Supplementary Online Content

von Krusenstiern L, Liu J, Liao E, et al; XIRIUS Part 1 Study Group; XOLARIS Study Group. Changes in retinal sensitivity associated with cotoretigene toliparvovec in X-linked retinitis pigmentosa with *RPGR* gene variations. *JAMA Ophthalmol*. Published online February 9, 2023. doi:10.1001/jamaophthalmol.2022.6254

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Background

Mutations in the *RPGR* gene account for the majority of XLRP cases.[1] The *RPGR* gene is alternatively spliced to produce 2 major isoforms: a full-length transcript ($RPGR^{Ex1\text{-}19}$) that is ubiquitously expressed in different tissues and a truncated transcript ($RPGR^{ORF1}$) that is highly expressed in the retina, localized to the connecting cilia of photoreceptors.[2-5] Mutations resulting in aberrant functioning of the retinitis pigmentosa (RP) guanosine triphosphate hydrolase regulator (RPGR) protein lead to the photoreceptor degeneration observed in XLRP.[3,5]

Cotoretigene toliparvovec (BIIB112/AAV8-RPGR) is a codon-optimized *RPGR* gene therapy delivered via the AAV8 vector, which contains recombinant complementary DNA encoding the $RPGR^{ORF15}$ protein. The intron 15 splice donor site has been disabled using a silent codon change to prevent aberrant splicing of the mRNA.[6] The vector genome (AAV8–codon-optimized RPGR, known as AAV8-RPGR) is constructed with a strong photoreceptor-specific expression cassette (a human rhodopsin kinase promoter), the codon-optimized human cDNA encoding $RPGR^{ORF15}$, and a bovine growth hormone polyadenylation sequence flanked by AAV2 inverted terminal repeats. The human cDNA encodes the entire $RPGR^{ORF15}$ retina-specific isoform of the RPGR protein. In addition to protecting against inadvertent DNA deletions during vector cloning, codon optimization stabilizes the RNA processing in the nucleus, minimizing mutations and allowing for increased levels of $RPGR^{ORF15}$ expression. [6] The *RPGR* construct was optimized so that the entire full-length protein is expressed, rather than a truncated variant, which could have detrimental effects on photoreceptor function at higher doses.[7]

eMethods

Inclusion and Exclusion Criteria

The XIRIUS Trial

Participants must have had active pathology clinically visible within the macular region in both eyes, as assessed by spectral domain optical coherence tomography at screening and defined as a measurable ellipsoid zone within the nasal and temporal border of any B-scan and not visible on the most inferior and superior B-scans. Participants must also have had best-corrected visual acuity (BCVA) as assessed by the Early Treatment Diabetic Retinopathy Study (ETDRS) chart in both eyes that met the appropriate criteria based on the cohort level: better than or equal to light perception in cohort 1, between 34 and 73 ETDRS chart letters in cohorts 2 and 3, and ≥34 ETDRS letters in cohorts 4 to 6. Participants were excluded from the study if they had a history of amblyopia in either eye, had any notable 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 received a gene/cell-based therapy at any time previously.

The XOLARIS Study

Eligible participants were aged ≥7 years with a genetically confirmed mutation in *RPGR* who were able to undergo ophthalmic examinations for up to 24 months. Participants must have had a BCVA in at least 1 eye of ≥34 letters as assessed by the ETDRS chart (equivalent to Snellen ≥6/60 or 2/200; decimal 0.1; LogMAR 1.0). Eligible participants must also have had a mean total retinal sensitivity in at least 1 eye of ≥0.1 decibels (dB) and ≤20 dB, as assessed by microperimetry. Individuals were excluded from the study if they had a history of amblyopia in the eligible eye, had any notable ocular or nonocular disease that could put them at risk or influence the study results, or had participated in a gene therapy trial or clinical trial within the past 12 weeks.

In the XOLARIS study, the study eye was defined as the worse eye. If BCVA was the same in both eyes at study entry, the right eye was designated as the study eye. Those with BCVA ≥34 letters in both eyes at baseline were selected on the basis of the BCVA value after the study eye and fellow eye were identified.

Procedures

In the XIRIUS trial, the dosed eye received a single, subretinal injection of cotoretigene toliparvovec via a 2-step procedure as described previously.[8,9] The suspension used to determine the dose was quantified using the linearized plasmid quantitative real-time PCR method.[10] Briefly, following a standard vitrectomy, a balanced salt solution was injected to create a subretinal bleb that migrated toward the fovea. The cotoretigene toliparvovec vector was then slowly injected into the subretinal space through the existing bleb. The other eye was designated as the "fellow eye." Selection of the study eye (ie, dosed eye) was made in conjunction with a surgical consultant and study sponsor before randomization and is generally the worse-affected eye. To minimize inflammation, all participants were given a 21-day course of oral corticosteroids starting 2 days before the planned date of surgery.

Safety Evaluations

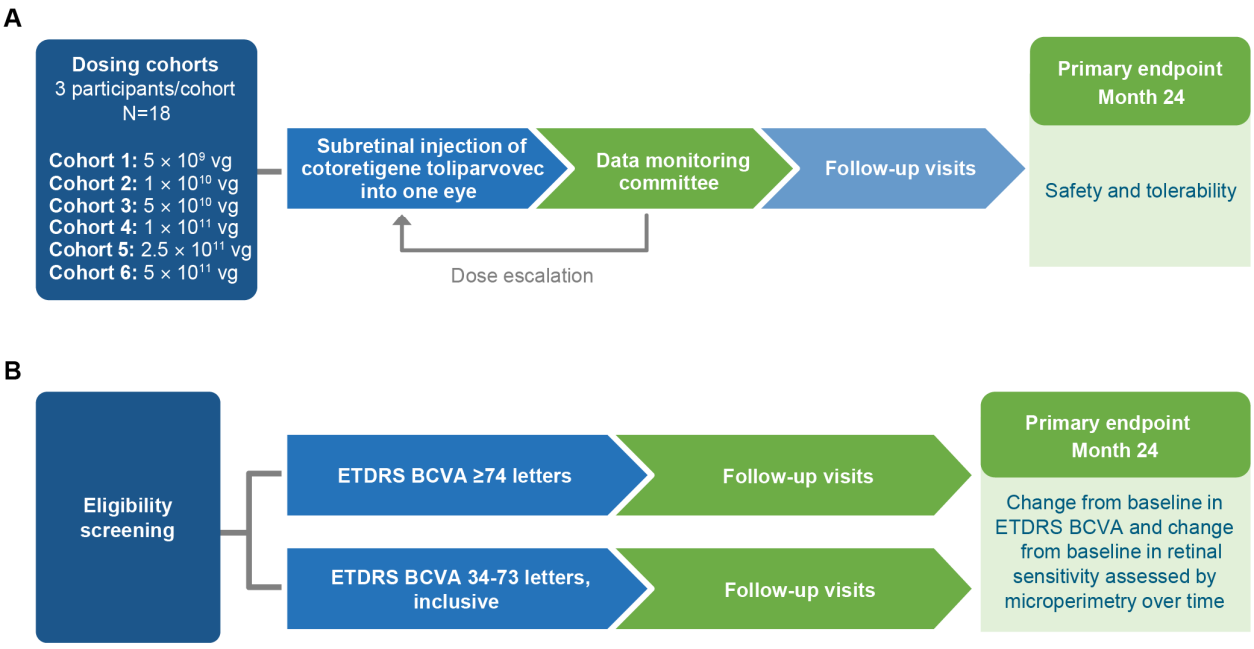
Dose-limiting toxicities were defined as any of the following events considered related to cotoretigene toliparvovec: a sustained (lasting ≥48 hours until recovery) decrease in BCVA of ≥30 letters on the ETDRS chart compared with baseline, a grade ≥3 vitreous inflammation, any clinically significant retinal damage observed that is not attributed to surgical complications, or any clinically relevant unexpected serious adverse reaction. Treatment-emergent adverse events were defined as adverse events starting on or after the day of surgery.

Data Reporting

The original XIRIUS study protocol was published on September 27, 2016; the first participant was dosed on March 16, 2017; and the study was completed on November 18, 2020.

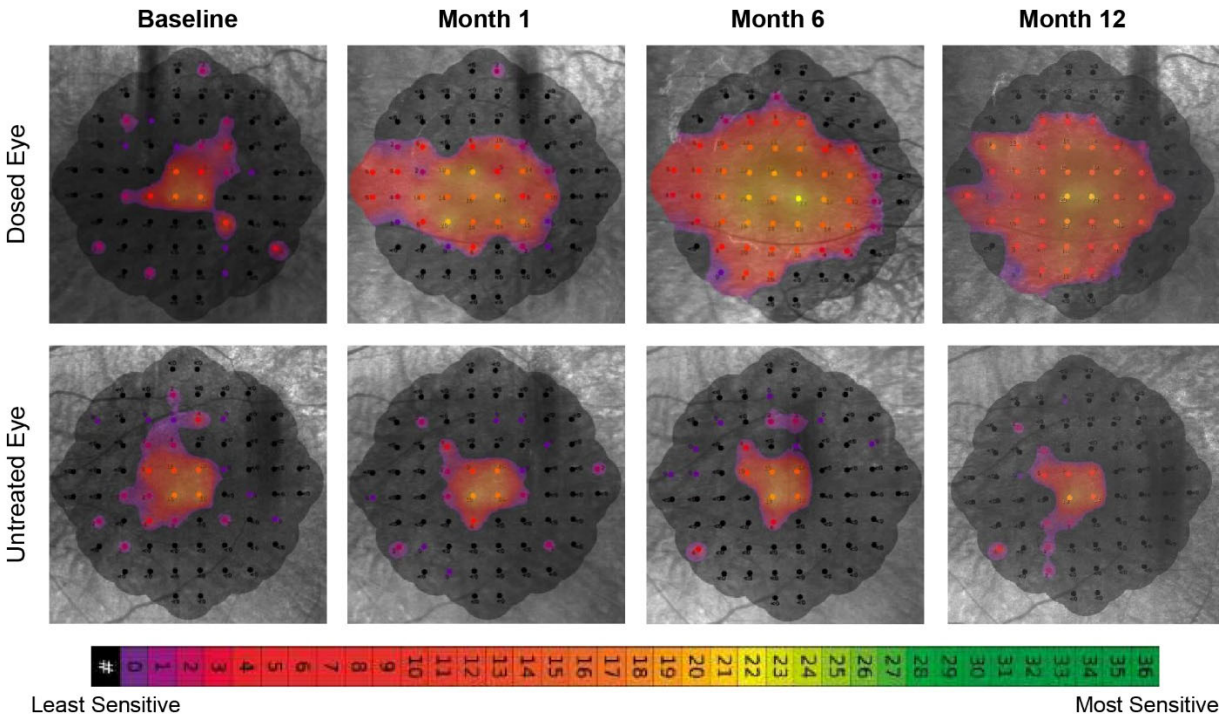
eAppendix 2. Discussion

Microperimetry is a unique tool that combines fundus imaging, retinal sensitivity mapping, and fixation analysis in one examination[11] and has been used over a decade as a powerful tool to detect and assess pathologies affecting the macular area, including RP.[12] The advantage of microperimetry over conventional perimetry is the ability to record and control a participant's fixation activity while measuring visual field, consequently eliminating errors caused by fixation losses. Microperimetry provides measures of visual function, including retinal imaging with measurements of macular sensitivity, scotomatous loci, and fixation stability.[11] Retinal sensitivity is a measure of photoreceptor function, and restored sensitivity in previously scotomatous loci following treatment may provide evidence of photoreceptor rescue.[13] Therefore, a microperimetry-derived endpoint was selected for establishing the efficacy of cotoretigene toliparvovec in this study.



eFigure 1. Study Design

The current analysis reports findings for **(A)** participants who received cotoretigene toliparvovec in Part 1 of the XIRIUS trial and **(B)** participants enrolled in the natural history study XOLARIS. BCVA, best-corrected visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; vg, vector genome.



eFigure 2. Microperimetry Image of a Participant From XIRIUS Part 1 Cohort 4 With Sustained Improvement in Retinal Sensitivity at 1 Month Sustained Through 12 Months

Dosed eye (top row) had a mean retinal sensitivity in 16 central loci of 3.8 decibels (dB) at baseline, which improved to 11.9 dB at 1 month, 16.4 dB at 6 months, and 13.6 dB at 12 months. Note the widening field of responsive loci in the dosed eye, visible as the central orange/red heat map, compared with baseline, in which many loci are scotomatous (nonresponsive to light; black spots). The untreated fellow eye (bottom row) had no change from baseline (4.3 dB) in mean retinal sensitivity in 16 central loci.

eAppendix 3. Study Groups

XIRIUS Part 1 Study Group

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XOLARIS Study Group

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