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Assessment of Visual Function with Cotoretigene Toliparvovec in X-Linked Retinitis Pigmentosa in the Randomized XIRIUS Phase 2/3 Study

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Purpose: Cotoretigene toliparvovec (BIIB112/AAV8-RPGR) is an investigational vector-based gene therapy designed to provide a full-length, codon-optimized retinitis pigmentosa GTPase regulator (RPGR) protein to individuals with *RPGR*-associated X-linked retinitis pigmentosa (XLRP). We assessed efficacy and tolerability of cotoretigene toliparvovec subretinal gene therapy.

Design: Part 2 of the XIRIUS trial (ClinicalTrials.gov identifier, NCT03116113) was a phase 2/3, 12-month, randomized (1:1:1) dose-expansion study.

Participants: Male patients ≥10 years of age with *RPGR*-associated XLRP were included.

Methods: Participants were randomized 1:1:1 to receive low-dose subretinal cotoretigene toliparvovec (5×10^{10} vector genomes/eye), high-dose cotoretigene toliparvovec (2.5×10^{11} vector genomes/eye) or to be an untreated control participant.

Main Outcome Measures: The primary end point was the percentage of participants meeting microperimetry responder criteria (≥ 7-dB improvement at ≥ 5 of 16 central loci). Secondary end points included change from baseline in retinal sensitivity at the central 16 loci and the entire 68 loci at 12 months and change from baseline in low-luminance visual acuity (LLVA) at 12 months, as well as the proportion of eyes with a ≥ 15-Early Treatment Diabetic Retinopathy Study ETDRS letter LLVA and ≥ 10-ETDRS letter LLVA change from baseline at month 12.

Results: Because of the impact of the COVID-19 pandemic, enrollment ended before reaching the initial target, leaving the trial underpowered. Twenty-nine participants were included (low-dose group, n = 10; high-dose group, n = 10; control group, n = 9). At month 12, the percentage of participants meeting microperimetry responder criteria was not significantly different between either cotoretigene toliparvovec group (low dose, 37.5% [$P = 0.3181$]; high dose, 25.0% [$P = 0.5177$]) and the control group (22.2%). However, the mean change from baseline in microperimetry sensitivity improved significantly with the low-dose group versus the control group at month 12 ($P = 0.0350$). Significant improvement in LLVA occurred in the low-dose group versus the control group at month 12 (33.3% difference [80% confidence interval, 14.7%−55.2%]; $P = 0.0498$). Three ocular-related serious adverse events (SAEs) occurred in the low-dose group versus 7 SAEs in the high-dose group.

Conclusions: The primary microperimetry end point was not met. Significant improvements in LLVA and mean microperimetry were observed compared with controls and fewer SAEs occured with low-dose compared with high dose cotoretigene toliparvovec.

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Inherited retinal disease is the most common cause of untreatable sight loss in young people,[1] and X-linked retinitis pigmentosa (XLRP) is one of the most common and severe genetic variants and invariably leads to blindness in young adults.[2,3] Male patients with XLRP typically experience impairment of night vision during childhood, accompanied by progressive peripheral constriction of the visual field.[2] Current clinical management approaches for XLRP are limited to supportive care and ameliorative measures.[2,4] Although several potential treatment strategies for XLRP are being investigated, including artificial vision devices, stem cell therapy, and gene therapy, currently no therapies for XLRP have been approved, highlighting a medical need

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for effective treatments to reduce the rate of disease progression.[2,4]

Mutations in the retinitis pigmentosa GTPase regulator (*RPGR*) gene account for almost all cases of XLRP, with prevalence of *RPGR*-associated XLRP estimated to be 3.4 to 4.4 per 100 000 male patients in Europe and the United States.[3,5] The *RPGR* gene is spliced alternatively to produce 2 major isoforms: $RPGR^{Ex1-19}$ (ubiquitously expressed in the retina and throughout the body) and $RPGR^{ORF15}$ (exclusively expressed in retinal photoreceptors).[3] Mutations resulting in aberrant functioning or altered localization of the RPGR protein lead to photoreceptor degeneration.[4,6]

Cotoretigene toliparvovec (BIIB112/AAV8-RPGR) is an investigational AAV8 vector–based gene therapy designed to provide a full-length, codon-optimized RPGR protein to individuals with *RPGR*-associated XLRP.[7,8] Because BIIB112 expresses the entire ORF15 region and with full glutamylation, it is predicted to enhance cone function as well as rod function (reduction of RPGR ORF15 glutamylation is associated with cone dystrophy).[9,10] Clinical evidence from the open-label, dose-escalation phase of the XIRIUS 1/2 study of cotoretigene toliparvovec ($n = 18$) demonstrated that therapeutic doses of cotoretigene toliparvovec–based gene therapy improved retinal sensitivity on mesopic microperimetry in adult male participants.[7,11] Administration of the 4 highest doses of cotoretigene toliparvovec ($n = 12$) resulted in some early (month 1 responders, 6/12 [50.0%]) and sustained (month 12 responders, 4/12 [33.3%]) improvements in retinal sensitivity.[7,11] Improvements in low-luminance visual acuity (LLVA), assessed by a gain of $\geq$ 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, were observed in study eyes versus untreated fellow eyes as early as month 1 (2/11 [18.2%] vs. 1/11 [9.1%]) and through 12 months of follow-up (3/11 [27.3%] vs. 1/11 [9.1%]).[7,11] Treatment with cotoretigene toliparvovec in the open-label dose-escalation study was well tolerated: most reported treatment-emergent adverse events (TEAEs) were mild and resolved, and no TEAE-related withdrawals occurred.[7,11] The randomized, controlled, XIRIUS phase 2/3 dose-expansion study assessed the efficacy and safety of cotoretigene toliparvovec subretinal gene therapy over 12 months in 29 male patients with *RPGR*-associated XLRP.

Methods

Study Design

XIRIUS (ClinicalTrials.gov identifier, NCT03116113) is a 2-part, first-in-human, multicenter trial of a single subretinal injection of cotoretigene toliparvovec in male participants with XLRP and a defect in the *RPGR* gene. Results from the XIRIUS phase 1, open-label dose-escalation study were reported previously.[11,12] The XIRIUS phase 2/3 study was a 12-month, controlled, dose-expansion study conducted at 8 sites in the United States and the United Kingdom that evaluated the safety, tolerability, and efficacy of subretinal injection of cotoretigene toliparvovec, as shown in Figure S1 (available at www.aaojournal.org). Eligible participants were randomized 1:1:1 to the high-dose (up to 2.5×10^{11} vector genomes/eye) or low-dose (up to 5×10^{10} vector genomes/eye) cotoretigene toliparvovec (up to 100 μl of vector suspension) or untreated control groups. The doses chosen for the study were in a range expected to be well tolerated based on previous studies and were comparable with doses in part 1 of XIRIUS associated with improvements in retinal sensitivity and reversal of visual field loss.[12] Standard block randomization was applied to generate the randomization and assign participants to treatment groups. Participants who were allocated to high-dose or low-dose cotoretigene toliparvovec underwent vitrectomy with detachment of the posterior hyaloid and received a single subretinal injection in the study eye.

Eligible participants were male patients $\geq$ 10 years of age with genetically confirmed XLRP caused by a pathogenic mutation in *RPGR*. Participants must have had a best-corrected visual acuity of $\geq$ 34 ETDRS letters and a mean total retinal sensitivity in the study eye of between $\leq$ 0.1 dB and $\leq$ 8 dB as assessed by microperimetry; preservation of outer photoreceptor structures in the macula on OCT was not required. Participants were excluded from the study if they had a history of amblyopia in either eye, had any significant ocular or nonocular disease that could put them at risk or influence the study results, had a contraindication to oral corticosteroids, were considered unsuitable for retinal surgery, had participated in another research study involving an investigational product within the past 12 weeks, or had previously received a gene or cell-based therapy. The original target enrollment for this study was 45 participants, which was not attained because of challenges associated with the coronavirus disease 2019 (COVID-19) pandemic in 2020 and the impending expiration of study drug. Therefore, a protocol was amended to enroll approximately 30 individuals in part 2.

The study was conducted in accordance with all applicable laws and regulations, including the International Conference on Harmonisation Guidelines for Good Clinical Practice and the relevant articles of the Declaration of Helsinki. Institutional review board or ethics committee approval was obtained at each participating study site. Written informed consent or assent forms were obtained.

Procedures

Before randomization, the study eye was assigned by selecting the generally worse-affected eye based on clinical assessment. The other eye was designated as the fellow eye. After a participant was randomized, a change in study eye designation was not permitted. Participants randomized to cotoretigene toliparvovec received a single subretinal injection in the study eye via a 2-step surgical procedure as described previously.[12,13] Because dosing required a surgical procedure, the sponsor, investigator, and participant were unmasked to the study procedure but were masked to the assigned dose level. All ophthalmic assessments at the screening or baseline visit and from month 3 onward were conducted by an assessor who was masked to study eye and dose versus the untreated control group. Up to 1 ml (40 mg) of triamcinolone was administered via a deep sub-Tenon approach to treated participants as needed at the time of surgery. Participants were also given a 9-week course of oral corticosteroid starting 3 days before surgery. A data monitoring committee was convened to review safety and assess the benefit-to-risk profile throughout the study.

Efficacy evaluation was based on macular integrity assessment microperimetry and LLVA. Mesopic macular integrity assessment microperimetry (CenterVue SpA) was conducted using a standard 10-2 grid. Low-luminance visual acuity was measured for both eyes using a standard ETDRS chart and a 2.0-log unit neutral density filter.

Safety evaluation was based on full ophthalmic examinations for both eyes and reporting of adverse events (AEs) throughout the study. The severity and relationship of AEs to the study drug or the

surgical procedure were assessed at the site by the investigator or a medically qualified designee.

Clinical Assessments

The primary efficacy end point was the proportion of study eyes with ≥ 7-dB improvement from baseline at ≥ 5 of the 16 central loci (not preselected) of the 10-2 grid (retinal sensitivity responder criterion) at 12 months, as assessed by macular integrity assessment microperimetry. Key secondary and other efficacy end points included the proportion of study eyes with ≥ 7-dB improvement from baseline at ≥ 5 of 16 central loci at 1, 2, 3, 6, and 9 months; the proportion of study eyes with ≥ 7-dB improvement from baseline at ≥ 5 of the entire 68 loci at 1, 2, 3, 6, 9, and 12 months; change from baseline in retinal sensitivity at the central 16 loci and the entire 68 loci at 1, 2, 3, 6, 9, and 12 months; change from baseline in LLVA at 1, 2, 3, 6, 9, and 12 months; and the proportion of eyes with a ≥ 15-ETDRS letter and ≥ 10-ETDRS letter change in LLVA from baseline at month 12, as shown in Figure S1. Because manually segmented OCT is considered experimental, the spectral-domain OCT structural end points were considered exploratory. The primary safety end point was the incidence of TEAEs over 12 months.

Statistical Analysis

The safety analysis set consisted of all randomized participants who received subretinal therapy when randomized to cotoretigene toliparvovec (under the 3-group schedule or a 2-group schedule from an earlier version of the protocol) or participants randomized to the untreated control group who attended visit 2—study day 0 via telephone. Efficacy was summarized in the intention-to-treat analysis set, which included all participants randomized under the 3-group schedule (Fig 2). Summary statistics were presented for both study and fellow eyes. For continuous efficacy end points and change from baseline, results were summarized using descriptive statistics. The proportion of study eyes with ≥ 7-dB improvement of retinal sensitivity from baseline at ≥ 5 of the 16 central loci (primary end point) was compared between dose groups (high-dose and low-dose cotoretigene toliparvovec vs. control groups) using the Fisher exact Boschloo test with a Berger-Boos correction[14] of $\beta = 0.001$. The primary hypothesis was tested using Hochberg's step-up method[15] with familywise error rate controlled at a 1-sided 0.10. The difference in proportions between dose groups was presented with confidence intervals (CIs) calculated using the Miettinen and Nurminen method.[16] Adverse events were summarized by system organ class and preferred term. For the mean change from the baseline in mean sensitivity and for best-corrected visual acuity and LLVA end points, multiple imputation on locus level followed by mixed-model repeated measure analysis were used to account for missing data.

Based on the Fisher exact Boschloo test (correction, $\beta = 0.001$; right-sided significance level of 0.10 to assess retinal sensitivity improvements only), a sample size of 10 participants from either dose group and 9 participants from the untreated control group provided approximately 87% power, assuming the dose group showed a 50% response rate, and the untreated group showed a 5% response rate.

Results

Study Population

Between October 19, 2018, and February 5, 2020, a total of 32 participants were randomized. The study was completed on November 18, 2020. Three of these participants were screened and received treatment under a previous version of the protocol via a 2-group randomization schedule and were included in the safety analysis set (Fig 2). Overall, 29 participants were enrolled in the phase 2/3 study, comprising the intention-to-treat analysis set (high-dose cotoretigene toliparvovec group, n = 10; low-dose cotoretigene toliparvovec group, n = 10; untreated control group, n = 9; Fig 2). Three participants (10.3%) discontinued the study before completion (high-dose group, n = 2; low-dose group, n = 1), all because of COVID-19 travel restrictions; loss of these 3 participants from the two treatment groups was not believed to have influenced the main conclusions of the study. All participants were male, ranging in age from 17 to 60 years; demographics generally were balanced across groups (Table 1). Baseline ocular characteristics were representative of individuals with XLRP and generally were balanced across treatment groups. The mean LLVA score in the study eye in the low-dose group was 39.30 ± 22.246 ETDRS letters. This score was slightly lower than those for the untreated group (50.22 ± 13.349 ETDRS letters) and the high-dose group (47.90 ± 18.975 ETDRS letters). These high standard deviation values highlight variability of LLVA scores across participants and treatment groups.

Efficacy

At month 12, no statistically significant difference was found in the proportion of participants meeting the protocol-defined microperimetry responder criteria between cotoretigene toliparvovec groups (low-dose group, 3/8 [37.5%; 80% CI, 14.7−65.5; $P = 0.3181$]; high-dose group, 2/8 [25.0%; 80% CI, 6.9−53.8; $P = 0.5177$]) and the control group (2/9 [22.2%; 80% CI, 6.1−49.0]; see Fig 3 for representative microperimetry images).

An exploratory analysis was conducted with the XIRIUS dataset using a new definition of microperimetry response recently recommended by the United States Food and Drug Administration: proportion of study eyes with ≥ 7-dB mean improvement from baseline at ≥ 5 preselected (before surgery, at the participant level) loci within the central 16 grid. The selection of loci at baseline in the area of central 16 grid was explored by selecting loci with baseline value of −1; −1, 0; −1, 0, 1; −1, 0, 1, 2; −1, 0, 1, 2; −1, 0, 1, 2, 3; −1, 0, 1, 2, 3, 4; and −1, 0, 1, 2, 3, 4, 5; respectively; and the number of loci is ≥ 5. Using these criteria, no statistically significant differences were found between treatment groups at month 12 per Hochberg procedure adjusting multiplicity between 2 doses, except for assessments within the baseline sensitivity ranges of −1, 0, 1, 2, 3, 4, 5, in which response rates in both of the cotoretigene toliparvovec−treated groups were higher than in the untreated group (nominal $P = 0.089$). No confirmed responders at month 12 were observed in the untreated group using this new definition of microperimetry response.

The change from baseline in microperimetry mean sensitivity in all 68 loci generally improved over time with low-dose cotoretigene toliparvovec (Fig 4). Across all 68 loci, an overall trend of higher sensitivity was found in

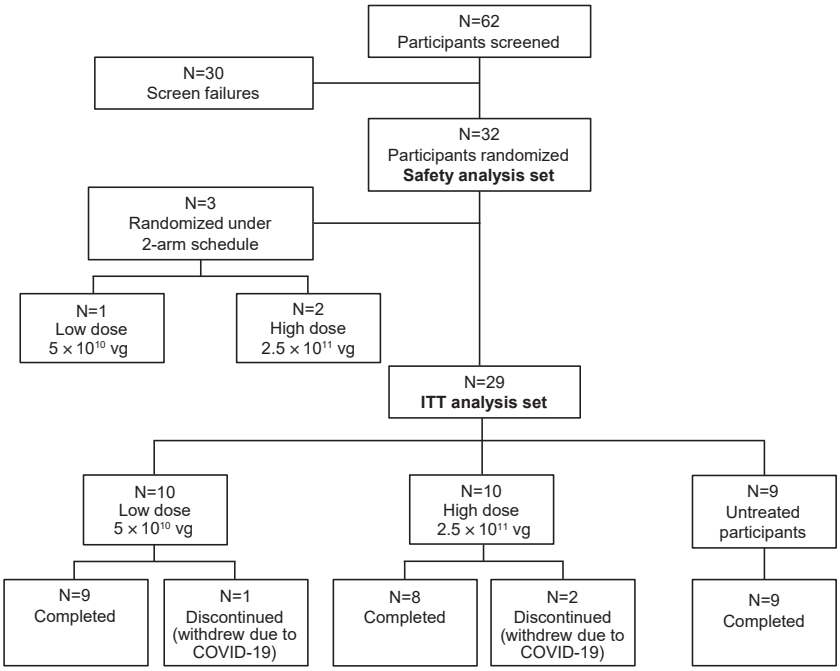

Figure 2. Flow diagram showing participant disposition in the XIRIUS phase 2/3 study. Three participants were screened under an earlier version of the protocol via a 2-group randomization schedule; these participants received treatment and were included in the safety analysis set (n = 32), but not the intention-to-treat (ITT) analysis set (n = 29). vg = vector genome. COVID-19 = coronavirus disease 2019.

the cotoretigene toliparvovec–treated groups compared with the untreated group (Fig 4A), with a nominally significantly (but potentially clinically meaningful) higher change from baseline in mean sensitivity in the low-dose group versus the untreated group at month 12 (2.79 dB [1.27–4.32 dB] vs. 0.11 dB [−0.63 to 0.85 dB]; P = 0.0350). The change from baseline in mean sensitivity in the 16 central loci was numerically higher in the low-dose group compared with the untreated group from month 1 to month 12; however, the treatment difference for the central 16 points only at month 12 (2.90 dB) was not statistically significant between the dose toliparvovec and untreated control group (2.79 dB [0.46–5.12 dB] vs. −0.11 [−2.18 to 1.96 dB]; P = 0.2092; Fig 4B). No statistically significant differences in microperimetry mean sensitivity across 68 loci were observed in the high-dose versus untreated groups at month 12 (0.89 dB [−0.68 to 2.46 dB]; nominal P = 0.5188; Fig 4A). The percentage change from baseline in microperimetry mean sensitivity across all 68 loci and at the central 16 loci for all treatment groups is shown in Figure S5 (available at www.aaojournal.org).

The proportion of study eyes with an increase in LLVA ETDRS letters from baseline generally trended higher in the cotoretigene toliparvovec–treated groups than in the untreated group over time (Fig 6). At month 12, the proportion of participants with LLVA gain of ≥ 15 ETDRS letters from baseline was significantly higher in the low-dose group (3/9 [33.3%; 80% CI, 12.9%–59.9%]) than in the control group (0% [80% CI, 0.0%–22.6%], nominal P = 0.0498; Fig 6A). A significant increase from baseline was found in LLVA gain of ≥ 15 ETDRS letters from months 1 to 3 in the high-dose versus the untreated group; no statistically significant difference was observed at month 12 (12.5% vs. 0%; nominal P = 0.3378; Fig 6A). Also, a numerically higher proportion of participants with LLVA gain of ≥ 10 ETDRS letters was observed from baseline at month 12 with the low-dose group (3/9 [33.3%; 80% CI, 12.9–59.9]) versus the untreated group (1/9 [11.1%; 80% CI, 1.2–36.8]; nominal P = 0.1631; Fig 6B), which did not attain statistical significance. No distinctive differences were found among these responders. In the high-dose group, 3 participants at months 1, 3, and 6 (30.0%, 30.0%, and 37.5%, respectively), 2 participants (28.6%) at month 9, and 1 participant (12.5%) at month 12 showed an LLVA gain of ≥ 10 ETDRS letters from baseline (Fig 6B).

A correlation (r = 0.6583) was observed between the change from baseline in retinal mean sensitivity in the 16 central loci and the change in LLVA in participants receiving cotoretigene toliparvovec over 12 months, as shown in Figure S7 (available at www.aaojournal.org). Generally, few meaningful differences between treatment groups were observed for other efficacy and exploratory outcomes, as summarized in the Supplemental Data and Table S2 (available at www.aaojournal.org).

Safety

The overall incidence of TEAEs was higher in cotoretigene toliparvovec–treated groups (100% in both groups) than in the untreated control group (55.6%; Table 3). Ocular TEAEs in the study eye were less common with the low-dose than the high-dose group (59 TEAEs vs. 130 TEAEs, respectively). Ocular inflammation-related TEAEs occurred in 12 participants (100%) in the high-dose group (31 TEAEs), 10

Table 1. Demographics and Baseline Characteristics

Parameter	Low-Dose Group	High-Dose Group	Untreated Control Group
Demographics*			
No. of participants (safety analysis set)	11	12	9
Age (yrs)	31 (24–50)	27 (17–37)	34 (18–60)
Race			
White	9 (81.8)	12 (100)	6 (66.7)
Black	1 (9.1)		
Asian	1 (9.1)		
Multiple/other			3 (33.3)
Hispanic or Latino ethnicity	3 (27.3)	5 (41.7)	2 (22.2)
Baseline characteristics			
No. of participants (ITT analysis set)	10	10	9
LLVA (ETDRS letters)			
Study eye	39.3 ± 22.25	47.9 ± 14.97	50.2 ± 13.35
Non–study eye	49.5 ± 19.13	51.6 ± 9.52	54.8 ± 11.87
BCVA (ETDRS letters)			
Study eye	65.9 ± 10.10	68.2 ± 8.99	68.8 ± 5.95
Non–study eye	68.5 ± 9.68	68.8 ± 6.99	72.9 ± 6.85
Microperimetry of 16 loci (dB)			
Study eye	7.4 ± 5.21	7.4 ± 3.10	6.8 ± 3.49
Non–study eye	9.1 ± 5.00	9.0 ± 3.58	9.6 ± 3.32
Microperimetry of 68 loci (dB)			
Study eye	2.5 ± 1.91	3.8 ± 2.14	2.2 ± 2.18
Non–study eye	3.3 ± 2.10	4.5 ± 2.33	3.3 ± 2.54

BCVA = best-corrected visual acuity; ETDRS = Early Treatment Diabetic Retinopathy Study; ITT = intention-to-treat; LLVA = low-luminance visual acuity.
Data are presented as no. (%), mean ± standard deviation, or mean (range), unless otherwise indicated.
*Based on the safety analysis set (n = 32).

participants (90.9%) in the low-dose group (16 TEAEs), and 0 participants in the untreated group. Approximately half of these ocular inflammation-related TEAEs (high-dose group, 18/31 TEAEs; low-dose group, 7/14 TEAEs) occurred within 30 days after treatment and most were recovered or resolved. No AEs led to study withdrawal, and no participants died during the study.

Ocular-related serious AEs (SAEs) were less common in the low-dose group (1 participant [9.1%]; 2 events of reduced visual acuity and 1 event of retinal detachment) than in the high-dose group (5 participants [41.7%]; 3 events of reduced visual acuity, 3 events of subretinal fluid, and 1 event of noninfective retinitis); none occurred in the untreated control group. All ocular SAEs occurred in the study eye and were considered to be related to the study drug, the study procedure, or both. Nine of the 10 SAEs were resolved or recovered by the end of the study. Among the 5 participants in the high-dose group who experienced 7 ocular-related SAEs, 1 participant experienced an SAE of subretinal fluid, which then caused an SAE of reduced visual acuity (25-ETDRS letter loss from baseline); this individual also experienced another SAE of reduced visual acuity (15-ETDRS letter loss) from days 280 to 356. Another participant experienced a 23-ETDRS letter decrease from a baseline SAE of reduced visual acuity related to subretinal inflammation that resolved with corticosteroid treatment. Two participants each experienced an SAE of subretinal fluid, 1 of which achieved resolution after subretinal fluid drainage and 1 of which achieved resolution after surgical reattachment of the retina. One participant reported an SAE of noninfective retinitis accompanied a reduction in retinal sensitivity assessed by microperimetry. The noninfective retinitis initially was treated with oral steroids, then with injectable steroids, and eventually resolved with sequelae; no improvement in microperimetry at month 12 was observed. Furthermore, 1 participant in the low-dose group experienced 3 ocular-related SAEs. An SAE of reduced visual acuity (26-ETDRS letter loss from baseline) occurred along with an SAE of retinal detachment related to both the study drug and study procedure. Another SAE of reduced visual acuity (16-ETDRS loss from baseline) that started at day 210 was ongoing at the end of the study.

Discussion

In the XIRIUS phase 2/3 study of cotoretigene toliparvovec subretinal gene therapy, the primary efficacy end point of this study, a single-point analysis of microperimetry improvement, was not met, with no significant differences observed between the proportion of study eyes with improved retinal sensitivity in the center grid at month 12 in cotoretigene toliparvovec–treated groups as compared with the untreated control group. Overall, the treatment effect size was small and not statistically significant, likely because of a higher than expected response rate in the untreated group (2/9 [22.2%]) using the protocol-specified primary end point. As previously reported in the XOLARIS natural history study, only 1 of 103 untreated

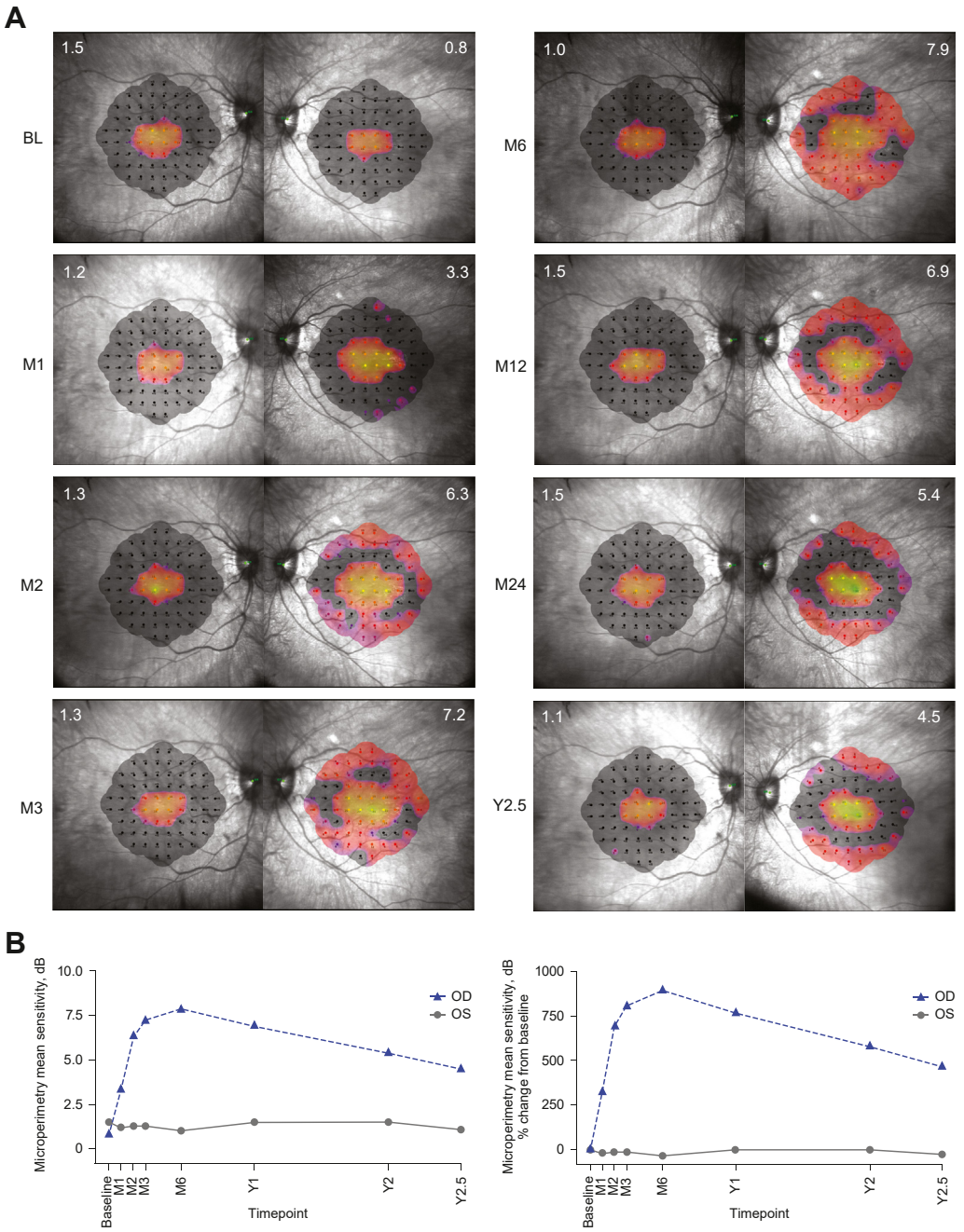

Figure 3. A, Microperimetry images of a representative study participant receiving subretinal gene therapy with BIIB112 to the left eye from baseline through 2.5 years. **B**, Graphs showing respective changes in microperimetry mean sensitivity values at each time point. In this patient, the entire macula area was detached and treated with the vector. Mean microperimetry values (decibels) are shown in white at the top of each image. The improvement in retinal function from baseline starts as early as month 1 and peaks at months 6 through 12. The visual field has returned at the edge of the microperimetry plot by month 2 and the ring extends beyond the recordable area; as such, the real gain is likely to be considerably greater than the numerical values shown. The combined improvement in both central retinal sensitivity and visual field in mesopic conditions can be attributed to rescue of cone function, which validates the use of a full-length retinitis pigmentosa GTPase regulator protein with full glutamylation in the current study. BL = baseline; M = month; MS = mean sensitivity; OD = right eye; OS = left eye; Y = year.

participants (1%) spontaneously achieved central retinal sensitivity response at 12 months.[11]

After the inception of the XIRIUS study, the United States Food and Drug Administration recommended a revised definition of microperimetry response for pivotal studies assessing inherited retinal disorders such as XLRP. Previously, responses were determined based on the proportion of eyes with a $\geq$ 7-dB improvement from baseline at $\geq$ 5 of the 16 central loci, and assessments carried a high false-positive rate. Under the new recommendations, response is defined as

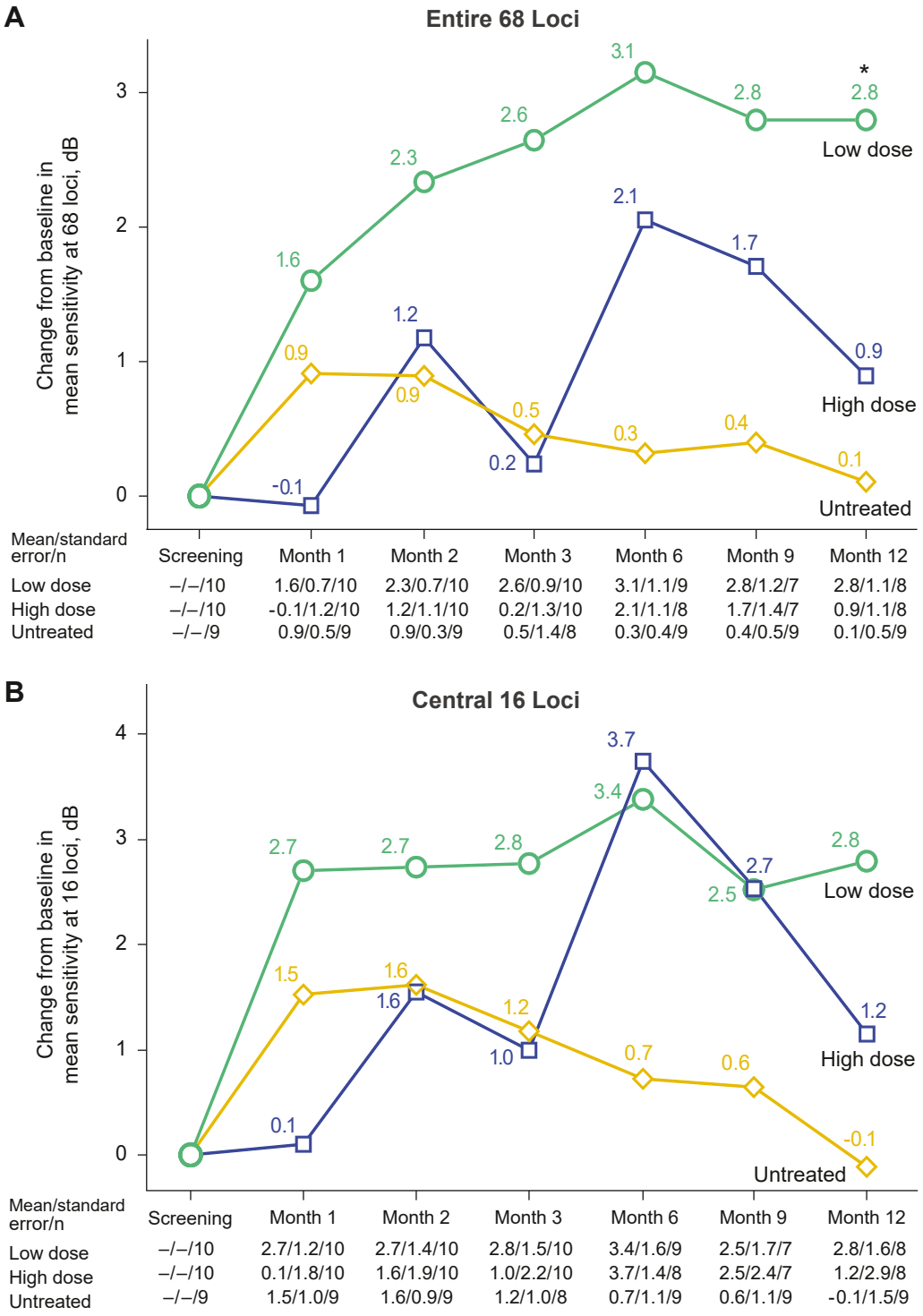

Figure 4. Line graphs showing the change from baseline in microperimetry mean sensitivity by visit at the (**A**) entire 68 or (**B**) central 16 loci. *Nominal $P = 0.0350$ versus untreated control group.

a mean of $\geq$ 7-dB improvement from baseline at $\geq$ 5 loci selected before surgery within the central 16-loci grid, confirmed at 2 visits separated by $\geq$ 3 months. To characterize changes in retinal sensitivity further, an exploratory analysis using this new definition was conducted with the XIRIUS dataset. After adjustment for multiplicity in this small cohort, no statistically significant differences were found between the cotoretigene toliparvovec–treated groups and the untreated group for any of the baseline sensitivity ranges at month 12, although a nominal treatment difference

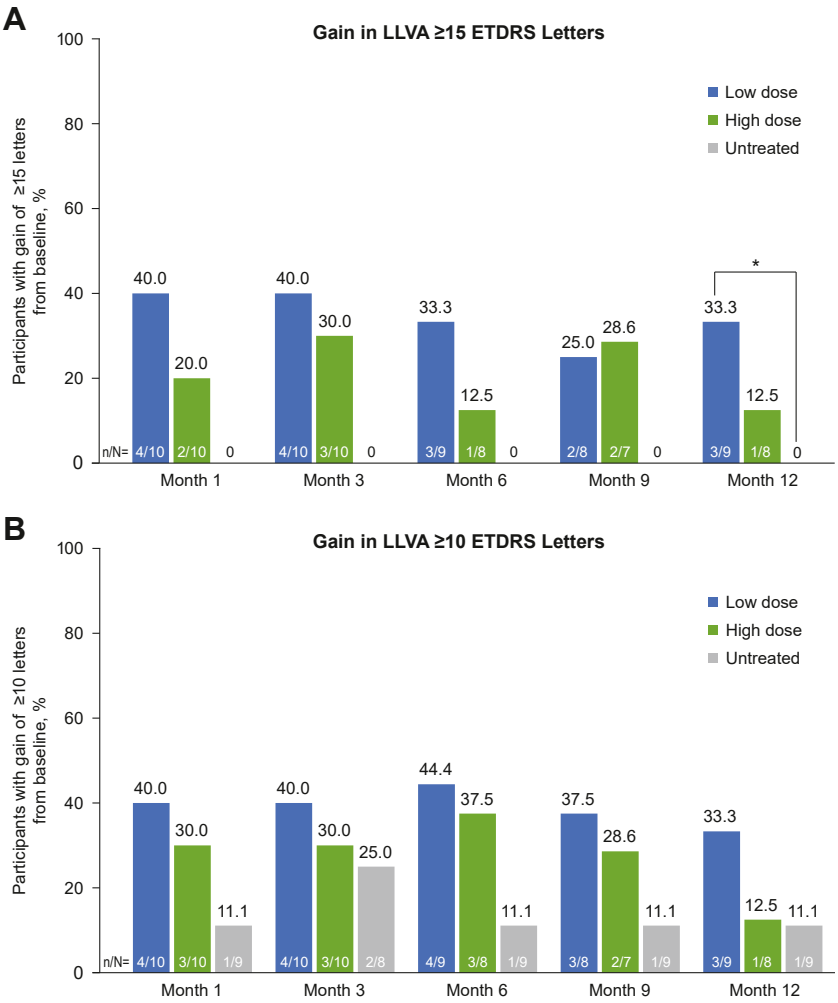

Figure 6. Bar graph showing the proportion of participants with low-luminance visual acuity (LLVA) gain of (**A**) ≥ 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters or **(B)** ≥ 10 ETDRS letters from baseline by visit. *Nominal $P = 0.0498$ versus untreated control group.

was observed for assessments within one of the baseline sensitivity ranges, with both of the treatment groups higher than in the untreated group. Notably, the 2 participants in the untreated control group categorized as responders using the protocol-defined end point would not have been considered responders at month 12 under the new definition of mean improvement of ≥ 7 dB. It should be noted that the 7-dB gain end point originally was developed for patients with glaucoma.[17] However, retinal ganglion cells cover much larger and overlapping receptive fields compared with individual photoreceptors. Hence, slight misalignments of the microperimetry grid at different visits will lead to variable responses, particularly at the edges of a centripetal outer retinal degeneration where photoreceptor function drops precipitously. This phenomenon would not occur with diseased retinal ganglion cells in glaucoma because hundreds of overlapping receptive fields correlate to each microperimetry stimulus point, and slight shifts in grid alignment would have a minimal effect on any given point.

Despite the lack of significant effect observed with the dichotomized primary end point in microperimetry, the continuous (mean) microperimetry end point and other measures of visual function suggested improvement with cotoretigene toliparvovec. Notably, improvements in mean microperimetry and LLVA were observed in both cotoretigene toliparvovec–treated groups (which reached statistical significance only in the low-dose group), despite patients undergoing macular detachment, which ordinarily would result in a reduction of visual acuity and retinal sensitivity. A trend of higher mean change from baseline in microperimetry retinal mean sensitivity across the 68-point grid in the low-dose group was observed compared with the untreated group that occurred as early as month 1, was sustained across all time points, and was statistically significant at month 12. It is unfortunate that this was not chosen as the primary end point.

Trends indicating improvement in LLVA over time were observed in the cotoretigene toliparvovec–treated groups, with a statistically significant gain of ≥ 15 ETDRS letters in the low-dose cotoretigene toliparvovec group versus the control group at month 12. Although the overall improvements were somewhat modest, these data support a growing body of evidence suggesting that LLVA may serve as a

Table 3. Summary of Treatment-Emergent Adverse Events in the Safety Analysis Set* (n = 32)

Parameter	Low-Dose Group (n = 11)		High-Dose Group (n = 12)		Untreated Control Group (n = 9)	
	No. (%)	*No. of Events*	*No. (%)*	*No. of Events*	*No. (%)*	*No. of Events*
TEAEs	11 (100)	97	12 (100)	160	5 (55.6)	14
Nonocular	8 (72.7)	30	9 (75)	26	5 (55.6)	9
Ocular	11 (100)	67	12 (100)	134	3 (33.3)	5
Study eye	10 (90.9)	59	12 (100)	130	1 (11.1)	2
Non–study eye	6 (54.5)	8	3 (25.0)	4	3 (33.3)	3
Serious TEAEs	3 (27.3)	5	5 (41.7)	8	1 (11.1)	1
Nonocular	2 (18.2)	2	1 (8.3)	1	1 (11.1)	1
Ocular	1 (9.1)	3	5 (41.7)	7	0	0
Study eye	1 (9.1)	3	5 (41.7)	7	0	0
Non–study eye	0	0	0	0	0	0
Study drug– and study procedure–related TEAEs	2 (18.2)	4	2 (16.7)	5	0	0
Nonocular	0	0	0	0	0	0
Ocular	2 (18.2)	4	2 (16.7)	5	0	0
Study eye	2 (18.2)	4	2 (16.7)	5	0	0
Non–study eye	0	0	0	0	0	0

TEAE = treatment-emergent adverse event.
*Includes 3 participants randomized under the 2-group schedule.

clinically relevant end point for the treatment of patients with XLRP. A review of publications summarizing the usefulness of clinical markers and research outcome measures in ophthalmic conditions demonstrated that LLVA enables early detection of retinal disease and may be a marker of disease progression.[18] Furthermore, LLVA seems to be an earlier clinical marker of change in central retinal function in *RPGR*-associated retinitis pigmentosa than standard visual acuity tests and also correlates with symptoms of night vision loss and shortening of the outer segments of foveal cone photoreceptors, which is of clinical relevance in XLRP.[18–20]

Reported TEAEs in the XIRIUS study were consistent with a population with XLRP who had undergone a vitrectomy and subretinal procedure.[21] The injection volume (up to 100 μl of vector suspension) has been well tolerated and injected without complication in many previous procedures. Fewer ocular-related SAEs occurred in the low-dose group (3 events) than in the high-dose group (7 events). Ocular SAEs that occurred in $\geq$ 1 participant were reduced visual acuity and subretinal fluid. All ocular SAEs were considered to be related to the study drug or the study procedure and nearly all resolved by the end of the study. The ocular-related SAE profile reported in XIRIUS part 2 generally was similar to that reported in part 1, in which 2 participants experienced SAEs of reduced visual acuity associated with ocular inflammation.[11] One participant experienced a reduction in best-corrected visual acuity by 16 ETDRS letters at month 9; ocular inflammation was observed in the dosed eye that was treated with corticosteroids. The SAE was not resolved by month 24, and return to baseline visual acuity was not expected because of loss of central photoreceptors subsequent to inflammation. Another participant experienced reduced visual acuity from days 133 to 154 that resolved with corticosteroid treatment.[11]

These TEAEs are all consistent with either inflammation to the adeno-associated virus vector (which is more likely to occur with the higher dose) or complications relating to the subretinal injection. Notably, these patients did not undergo a fluid–air exchange at the end of the procedure after the vector subretinal injection. This intervention almost certainly would help to reattach the retina and is standard in the administration of voretigene neparvovec-rzyl for treatment of *RPE65* mutations. However, it should be noted that the goal in XLRP is to target photoreceptors in the central macula and not the retinal pigment epithelium. Therefore, a substantial risk of pneumatic displacement of vector suspension away from the central macula exists if air is present in the vitreous cavity. It is likely that further refinements to the subretinal injection technique, such as using a robotic system to slow down the infusion and thereby limit the size of the retinal detachment,[22] will improve the surgical safety profile of this treatment after approval.

During execution of the phase 2/3 study, several external challenges were encountered and decreased the sample size. An issue with availability of cotoretigene toliparvovec (caused in part by manufacturing demands for COVID-19 vaccines) led to reduced enrollment of 29 participants (against the target of 45 participants), which ultimately decreased statistical power to detect treatment effects because of a smaller analysis population. Further, 3 participants did not complete the study because of COVID-19 travel restrictions. Additionally, increased incidence of inflammation-related AEs with cotoretigene toliparvovec may have affected treatment-related improvements in retinal sensitivity. Similar results were reported in XIRIUS part 1, in which 2 participants who received higher vector doses (1×10^{11} vector genomes) showed no measurable improvement in microperimetry and also experienced complications related to retinal inflammation. Taken together, these findings suggest that a complex relationship may exist between the stage of retinal degeneration, vector dose, and inflammation-related effects that warrants further exploration.[12] Moreover, a longer observation time (beyond 12 months) was not

feasible but also may have allowed for further characterization of the potential sustained therapeutic benefits of cotoretigene toliparvovec observed in XIRIUS part 1.[11]

As of late 2022, two other gene therapy trials for XLRP caused by mutations in *RPGR* are ongoing, using different therapeutic vectors than cotoretigene toliparvovec (ClinicalTrials.gov identifiers, NCT03252847 and NCT03316560).[3] It is important to note that the codon-optimized RPGR sequence in the current XIRIUS study encoded the entire full-length protein with no deletions in the ORF15 region. Through post-translational glutamylation, the RPGR ORF15 region is known to be critical for cone function.[9,10] Hence, the observed restoration of cone responses (as evidenced by improved mean microperimetry and trend toward LLVA improvement) provides additional evidence that human cones respond favorably to *RPGR* gene replacement. This is relevant because, until now, a gain in cone function has not yet been observed in the rod-dominant rodent or canine retina models after *RPGR* gene therapy, only slowing of cone degeneration, which would be predicted to occur indirectly simply through preservation of rod function.[8,23,24]

Therefore, the findings from this XIRIUS study provide insight into potential treatment options for XLRP. Because other investigational therapies are on the horizon, this work adds to the emerging body of evidence on gene therapy potentially to help reduce the rate of progression of this rare but debilitating condition.

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*A list of members of the XIRIUS Study Group appears in the Appendix (available at www.aaojournal.org).

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To request access to available data, please visit https://vivli.org/. The individual participant data collected during the trial, which supports the research proposal, will be available to qualified scientific researchers after anonymization and on approval of the research proposal.

HUMAN SUBJECTS: Human subjects were included in this study. The study was conducted in accordance with all applicable laws and regulations, including the International Conference on Harmonisation Guidelines for Good Clinical Practice and the relevant articles of the Declaration of Helsinki. Institutional Review Board (IRB)/Ethics Committee approval was obtained. Written informed consent/assent forms were obtained at each participating study site.

No animal subjects were included in this study.

Author Contributions:

Conception and design: MacLaren

Analysis and interpretation: Lam, Pennesi, Kay, Panda, Gow, Zhao, MacLaren

Data collection: Lam, Pennesi, Kay, MacLaren

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Overall responsibility: Lam, Pennesi, Kay, Panda, Gow, Zhao, MacLaren

Abbreviations and Acronyms:
AE = adverse event; **CI** = confidence interval; **COVID-19** = coronavirus disease 2019; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **LLVA** = low-luminance visual acuity; **RPGR** = retinitis pigmentosa GTPase regulator; **SAE** = serious adverse event; **TEAE** = treatment-emergent adverse event; **XLRP** = X-linked retinitis pigmentosa.

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