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# Study design and baseline characteristics for the reflect gene therapy trial of m.11778g>A/*ND4*-LHON

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## ABSTRACT

**Objective** REFLECT is the first randomised, double-masked, placebo-controlled multicentre phase 3 clinical trial that evaluated the efficacy and safety of bilateral intravitreal (IVT) injection of lenadogene nolparvovec in subjects with Leber hereditary optic neuropathy carrying the m.11778G>A mutation.

**Methods and analysis** A total of 98 subjects were enrolled with vision loss of ≤12 months. The subjects were randomised to one of two treatment arms with all subjects receiving an intravitreal (IVT) injection of lenadogene nolparvovec in their first affected eye and the second-affected eye randomised to receive IVT of either lenadogene nolparvovec or placebo.

**Results** The majority of subjects were male with a mean duration of vision loss of 8.3 months. All but one subject experienced bilateral loss of vision at the time of injection. The mean best-corrected visual acuity of first-affected eyes was worse compared with second/not-yet-affected eyes. Analysis of retinal anatomical parameters showed increased thinning in the first-affected eyes when compared with the second/not-yet-affected eyes with both treatment arms showing significant changes compared with unaffected individuals.

**Conclusion** The REFLECT trial is the third and the largest phase 3 clinical study evaluating lenadogene nolparvovec in m.11778G>A Leber hereditary optic neuropathy (LHON) subjects. The observed demographics in REFLECT are consistent with previous reports in LHON subjects in the acute and dynamic phases of LHON disease. Combined with the visual function and anatomical parameters obtained in the previous RESCUE and REVERSE trials, REFLECT has provided a uniformly collected data set that should help direct future LHON clinical trials.

## INTRODUCTION

Leber hereditary optic neuropathy (LHON), is a rare, maternally inherited mitochondrial genetic disease that typically causes severe bilateral visual loss from isolated optic neuropathies.[1] Three primary point mutations in the mitochondrial DNA (mtDNA) at nucleotide positions m.3460G>A, m.11778G>A and

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The efficacy and safety of a unilateral intravitreal injection of lenadogene nolparvovec was evaluated in subjects with Leber hereditary optic neuropathy (LHON) carrying the m.11778G>A mutation in two previous phase 3 clinical studies.

### WHAT THIS STUDY ADDS

⇒ The REFLECT trial is the only study to date evaluating the efficacy and safety of bilateral intravitreal injection of lenadogene nolparvovec in LHON subjects with the m.11778G>A mutation. REFLECT provides a large, homogeneous cross-sectional data set of visual function and retinal anatomic measurements collected during the acute and dynamic phases of the disease.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of REFLECT when combined with RESCUE and REVERSE provide important information on the inferred natural history of LHON that should help guide future research in subjects with the m.11778G>A mutation.

m.14484T>C, occurring, respectively, in the *ND1, ND4* and *ND6* genes, have been identified as causative of LHON in approximately 90% of subjects. The primary mutations are necessary, but not sufficient, to cause vision loss, with approximately 20%–50% of male and 4%–10% of female carriers expressing the clinical disease, resulting in a male predominance of more than 80% in most pedigrees.[1] Subjects are usually affected between the ages of 15 and 35 years, but symptomatic LHON has been reported in molecularly confirmed individuals as young as age 2 to as old as age 87.[2–4] LHON classically manifests with acute to subacute, bilateral, painless central vision loss, with dyschromatopsia, central
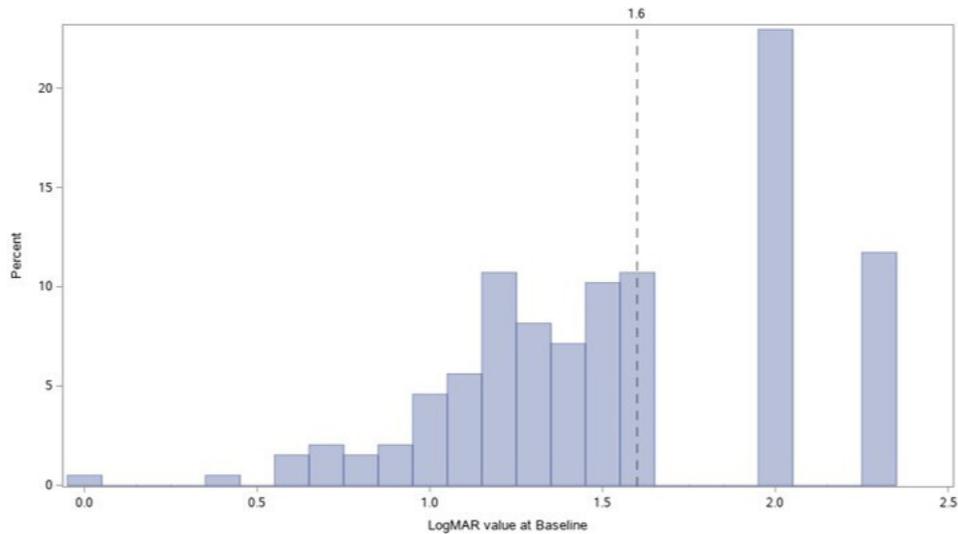
**Figure 1** Histogram of best-corrected visual acuity values in logMAR at baseline (n=196 eyes). logMAR, logarithm of the minimal angle of resolution.

or cecocentral visual field defects, and often swollen-appearing hyperaemic optic discs.[5]

The REFLECT study (ClinicalTrials.gov NCT03293524) is a phase 3, international, multicentre, randomised, double-masked, placebo-controlled, clinical trial of *ND4*-LHON patients with vision loss ≤1 year in one or both eyes assessing the effect of bilateral intravitreal (IVT)

injection of the gene therapy lenadogene nolparvovec. Lenadogene nolparvovec has been investigated previously in four clinical studies in 91 patients affected by vision loss from *ND4*-LHON: the REVEAL study, a phase 1/2a dose escalation open label study,[6] and the RESCUE and REVERSE phase 3 randomised, sham-controlled pivotal studies.[7-9] Patients who completed the RESCUE and
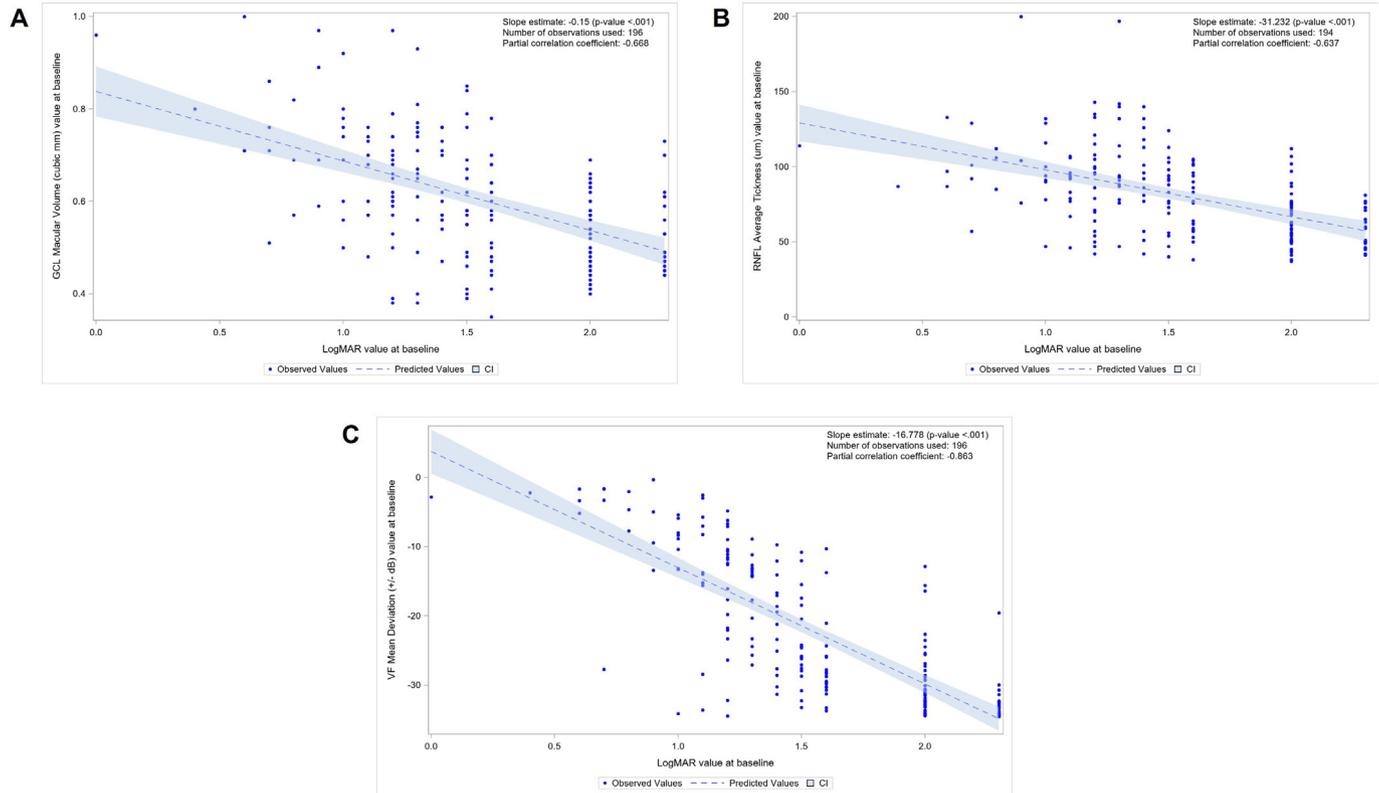


**Figure 2** (A) Regression analysis between ganglion cell layer (GCL) macular volume and best-corrected visual acuity (BCVA) in logMAR at baseline (n=196 eyes). (B) Regression analysis between average retinal nerve fibre layer (RNFL) thickness and BCVA in logMAR at baseline (n=196 eyes). (C) Regression analysis between humphrey visual field (HVF) mean deviation and BCVA in logMAR at baseline (n=196 eyes). logMAR, logarithm of the minimal angle of resolution.
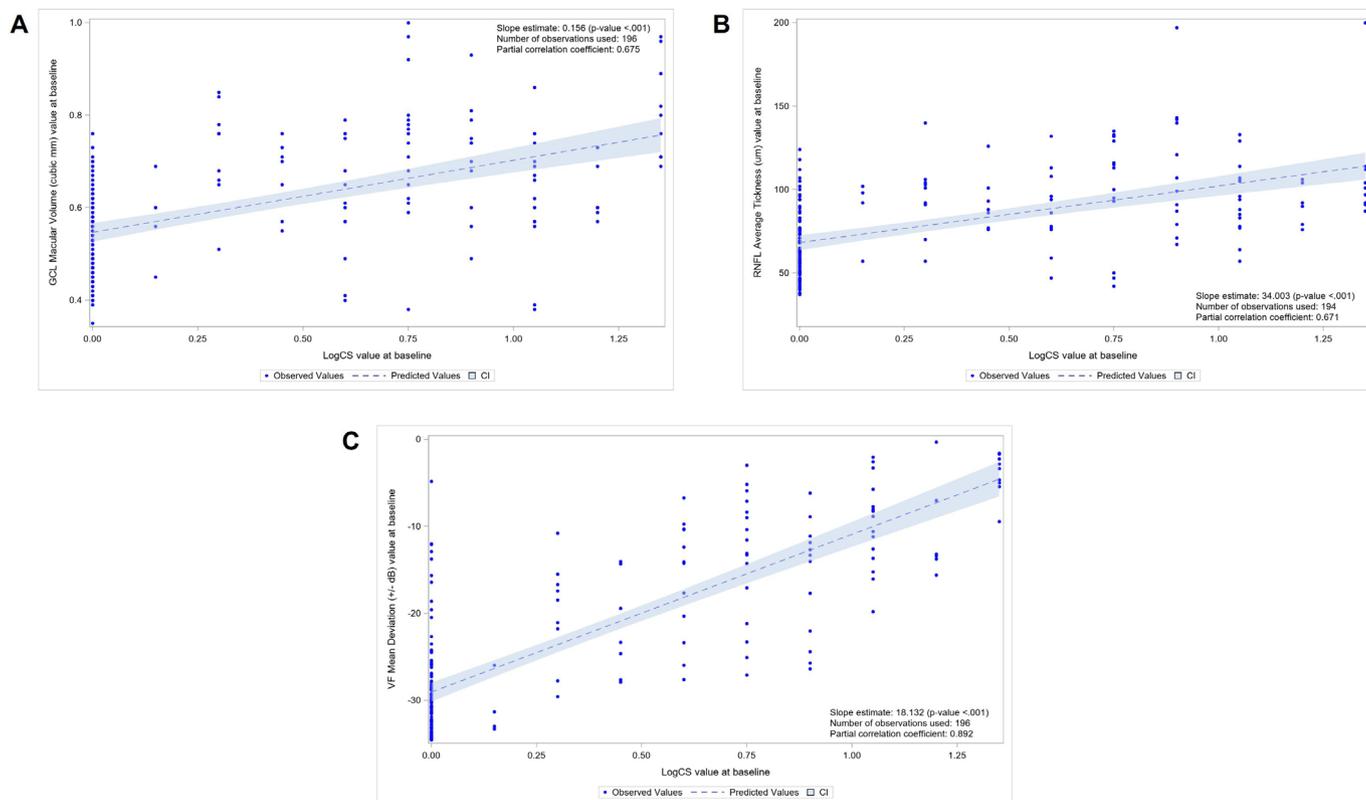
**Figure 3** (A) Regression analysis between ganglion cell layer (GCL) macular volume and logarithm of contrast sensitivity (LogCS) at baseline (n=196 eyes). (B) Regression analysis between average retinal nerve fibre layer (RNFL) thickness and LogCS at baseline (n=196 eyes). (C) Regression analysis between humphrey visual field (HVF) mean deviation and LogCS at baseline (n=196 eyes).

REVERSE studies are currently followed in an ongoing extension study, RESTORE.[10] The baseline characteristics of the 76 *ND4*-LHON patients from the RESCUE and REVERSE clinical trials presenting within 1 year of vision loss were recently published.[11] This report describes the trial design of REFLECT and provides a cross-sectional analysis of the baseline characteristics of visual function and structural parameters in the 98 enrolled *ND4*-LHON subjects.

## METHODS
### Study rationale
Lenadogene nolparvovec was investigated in four clinical studies (REVEAL, RESCUE, REVERSE, RESTORE) where it was exclusively administered as a unilateral IVT injection. However, LHON is a bilateral disease that typically affects both eyes of a patient in a sequential manner. The REFLECT study was therefore constructed to assess the efficacy and safety of bilateral IVT of lenadogene nolparvovec in *MT-ND4* LHON subjects.

### Study design
REFLECT (ClinicalTrials.gov NCT03293524) is a phase 3, international, multicentre, randomised, double-masked (for the primary analysis up to 1.5 years post-treatment), placebo-controlled, clinical trial conducted in 13 centres located across 7 countries (Belgium, France, Italy, Spain,

Taiwan, United Kingdom (1 centre per country) and USA (7 centres)). Eligible subjects, LHON patients with vision loss ≤1 year in one or both eyes caused by the m.11778G>A mutation in the *ND4* gene, were randomised into one of two treatment arms, with all patients receiving an IVT of lenadogene nolparvovec in their first affected eye and the second-affected eye randomised to receive IVT of either lenadogene nolparvovec or placebo (online supplemental figure 1). The primary efficacy analysis is the comparison of the change from baseline in best-corrected visual acuity (BCVA) reported at 1.5 years post-treatment between the second affected/not-yet-affected eyes receiving lenadogene nolparvovec and placebo.

The study was conducted in accordance with the principles and requirements of the International Conference on Harmonisation Good Clinical Practice and adhered to the ethical principles outlined in the Declaration of Helsinki. An independent Data Safety Monitoring Board periodically reviews study data to ensure the continued safe conduct of the trial and protection of subjects.

### Patient and public involvement
Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.
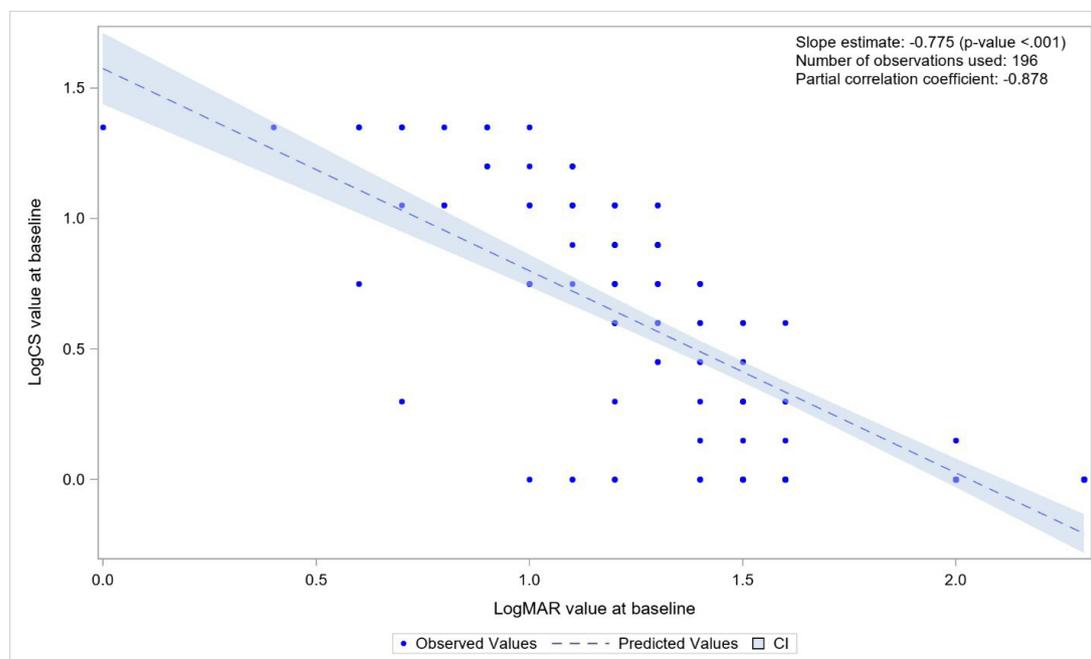
**Figure 4** Regression analysis between logarithm of contrast sensitivity (LogCS) and best-corrected visual acuity (BCVA) in logMAR at baseline (n=196 eyes). LogMAR, logarithm of the minimal angle of resolution.

### Study population

The planned number of subjects to be included in the study was 90 overall (45 in each treatment arm) based on a treatment effect of 0.3 logarithm of the minimal angle of resolution (LogMAR) in the change from baseline to 1.5 years in BCVA, an SD of 0.50 and a power of 80% (alpha=0.05, two sided).

### Inclusion and exclusion criteria

To be eligible for the REFLECT study, subjects needed to be 15 years of age or older with documented vision loss, to any extent, in at least one eye, documentation of the m.11778G>A mutation, duration of vision loss ≤365 days and a visual acuity of at least hand motion (HM) in each affected eye (online supplemental table 1). Patients using idebenone at screening were required to discontinue this treatment at least 7 days prior to inclusion. Exclusion criteria included contraindications to IVT, previous treatment with an investigational medicinal drug, ocular surgery within 90 days prior or IVT within 30 days prior, or history of recurrent uveitis or active ocular inflammation, and other causes of visual loss (eg, other optic neuropathies, macular disease) not attributable to LHON.

### Assessments at baseline

Demographic characteristics were collected before treatment, along with visual function and anatomic parameters. Ophthalmologic assessments included BCVA, slit-lamp biomicroscopy, Goldmann applanation tonometry, funduscopy, contrast sensitivity (CS), automated perimetry, spectral-domain optical coherence tomography (SD-OCT) and colour fundus photography.

The BCVA was assessed using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts at 1 or 4 m. Subjects who could not read at least three letters on a single line on the ETDRS chart at 1 m were tested for their ability to count the assessor's fingers (CF), detect HM, and perceive light (light perception (LP)/no LP (NLP)). All BCVAs were expressed in LogMAR according to a standard conversion scale for on-chart values. Off-chart BCVA were assigned LogMAR values of +2.0 and +2.3, respectively, for CF and HM eyes, according to the validated Lange scale,[12] and +4.0 and +4.5, respectively, for LP and NLP eyes.

CS was assessed with the Pelli-Robson Low Vision Contrast Sensitivity chart and expressed as a logarithm (LogCS).[13] The Contrast Sensitivity score was defined as the last contrast triplet for which at least two of the three letters were read correctly. On-chart eyes were those able to read at least two letters of the first triplet on the chart and had a CS value expressed in logarithm (LogCS). Off-chart eyes were those unable to read at least two letters of the first triplet on the chart and were assigned a LogCS of 0 (worst possible score).

Standard, automated visual field assessment was obtained with an Humphrey Visual Field (HVF) Analyzer II (Carl Zeiss Meditec) using the 30–2 SITA Fast strategy. The HVF test was repeated if considered unreliable (ie, fixation losses ≥15%, false-positive errors ≥20% or false-negative errors ≥33%). The following parameters were collected: mean deviation (MD), pattern SD (PSD) and foveal threshold sensitivity (FT). If HVF foveal threshold was off then it was set to missing, and if the measured foveal threshold was <0, it was set to 0.

**Table 1** Comparison of baseline characteristics of visual function and retinal anatomy in the rescue, reflect and reverse studies

| | Rescue | Reflect | Reverse |
|---|---|---|---|
| N subjects | 39 | 98 | 37 |
| N males (%) | 32 (82) | 78 (79.6) | 29 (78.4) |
| Age (years) at screening | | | |
| Mean (SD) | 36.3 (16.5) | 32.1 (13.8) | 34.2 (15.2) |
| Min; max | 15; 69 | 15; 74 | 15; 67 |
| Duration of vision loss (days) | | | |
| N eyes | 73 | 196 | 74 |
| Mean (SD) | 112.1 (42.8) | 222.8 (103.3) | 271.0 (59.5) |
| Min; max | 24; 179 | 0; 363 | 181; 364 |
| N eyes | 78 | 196 | 74 |
| BCVA (LogMAR) | | | |
| Mean (SD) | 1.29 (0.56) | 1.55 (0.47) | 1.61 (0.46) |
| Min; max | −0.20; 2.51 | 0 ; 2.3 | 0.70; 3.17 |
| Contrast sensitivity (LogCS) | | | |
| Mean (SD) | 0.62 (0.54) | 0.37 (0.46) | 0.30 (0.43) |
| Min; max | 0; 1.65 | 0; 1.35 | 0; 1.50 |
| GCL macular volume (mm$^3$)* | | | |
| Mean (SD) | 0.73 (0.17) | 0.62 (0.1) | 0.53 (0.066) |
| Min; max | 0.50; 1.28 | 0.35; 1.12 | 0.42; 0.72 |
| PMB RNFL thickness (µm)* | | | |
| Mean (SD) | 34.8 (14.4) | 30.30 (13.8)† | 23.4 (6.7) |
| Min; max | 21.0; 123.0 | 10.0; 126.0 | 1.5; 39.5 |
| RNFL temporal quadrant (µm)* | | | |
| Mean (SD) | 49.6 (20.9) | 39.66 (23.0)† | 28.2 (8.0) |
| Min; max | 24.5; 147.5 | 16.0; 183.0 | 13.5; 62.5 |
| Average RNFL thickness (µm)* | | | |
| Mean (SD) | 99.1 (18.2) | 82.16 (28.98)† | 69.7 (18.1) |
| Min; max | 47.0; 162.0 | 37; 200 | 39.5; 116.5 |
| ETDRS total macular volume (mm$^3$)* | | | |
| Mean (SD) | 8.40 (0.47) | 8.00 (0.5) | 7.83 (0.36) |
| Min; max | 7.54; 9.58 | 6.2; 9.5 | 6.54; 8.75 |

*Average of screening and baseline values.
†A total of 194 eyes (missing data for 2 eyes).
BCVA, best-corrected visual acuity; ETDRS, Early-Treatment Diabetic Retinopathy Study; GCL, ganglion cell layer; LogCS, logarithm of contrast sensitivity; LogMAR, logarithm of the minimal angle of resolution; PMB, papillomacular bundle; RNFL, retinal nerve fibre layer.

SD-OCT was performed using a Spectralis OCT (Heidelberg Engineering). Ganglion cell layer (GCL), temporal quadrant retinal nerve fibre layer (RNFL) thickness, papillomacular bundle RNFL thickness and average RNFL thickness were measured for the optic nerve and posterior pole as per standard protocols included in the Spectralis software. The OCT assessments were performed using triplicate scans of high quality (Q values >20). Borders of the retinal layers were manually adjusted when automated segmentation errors were detected.

BCVA, HVFs and SD-OCT exams were centrally reviewed, quality checked and graded by a central ophthalmology reading centre (William H. Annesley Jr. EyeBrain Centre (AEBC), Thomas Jefferson University). The AEBC was masked for all analyses.

### Statistical analyses

Statistical analyses were carried out using SAS, software V.9.4 (SAS Institute). Summary statistics for continuous variables were described using N, mean, SD and range. The eyes with no vision loss were considered to have a

duration of vision loss equal to 0. The predefined baseline for visual function (BCVA, CS, HVF) and anatomic parameters (OCT) was the last available assessment before treatment; for anatomic metrics, the average of values measured at screening and inclusion visits was also calculated.

Regressions between baseline parameters were performed with an analysis of covariance (ANCOVA) model which includes terms treatment as a fixed effect and with repeated values for eye. The partial correlation coefficient was performed to quantify the impact of one variable on a second variable. From the ANCOVA model the correlation is calculated as:

$$\widehat{\rho}ICC = \frac{z/\sqrt{m}}{\sqrt{(1+\frac{2}{m})}} \text{ where } z = \widehat{\beta}/\sqrt{\widehat{var(\beta)}} \text{ for m subjects. } \widehat{\beta} \text{ and }$$

$\widehat{var}(\widehat{\beta})$ are the model estimates and variances.[14]

## RESULTS
### Disposition
A total of 108 patients were screened and 98 were included, randomised, and treated from March 2018 to June 2019.

### Demographic characteristics
Of the 98 included *ND4*-LHON patients, 78 (79.6%) were male and the average age at disease onset was 31.5 years (range 14–73 years) (online supplemental table 2). Ten subjects (10.2%) were between 15 and 18 years of age, and 5 subjects (5.1%) were at least 60 years old. There were 15 subjects (15.3%) from Asia and the remaining subjects were from either the USA (56 subjects, 57.1%) or Europe (27 subjects, 27.5%). Half of patients reported alcohol consumption and approximately one in five subjects were current smokers. Prior idebenone treatment was reported by 14 subjects (14.3%), and all discontinued the treatment at least 7 days prior to inclusion, consistent with protocol requirements.

### Disease characteristics
Duration of vision loss at baseline averaged 8.3 months (range of 1.7–11.9), consistent with inclusion criteria requiring vision loss ≤1 year. Among the 98 patients, one subject had unilateral disease (only one eye affected) at the time of treatment with the fellow eye having a BCVA of 20/20 at baseline (online supplemental table 3).

The mean BCVA of the first-affected eyes was worse than that of the second affected eyes (1.64 LogMAR vs 1.47 LogMAR) (online supplemental table 2). More than one third of eyes were off-chart at baseline (68 eyes; 34.7%) and 25 subjects had both eyes off-chart. The distribution of BCVAs for all eyes is provided in figure 1.

A total of 89 eyes (45.4%) were off-chart for CS at enrolment (these eyes could not correctly read 2 letters at the maximum contrast possible on the Pelli-Robson chart). The mean Log CS was 0.37, with a range from 0 to 1.35 LogCS; off-chart eyes were scored 0 for LogCS (online supplemental table 2).

The mean (SD) MD and PSD for HVF 30–2 testing in all eyes were −22.31 (10.3) dB and 7.02 (3.5) dB, respectively.

LogMAR and HVF MD showed no significant correlation with tobacco and alcohol consumption (online supplemental tables 4 and 5).

Retinal anatomy analyses revealed that the average values of retinal layers of interest were consistently thinner for the first affected eyes when compared with the second affected eyes, with both differing significantly from population norms (online supplemental table 2).

### Association between baseline parameters
At baseline, a correlation was observed between the GCL macular volume and the BCVA: the less affected BCVAs were associated with larger GCL macular volumes (correlation coefficient; r=−0.668) (figure 2). A similar relationship was observed between the average RNFL thickness and the BCVA (r=−0.637) (figure 2).

As observed for the OCT values, the baseline visual field measurements, assessed by the HVF MD, were related to the baseline BCVA: the less affected BCVAs were correlated with less severe MD (r=−0.863) (figure 2).

Similarly, better LogCS values were associated with lesser reductions in GCL macular volume (r=0.675), average RNFL thickness (r=0.671) and HVF MD (r=0.892) (figure 3).

There was also a correlation between LogMAR and LogCS values at baseline (r=−0.878) (figure 4).

## DISCUSSION
We present a large, homogeneous dataset of visual function and retinal anatomic measurements collected during the first year following visual loss in LHON subjects carrying the m.11778G>A mutation in the *ND4* gene. These results provide detailed information on the first two clinical stages of the disease (subacute (<6 months from onset) and dynamic (6–12 months)) prior to onset of the chronic stage.[15] To our knowledge, the REFLECT population is the largest *ND4*-LHON cohort studied in a randomised, double masked, controlled study, providing meaningful information on the inferred natural history of disease, using cross-sectional analysis on 98 patients (196 eyes). The REFLECT demographic characteristics are aligned with the typical *ND4*-LHON population, as described in the literature.[16–18] Our results confirm the male predominance (80%) and a preponderance of young adults (median age: 28.0 years), recognising that our study only included patients at least 15 years of age. Visual impairment of the second eye occurred quite rapidly, on average approximately 2 months after the first eye. Consistently, the first affected eyes, with a longer duration of vision loss at baseline, had worse visual function and anatomic parameters than the more recently second affected eyes. The difference in HVF parameters was less striking between the first and

second affected eyes but was still present. There was no discernible influence of smoking or alcohol consumption on baseline BCVA or HVF MD.

There was a correlation between anatomic parameters and BCVA at baseline using the cross-sectional analyses over the 1-year postvision loss . In addition, the correlation was even stronger between visual function parameters: HVF and BCVA . The same magnitude of correlation of OCT parameters and HVF MD with LogCS was observed, with again a stronger correlation with HVF MD. Furthermore, a correlation between BCVA and LogCS was present, as both parameters showed a comparable magnitude of correlation with OCT parameters and HVF MD .

RESCUE and REVERSE (ClinicalTrial.gov NCT02652767and NCT026527080)[7] [8] were two randomised, double masked, sham-controlled, multi-centre, international clinical trials assessing the benefit of a single IVT of lenadogene nolparvovec in 76 patients. In RESCUE, one or both eyes could be affected by vision loss provided the duration of vision loss was ≤6 months in the first (or only) affected eye at screening. In REVERSE, both eyes had to be affected by vision loss for 6–12 months at time of screening. To be included in either study, LHON subjects had to harbour the m.11778G>A mutation, be at least 15 years old at enrolment, and have vision of CF or better in both eyes. Baseline data analyses of these two studies were recently published.[11] The REFLECT demographic characteristics are comparable to the REVERSE and RESCUE populations' characteristics (table 1). The mean time for inclusion and treatment for RESCUE, REFLECT and REVERSE subjects was 112, 223 and 271 days, respectively, in alignment with the inclusion criteria of each trial. As expected, the difference in duration of vision loss was associated with a consistent gradient of impairment of visual function and anatomic parameters at baseline across the three clinical studies populations (worse in REVERSE, intermediate in REFLECT, better in RESCUE) (table 1). In addition, the one REFLECT subject with unilateral vision loss at baseline had similar structural and functional characteristics to four patients with unilateral vision loss in RESCUE (online supplemental table 6).

The limitations of our analyses are related to the cross-sectional nature of the data, as subjects naive to LHON treatment were subsequently administered gene therapy and not followed longitudinally as untreated individuals. However, the data assembled from the REFLECT study provide a valuable landscape of the clinical characteristics of LHON patients harbouring the m.11778G>A mutation in *MT-ND4* within 1 year of the onset of vision loss. In addition, when combined with the baseline data from the RESCUE and REVERSE trials, these data improve our understanding of the subacute and dynamic phases of vision loss from *ND4*-LHON and provide uniformly collected anatomic and functional information to guide future interventions.

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**Supplemental Table 1: Main Inclusion and Exclusion Criteria**

**Inclusion criteria**

- Age 15 years or older

- Clinically manifested vision loss in at least one eye, due to *MT-ND4* LHON

- Documented results of genotyping showing the presence of the m.11778G>A mutation in the *ND4* gene and absence of other primary LHON-associated mutations (m.3460G>A or m.14484T>C)

- Duration of vision loss ≤1 year in each affected eye

- At least Hand Motion visual acuity in each eye

- Negative test for human immunodeficiency virus infection

- Negative pregnancy test for women of childbearing potential

- Female subjects (if of childbearing potential) had to agree to use effective methods of birth control and male subjects had to agree to use condoms, for up to 6 months after treatment administration

- Discontinuation of idebenone treatment at least 7 days before inclusion. Concomitant treatment with idebenone was not authorized during the study

**Exclusion criteria**

- Any known allergy or hypersensitivity to lenadogene nolparvovec

- Contraindication to IVT in any eye

- Previous treatment with an investigational medicinal drug (except idebenone if discontinued at least 7 days before inclusion)

- Ocular surgery of clinical relevance (per investigator assessment) within 90 days preceding the screening

- IVT performed in any eye within 30 days prior screening

- Disorders of the eye or adnexa other than LHON which may interfere with ophthalmologic assessments (including SD-OCT) during the study

- Mutations other than m.11778G>A in the *ND4* gene known to cause pathology of the optic nerve, retina or afferent visual system

- Systemic or ophthalmologic disorders other than LHON known to be associated with visual dysfunction or for which associated treatment(s) may cause visual dysfunction

- History of recurrent uveitis or active ocular inflammation

- Presence of alcoholism or drug abuse (excluding nicotine)

IVT, intravitreal; LHON, Leber Hereditary Optic Neuropathy; SD-OCT, Spectral-Domain Optical Coherence Tomography

**Supplemental Table 2: Baseline Characteristics of REFLECT Patients**

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| | N=98 subjects (196 eyes) | |
| --- | --- | --- |
| | Mean (SD) or n (%) | Range |
| **DEMOGRAPHIC CHARACTERISTICS** | | |
| Gender, Male, n | 78 (79.6%) | |
| Age at onset of the disease (years) | 31.5 (13.8) | 14; 73 |
| Age group at onset of the disease, n | | |
| <15 years | 4 (4.1%) | |
| 15-18 years | 9 (9.2%) | |
| 18-60 years | 80 (81.6%) | |
| ≥60 years | 5 (5.1%) | |
| Age at screening (years) | 32.1 (13.8) | 15; 74 |
| Age group at screening, n | | |
| 15 – 18 years | 10 (10.2%) | |
| 18 – 60 years | 83 (84.7%) | |
| ≥60 years | 5 (5.1%) | |
| Study region (location of study sites), n | | |
| Asia | 15 (15.3%) | |
| Europe | 27 (27.6%) | |
| United States | 56 (57.1%) | |
| Current tobacco smoking, n | 21 (21.4%) | |
| Current alcohol consumption, n | 48 (49.0%) | |
| Blood Pressure | | |
| Systolic Blood pressure (mm Hg) | 127.7 (14.61) | 92, 166 |
| Diastolic Blood Pressure (mm Hg) | 80.1 (10.83) | 54, 110 |
| Medical history, n[1] | | |
| Anxiety | 15 (15.3%) | |
| Hypertension | 14 (14.3%) | |
| Cataract | 13 (13.3%) | |
| **DISEASE CHARACTERISTICS** | | |
| Duration of vision loss at baseline (months) | 8.30 (3.2) | 1.7; 11.9 |
| Inter-eye delay of vision loss (months) | 1.95 (1.98) | 0; 8.7 |
| Simultaneous vision loss of both eyes | 24 (24.49%) | |
| Bilateral vision loss at baseline[2], n | 97 (99.0%) | |
| Prior idebenone treatment[3], n | 14 (14.3%) | |
| **Best Corrected Visual acuity (BCVA)** | | |

| | | |
|---|---|---|
| BCVA of all eyes | 1.55 (0.47) | 0; 2.30 |
| BCVA of first-affected eye (LogMAR) | 1.64 (0.45) | 0.60; 2.30 |
| BCVA of second/not-yet-affected eye (LogMAR) | 1.47 (0.48) | 0.00; 2.30 |
| Better seeing-eye, n | | |
| First affected eye | 27 (27.6%) | |
| Second/not-yet-affected eye | 71 (72.4%) | |
| Eyes off-chart at baseline (BCVA >1.6 LogMAR) | | |
| First-affected eye | 40 (40.8%) | |
| Second/not-yet-affected eye | 28 (28.6%) | |
| Both eyes off-chart | 25 (25.5%) | |
| **Intraocular Pressure (IOP)** | 14.92 (2.9) | 6; 22 |
| **Contrast sensitivity (LogCS)** | | |
| LogCS of all eyes | 0.37 (0.46) | 0; 1.35 |
| LogCS of first affected eye | 0.31 | 0; 1.35 |
| LogCS of second/not-yet-affected affected eye | 0.44 | 0; 1.35 |
| **Humphrey Visual Field** | | |
| HVF mean deviation (MD) (dB) | | |
| HVF MD of all eyes (dB) | -22.31 (10.3) | -34.6; -0.3 |
| HVF MD of first affected eye (dB) | -23.25 | -34.6; -0.3 |
| HVF MD of second affected eye (dB) | -21.37 | -34.5; -1.6 |
| HVF pattern standard deviation (PSD) (dB) | | |
| HVF PSD of all eyes (dB) | 7.02 (3.5) | 1.4; 14.0 |
| HVF PSD of first affected eye (dB) | 6.91 | 1.5; 14.0 |
| HVF PSD of second affected eye (dB) | 7.13 | 1.4; 13.6 |
| HVF foveal threshold (FT) (dB)[4] | | |
| HVF FT of all eyes (dB) | 6.05 (10.1) | 0; 43.0 |
| HVF FT of first affected eye (dB) | 5.86 | 0; 36.0 |
| HVF FT of second affected eye (dB) | 6.24 | 0; 43.0 |
| **STRUCTURAL PARAMETERS (SD-OCT)[5]** | | |
| GCL macular volume ($mm^3$) | | |
| GCL macular volume of all eyes ($mm^3$) | 0.60 (0.1) | 0.35; 1.00 |
| GCL macular volume of first affected eye ($mm^3$) | 0.58 | 0.38; 0.93 |
| GCL macular volume of second affected eye ($mm^3$) | 0.63 | 0.35; 1.00 |
| Papillo-Macular Bundle (PMB) RNFL thickness ($\mu m$) | | |
| PMB RNFL thickness of all eyes ($\mu m$)[6] | 29.5 (13.4) | 10.0; 126.0 |
| PMB RNFL thickness of first affected eyes ($\mu m$) | 28.17 | 10.0; 126.0 |

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| | | |
|---|---|---|
| PMB RNFL thickness of second affected eyes (µm) | 30.77 | 10.0; 101.0 |
| Temporal Quadrant RNFL thickness (µm)[6] | | |
| Temporal Quadrant RNFL thickness of all eyes (µm) | 38.38 (23.0) | 19.0; 183.0 |
| Temporal Quadrant RNFL thickness of first affected eyes (µm) | 34.99 | 19.0; 183.0 |
| Temporal Quadrant RNFL thickness of second affected eyes (µm) | 41.69 | 19.0; 162.0 |
| Average RNFL thickness (µm)[6] | | |
| Average RNFL thickness of all eyes (µm) | 80.85 (28.97) | 37; 200 |
| Average RNFL thickness of first affected eyes (µm) | 76.09 | 38; 197 |
| Average RNFL thickness of second affected eyes (µm) | 75.50 | 37; 200 |
| ETDRS macular volume (mm³) | | |
| ETDRS macular volume of all eyes (mm³) | 7.91 (0.5) | 6.8; 9.5 |
| ETDRS macular volume of first affected eyes (mm³) | 7.91 | 6.8; 9.5 |
| ETDRS macular volume of second affected eyes (mm³) | 8.03 | 6.2; 9.5 |

Data presented are mean (SD) and range or counts (%) where appropriate.

[1] Medical history occurring in more than 10% of subjects

[2] One subject was unilaterally affected (only one eye with drop of vision) at baseline

[3] Use of idebenone within 30 days prior to intravitreal injection of the study treatment and discontinued prior to study treatment. Idebenone had to be discontinued at least 7 days prior to inclusion and was prohibited during the study.

[4] 186 eyes (missing data for 5 eyes)

[5] Average of screening and baseline values

[6] 194 eyes (missing data for 2 eyes)

Better-seeing eye: The better-seeing and worse-seeing eye of each subject were determined based on the baseline vision testing performed at inclusion. A pre-defined algorithm for determining the better- and worse-seeing eyes was utilized as follows: 1) LogMAR BCVA (Criterion 1): The eye with the better (i.e., lower) LogMAR BCVA was the better-seeing eye. If both eyes had an equal LogMAR acuity, the second criterion was utilized; 2) SD-OCT parameters (Criterion 2; used if there was no inter-eye difference based on Criterion 1); 3) Log of Contrast Sensitivity (LogCS) (Criterion 3; used if there was no inter-eye difference in criteria 1 or 2). The eye with the better LogCS score was the better-seeing eye; 4) If the better-seeing eye could not be determined based on criteria 1 through 3, the selection of the better-seeing eye was based on the subject's opinion.

BCVA, Best Corrected Visual Acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; GCL, Ganglion Cell Layer; HVF, Humphrey Visual Field; LogCS, Logarithm of Contrast Sensitivity; LogMAR, Logarithm of the Minimal Angle of Resolution; n, number of subjects; RNFL, Retinal Nerve Fiber Layer; SD, Standard Deviation; SD-OCT, Spectral-Domain Optical Coherence Tomography.

**Supplemental Table 3. Individual data of a female patient aged between 35 and 40 years with no vision loss in one eye at baseline. The duration of vision loss in the affected eye (left) was 70 days.**

| | | Period | Affected eye | Unaffected eye |
|---|---|---|---|---|
| **BCVA (LogMAR) (Snellen)** | | Screening | 0.7 20/100 | 0 20/20 |
| | | Baseline | 0.7 20/100 | 0 20/20 |
| **CS (LogCS)** | | Screening | 1.20 | 1.65 |
| | | Baseline | 1.05 | 1.35 |
| **HVF (dB)** | **MD** | Screening | -2.64 | -4.05 |
| | | Baseline | -3.28 | -2.82 |
| | **PSD** | Screening | 1.86 | 2.44 |
| | | Baseline | 4.17 | 1.59 |
| **SD-OCT** | **GCL Macular Volume (mm³)** | Screening | 0.84 | 0.96 |
| | | Baseline | 0.86 | 0.96 |
| | **PMB RNFL Thickness (µm)** | Screening | 53 | 63 |
| | | Baseline | 88 | 68 |
| | **RNFL Quadrant Temporal (µm)** | Screening | 82 | 87 |
| | | Baseline | 111 | 90 |
| | **RNFL Average Thickness (µm)** | Screening | 120 | 112 |
| | | Baseline | 129 | 114 |
| | **ETDRS Total Macular Volume (mm³)** | Screening | 9.49 | 9.24 |
| | | Baseline | 9.47 | 9.22 |

BCVA, best-corrected visual acuity; CS, contrast sensitivity; ETDRS, Early-Treatment Diabetic Retinopathy Study; GCL, ganglion cell layer; HVF, Humphrey visual field; logMAR, logarithm of the minimal angle of deviation; MD, mean deviation; PMB, papillomacular bundle; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD-OCT, spectral-domain optical coherence tomography.

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**Supplemental Table 4. Logarithm of the Minimal Angle of Resolution (LogMAR) and Humphrey visual field (HVF) Mean Deviation (MD) according to tobacco consumption.**

| | LogMAR | | | |
|---|---|---|---|---|
| **Tobacco** | **Mean** | **SD** | **Min** | **Max** |
| **Current** | 1.59 | 0.41 | 0.40 | 2.00 |
| **Former** | 1.43 | 0.49 | 0 | 2.30 |
| **Never** | 1.61 | 0.48 | 0.60 | 2.30 |
| | HVF MD | | | |
| **Tobacco** | **Mean** | **SD** | **Min** | **Max** |
| **Current** | -23.26 | 9.08 | -33.96 | -2.22 |
| **Former** | -18.71 | 10.27 | -33.40 | -2.06 |
| **Never** | -23.97 | 10.39 | -34.55 | -0.31 |

SD, standard deviation

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**Supplemental Table 5. Logarithm of the Minimal Angle of Resolution (LogMAR) and Humphrey visual field (HVF) Mean Deviation (MD) according to alcohol consumption.**

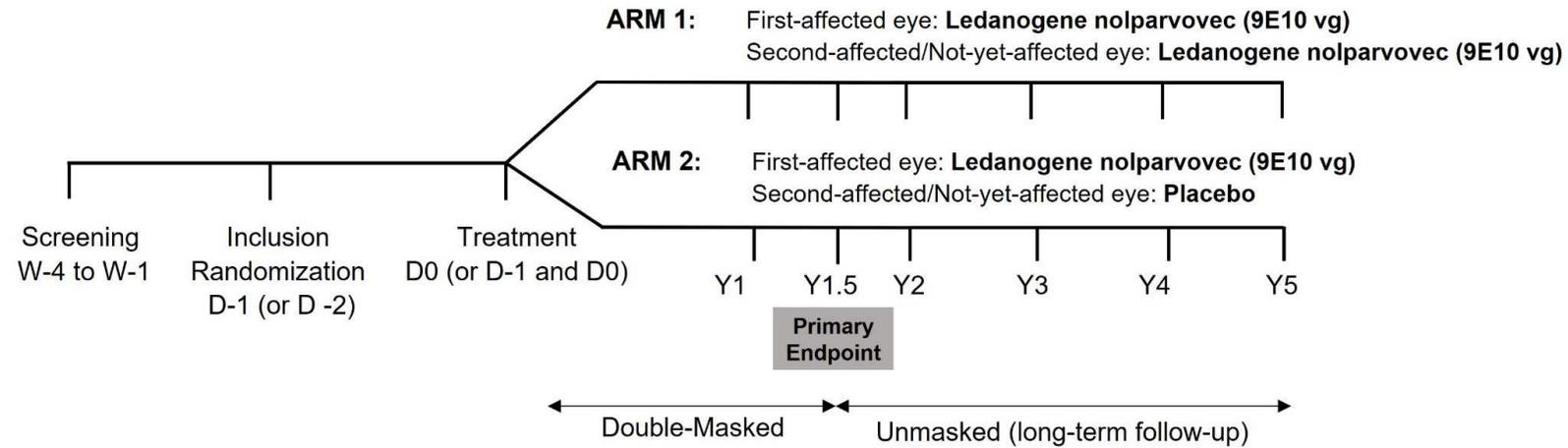| | LogMAR | | | |
|---|---|---|---|---|
| **Alcohol** | **Mean** | **SD** | **Min** | **Max** |
| **Current** | 1.51 | 0.45 | 0 | 2.30 |
| **Former** | 1.54 | 0.48 | 0.40 | 2.30 |
| **Never** | 1.63 | 0.50 | 0.60 | 2.30 |
| | HVF MD | | | |
| **Alcohol** | **Mean** | **SD** | **Min** | **Max** |
| **Current** | -21.24 | 10.03 | -34.15 | -2.06 |
| **Former** | -20.87 | 10.90 | -34.55 | -0.31 |
| **Never** | -25.14 | 9.89 | -34.48 | -1.65 |

SD, standard deviation

**Supplemental Table 6. Individual data of all 5 patients in RESCUE and REFLECT studies with one eye with no vision loss at baseline**

| | | | RESCUE | | | | REFLECT |
|---|---|---|---|---|---|---|---|
| | | | Subject #12 | Subject #15 | Subject #22 | Subject #26 | Subject |
| Age range (years) | | | 20-25 | 20-25 | 25-30 | 30-35 | 35-40 |
| Gender | | | F | M | F | M | F |
| Duration of vision loss in *affected* eye (days) | | | 155 | 90 | 57 | 49 | 70 |
| Unaffected eye | | | Left | Right | Left | Right | Right |
| BCVA (LogMAR) (Snellen) | | *Screening* | -0.2 20/13 | -0.3 20/10 | 0.0 20/20 | -0.1 20/16 | 0 20/20 |
| | | *Inclusion* | -0.2 20/13 | -0.2 20/13 | -0.1 20/16 | -0.1 20/16 | 0 20/20 |
| CS (LogCS) | | *Screening* | 1.65 | 1.65 | 1.65 | 1.65 | 1.65 |
| | | *Inclusion* | 1.35 | 1.65 | 1.5 | 1.65 | 1.35 |
| HVF (dB) | MD | *Screening* | NR | NR | NR | -1.14 | -4.05 |
| | | *Inclusion* | NR | -2.4 | -1.9 | NR | -2.82 |
| | PSD | *Screening* | NR | NR | NR | 1.87 | 2.44 |
| | | *Inclusion* | NR | 1.6 | 1.29 | NR | 1.59 |
| SD-OCT | GCL Macular Volume (mm$^3$) | *Screening* | 1.06 | 1.28 | 1.06 | 1.27 | 0.96 |
| | | *Inclusion* | 1.07 | 1.27 | 1.01 | 1.24 | 0.96 |
| | ETDRS Total Macular Volume (mm$^3$) | *Screening* | 8.44 | 9.53 | 8.72 | 9.34 | 9.24 |
| | | *Inclusion* | 8.48 | 9.63 | 8.69 | 9.31 | 9.22 |
| | PMB RNFL Thickness (µm) | *Screening* | 62 | 118 | 66 | 65 | 63 |
| | | *Inclusion* | 64 | 128 | 69 | 69 | 68 |
| | RNFL Quadrant Temporal (µm) | *Screening* | 88 | 141 | 81 | 80 | 87 |
| | | *Inclusion* | 88 | 154 | 85 | 85 | 90 |
| | RNFL Average Thickness (µm) | *Screening* | 107 | 143 | 95 | 118 | 112 |
| | | *Inclusion* | 108 | 149 | 97 | 121 | 114 |

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BCVA, best-corrected visual acuity; CS, contrast sensitivity; ETDRS, Early-Treatment Diabetic
Retinopathy Study; GCL, ganglion cell layer; HVF, Humphrey visual field; logMAR, logarithm of the
minimal angle of deviation; MD, mean deviation; NR, not reliable; PMB, papillomacular bundle; PSD,
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**Supplemental Figure 1. REFLECT study design.**



Study treatment was administered on the same day (Day 0) with two separate procedures or on two consecutive days (Day -1 and Day 0) at the investigator's discretion. In all cases, the first intravitreal injection had to be performed the day following inclusion. Subjects were allocated 1:1 to the two arms. If vision loss was simultaneous, one eye was randomly designated as first-affected. D, day; vg, vector genomes; W, week; Y, year