion cell dysfunction in asymptomatic G11778A: Leber hereditary optic neuropathy. Invest Ophthalmol Vis Sci. 2014;55:841–8.

46. Hwang TJ, Karanjia R, Moraes-Filho MN, et al. Natural history of conversion of Leber's hereditary optic neuropathy: a prospective case series. Ophthalmology. 2017;124:843–50.

47. Committee For Medicinal Products For Human Use (CHMP). Guideline on Clinical Trials in Small Populations. CHMP/EWP/83561/2005. 2006. https://www.ema.europa.eu/en/clinical-trials-small-populations-scientific-guideline.

48. Committee for Proprietary Medicinal Products (CPMP). Note For Guidance on Choice of Control Group in Clinical Trials: ICH Topic E10. CPMP/ICH/364/96. 2001. https://www.ema.europa.eu/en/ich-e10-choice-control-group-clinical-trials-scientific-guideline.

49. Food and Drug Administration. Rare Diseases: Natural History Studies for Drug Development. Draft Guidance. 2019. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/rare-diseases-natural-history-studies-drug-development.

50. Goring S, Taylor A, Müller K, et al. Characteristics of non-randomised studies using comparisons with external controls submitted for regulatory approval in the USA and Europe: a systematic review. BMJ Open. 2019;9:e024895.

51. Guy J, Feuer WJ, Davis JL, et al. Gene therapy for Leber hereditary optic neuropathy: low- and medium-dose visual results. Ophthalmology. 2017;124:1621–34.

52. Lam BL, Feuer WJ, Davis JL, et al. Leber hereditary optic neuropathy gene therapy: adverse events and visual acuity results of all patient groups. Am J Ophthalmol. 2022;241:262–71.

53. Llòria X, Tomasso L, Klopstock T, LEROS Study Group. Long-term efficacy and safety of idebenone in patients with LHON in the chronic phase: results from the LEROS study [Meeting Abstract]. Invest Ophthalmol Vis Sci. 2022;63:1581-A0370.

54. Csaky K, Ferris F, Chew EY, Nair P, Cheetham JK, Duncan JL. Report from the NEI/FDA endpoints workshop on age-related macular degeneration and inherited retinal diseases. Invest Ophthalmol Vis Sci. 2017;58:3456–63.

55. Yuan J, Zhang Y, Liu H, et al. Seven-year follow-up of gene therapy for Leber's hereditary optic neuropathy. Ophthalmology. 2020;127:1125–7.

56. Chuenkongkaew W. Leber's hereditary optic neuropathy in Thailand. Jpn J Ophthalmol. 2001;45:665–8.

57. Mashima Y, Kigasawa K, Shinoda K, Wakakura M, Oguchi Y. Visual prognosis better in eyes with less severe reduction of visual acuity one year after onset of Leber hereditary optic neuropathy caused by the 11,778 mutation. BMC Ophthalmol. 2017;17:192.

58. Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. Prog Retin Eye Res. 2004;23:53–89.

59. Yu-Wai-Man P, Griffiths PG, Chinnery PF. Mitochondrial optic neuropathies—disease mechanisms and therapeutic strategies. Prog Retin Eye Res. 2011;30:81–114.