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Phase 1/2 AAV5-hRKp.RPGR (Botaretigene Sparoparvovec) Gene Therapy: Safety and Efficacy in RPGR-associated X-linked Retinitis Pigmentosa

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Highlights

- AAV5-hRKp.*RPGR* is a gene therapy evaluated for *RPGR*-XLRP
- This is a phase 1/2 open-label, dose escalation and randomized dose expansion study
- 45 participants received treatment (escalation: 10; confirmation: 3; expansion: 32)
- AAV5-hRKp.*RPGR* was safe and well-tolerated, with no dose-limiting events
- AAV5-hRKp.*RPGR* treatment showed improvements in several efficacy assessments

Phase 1/2 AAV5-hRKp.*RPGR* (Botaretigene Sparoparvovec) Gene Therapy: Safety and Efficacy in *RPGR*-associated X-linked Retinitis Pigmentosa

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Abstract (word limit: 250; current count: 249)

Purpose: To assess the safety and efficacy of AAV5-hRKp.*RPGR* in participants with retinitis pigmentosa GTPase regulator (*RPGR*)-associated X-linked retinitis pigmentosa (XLRP).

Design: Open-label, phase 1/2 dose escalation/expansion study (NCT03252847).

Methods: Males (≥5 years old) with XLRP-*RPGR* were evaluated. In the dose escalation phase, subretinal AAV5-hRKp.*RPGR* (low: 1.0 x 10^{11} vg/ml; intermediate: 2.0 x 10^{11} vg/ml; high: 4.0 x 10^{11} vg/ml) was administered to the poorer-seeing eye (n = 10). Dose confirmation (intermediate dose) was carried out in 3 pediatric participants. In the dose expansion phase, 36 participants were randomized 1:1:1 to immediate (low or intermediate dose) or deferred (control) treatment. The primary outcome was safety. Secondary efficacy outcomes included static perimetry, microperimetry, vision-guided mobility, best corrected visual acuity, and contrast sensitivity. Safety and efficacy outcomes were assessed for 52 weeks for immediate treatment participants and 26 weeks for control participants.

Results: AAV5-hRKp.*RPGR* was safe and well tolerated, with no reported dose-limiting events. Most adverse events (AEs) were transient and related to the surgical procedure, resolving without intervention. Two serious AEs were reported with immediate treatment (retinal detachment, uveitis). A third serious AE (increased intraocular pressure) was reported outside the reporting period. All ocular inflammation–related AEs responded to corticosteroids. Treatment with AAV5-hRKp.*RPGR* resulted in improvements in retinal sensitivity and functional vision

compared with the deferred group at Week 26; similar trends were observed at Week 52.

Conclusions: AAV5-hRKp.*RPGR* demonstrated an anticipated and manageable AE profile through 52 weeks. Safety and efficacy findings support investigation in a phase 3 trial.

Key Words: retinitis pigmentosa; X-linked retinitis pigmentosa; retinitis pigmentosa GTPase regulator; gene therapy, clinical trial

Introduction

Retinitis pigmentosa (RP) encompasses a group of inherited degenerative diseases of the retina that affect approximately 1 in 3000 to 4000 persons.[1,2] Affected individuals initially experience nyctalopia followed by progressive visual field (VF) constriction leading to tunnel vision, legal blindness, and often, complete blindness.[3,4]

In the United States, approximately 6% to 16% of patients with RP have X-linked RP (XLRP), an especially severe form of RP.[5] In patients with XLRP, deterioration of visual function tends to progress more rapidly than with other forms of RP.[6,7] The aggressiveness and severity of XLRP greatly impact patients' ability to carry out activities of daily living and school or work, reducing their quality of life and imposing a heavy psychosocial burden.[5]

Disease-causing variants in the RP GTPase regulator (*RPGR*) gene (OMIM 312610) are responsible for the disease in 70% to 90% of people with XLRP (*RPGR*-XLRP).[8-10] *RPGR* variants that affect the retina are predominantly found in the *RPGR* exon open reading frame 15 (ORF15).[11] RPGR is thought to directly or indirectly act on ciliary regulatory signaling and has been localized to the outer segments of rod and cone photoreceptor cells, as well as to motile cilia of airway epithelia.[12] Disease-causing variants in *RPGR* lead to photoreceptor dysfunction, death and retinal degeneration.[1]

There are currently no approved treatments for *RPGR*-XLRP. There is limited evidence supporting the use of antioxidant supplementation (e.g., vitamin A palmitate, docosahexaenoic acid, lutein), with management being primarily supportive and directed to associated ocular comorbidities (i.e., cataract and cystoid macular edema).[9,13]

Gene augmentation therapy for patients with *RPGR*-XLRP is a promising therapeutic strategy since most cases of the condition are associated with loss of function sequence variants.[14] Replication-deficient, recombinant, adeno-associated viral (AAV) vectors have been extensively studied in ophthalmology for therapeutic intervention in a myriad of retinal diseases.[9,15,16] Preclinical data from mouse models of *RPGR*-XLRP support the rationale for using gene therapy with a shortened human *RPGR-ORF15* replacement gene, in which one-third of a purine-rich repetitive sequence of the ORF15 exon is removed.[17,18] *RPGR-ORF15* is driven by the rhodopsin kinase (RK) promoter and has been expressed at levels similar to or

slightly above that seen for RPGR protein in a normal human retina.[19] This has also been demonstrated in human retinal organoids derived from patients carrying *RPGR* variants.[20] Moreover, replacement gene therapy with *RPGR-ORF15* is sufficient to rescue photoreceptor degeneration in *RPGR*-deficient mice.[17,18]

RPGR-XLRP is a severe form of retinal degeneration without established treatment. Preclinical trials of gene augmentation strategies are promising. The primary and secondary objectives of this study were to assess the safety and efficacy of subretinal AAV5-hRKp.*RPGR* gene replacement therapy in males with *RPGR*-XLRP.

Methods

Study design

This open-label, multicenter, phase 1/2 dose escalation, dose confirmation, and randomized, controlled dose expansion clinical trial (ClinicalTrials.gov Identifier: NCT03252847) was conducted at 5 sites across the United States and the United Kingdom (**Figure 1**). The study was initiated on July 31, 2017 (first patient screened) and was completed on November 18, 2021 (last observation of the last participant). This study was approved by an independent ethics committee or institutional review board at all participating sites. The trial adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with Good Clinical Practices guidance and applicable laws and regulations. Participants provided written informed consent, with the guidance of their parent or guardian where appropriate.

In the dose escalation phase, adult participants (≥16 years of age in the United Kingdom and ≥18 years of age in the United States) were administered 1 of 3 doses

of AAV5-hRKp.*RPGR* in the worse seeing eye (low: 1.0 x 10^{11} vg/ml; intermediate: 2.0 x 10^{11} vg/ml; high: 4.0 x 10^{11} vg/ml) in cohorts of 3 participants at a time to identify the maximum tolerated dose. Based on review of safety data by an independent data monitoring committee (IDMC), the intermediate dose was recommended for administration in the dose confirmation phase in pediatrics (≥5 years of age and <16 years in the United Kingdom or <18 years in the United States). In the subsequent dose expansion phase, both adults and children were eligible to participate, with only adults enrolled. Participants were randomized in a 1:1:1 ratio to the low dose, intermediate dose, or deferred (concurrent control) group (randomized for treatment with either low dose or intermediate dose after 26 weeks from baseline). The eye receiving treatment was also randomized in a 1:1 ratio. Participants were considered to have completed the study if they completed the Week 52 visit for the treatment group, and if they completed the Week 26 visit (last follow-up) for the concurrent control group. Post treatment results for deferred (concurrent control) participants who received study treatment at Week 26 are not reported herein.

Participant eligibility

Eligible participants were males ≥5 years of age, with a diagnosis of XLRP (confirmed by a retina specialist) associated with a disease-causing missense or null variant in *RPGR* confirmed in an accredited laboratory, and had relatively symmetrical retinal disease, which was defined as a <15 letter best corrected visual acuity (BCVA) difference between eyes. Other key inclusion criteria were evidence of relative preservation of retinal structure at the macula as determined by the presence of a discernable ellipsoid zone seen on spectral-domain optical coherence

tomography; evidence of impaired navigation in dim illumination on mobility assessment in both eyes, with each eye tested monocularly, as determined by a mobility assessment completion time of ≥12 seconds at low illumination (either 1 or 4 lux); and the ability to undertake age-appropriate clinical assessments. Exclusion criteria are presented in the **Supplementary Methods**.

Study treatment

AAV5-hRKp.*RPGR* consists of a recombinant AAV (rAAV) capsid of serotype 5 containing a linear single strand of human *RPGR-ORF15* deoxyribonucleic acid (DNA) with a 378-base pair (in frame) deletion in the purine-rich tract in the ORF15 region. The transgene is driven by a fragment of the human RK promoter, flanked by inverted terminal repeats of AAV serotype 2. In the dose escalation and dose confirmation phases, AAV5-hRKp.*RPGR* was administered to the poorer-seeing eye, as identified by the participant and investigator; in the expansion phase of the study, AAV5-hRKp.*RPGR* was administered to 1 randomized eye. Prior to surgery, participants received age- and weight-appropriate oral steroid prophylaxis. See the **Supplementary Methods** for more information. All participants underwent a 3-port pars plana vitrectomy followed by injection of ≤1 mL of AAV5-hRKp.*RPGR* vector suspension using a fine cannula through small retinotomies into the subretinal space, resulting in a transient retinal detachment (bleb). The contralateral eye was left untreated. Perioperative surgical antibiotics, steroids, and proton pump inhibitors were administered per study protocol according to local practices.

Endpoints and assessments

The primary objective was to assess the safety of AAV5-hRKp.*RPGR*. The primary safety endpoint was defined as the absence of any of the 5 following dose-limiting events (DLEs) being deemed to be possibly related to AAV5-hRKp.*RPGR* and occurring during the 9 weeks following surgery: 1) reduction in visual acuity (VA) by ≥15 Early Treatment Diabetic Retinopathy Study (ETDRS) chart letters, 2) severe unresponsive inflammation, defined according to Standardization of Uveitis Nomenclature Working Group grading system (i.e., anterior chamber cells 3+, anterior chamber flare 3+, or vitreous haze 3+ that fails to improve by 2 steps [or to grade 0] during a 6-week period),[21,22] 3) infective endophthalmitis, 4) ocular malignancy, and 5) grade 3 or above non-ocular serious adverse reaction.

In the dose escalation phase, the IDMC reviewed DLE data from a minimum of 9 weeks of follow-up from each cohort of participants, before recommending the next dose to be assessed in a further cohort of patients. Safety assessments were also based on adverse events (AEs), clinical laboratory tests, vital signs, and physical examinations. Ocular examination was conducted using slit lamp biomicroscopy to assess intraocular inflammation and anatomical integrity. Safety assessments were performed at all visits from screening through Week 52.

The secondary objectives were to examine if participants who received AAV5-hRKp.*RPGR* experienced slowing or halting of progressive deterioration in retinal structure or visual function, as well as improvement in retinal function, visual function, and quality of life. Secondary efficacy endpoints were grouped into 3 visual domains: functional vision, retinal function, and visual function. Quality of life data will be presented in a separate publication. Efficacy assessments for functional vision

included the vision-guided mobility assessment (VMA), which evaluated the ability of participants to navigate through a maze at various illumination levels (1 lux: deep twilight; 4 lux: residential street lighting; 16 lux: twilight; 64 lux: car park). Vision-guided mobility was conducted at screening and at Weeks 13, 26, and 52. Efficacy assessments for retinal function included the proportion of responders in static perimetry testing with the Octopus 900 device (Haag-Streit AG, Köniz, Switzerland). Responders were defined as participants with ≥5 of the same loci showing a ≥7 decibel (dB) improvement from baseline at two time points. In addition, mean retinal sensitivity (MRS) on Octopus static perimetry in the central 10° excluding scotomata, was calculated. Microperimetry data were captured using the Macular Integrity Assessment (MAIA) device (CenterVue, Padova, Italy), which tracks a participant's fixation activity while measuring their retinal sensitivity and minimizing errors caused by fixation losses. MRS with scotopic microperimetry was assessed in a subset of participants using a red stimulus under dark-adapted conditions, which primarily measures the sensitivity of dark-adapted cones in the central retina. Retinal function assessments were performed at baseline and at Weeks 13, 26, and 52. Visual function assessments included ETDRS BCVA, and contrast sensitivity (CS), measured using the Pelli-Robson chart.[23,24] VA was assessed at screening, baseline, and at all time points through Week 52. CS was assessed at screening, baseline, and Weeks 13, 26, 39, and 52. Due to the impact of the COVID-19 pandemic, participants missed certain efficacy assessments.

A post hoc sensitivity analysis included participants with static perimetry data considered reliable (reliability factor ≤19),[25] and an MRS range of ≥2 to ≤20 dB; these criteria are consistent with those being used in the phase 3 study

(NCT04671433). This sensitivity analysis was conducted by pooling data from the treated eye in participants from the dose escalation and dose expansion phases (low and intermediate doses) compared with data from the untreated eyes of deferred (concurrent control) participants prior to treatment.

Statistical analysis

Given that the study was conducted to evaluate safety and assess potential indicators of efficacy of AAV5-hRKp.*RPGR*, it was not powered for efficacy and there was no formal sample size calculation; and therefore, nominal 2-sided *P*-values ($\alpha<0.05$) are provided for descriptive purposes only and should be interpreted with caution.

Safety analyses included all participants who were administered AAV5-hRKp.*RPGR* (regardless of study phase), and all control participants who completed their last baseline assessment during the deferred period prior to treatment (Safety Analysis Set). Efficacy analyses included all treated participants who completed ≥1 baseline and 1 post baseline visit after AAV5-hRKp.*RPGR* administration, and all control participants who completed ≥1 baseline and 1 post baseline visit prior to AAV5-hRKp.*RPGR* administration (Full Analysis Set). In this report, efficacy results are presented with pooled treatment (low and intermediate doses) participants from both the dose escalation and randomized dose expansion groups compared with participants from the control (prior to treatment) group. Methods describing the pooling of efficacy results are detailed in **Supplementary Methods**.

Results

Participant disposition and exposure

Overall, 49 participants were enrolled in the study: 10 adult participants in the dose escalation phase (low dose: n = 3; intermediate dose: n = 4; high dose: n = 3), 3 pediatric participants (ages 11, 14, and 15 years) in the dose confirmation phase (intermediate dose, n = 3), and 36 adult participants in the randomized, controlled dose expansion phase (low dose: n = 8; intermediate dose: n = 11; control: n = 13; discontinued prior to AAV5-hRKp.*RPGR* administration: n = 4; **Supplementary Figure 1**). A participant assigned to the intermediate dose group of the dose expansion phase inadvertently received treatment with the high dose. Safety analyses were performed according to actual treatment received and efficacy analyses were performed according to assigned treatment group. Therefore, this participant was included with the other 3 participants who received the high dose for the safety analysis, and with the intermediate dose group for the efficacy analysis.

Overall, 45 of the 49 enrolled participants were administered AAV5-hRKp.*RPGR* and were included in the Safety Analysis Set. Of these, a total of 43 completed the study; 2 discontinued the study prior to completing the Week 52 visit (1 in the intermediate dose group due to mental health issues and 1 in the high dose group due to unspecified reasons).

Baseline demographics and clinical characteristics

The mean age of all participants was 28.1 (range: 11, 61) years and the majority (n = 41; 91.1%) were White (**Table 1**). The degree of VF loss as reflected by percentages of participants with baseline MRS <10 dB was similar across expansion phase groups.

Safety

Subretinal administration of AAV5-hRKp.*RPGR* was safe and well tolerated throughout the study. The mean volume of AAV5-hRKp.*RPGR* administered across all 45 participants was 0.42 mL (median: 0.50 mL; range: 0.10, 0.80 mL). There were no DLEs related to administration. No treatment-emergent AEs (TEAEs) leading to study discontinuation were reported throughout the study.

Overall, 37/45 participants experienced ≥1 TEAE, including all participants in the treatment cohorts and 5/13 (38.5%) in the control group (**Supplementary Table 1**). Across all study phases, the most frequently reported TEAEs were in the eye disorder system organ class (SOC), with the most frequent events for participants treated with low, intermediate, and high doses being conjunctival hemorrhage (n = 8 [72.7%], n = 11 [64.7%], n = 4 [100], respectively), reduced VA (n = 6 [54.5], n = 8 [47.1%], n = 2 [50.0%], respectively), and the presence of anterior chamber cells (n = 4 [36.4%], n = 9 [52.9%], n = 3 [75.0%], respectively; **Supplementary Table 2**).

Most participants in the treatment and control cohorts had TEAEs considered mild (n = 11; 29.7%) or moderate (n = 22; 59.5%) in severity; 4 (10.8%) participants reported TEAEs considered severe (**Supplementary Table 1**). Among all participants in the treatment groups, 59.4% (19/32) reported an AE deemed related to AAV5-

hRKp.*RPGR*. Nearly all (31/32; 96.9%) participants in the treatment groups reported an AE considered related to surgery. These AEs were transient and resolved without intervention.

Overall, 2 serious AEs (SAEs) were reported in the immediate treatment cohort. One SAE of retinal detachment was reported in 1 participant in the low dose group of the dose escalation phase on Day 8. This SAE was deemed severe and related to surgery. The event resolved after surgical correction on Day 10 with no sequelae. An SAE of uveitis was reported in another participant in the low dose group of the dose escalation phase on Day 33. This SAE was considered severe and related to AAV5-hRKp.*RPGR* treatment. The event was ongoing at the time of study completion. A third SAE of increased intraocular pressure (IOP) was reported outside of the reporting period in a participant in the deferred (concurrent control) group. This SAE was also considered severe. Two additional severe AEs were reported in the immediate treatment cohort, chorioretinitis (intermediate dose group) and uveitis (high dose group); these events were not reported as serious.

Ocular inflammation was a principal AE of interest. Overall, 25/45 (55.6%, all in the treatment cohorts) participants experienced ≥1 treatment-emergent ocular inflammation–related AE (**Supplementary Table 3**). Most ocular inflammation–related events were mild or moderate in severity. In addition to the participant detailed with an SAE of uveitis, 2 participants (1 in the intermediate dose group in the dose confirmation phase and 1 in the high dose group in the dose escalation phase) experienced severe ocular inflammation–related events (chorioretinitis and

severe uveitis, respectively); both events were recovering or resolved at study completion.

Following the implementation of a modified prophylactic corticosteroid regimen (addition of sub-Tenon's capsule injection of triamcinolone acetonide) in the dose expansion phase, there was a corresponding reduction in the frequency and severity of inflammation-related AEs (**Figure 2**). Fifty percent (8/16) of the participants who received sub-Tenon triamcinolone experienced an ocular inflammation–related AE, with all events considered mild; almost 90% (26/29) of participants who did not receive sub-Tenon triamcinolone experienced an ocular inflammation–related AE, with 55.2%, 24.1%, and 10.3% considered mild, moderate, and severe, respectively.

Increased IOP was reported in 16/45 (35.6%) participants, all in the treatment cohorts (low dose, n = 5; intermediate dose, n = 9; high dose, n = 2). In 2 participants, the increased IOP event was considered related to AAV5-hRKp.*RPGR* treatment (low dose, n = 1; intermediate dose, n = 1). In 4 participants, the increased IOP event was considered related to surgery (low dose, n = 2; intermediate dose, n = 2). All events of increased IOP were treated medically with standard of care or observation and resolved. As mentioned previously, one SAE of increased IOP occurred outside the reporting period in the control group. This event was treated medically, but ultimately required surgical intervention and resolved on the day of the surgical procedure.

Hematology and chemistry laboratory analysis revealed clinically relevant increases in leukocytes, neutrophils, monocytes, and plasma glucose, increases and

decreases in lymphocytes, and decreases in chloride, urate, and phosphate. These findings are consistent with the tapering course of systemic corticosteroids required per protocol. Elevations of alanine transaminase (ALT) were seen in a few early participants; however, ALT did not move in a singular direction when analyzed across all study participants; increases in ALT considered clinically significant by the investigator were reported as TEAEs. None were considered related to AAV5-hRKp.*RPGR*.

Efficacy

Functional vision

Improvements in functional vision were observed with AAV5-hRKp.*RPGR* administration in participants from the treatment (pooled low and intermediate dose) groups from the dose escalation and dose expansion phases when compared to participants from the control group. Nominal improvements in changes in walk time in the VMA favoring AAV5-hRKp.*RPGR* treatment were seen at the lower lux levels (1 to 16 lux; **Supplementary Video 1**). Given the better-preserved cone function anticipated in this population, a treatment effect was not expected at the higher lux levels, such as 64 lux (**Figure 3**). The least square (LS) mean differences at Week 26 between the treatment participants and the control participants at 1 lux, 4 lux, 16 lux, and 64 lux were –36.96 (95% CI: –68.10, –5.82; nominal $P = 0.022$), –19.48 (95% CI: –39.62, 0.65; nominal $P = 0.057$), –5.58 (95% CI: –10.85, –0.31; nominal $P = 0.039$), and –2.16 (95% CI: –5.18, 0.86; nominal $P = 0.153$) seconds, respectively. The LS mean changes from baseline at Week 52 in the treatment groups at 1 lux, 4 lux, 16 lux, 64 lux were –22.10 (95% CI: –30.12, –14.08), –4.32 (95% CI: –15.50,

6.85), 0.07 (95% CI: –1.45, 1.58), and –0.68 (95% CI: –1.70, 0.33) seconds, respectively.

Retinal function

Retinal function assessments showed improvements in participants treated with AAV5-hRKp.*RPGR* compared to participants in the control group. At Week 26, the percentage of responders in point-by-point static perimetry analysis within the full (photopic) VF was 26.1% for the treatment participants compared to 20.0% for the control (odds ratio: 1.41; 95% CI: 0.23, 8.61; *P* = 1.000; **Table 2**). Although there was no control group at Week 52, the percentage of responders in the point-by-point static perimetry analysis increased to 47.8% for the treatment participants.

Similarly, the MRS within the central 10˚, excluding scotomata, showed static perimetry improvements with AAV5-hRKp.*RPGR* administration; the LS mean change from baseline was 2.41 dB (95% CI: 1.62, 3.20) for the treatment participants and 0.45 dB (95% CI: –0.66, 1.56) for the control participants at Week 26 (**Table 3**). The LS mean difference between the treatment participants and the control participants at Week 26 was 1.96 dB (95% CI: 0.59, 3.34; nominal *P* = 0.006). At Week 52, the LS mean change from baseline was 2.13 dB (95% CI: 1.46, 2.80) for the treatment participants.

MRS in scotopic microperimetry also showed improvements, suggesting AAV5-hRKp.*RPGR* improved cone function under dark-adapted conditions. The LS mean change from baseline at Week 26 was 0.88 dB (95% CI: 0.35, 1.41) for the treatment participants and –0.15 dB (95% CI: –0.97, 0.66) for the control participants (**Table 3**).

The LS mean difference between the treatment participants and the control participants at Week 26 was 1.06 dB (95% CI: 0.05, 2.07; nominal *P* = 0.041). At Week 52, the LS mean change from baseline was 0.79 dB (95% CI: 0.15, 1.43) for the treatment participants.

Visual function

Improvements in visual function were observed with AAV5-hRKp.*RPGR* administration in participants from the treatment (pooled low and intermediate dose) groups when compared to participants from the control group. BCVA assessments showed stability with AAV5-hRKp.*RPGR* treatment (**Table 3**). At Week 26, the LS mean change from baseline in number of ETDRS letters was 0.59 (95% CI: –1.19, 2.37) for treatment participants and –3.05 (95% CI: –5.58, –0.52) for the control participants. The LS mean difference between the treatment participants and the control participants at Week 26 was 3.39 (95% CI: 0.22, 6.56; nominal *P* = 0.037). At Week 52, the LS mean change from baseline was 0.40 (95% CI: –1.51, 2.30) for the treatment participants.

For CS, the LS mean change from baseline was 0.03 LogCS (95% CI: –0.03, 0.10) for the treatment participants and –0.05 LogCS (95% CI: –0.14, 0.04) for the control participants at Week 26. There was little difference in LS means between the treatment group and the control group at Week 26 for CS (0.07; 95% CI: –0.03, 0.18; nominal *P* = 0.173). The LS mean change from baseline at Week 52 was –0.03 LogCS (95% CI: –0.10, 0.04; **Table 3**) for the treatment participants.

Post hoc sensitivity analysis

When applying the phase 3 criteria for static perimetry MRS, which included only participants with data considered reliable (reliability factor: ≤19) and a baseline MRS range of ≥2 to ≤20 dB, the sensitivity analysis demonstrated improvements in walk time, point-by-point responder analysis, and MRS.

In the VMA, the LS mean differences in change from baseline at Week 26 between the treatment participants and the control participants at 1 lux, 4 lux, 16 lux, and 64 lux were –36.97 seconds (95% CI: –71.98, –1.96; nominal *P* = 0.040), –17.88 (95% CI: –38.19, 2.44; nominal *P* = 0.082), –4.06 (95% CI: –7.67, –0.44; nominal *P* = 0.029), and –1.52 (95% CI: –4.29, 1.25; nominal *P* = 0.269), respectively.

In the point-by-point responder analysis of static perimetry at Week 26, the proportion of responders was 23.8% (5/21) for the treatment participants compared to 0% (0/8) for the control participants (odds ratio: 5.67; 95% CI: 0.28, 115.1; *P* = 0.283), with the number of responders increasing to 47.6% (10/21) of the treatment participants at Week 52.

Sensitivity analyses of static perimetry MRS within the central 10˚, excluding scotomata, as well as scotopic microperimetry MRS, were also supportive, with LS mean differences in change from baseline at Week 26 between the treatment participants and the control participants of 2.39 dB (95% CI: 0.94, 3.83; *P* = 0.002) and 1.06 dB (95%: 0.05, 2.07; *P* = 0.041), respectively.

Discussion

This study of 49 males with *RPGR*-XLRP assessed the safety and efficacy of subretinal AAV5-hRKp.*RPGR* gene therapy. Subretinal delivery of AAV5-hRKp.*RPGR* was observed to be feasible, safe, and well tolerated. Safety was generally as expected, with 2 SAEs and 4 severe AEs reported in the immediate treatment cohort. An additional severe SAE was reported outside of the reporting period. Overall, reported AEs were primarily related to ocular inflammation and were deemed related to both the surgical procedure and to AAV5-hRKp.*RPGR*. All ocular inflammation–related AEs responded to treatment with an extended steroid course. Moreover, the introduction of an augmented regimen with sub-Tenon's capsule corticosteroid treatment at the end of surgery reduced the frequency and severity of ocular inflammation-related AEs. Longer-term safety data of AAV5-hRKp.*RPGR* will continue to be collected.

In the randomized, controlled expansion phase (n=32) comparing treated participants to concurrent control participants, improvements were seen in several assessments at Week 26, including functional vision (VMA, shorter walk time after treatment); retinal function (MRS within the central 10˚ in static perimetry and MRS in scotopic microperimetry); and visual function (BCVA, higher number of ETDRS letters seen after treatment). VMA-type assessments for measuring functional vision have been used previously to demonstrate the effectiveness and durability of a therapeutic intervention.[26,27] A Multi-Luminance Mobility Test, similar to the VMA, was used to assess the efficacy of the recombinant AAV vector voretigene neparvovec-rzyl (AAV2-hRPE65v2), the only Food and Drug Administration– and European Medicine Agency–approved retinal gene therapy.[26,27]

At Week 52, increasing or sustained trends of improvement for all perimetry endpoints were observed. The untreated deferred (concurrent control) group was evaluated up to Week 26, so there was no randomized control for the immediate treatment group at Week 52. Moreover, when the static perimetry data were analyzed by applying the MRS criteria being used in the phase 3 study (NCT04671433), which included only participants with data considered reliable (reliability factor: ≤19) and a baseline MRS range of ≥2 to ≤20 dB, the post hoc sensitivity analyses supported—and further strengthened—the efficacy outcomes observed at Weeks 26 and 52. Presently, *RPGR*-XLRP is an incurable genetic disease, in which patients experience almost certain blindness by the fourth decade of life. A gene therapy–based agent has the potential to change the treatment landscape for the disease. The safety and efficacy results reported herein support further efficacy evaluation of AAV5-hRKp.*RPGR* administration. It is important to note that this study was conducted to evaluate safety and assess potential indicators of efficacy of AAV5-hRKp.*RPGR*, and thus was not powered for efficacy. There was no formal sample size calculation, and therefore, nominal 2-sided *P*-values ($\alpha<0.05$) are provided for descriptive purposes only and should be interpreted with caution.

Two other clinical programs have assessed subretinal AAV-mediated gene therapy in *RPGR*-XLRP. Biogen conducted a phase 2/3 study (XIRIUS trial; NCT03116113) which demonstrated subretinal delivery of BIIB112 (cotoretigene toliparvovec), an AAV8 vector expressing full-length *RPGR,* to be well tolerated in male participants with *RPGR*-XLRP.[28] However, the study failed to meet its primary endpoint (i.e., statistically significant improvement in the proportion of treated study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed

by microperimetry).[28] However, results from a recent post hoc analysis of retinal sensitivity following BIIB112 treatment in 18 patients from the XIRIUS trial compared with 103 patients from the Natural History of the Progression of XLRP (XOLARIS) trial suggest additional clinical trials may be warranted as early and sustained improvements in visual function through 12 months were observed.[29] In another phase 2 study from Applied Genetic Technologies Corporation (Skyline trial; NCT03316560), participants with *RPGR*-XLRP received subretinal administration of low or high dose rAAV2tYF-GRK1-RPGR, a recombinant AAV2 vector expressing a codon optimized full-length human *RPGR* cDNA.[30]

Given the encouraging safety and efficacy data from the current study, a phase 3 randomized, controlled study of AAV5-hRKp.*RPGR* (NCT04671433) has completed enrollment and treatment of participants with *RPGR*-XLRP at study sites in Belgium, Canada, Denmark, France, Israel, Italy, the Netherlands, Spain, Switzerland, the United Kingdom, and the United States. The primary endpoint being evaluated is change from baseline to Week 52 in VMA, with secondary endpoints including assessments of retinal function, visual function, functional vision, safety, and tolerability.

Limitations

The phase 1/2 AAV5-hRKp.*RPGR* gene therapy study reported here was conducted in a prospective manner with a large randomized, controlled expansion phase, however certain limitations are expected. The prospective nature of the study limited the follow-up of the current report to 52 weeks. Understanding of the safety and efficacy of AAV5-hRKp.*RPGR* may be limited due to the lack of longer-term data.

Second, some of the visual assessments used in the study are not routine to clinical practice and may not mirror real-world outcomes. Thirdly, the concurrent control group in the expansion phase received treatment at Week 26 and could not be used as an untreated comparator at Week 52. Lastly, due to the COVID-19 pandemic, some participants missed certain efficacy assessments.

Conclusions

This phase 1/2 study demonstrated that subretinal delivery of AAV5-hRKp.*RPGR* is safe and well tolerated. At Week 26, improvements were observed in functional vision, retinal function, and visual function in treated participants compared to untreated controls. These data support the conduct of the ongoing phase 3 study of AAV5-hRKp.*RPGR* gene therapy in patients with *RPGR*-XLRP.

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Figure Legends

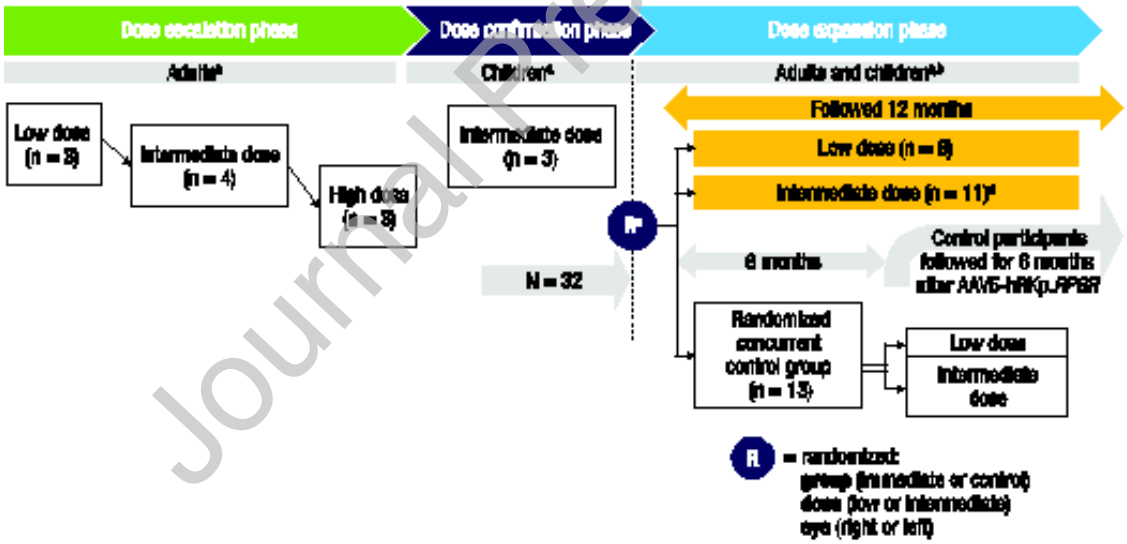

Figure 1. Study design.

An open-label, multicenter, phase 1/2 dose escalation, dose confirmation, and randomized, controlled dose expansion trial of recombinant adeno-associated virus vector (AAV5-hRKp.*RPGR*) for gene therapy of adults and children with *RPGR*-

associated XLRP (NCT03252847). This study was conducted at 5 sites in the United States and United Kingdom.

RPGR, retinitis pigmentosa GTPase regulator.

[a]Eligible adults were ≥16 years of age in the United Kingdom and ≥18 years of age in the United States; eligible children were ≥5 years of age and <16 years in the United Kingdom or <18 years in the United States.

[b]While adults and children were eligible to participate in the dose expansion phase of the study, only adults were enrolled.

[c]1:1:1 randomization.

[d]One participant assigned to the intermediate dose group of the dose expansion phase was inadvertently treated with the high dose.

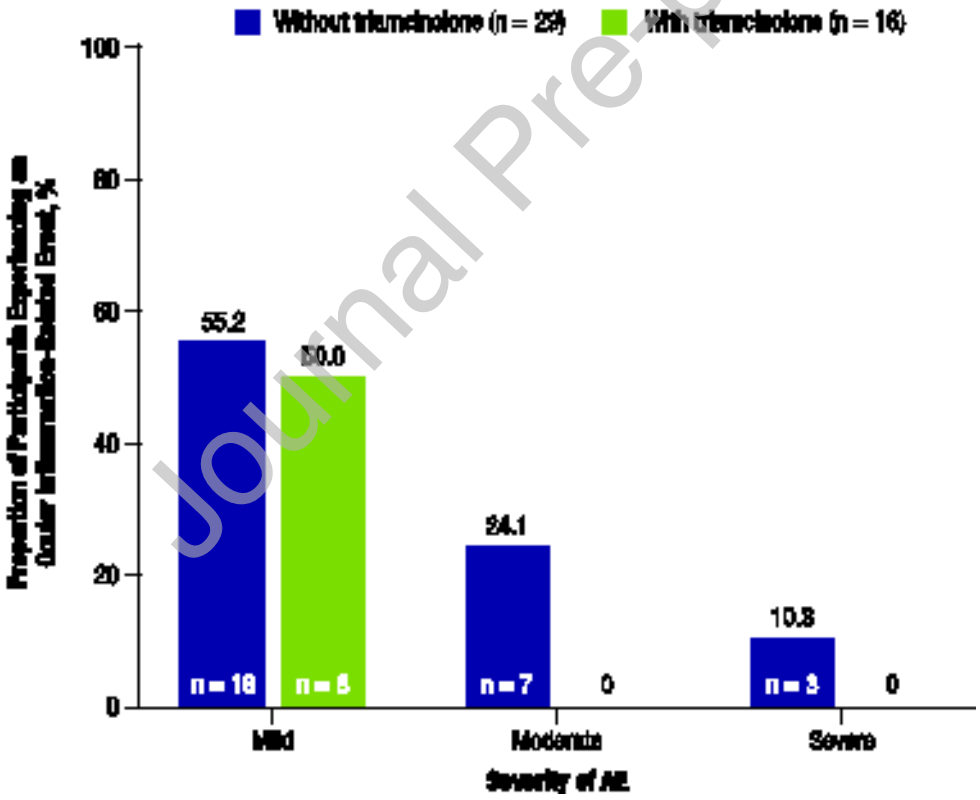

Figure 2. Participants with ocular inflammation–related AEs by severity of AE.[a,b]

Following implementation of a modified prophylactic steroid regiment in the expansion phase of the study, a reduction in inflammation-related AEs was observed.

AE, adverse event.

[a]A sub-Tenon's capsule injection of triamcinolone was administered at the end of surgery as add-on therapy to standard steroid prophylaxis to help control ocular inflammation.

[b]Includes data throughout the treatment period including 6-months following treatment.

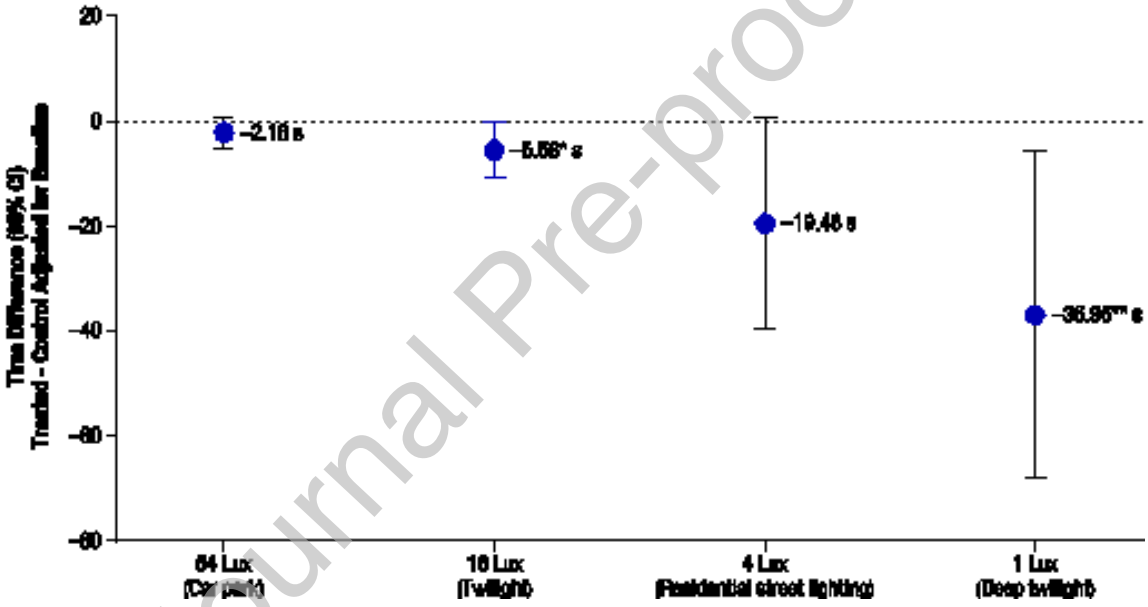

Figure 3. Walk time at Week 26 compared to baseline.

Significant improvements in walk time compared to baseline were observed at Week 26.

Pooled low/intermediate: 1 lux (n = 5); 4 Lux (n = 7); 16 Lux (n = 6); 64 Lux (n = 6).

Concurrent control: 1 lux (n = 10); 4 Lux (n = 12); 16 Lux (n = 11); 64 Lux (n = 11).

CI, confidence interval.

*Nominal *P* = 0.039.

**Nominal *P* = 0.022.

Table 1. Baseline Demographics and Characteristics

	Dose escalation phase			Dose confirmation phase	Dose expansion phase				
	Low n = 3	Intermediate n = 4	High n = 3	Intermediate n = 3	Low n = 8	Intermediate n = 10	High [a] n = 1	Control n = 13	Total N = 45
Mean age (range), years	27.0 (24, 30)	24.8 (19, 29)	21.0 (18, 24)	13.3 (11, 15)	31.1 (18, 60)	30.4 (22, 47)	20.0 (20, 20)	31.5 (19, 61)	28.1 (11, 61)
Male, n (%)	3 (100)	4 (100)	3 (100)	3 (100)	8 (100)	10 (100)	1 (100)	13 (100)	45 (100)
Race, n (%)									
Black or African American	0	0	1 (33.3)	0	0	0	0	0	1 (2.2)
White	3 (100)	3 (75)	2 (66.7)	3 (100)	7 (87.5)	9 (90)	1 (100)	13 (100)	41 (91.1)
Other	0	1 (25)	0	0	1 (12.5)	1 (10)	0	0	3 (6.7)
Ethnicity, n (%)									
Hispanic or Latino	0	0	0	0	0	1 (10)	0	2 (15.4)	3 (6.7)
Not Hispanic or Latino	3 (100)	4 (100)	3 (100)	3 (100)	8 (100)	9 (90)	1 (100)	11 (84.6)	42 (93.3)
Mean BMI (range), kg/m^2	25.1 (20.5, 28.7)	26.3 (24.1, 28.7)	24.7 (21.1, 28.8)	19.8 (17.1, 21.4)	29.2 (21.6, 37.1)	29.1 (20.6, 35.8)	25.9 (25.9, 25.9)	26.2 (19.7, 31.5)	26.8 (17.1, 37.1)
Retinal sensitivity [*], n (%), dB									
≤10 dB	3 (100)	4 (100)	2 (66.7)	1 (33.3)	5 (62.5)	7 (70.0)	0	11 (84.6)	33 (73.3)
>10 dB	0	0	1	2 (66.7)	3	3 (30.0)	1 (100)	2 (15.4)	12

			(33.3)		(37.5)				(26.7)

BMI, body mass index; dB, decibel; *Octopus 900 full-field static perimetry.
[a]One participant assigned to the intermediate dose group of the dose expansion phase was inadvertently treated with the high dose.

Table 2. Responders in Point-by-Point Data in Static Perimetry Within the Full Visual Field

	Full Analysis Set – dose escalation + dose expansion[a]	
	Low + intermediate dose	Control
Responder[b], n/N (%)		
Week 26[c]	6/23 (26.1)	2/10 (20.0)
Week 52[d]	11/23 (47.8)	—[e]

[a]The Full Analysis Set was comprised of all treatment participants who completed ≥1 baseline visit and 1 visit after AAV5-hRKp.*RPGR* administration, and all control participants who completed ≥1 baseline visit and 1 post baseline visit prior to AAV5-hRKp.*RPGR* administration.

[b]Responder criteria: At least a 7 dB improvement from baseline in ≥5 individual loci, with the same 5 loci showing improvement at 2 time points following treatment.

[c]Week 26: number of participants who completed assessments at both Week 26 and Week 13.

[d]Week 52: number of participants who completed assessments at Week 52 and ≥1 visit prior to Week 52.

[e]For control participants, this table only summarizes data prior to AAV5-hRKp.*RPGR* administration and serves as a control group. These participants were treated after Week 26. There are no Week 52 data for these participants.

Table 3. Retinal and Visual Function Efficacy Assessments

	Full Analysis Set – dose escalation + dose expansion[a]	
	Low + intermediate	Control
Retinal function assessments		
Static perimetry (MRS 10°), dB		
Baseline		
N	24	13
Mean (SD)	18.91 (3.99)	17.36 (5.06)
Week 26		
N	24	13
LS mean change from baseline (±95% CI)	2.41 (1.62, 3.20)	0.45 (–0.66, 1.56)
LS mean treated – control difference	1.96 (0.59, 3.34)*	—

(±95% CI)[b]		
Week 52		
N	23	—
LS mean change from baseline (±95% CI)	2.13 (1.46, 2.80)	—
Microperimetry (MRS-scotopic red), dB		
Baseline		
N	21	7
Mean (SD)	0.81 (1.13)	0.76 (1.08)
Week 26		
N	15	7
LS mean change from baseline (±95% CI)	0.88 (0.35, 1.41)	–0.15 (–0.97, 0.66)
LS mean treated – control difference (±95% CI)[b]	1.06 (0.05, 2.07)**	
Week 52		
N	17	—
LS mean change from baseline (±95% CI)	0.79 (0.15, 1.43)	—
Visual function assessments		
BCVA, number of ETDRS letters		
Baseline		
N	26	13
Mean (SD)	67.8 (9.56)	71.1 (8.89)
Week 26		
N	25	13
LS mean change from baseline (±95% CI)	0.59 (–1.19, 2.37)	–3.05 (–5.58, –0.52)
LS mean treated – control difference (±95% CI)	3.39 (0.22, 6.56)***	—
Week 52		
N	23	—
LS mean change from baseline (±95% CI)	0.40 (–1.51, 2.30)	—
CS, LogCS		
Baseline		
N	26	13
Mean (SD)	1.25 (0.39)	1.14 (0.36)
Week 26		
N	22	13
LS mean change from baseline (±95% CI)	0.03 (–0.03, 0.10)	–0.05 (–0.14, 0.04)
LS mean treated – control difference (±95% CI)	0.07 (–0.03, 0.18)	—
Week 52		
N	23	—

LS mean change from baseline (±95% CI)	–0.03 (–0.10, 0.04)	—

BCVA, best corrected visual acuity; CI, confidence interval; CS, contrast sensitivity; dB, decibel; LS, least square; MRS, mean retinal sensitivity; SD, standard deviation.

[a]The Full Analysis Set was comprised of all treatment participants who completed ≥1 baseline visit and 1 visit after AAV5-hRKp.*RPGR* administration, and all control participants who completed ≥1 baseline visit and 1 post baseline visit prior to AAV5-hRKp.*RPGR* administration.

[b]Adjusted for baseline, 2-sided nominal *P* value.

*Nominal *P* = 0.006.

**Nominal *P* = 0.041.

***Nominal *P* = 0.037.

Author Statements

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Table of Contents Statement *(limit: 75 words; current 75 words)*

This phase 1/2, open-label trial (NCT03252847) investigated the safety and efficacy of subretinal AAV5-hRKp.*RPGR* gene therapy in participants with retinitis pigmentosa GTPase regulator (RPGR)-associated X-linked retinitis pigmentosa (XLRP). AAV5-hRKp.*RPGR* demonstrated an acceptable safety and tolerability profile, with most adverse events (AEs) related to the surgical procedure and resolving without intervention. Treatment with AAV5-hRKp.*RPGR* resulted in improvements in functional vision and retinal sensitivity compared with the deferred control group, supporting investigation in a phase 3 trial.