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Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial

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SR, JB, JAW, DCC, Z-FY, JW, BPL, KAH, and AMM contributed to trial design. SR, JB, JAW, DCC, JP, SM, DC, KAM, TLK, HR, MD, XZ, ES, WP, CJ, AD, KW, KAH, and AMM contributed to trial implementation. SR, JB, ES, AD, FS, BPL, and AMM referred potential participants to the study. SR, EHS, VBM, JAH, DG, and AMM contributed to surgical delivery of voretigene neparvovec. SR, JB, SM, DC, KAM, JW, JP, TLK, HR, MD, LR, AD, LAG, FPH, LD, XZ, WP, MW, and AMM contributed to patient assessments, data collection, or both. Z-FY, AT, and JWi provided statistical analysis and interpretation of the data. JP and OE provided statistical support. All authors contributed to development of the initial manuscript or provided critical content revisions, and approved the final manuscript.

Declaration of interests

SR's institution has received grants from Spark Therapeutics and he has provided presentations on behalf of Spark Therapeutics. DC and AD have received grants from Spark Therapeutics. JB has received grants from the Foundation Fighting Blindness, the National Institutes of Health, and Spark Therapeutics; non-financial support from the Center for Advanced Retinal and Ocular Therapeutics, University of Pennsylvania, and the FM Kirby Foundation; has a provisional patent pending and a US patent licensed to Spark Therapeutics for which she has waived financial interest; and is a coauthor on a copyrighted visual function questionnaire used in the present study. JAW is an employee of and has equity/options in Spark Therapeutics; has received grants from the National Institutes of Health (specifically for Clinical and Translational Research Centre services); and has a patent pending pertaining to the primary endpoint measure licensed to Spark Therapeutics. DCC is an employee of Spark Therapeutics and has a patent pending pertaining to the primary endpoint measure licensed to Spark Therapeutics. Z-FY, AT, and JWi are employees of Statistics Collaborative, which provides statistical and regulatory consulting to Spark Therapeutics. JP is an employee of Westat, which was contracted by Spark Therapeutics. SM is a clinical coordinator of a study sponsored by Spark Therapeutics, receives salary support from the Center for Cellular and Molecular Therapeutics (CCMT) at The Children's Hospital of Philadelphia, and has a patent pending licensed to Spark Therapeutics. KAM is a clinical coordinator of a study sponsored by Spark Therapeutics and received salary support from CCMT at Children's Hospital of Philadelphia. JWa, TLK, and MD have received salary support from Spark Therapeutics and grants from the Carver Center for Macular Degeneration and Children's Hospital of Philadelphia; TLK has received travel support from Spark Therapeutics. JAH has received consulting fees from Merck and Kalvista, grants from ThromboGenics, and serves on boards for Janssen and Celgene. ES's institution has received grants from Children's Hospital of Philadelphia and Spark Therapeutics. EHS has received grants from Oxford Biomedica. KW is an employee of and has equity/options with Spark Therapeutics and has received grants from the National Institutes of Health. FS has received grants from Regione Campania and serves on boards for Sanofi, Dompe Farmaceutici, and Spark Therapeutics. JFW has a patent and a patent pending, both licensed to Spark Therapeutics. KAH is an employee of Spark Therapeutics and has a patent pending pertaining to the primary endpoint measure. AMM has received grants from Spark Therapeutics, the National Institutes of Health, and the Foundation Fighting Blindness; non-financial support from the Center for Advanced Retinal and Ocular Therapeutics, University of Pennsylvania, and the FM Kirby Foundation; and has a provisional patent pending and a US patent licensed to Spark Therapeutics for which he has waived financial interest. OE, HR, LR, LAG, FPH, LD, XZ, VBM, WP, MW, CJ, DG, and BPL declare no competing interests.

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Summary

Background—Phase 1 studies have shown potential benefit of gene replacement in *RPE65*-mediated inherited retinal dystrophy. This phase 3 study assessed the efficacy and safety of voretigene neparvovec in participants whose inherited retinal dystrophy would otherwise progress to complete blindness.

Methods—In this open-label, randomised, controlled phase 3 trial done at two sites in the USA, individuals aged 3 years or older with, in each eye, best corrected visual acuity of 20/60 or worse, or visual field less than 20 degrees in any meridian, or both, with confirmed genetic diagnosis of biallelic *RPE65* mutations, sufficient viable retina, and ability to perform standardised multi-luminance mobility testing (MLMT) within the luminance range evaluated, were eligible. Participants were randomly assigned (2:1) to intervention or control using a permuted block design, stratified by age (<10 years and ≥10 years) and baseline mobility testing passing level (pass at ≥125 lux *vs* <125 lux). Graders assessing primary outcome were masked to treatment group. Intervention was bilateral, subretinal injection of $1{\cdot}5\times10^{11}$ vector genomes of voretigene neparvovec in 0·3 mL total volume. The primary efficacy endpoint was 1-year change in MLMT performance, measuring functional vision at specified light levels. The intention-to-treat (ITT) and modified ITT populations were included in primary and safety analyses. This trial is registered with ClinicalTrials.gov, number NCT00999609, and enrolment is complete.

Findings—Between Nov 15, 2012, and Nov 21, 2013, 31 individuals were enrolled and randomly assigned to intervention (n=21) or control (n=10). One participant from each group withdrew after consent, before intervention, leaving an mITT population of 20 intervention and

nine control participants. At 1 year, mean bilateral MLMT change score was 1·8 (SD 1·1) light levels in the intervention group versus 0·2 (1·0) in the control group (difference of 1·6, 95% CI 0·72–2·41, p=0·0013). 13 (65%) of 20 intervention participants, but no control participants, passed MLMT at the lowest luminance level tested (1 lux), demonstrating maximum possible improvement. No product-related serious adverse events or deleterious immune responses occurred. Two intervention participants, one with a pre-existing complex seizure disorder and another who experienced oral surgery complications, had serious adverse events unrelated to study participation. Most ocular events were mild in severity.

Interpretation—Voretigene neparvovec gene replacement improved functional vision in *RPE65*-mediated inherited retinal dystrophy previously medically untreatable.

Funding—Spark Therapeutics.

Introduction

Inherited retinal dystrophies are a group of rare blinding conditions caused by mutations in any one of more than 220 different genes.[1] The most common clinical subgroup is retinitis pigmentosa, a disorder characterised by reduced ability to perceive light and progressive loss of visual field. A less common but more severe inherited retinal dystrophy, Leber congenital amaurosis, a retinitis pigmentosa subtype, is further characterised by earlier onset, more rapid progression, and nystagmus. Mutations in any one of at least 19 different genes can cause Leber congenital amaurosis.[2,3]

The *RPE65* gene encodes all-trans retinyl ester isomerase, an enzyme crucial to the retinoid cycle. Biallelic mutations in this gene, which disrupt the visual cyde, can be described as Leber congenital amaurosis type 2, retinitis pigmentosa type 20, early-onset retinal dystrophy, and other clinical labels for severe rod-mediated inherited retinal dystrophies, which all eventually progress to complete blindness.[4–7] Proof of principle of gene augmentation therapy for *RPE65*-mediated conditions was established in canine and murine animal models using a recombinant adeno-associated virus (AAV), showing that the biochemical blockade of the visual cycle due to RPE65 deficiency could be corrected.[8–11] After additional efficacy, safety, and dosing studies in large animals, human clinical trials have further supported the utility of gene therapy for *RPE65*-mediated inherited retinal dystrophy.[12–14]

One of these phase 1 trials,[13,15–17] done at the Children's Hospital of Philadelphia, showed safe and stable improvement in retinal and visual function in all 12 participants. These individuals received unilateral, subretinal injections of AAV2-hRPE65v2 (voretigene neparvovec) in their worse-seeing, non-preferred eye in a dose-escalation study, with doses from $1{\cdot}5\times10^{10}$ to $1{\cdot}5\times10^{11}$ vector genomes (vg).[13,15] Most of these participants showed improved light sensitivity, navigational abilities, or visual acuity. A follow-on study, in which 11 of these 12 participants underwent injection of the contralateral eye at the dose of $1{\cdot}5\times10^{11}$ vg, demonstrated the safety of contralateral eye injection, as well as gains in visual and retinal function in the second eye.[18] This improvement has remained durable over at least 3 years, with observation ongoing.[18,19] In addition, a subset of participants in this study was enrolled in a separate functional magnetic resonance imaging study[20] that showed

increased activation of the visual cortex and evidence of improved function and structure of the visual pathways after intervention. Other *RPE65* gene therapy trials, which were administered to only one eye per individual using different gene constructs, vector formulations, or surgical approaches, have shown improvements in retinal function but variable durability of effect.[21–25]

Over time, patients with untreated *RPE65*-mediated inherited retinal dystrophy lose the ability to detect light of any intensity. Independent navigation becomes severely limited, and vision-dependent activities of daily living are impaired. Currently, no approved pharmacological treatment is available, although phase 1b trials of oral 9-cis-retinyl acetate have shown transient increases in Goldmann visual fields area in some participants.[26,27] Here, we report the design, conduct, and safety and efficacy results of a phase 3 study of sequential, bilateral, subretinal administration of voretigene neparvovec in participants with *RPE65*-mediated inherited retinal dystrophy. To our knowledge, this is the first randomised, controlled, phase 3 study of a gene therapy for a genetic disease.

Methods

Study design and participants

This randomised, controlled, open-label, phase 3 study was done with five surgeons at two sites in the USA (Children's Hospital of Philadelphia, Philadelphia, PA, and University of Iowa, Iowa City, IA). The study protocol and individual institutional informed consent documents were reviewed and approved by The Committees for the Protection of Human Subjects at Children's Hospital of Philadelphia and The University of Iowa Carver School of Medicine.

Participants were recruited to the study through posting on Clinicaltrials.gov, and through discussions with physicians who care for patients with inherited retinal dystrophies. Individuals aged 3 years or older with a confirmed genetic diagnosis of biallelic *RPE65* gene mutations were eligible for enrolment if both eyes had visual acuity of 20/60 or worse or visual field less than 20 degrees in any meridian, or both; they had sufficient viable retinal cells as determined by retinal thickness on spectral domain optical coherence tomography (>100 microns within the posterior pole), fundus photography, and clinical examination; and they were able to perform a standardised multi-luminance mobility test (MLMT) within the luminance range evaluated, but unable to pass the MLMT at 1 lux, the lowest luiminance level tested. Individuals were excluded if they had participated in a previous gene therapy or investigational drug study, used high-dose (>7500 retinol equivalent units [or >3300 IU] per day of vitamin A) retinoid compounds in the past 18 months, had intraocular surgery in the past 6 months, had known sensitivity to medications planned for use in the peri-operative period, or had ocular or systemic conditions that would interfere with study interpretation. Women who were pregnant or any participants unwilling to use effective contraception for 4 months after vector administration were also ineligible. Figure 1 summarises the trial design and endpoints.

All participants provided consent or parental permission and assent was obtained, as applicable. An independent data safety and monitoring board oversaw the study and ensured

data integrity. Monitoring and data management were done by an independent party (Westat, Rockville, MD, USA), as were statistical analyses (Statistics Collaborative, Washington, DC, USA). A study audit plan, led by an independent party (ClinAudits, Kinnelon, NJ, USA), provided additional study oversight.

Randomisation and masking

A randomisation list was generated under the direction of the independent party biostatistician such that each enrolled participant would be assigned to either intervention or control. This list was created before enrollment began and contains the entire sequence of random assignments used serially during the study. Within each age group (<10 years and ≥10 years) and baseline mobility testing passing level (pass at ≥125 lux *vs* <125 lux), the randomisation block size was 3 with 2:1 assignments of intervention to control. Within each stratum, randomised blocks (block size of 3) governed the allocation to treatment group (see appendix for further detail)]. Graders assessing primary outcome were affiliated with an independent reading center, and were masked to treatment group by providing video files to them as coded files that did not reference date or assignment group (see appendix). Orientation and mobility assessors were also masked to treatment group. All other people involved in the trial were aware of group assignment.

Procedures

Voretigene neparvovec is an AAV2 vector containing human RPE65 cDNA with a modified Kozak sequence engineered at the translational start site, under control of a hybrid chicken β-actin promoter with a cytomegalovirus enhancer. After generation by transfection of HEK293 cells, the vector is purified to substantially remove empty capsids, and a surfactant is added to prevent subsequent vector loss during storage and administration. Participants randomly assigned to the intervention group received 1 mg/kg per day of prednisone orally for 7 days, at a maximum dose of 40 mg/day regardless of weight, beginning 3 days before their first injection. Prednisone was tapered until 3 days before injection of the second eye, when the steroid regimen was repeated.

Under general anaesthesia, subretinal injection of $1{\cdot}5\times10^{11}$ vg voretigene neparvovec in a total subretinal volume of 0·3 mL was performed on the first assigned eye, established by determining worse function by visual acuity or subject preference, or both. Standard vitreoretinal techniques for subretinal surgery were used, including a three-port pars plana posterior cortical vitrectomy, as in the phase 1 studies.[15] The second eye was injected 6–18 days after the first procedure. Full details of the subretinal injection procedure are included in the appendix.

Safety clinical evaluations were done for the first eye at days 1 and 3 after the first injection, and for the second eye at days 1, 3, and 14 after the second injection. Efficacy assessments were limited to 30, 90, 180, and 365 days after second injection.

Participants randomly assigned to the control group did not receive voretigene neparvovec, but participated in the same efficacy outcome testing as did the intervention group. The control group became eligible to receive voretigene neparvovec 1 year after their baseline

evaluations, provided they still met all eligibility criteria. At this point, they received bilateral administrations according to the same protocol as the intervention group.

Participants were assessed with MLMT at baseline, with subsequent assessments at 30, 90,180, and 365 days after randomisation (control group) or after the second injection (intervention group). In response to the need for a relevant, reliable, and clinically meaningful measure of functional vision in these low-vision participants with nyctalopia, members of the sponsor and study team, with input from the US Food and Drug Administration, developed the MLMT. This endpoint demonstrated construct and content validity in a concurrent non-interventional study[28] that characterised it in detail. This 5-foot by 10-foot course surrounded by a 1-foot border (1·52 m×3·05 m×0·3 m) evaluates an individual's ability to navigate a marked path, while avoiding obstacles in or adjacent to the path, negotiating raised steps, and identifying a door, all while relying on vision. The MLMT was designed to quantify participants' ability to navigate around these obstacles in varying environmental illuminations, including very low light levels, integrating aspects of visual acuity, visual field, and light sensitivity. A normally sighted ambulatory person would be able to complete the course at 1 lux with no or minimal errors. Passing (at any light level) is defined as completion of the course at the specified light level with fewer than four errors (corresponding to an accuracy score of <0·25) and within 3 min. The test has 12 configurations to reduce learning effect. After 40 min of dark adaptation, participants completed the course with one eye patched, then completed a new configuration with the other eye patched, and then again using both eyes. This process was repeated at for at least two light levels (one failing, one passing) or up to a maximum of seven levels if required to identify the failing and passing levels for each eye-patching condition, progressing from lower to higher luminance levels that were controlled with a customised dimmer panel and measured in lux with calibrated light metres at five locations of the course before each testing session. Each light level was assigned a discrete lux score from 0 to 6, with lower light levels corresponding to higher lux scores. Testers did not provide verbal or physical cues, although they did guide participants back to the course if they stepped off the path or were at risk for injury.

Participants were assessed on visual and retinal function at baseline, and 30, 90, 180, and 365 days after randomisation (control) or second injection (intervention) using full-field light sensitivity threshold (FST) testing, done using both white light and chromatic stimuli to probe potential differential effects on rod versus cone photoreceptors; visual field testing by Goldmann perimetry for kinetic fields; Humphrey computerised testing for macular static fields with foveal sensitivity thresholds (method details in the appendix); contrast sensitivity testing, and pupillary light reflex (PLR). Participants (or the parents or guardians of paediatric participants) also completed a visual function questionnaire designed to assess activities of daily living relevant to visual deficits due to *RPE65* gene mutations. This questionnaire contained 25 questions with numerical answers from 0 (worst vision) to 10 (best vision), and has not been validated. Participants were also given in-home orientation and mobility assessments at baseline and 1 year after randomisation (control group) or second injection (intervention group). These functional home-based assessments were conducted and evaluated by orientation and mobility specialists independent from the study teams and the sponsor. They were designed to document the functional visual abilities of the

participants in each of the following domains: self-report, functional visual field, basic visual skills, illumination, orientation, and mobility.

Safety and efficacy will be monitored for at least 5 years through assessments at annual visits, including safety, mobility testing, and retinal and visual function testing, and for 15 years via questionnaires at annual visits or telephone visits.

Outcomes

The primary efficacy endpoint, designed to measure the effect of intervention on functional vision, was the change in bilateral MLMT performance (change in lux score for the lowest passing light level) at 1 year relative to baseline. Baseline testing established the lowest level of illumination at which each participant could pass the MLMT. A positive change score indicates passing the MLMT at a lower light level. Audio and video recordings of MLMT were independently graded by two masked, trained reviewers and an adjudicator, if needed, at a separate time and location from the testing. Participants were evaluated for accuracy and speed on MLMT.

Secondary efficacy endpoints were FST testing averaged over both eyes, MLMT testing of the assigned first eye, and best-corrected visual acuity (BCVA) averaged over both eyes. FST, a measure of visual function generally assessed per eye, was chosen to test the underlying physiologic function of the rod photoreceptors predominantly affected by *RPE65* mutations. FST measures the lowest illumination perceived—light sensitivity—over the entire visual field, is therefore unaffected by nystagmus, and is useful over a wide range of visual impairment. FST test-retest variability has been stated as 0·3 log[29] and a meaningful change has been suggested as 10 dB or 1 log. For visual acuity, a meaningful change is generally understood to be greater than three lines (15 letters, 0·3 LogMAR) on the Early Treatment Diabetic Retinopathy Study eye chart.[30]

Additional efficacy endpoints were visual field testing by Goldmann perimetry for kinetic fields and Humphrey computerised testing for macular static fields with foveal sensitivity thresholds, score on the visual function questionnaire, contrast sensitivity, pupillary light reflex (PLR), and in-home orientation and mobility assessments. Both kinetic and static visual field tests were chosen as exploratory endpoints to evaluate alterations in function of different regions of the retina. The visual function questionnaire was a patient-reported outcome (administered separately to subjects and to parents/guardians of younger subjects) designed to assess activities of daily living relevant to visual deficits due to *RPE65* gene mutations.

Safety assessments included physical and ophthalmic examination, clinical laboratory assessments, immunology testing, and reporting of ocular and non-ocular adverse events. Physical examinations were done at baseline and 1 year after intervention or randomisation. Immunology and clinical laboratory testing was done at baseline, 30 days, 90 days, and 1 year after intervention.

When this trial was initially organised, the primary endpoint was the difference between the intervention and the control groups at the 1-year timepoint in a composite score on the

MLMT, consisting of performance with both eyes (bilateral testing condition), with the right eye only, and with the left eye only, referred to as the sum score. Discussions with regulatory agencies influenced the eventual choice of endpoints. These were agreed to and outlined in the statistical analysis plan (on Aug 24, 2015) prior to database lock and data analysis. Additional detail of these discussions is in the appendix.

Statistical analysis

The simulated power to detect a clinically meaningful difference, based on a sample of 16 intervention participants and eight concurrent controls and a type I error rate of 0·05, was greater than 99%. We based this result on previous phase 1 data scored in a manner consistent with MLMT scoring for the phase 3 trial. Recruitment was designed to stop when at least 18 intervention participants had been injected in the second eye and at least nine participants were in the control group (27 evaluable participants), which could have required randomisation of up to four additional eligible participants (or up to 31 evaluable participants in total) due to the four randomisation strata. A change score of 1 or more lux levels was considered a clinically meaningful improvement from baseline. Control participants were predicted to have a mean change score of 0 because, although the condition is inexorably degenerative, progression over 1 year is typically slow.

We used descriptive statistics (mean, median, IQR, and range for continuous variables; counts and percentages for categorical variables) to summarise the observed distribution within each treatment arm for all outcomes. We present SDs for observed means and 95% CIs for modelled means. We used a permutation test to analyse performance on the MLMT, as measured by the change score at 1 year compared with baseline. We used the Wilcoxon rank-sum test and an exact method for calculating the p value for the observed test statistic. Separate models for FST and BCVA assessed the magnitude of the difference in response by comparing tests at 1 year with baseline. A longitudinal repeated measures model used the vector of observed measurements at each study visit as the outcome; time (study visit), treatment group, and their interaction as categorical covariates; an unstructured within-participant correlation; and a linear contrast to estimate the change from baseline to 1 year. For visual field and visual function questionnaire parameters, we calculated an observed two-sided p value from a Wilcoxon rank-sum test. For statistical tests, we considered $p<0·05$ as statistically significant.

We defined the intention-to-treat (ITT) population as all randomised participants; the modified ITT (mITT) population excluded any participant removed from the study on the day of randomisation and before any intervention. Analyses for primary and secondary efficacy endpoints included prespecified summaries on the full ITT and mITT populations. Analyses for exploratory efficacy endpoints used all available participants without imputation. Results describe observed values; two-sided p values test the difference between the two treatment arms for modeled results. Adverse event summaries use the safety (mITT) population, defined as all participants exposed to vector in the intervention group and all those in the control group who did not withdraw before baseline. Presented outcomes and their descriptive analyses were prespecified in a statistical analysis plan. Visual acuity was a prespecified outcome. The prespecified scale of so-called off-chart low BCVA acuity

(acuities so low as to be unmeasurable with available vision charts) was that adapted by Holladay;[31] however, we also report visual acuity measured by the scale adapted by Lange.[32] Formal comparisons (p values) were prespecified for the primary and secondary outcomes. All other p values are post hoc and denoted as such.

We generated all figures, summaries, and statistical analyses using SAS (version 9.4) and R. The study is registered on ClinicalTrials.gov, NCT00999609.

Role of the funding source

The study sponsor (originally Center for Cellular and Molecular Therapeutics at Children's Hospital of Philadelphia, then Spark Therapeutics) collaborated with the study investigators in the design of the study, the writing of the manuscript, and the decision to submit the paper for publication. The sponsor participated in the analysis and interpretation of group data, but did not participate in collection of data from individual subjects. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 15, 2012, and Nov 21, 2013, 36 participants consented to screening, and 31 passed screening and were randomly assigned to intervention (21 participants) or control (10 participants), comprising the ITT population. The groups were similar in age and sex at screening (table 1). Baseline MLMT passing level was not completely balanced between the two groups (table 1). With four strata and a small trial, these results are not unexpected. One participant from each group withdrew after consent; neither received voretigene neparvovec, leaving 20 intervention and nine control participants for the mITT and safety analysis population (figure 2).

Most deviations were procedure-related, generally due to departures from the manual of procedures or standard operating procedures. The most substantial protocol deviation was an eligibility violation in one participant who passed screening mobility test runs at 1 lux, discovered after the participant received bilateral vector administrations. We excluded this participant from per-protocol analyses and we did an additional sensitivity analysis, in which the screening MLMT performance was carried forward; study outcomes were not affected in any of these analyses.

At 1 year, the mean of the bilateral MLMT change score for the ITT population was 1·8 (SD 1·1) in the intervention group and 0 · 2 (1 · 0) in the control group, for a difference of 1 · 6 (95% CI 0 · 72–2 · 41, p=0 · 0013; table 2). Monocular MLMT change scores were similar to the bilateral scores (table 2). The response to bilateral administration of voretigene neparvovec in the mITT population was rapid (figure 3). Mean MLMT lux score improved by the day 30 visit and remained stable throughout 1 year. 13 (65%) of the 20 mITT intervention participants passed MLMT at the lowest luminance level tested (1 lux) at 1 year, demonstrating the maximum MLMT improvement possible. By contrast, no control participants passed MLMT at 1 lux at 1 year (appendix).

Mean FST (white light [reported as log10(cd.s/m^2)] averaged over both eyes) in the intervention group showed a rapid, greater than 2 log units improvement by day 30 in light sensitivity that remained stable over 1 year (figure 3). The control group showed no meaningful change in this measure over 1 year. The difference of −2·11 (95% CI −3·19 to −1·04) between the intervention and control groups (ITT) was significant (p=0·0004). Generally, participants with improvements in MLMT (18 [90%] of 20 receiving voretigene neparvovec) also showed improvements in FST; 12 MLMT responders improved by more than 1 log10 in FST testing. One MLMT responder had missing FST data due to inability to complete testing at baseline because of attentional limitations (4 years old at study enrollment).

BCVA, averaged over both eyes (with the scale adapted from Holladay[31] used to assign values for off-chart acuities) showed a numerical improvement between the intervention and control groups (figure 4). The observed LogMAR changes reflect a mean improvement of 8 · 1 letters on the eye chart for intervention participants versus a mean gain of 1·6 letters for control participants, which was not significant. The modelled mean change across both eyes in the ITT population decreased by 0 · 16 LogMAR from baseline for the intervention group and increased by 0·01 LogMAR for the control group, leading to a treatment difference of −0·16 LogMAR (95% CI −0·41 to 0·08, p=0·17).

For the mITT population, using the scale adapted from Lange and colleagues[12] for off-chart acuities, intervention participants showed a significant 9·0-letter improvement versus a 1 · 6-letter improvement in control subjects averaged over both eyes (difference of 7·4 letters, 95% CI 0·1 to 14·6, post-hoc p=0·0469; figure 4). This post-hoc visual acuity analysis was requested by regulators and by the study data safety monitoring board. On average, the assigned first eye improved by 10·5 letters for intervention participants versus 2·1 letters for control participants (difference of 8·4 letters, 95% CI −0·3 to 17·1, post-hoc p=0·0592). Of the assigned first eyes, six (30%) of 20 intervention participants and no control participants gained 15 or more letters (≥0·3 LogMAR) at 1 year. Assigned second eyes improved by a mean of 7·5 letters in intervention participants versus 1·1 letters in control participants (difference of 6·4 letters, 95% CI −0·8 to 13·6, post-hoc p=0·0809). Four (20%) of 20 assigned second eyes had an improvement of at least 15 letters versus none of the control participant eyes. At the 1-year visit, one intervention participant, whose baseline visual acuity in the first assigned eye was profoundly reduced at 1·95 LogMAR (approximately 20/1783 on Snellen chart across multiple tests), lost 2 · 05 LogMAR using the scale adapted from Holladay and 0·65 LogMAR using the scale adapted from Lange in the assigned first eye. This participant was also one of only two participants (both in the intervention group) with off-chart BCVA measurements after the immediate postoperative period, and the only intervention participant whose MLMT performance did not improve (appendix).

Visual field results are summarised in table 3. Most participants were able to perform Goldmann perimetry using the smaller III4e target. Mean sum total degrees of Goldmann visual field (III4e) nearly doubled in the intervention group and decreased in the control group (table 3). Likewise, macula sensitivity threshold on Humphrey visual field testing increased in the intervention group, whereas no meaningful change occurred in the control

group (table 3). No statistical difference between groups appeared in Humphrey foveal sensitivity threshold. Visual function questionnaire results are described in the appendix.

No product-related serious adverse events and no deleterious immune responses occurred. Two participants in the intervention group, one with a pre-existing complex seizure disorder and another who experienced complications from oral surgery, had serious adverse events unrelated to study participation. Most ocular events were mild in severity (table 4). The most common ocular adverse events were transient mild ocular inflammation, transient elevated intraocular pressure, and intraoperative retinal tears (table 4). Systemic adverse events are provided in the appendix.

Discussion

In this randomised, controlled trial of voretigene neparvovec—to our knowledge, the first randomised phase 3 gene therapy trial for a genetic disease—bilateral vector administration led to clinically meaningful and statistically significant improvements in ability to navigate independently in low-to-moderate light conditions, as shown by change in MLMT score in the intervention group compared with controls. The intervention group also showed marked improvement in FST. Both these improvements reflect restoration of RPE65 enzymatic activity, crucial for light perception. Improvements in both navigational abilities and light sensitivity were evident within the first 30 days after subretinal delivery and remained stable for 1 year, as they have for at least 3 years in the participants of the phase 1 follow-on trial.[18] Approximately two thirds of intervention participants achieved the maximum MLMT improvement possible, the ability to pass at 1 lux. Likewise, improvements in visual fields were also apparent soon after intervention, and also persisted throughout the 1 year follow-up.

Individuals with inherited retinal dystrophy due to autosomal recessive mutations in *RPE65* can present with visual impairment at a range of ages, from infancy to adolescence, with the most common presentation being nyctalopia, often at an early age. The disease inexorably progresses to near-total blindness as early as the preschool years or as late as the third decade of life. However, despite the absence of functional RPE65 isomerohydrolase and the subsequent inability of the retinal pigment epithelium cells to provide sufficient 11-cis retinal to the photoreceptors, the photoreceptors degenerate slowly, so that phenotypic recovery is possible through restoration of the missing enzyme to the retinal pigment epithelium cells.[18] 11-cis retinal is essential for phototransduction in both rod and cone photoreceptors, with the latter able to use alternative pathways.[33,34]

Improvement in BCVA was not necessarily expected following voretigene neparvovec administration because BCVA is a measure of foveal, cone-mediated function. Therefore, BCVA was not the primary target of the intervention in this rod-mediated disease. Furthermore, voretigene neparvovec did not come into contact with the fovea of all participants. Nonetheless, the BCVA data showed some evidence of improvement. The limited nature of the improvements in BCVA might be due to many factors, such as decreased cone health due to erratic cone opsin trafficking attributable to absence of 11-cis retinal.[35]

The increase in sum total degrees of visual field on Goldmann testing indicates an enlarged total area of retinal sensitivity, attributable to increased photoreceptor function, corresponding with improved peripheral vision that probably contributes to observations of improved navigational abilities. A static macula sensitivity threshold test was introduced, both to potentially evaluate efficacy, and to evaluate possible toxicity to the macular area related to the administration procedure or vector. This central 4° field includes a foveal sensitivity threshold that tests mainly the cone-only area of the foveola. We did not find significant toxicity in terms of macular dysfunction; indeed, group statistics show an improvement. Additionally, we observed no diminution in foveal sensitivity threshold in the group as a result of voretigene neparvovec.

Humphrey macula sensitivity threshold was increased in the intervention group, but Humphrey foveal sensitivity threshold was not. This result might be because, in many cases, the central macula (fovea) was not targeted by the subretinal injection of voretigene neparvovec.[36] Moreover, restoration of 11-cis retinal might not be as efficient in the fovea because this region exclusively contains cone photoreceptors, whereas voretigene neparvovec is believed to predominantly target retinal pigmented epithelium, the cells that supply 11-cis retinal to rod photoreceptors.[37,38] Additionally, the intervention group had foveal sensitivity levels closer to normal levels,[39] which might have limited the potential for improvement on this measure.

Safety outcomes in the 1-year observation period for this study did not identify any unacceptable barriers to administration of voretigene neparvovec. Specifically, no vector-related adverse events occurred, and the adverse events related to the procedure were mostly transient, mild in nature, or treatable (eg, cataracts). One participant experienced a loss of visual acuity in the first assigned eye (appendix).

Two aspects of the trial design merit comment. First, regarding the choice of controls, some inherited diseases have extensive literature on their natural history, making historical controls scientifically feasible. For most inherited retinal dystrophies, the requisite natural history data are not available. Use of an uninjected contralateral eye as control is almost ideal (same mutation at same level of disease progression), but this study design does not reflect what is likely to be the pattern of clinical use of the product (ie, bilateral administration) and does not allow assessment of systemic effects. The second aspect involves the choice of a 2:1 randomisation design, and is discussed in the appendix.

The current study protocol and vector have several noteworthy features. First, we introduced several innovations to the surgical procedure at early stages to reduce risks related to vector administration.[13] These changes resulted in low complication rates and excellent success of subsequent operating surgeons. Complications were likewise rare even when the fovea was involved with the subretinal injection. Second, we used a perioperative immunomodulatory regimen to reduce risks related to immune response. Third, the vector used in this study had been extensively optimised for strength of the promoter, engineering of an optimised Kozak sequence, inclusion of surfactant in the final formulation, and removal of empty capsids from the final product.[11,15,39–43] Fourth, we designed a novel measurement method that had high power for this small trial. The method quantifies the gradation of improvement, using

the MLMT framework and its different lighting conditions, into a continuous metric. The method was validated in a separate mobility testing validation study.[28]

Our study has several limitations. First, *RPE65*-mediated inherited retinal dystrophy is rare. Our study had no participants under the age of 4 years, although per the protocol 3-year-olds could have been eligible. Moreover, the study does not yield insight into the potential effect of voretigene neparvovec on the vision of patients whose baseline visual function or functional vision are better than that specified by the protocol. Furthermore, the treatment was open label, because the ethical administration of sham subretinal surgery to a paediatric population is questionable; however, graders for the primary endpoint were masked to treatment group. Another limitation is the possibility that BCVA averaged across both eyes was underestimated relative to traditional bilateral BCVA (which was not done) due to the averaging method used, which underweighted the better-seeing eye. Finally, both the novel primary endpoint developed to evaluate this patient population, and the characteristics of their visual impairment, are unfamiliar to most clinicians. Therefore, it is notable that more conventional measures of retinal and visual function, such as FST and visual field, support the findings on the MLMT.

The data presented here, which add to the evolving safety and efficacy profile of voretigene neparvovec, show improved light sensitivity, visual fields, and navigational ability under dim lighting conditions in patients with *RPE65*-mediated inherited retinal dystrophy, a population with no approved pharmacological treatment options. Data from the follow-on phase 1 study suggest that this effect might last at least 3 years;[18] observation is ongoing. These results underscore the need for access to genetic screening to identify patients with inherited retinal dystrophy who might benefit from this and other potential future gene therapies.

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Research in context

Evidence before this study

We searched PubMed from inception to Dec 1, 2015, for all Studies on *RPE65* mutations, their association with inherited retinal diseases, particularly Leber congenital amaurosis and retinitis pigmentosa, and all related animal and human preclinical and clinical treatment trials. Search terms included “RPE65”, “gene therapy”, “Leber congenital amaurosis”, “retinitis pigmentosa”, “AAV”, “gene therapy”, and combinations thereof. We also searched the RetNet Retinal Information Network for genes and loci causing inherited retinal dystrophies, and ClinicalTrials.gov for related past and current clinical trials. Proof-of-principle of gene augmentation therapy for *RPE65*-mediated disease was established in animal models using a recombinant adeno-associated viral vector. Phase 1 and 2 human clinical trials further supported the utility of gene therapy for *RPE65*-mediated inherited retinal dystrophy, although the gene constructs, vector formulations, and surgical approaches used by different programmes have had variable durability of effect.

Added value of this study

To our knowledge, this trial is the first randomised, controlled phase 3 study of a gene therapy for *RPE65*-mediated inherited retinal dystrophy. It demonstrates the efficacy of voretigene neparvovec, the first gene therapy potentially available for a type of visual impairment that is otherwise untreatable with established classes of therapeutics. The data presented here, added to the evolving broader safety and efficacy profile of adeno-associated virus administration to the subretinal space, demonstrate improved light sensitivity, visual fields, and functional vision under dim lighting conditions in individuals with *RPE65*-mediated inherited retinal dystrophy, a population that currently has no approved pharmacological treatment options. Participants in this programme’s phase 1 studies have had stable improvement, on average, in retinal and visual function that has generally remained durable for 3 or more years to date.

Implications of all the available evidence

We have done the first randomised, controlled phase 3 study for a category of disease that currently has no pharmacological treatment. Our study provides support for a gene-based approach to treatment of a rare genetic cause of blindness. In addition to its relevance to patients with *RPE65*-mediated inherited retinal dystrophy, this study provides a foundation for a novel treatment paradigm that might be applicable to other causes of inherited blindness. The manufacturing techniques optimised in the voretigene neparvovec clinical programme might potentially be applied to the treatment of other genetic diseases, advancing the field of gene therapy.

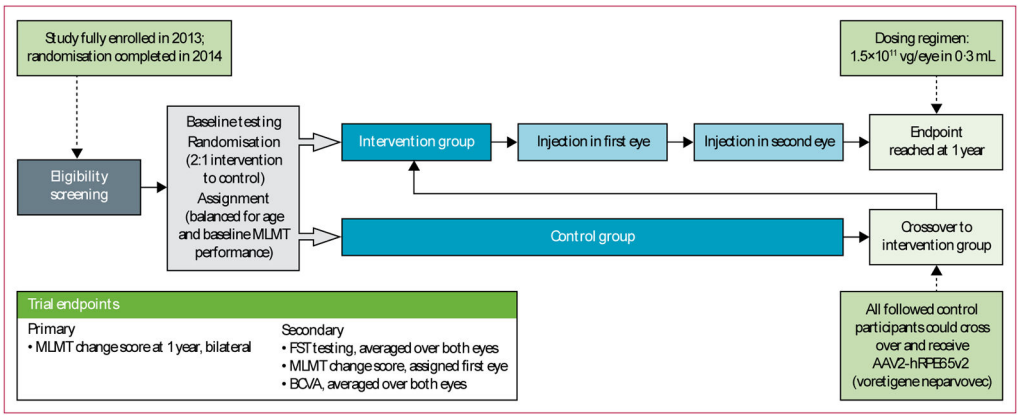

Figure 1. Phase 3 trial design

Visual field and a visual function questionnaire were additional, protocol-specified efficacy endpoints. vg=vector genomes. MLMT=multi-luminance mobility test. BCVA=best-corrected visual acuity. FST=full-field light sensitivity threshold.

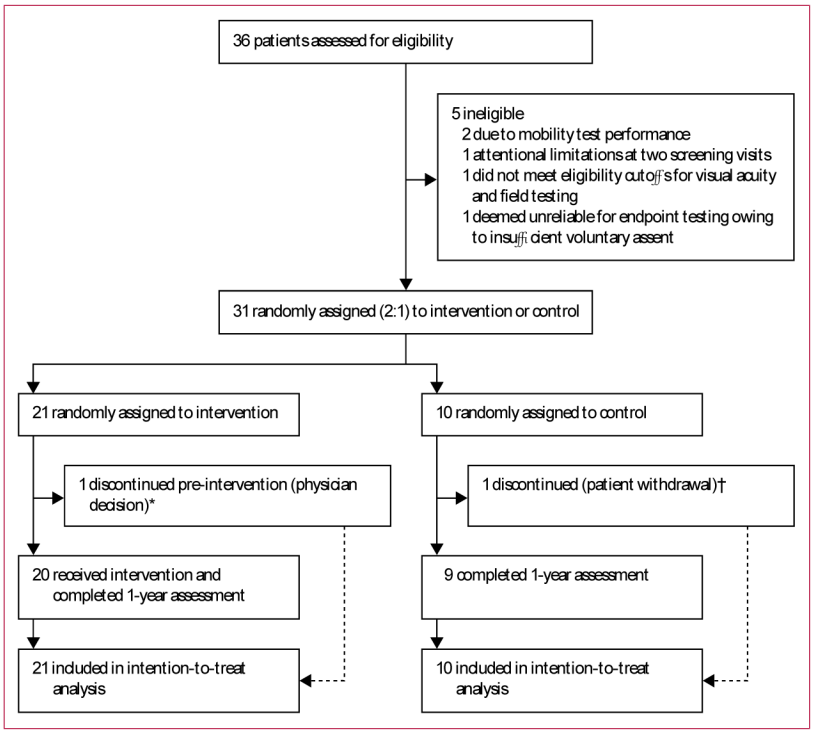

Figure 2. Trial profile

*Baseline optical coherence tomography findings included severe retinal atrophy or degeneration, with an almost complete absence of the photoreceptor layer in the macular area. The discontinuation decision was made before either the participant or the physician had been informed of the treatment assignment. †The participant discontinued due to personal reasons, and this decision was made before either the participant or the physician had been informed of the treatment assignment.

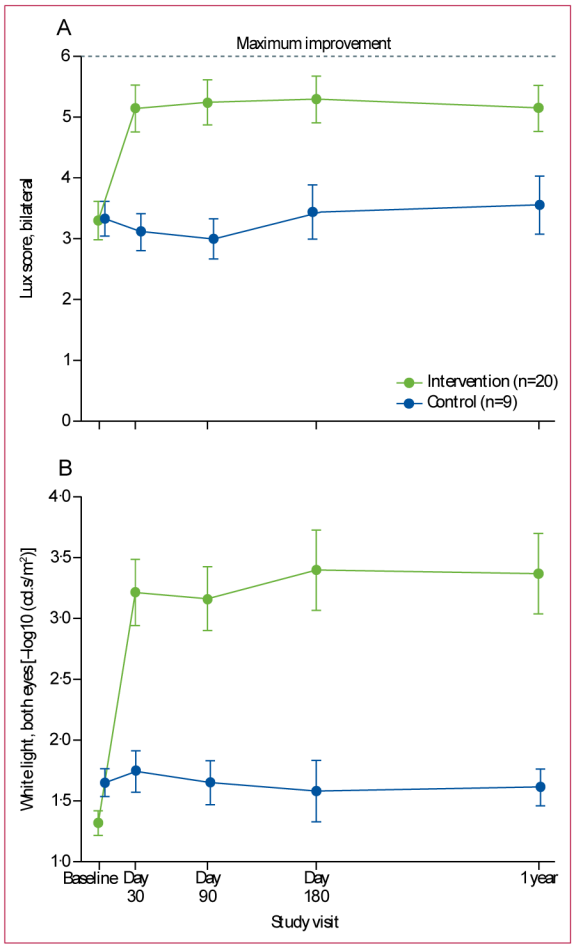

Figure 3. Mean bilateral MLMT lux score and white light FST for mITT population

(A) Mean bilateral MLMT lux score (p=0·0038) and (B) mean white light FST (both eyes; p=0·0004) by treatment arm and study visit for the mITT population. Bars denote SEs. FST=full-field light sensitivity threshold. mITT=modified intention-to-treat. MLMT=multi-luminance mobility test.

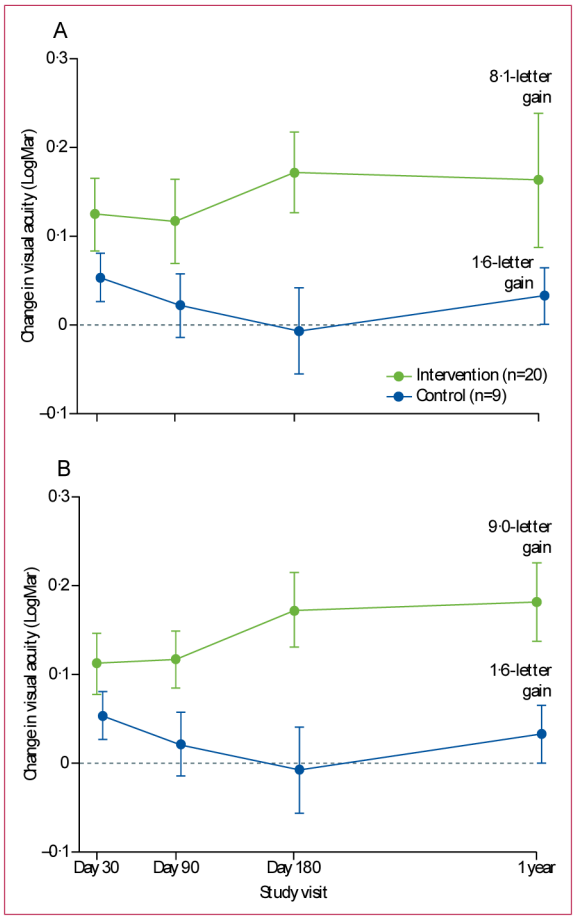

Figure 4. Mean change in BCVA in mITT population

Mean change (LogMAR; baseline minus each study visit) in BCVA (both eyes) by treatment arm and study visit using (A) Holladay (post-hoc p=0·27) and (B) Lange (post-hoc p=0·0469) for off-chart acuities in the mITT population. Bars denote SEs. BCVA=best-corrected visual acuity. mITT=modified intention-to-treat.

Table 1

Demographics of intention-to-treat population at baseline

	Intervention (n=21)	Control (n=10)	Total (n=31)
Age at randomisation (years)			
Mean (SD)	14·7 (11·8)	15·9 (9·5)	15·1 (10·9)
Median (IQR)	11 (6–18)	14 (9–24)	11 (6–20)
Range	4–44	4–31	4–44
Sex			
Female	12 (57%)	6 (60%)	18 (58%)
Male	9 (43%)	4 (40%)	13 (42%)
Age group (years) *			
<10	9 (43%)	4 (40%)	13 (42%)
≥10	12 (57%)	6 (60%)	18 (58%)
Race			
White	14 (67%)	7 (70%)	21 (68%)
Asian	3 (14%)	2 (20%)	5 (16%)
American Indian or Alaska Native	2 (10%)	1 (10%)	3 (10%)
Black or African American	2 (10%)	0	2 (6%)
Ethnicity			
Hispanic or Latino	5 (24%)	1 (10%)	6 (19%)
Other	16 (76%)	9 (90%)	25 (81%)
MUMT passing level *			
<125 lux	12 (57%)	4 (40%)	16 (52%)
≥125 lux	9 (43%)	6 (60%)	15 (48%)

MLMT=Multi-luminance Mobility Test.

*Randomisation strata.

Table 2

MLMT change scores at year 1 compared with baseline in intention-to-treat population

	Intervention (n=21)	Control (n=10)	Difference (95% CI)	Permutation test p value
Both eyes				
Mean (SD)	1·8 (1·1)	0·2 (1·0)	1·6 (0·72 to 2·41)	0·0013
Range	0 to 4	−1 to 2	··	··
Median (IQR)	2 (1 to 3)	0 (−1 to 1)	··	··
First eye				
Mean (SD)	1·9 (1·2)	0·2 (0·6)	1·7 (0·89 to 2·52)	0·0005
Range	0 to 4	−1 to 1	··	··
Median (IQR)	2 (1 to 3)	0 (0 to 1)	··	··
Second eye				
Mean (SD)	2·1 (1·2)	0·1 (0·7)	2·0 (1·14 to 2·85)	0·0001
Range	0 to 5	−1 to 1	··	··
Median (IQR)	2 (1 to 3)	0 (0 to 1)	··	··

Difference is intervention–control. MLMT=multi-luminance mobility test.

Table 3

Goldmann perimetry and Humphrey computerised testing

	Intervention (n=21)			Control (n=10)			1 year	
	Baseline	1 year	Change	Baseline	1 year	Change	Difference at 1 year (95% CI) Intervention-control	Post-hoc p value
Goldmann visual field III4e (sum total degrees)								
n	20	20	19	10	9	9		
Mean (SD)	332·9 (413·3)	673·9 (423·7)	302·1 (289·6)	427·1 (372·0)	397·8 (367·3)	−76·7 (258·7)	378·7 (145·5 to 612·0)	0·0059
Range	0 to 1418	0 to 1405	·59 to 820	0 to 1042	45 to 1144	−641 to 218	··	··
Median (IQR)	153 (53 to 469)	592 (287 to 1045)	257 (19 to 520)	372 (109 to 686)	349 (105 to 474)	−4 (−186 to 31)	··	··
Humphrey visual field, foveal sensitivity (dB)								
n	20	20	19	10	9	9	··	··
Mean (SD)	22·4 (6·8)	25·8 (9·1)	2·4 (9·7)	17·6 (8·9)	21·5 (8·9)	2·3 (5·3)	0·04 (−7·1 to 7·2)	0·18
Range	5 to 32	0 to 37	−24 to 18	3 to 28	6 to 31	−3 to 16	··	··
Median (IQR)	24 (19 to 27)	30 (21 to 32)	5 (−1 to 7)	17 (11 to 27)	26 (17 to 28)	2 (−1 to 3)	··	··
Humphrey visual field, macula threshold (dB)								
n	20	20	19	10	9	9	··	··
Mean (SD)	16·1 (5·5)	24·0 (8·0)	7·7 (6·2)	14·4 (8·0)	15·8 (7·4)	−0·2 (1·7)	7·9 (3·5 to 12·2)	0·0005
Range	8 to 26	2 to 32	−8 to 19	0 to 22	2 to 25	−2 to 3	··	··
Median (IQR)	15 (12 to 21)	28 (19 to 29)	8 (4 to 13)	16 (10 to 22)	16 (13 to 21)	−1 (−1 to 1)	··	··

Table 4

Treatment-emergent ocular adverse events in the intervention group in 1 year (mITT)

	Participants (n=20)	Number of events	Severity	Outcome
Elevated intraocular pressure	4 (20%)	5	Mild	Recovered or resolved
Cataract	3 (15%)	4	Mild	Ongoing in two participants; recovered or resolved in one participant (following extraction)
Retinal tear	2 (10%)	2	Mild to moderate	Recovered or resolved (following laserpexy)
Eye inflammation	2 (10%)	6	Mild	Recovered or resolved
Conjunctival cyst	1 (5%)	1	Mild	Recovered or resolved
Conjunctivitis viral	1 (5%)	1	Mild	Recovered or resolved
Eye irritation	1 (5%)	1	Moderate	Recovered or resolved
Eye pain	1 (5%)	1	Mild	Recovered or resolved
Eye pruritus	1 (5%)	1	Moderate	Ongoing
Eye swelling	1 (5%)	1	Mild	Recovered or resolved
Foreign body sensation in eyes	1 (5%)	1	Mild	Recovered or resolved
Iritis	1 (5%)	1	Mild	Recovered or resolved
Macular hole/degeneration	1 (5%)	2*	Mild to moderate	Recovered or resolved with sequelae; recovered or resolved
Maculopathy/epiretinal membrane	1 (5%)	2	Mild	Ongoing
Pseudopapilledema†	1 (5%)	1	Mild	Recovered or resolved
Retinal hemorrhage	1 (5%)	1	Mild	Recovered or resolved

One participant in the control group experienced 1 event of photopsia, which was classified as mild and resolved without sequelae. mITT=modified intention to treat.

*In the same eye of a single subject, a full-thickness macular hole spontaneously resolved (with sequelae) to thinning, which subsequently resolved (without sequelae). This was classified as two adverse events, but occurred in the same clinical course of events.

†Disc elevation unrelated to increased intracranial pressure or optic nerve oedema.