Protocol Title: A Phase I/IIa Study of *RS1* Ocular Gene Transfer for X-linked Retinoschisis

Abbreviated Title: XLRS2

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Site Principal Investigator:

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Contracted Laboratory: [redacted]

Human Research Protections Program Investigator and Staff Training:

"Just in time" human subjects protection training courses are required for investigators and staff participating on this protocol: None.

Total Requested Accrual:

1. Up to 24 male participants with XLRS.
2. No healthy volunteers.

Project Uses Ionizing Radiation: ☒ No ☐ Yes

IND/IDE: ☐ No ☒ Yes

Drug/Device/#: _____16223_____

Sponsor: [redacted]

Sponsor Contact: [redacted]

Durable Power of Attorney:	☒ No ☐ Yes
Multi-institutional Project:	☒ No ☐ Yes
Data and Safety Monitoring Board:	☐ No ☒ Yes
Technology Transfer Agreement:	☐ No ☒Yes
Confidential Disclosure Agreement:	☐ No ☒Yes
Samples Stored For Future Use:	☐ No ☒ Yes
Flesch-Kincaid Reading Level of Consent Form:	8.3

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PROTOCOL ABBREVIATION LIST

AC	Anterior Chamber
AAV	Adeno-associated Virus
ALT	Aspartate Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
b/a	b-wave/a-wave ratio
BCVA	Best-corrected Visual Acuity
BD	Becton-Dickinson
BUN	Blood Urea Nitrogen
CAI	Carbonic Anhydrase Inhibitors
CBC	Complete Blood Count
CSA	Cyclosporine
CTCAE	Common Terminology Criteria for Adverse Events
DEC	Deputy Ethics Commissioner
DM	Diabetes Mellitus
DMC	Data and Safety Monitoring Committee
DoH	Declaration of Helsinki
ERG	Electroretinography
ETDRS	Early Treatment Diabetic Retinopathy Study
EVA	Electronic Visual Acuity
FA	Fluorescein Angiography
FACS	Fluorescence Analysis Cell Sorting
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GLP	Good Laboratory Practices
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HED	Human equivalent dose
HIV	Human Immunodeficiency Virus
IOP	Intraocular Pressure
IRB	Institutional Review Board
IRBP	Interphotoreceptor Retinoid-binding Protein
IV	Intravenous

IVT	Intravitreal
IP	Investigational Product
LPL	Lipoprotein Lipase
MFD	Maximal Feasible Dose
mL	Milliliter
MOP	Manual of Procedures
MPW	Medical Pathological Waste
NEI	National Eye Institute
NeoR	Neomycin Resistance
NIH	National Institutes of Health
NOAEL	No-observed-adverse-effect level
NOEL	No observed effect level
NZW	New Zealand White
OCT	Optical Coherence Tomography
OD	Right Eye
OS	Left Eye
OU	Both Eyes
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PPD	Purified Protein Derivative
RPE	Retinal Pigment Epithelium
RPR	Rapid Plasma Reagin
RS1	Retinoschisin
Rs1-KO	Retinoschisin Knockout
SAE	Serious Adverse Event
SR	Sub-retinal
ULN	Upper Limit of Normal
UP	Unanticipated Problem
Vg	Vector Genomes
XLRS	X-linked Retinoschisis

PRÉCIS

Objective: To evaluate the safety and tolerability of ocular AAV-*RS1* vector (AAV8-scRS/IRBP-hRS) gene transfer to the retina of participants affected with X-linked juvenile retinoschisis (XLRS).

Study Population: Male participants affected with XLRS will receive ocular gene transfer. A maximum of up to 24 participants may be enrolled.

Design: This is a Phase I/IIa, prospective, dose escalation, single-center study. One eye of each participant will receive the AAV-*RS1* gene vector application by intravitreal injection. Participants will be closely monitored in conjunction with DMC oversight. Participants will be followed for 18 months after which they will continue to be followed for 5 years after enrollment, or per FDA requirements, for further safety analysis.

Outcome Measures: The primary outcome is the safety of ocular AAV-*RS1* vector as determined from assessment of retinal function, ocular structure and occurrence of adverse events and laboratory tests. Secondary outcomes include changes in visual function, electroretinogram (ERG) responses, visual field measurements, retinal imaging with optical coherence tomography (OCT), and the formation of anti-AAV and anti-RS1 antibodies.

Statistics: No formal sample size calculations are used in this Phase I/IIa dose-escalation study.

1.0 INTRODUCTION/SCIENTIFIC RATIONALE

1.1 Juvenile Retinoschisis Disease

Juvenile retinoschisis is a genetic X-linked recessive retinal disease (XLRS) that affects 1:5000 to 1:25000 males worldwide and is one of the more common causes of vision loss from juvenile macular degeneration affecting young men [1]. To-date there is no approved treatment for XLRS.

1.1.1 Disease Onset

XLRS-affected males generally present during childhood and come to medical attention by early grade school years. The condition manifests clinically with cystic changes in the macula and loss of visual acuity. Clinical retinoschisis changes extend into the peripheral retina and cause lamellar splitting through multiple retinal planes, both in the nerve fiber layer at the retinal surface and also deeper in the retina. Peripheral retinoschisis occurs most commonly in the infero-temporal retina [2]. About half of affected males have clinical evidence of peripheral retinoschisis [3] and upwards of 10% of cases have full thickness retinal detachment of peripheral schisis. Optical coherence tomography (OCT) has increased the clinical detection considerably [4], and by using this method peripheral schisis has been detected in the majority of XLRS males [5].

1.1.2 Progression

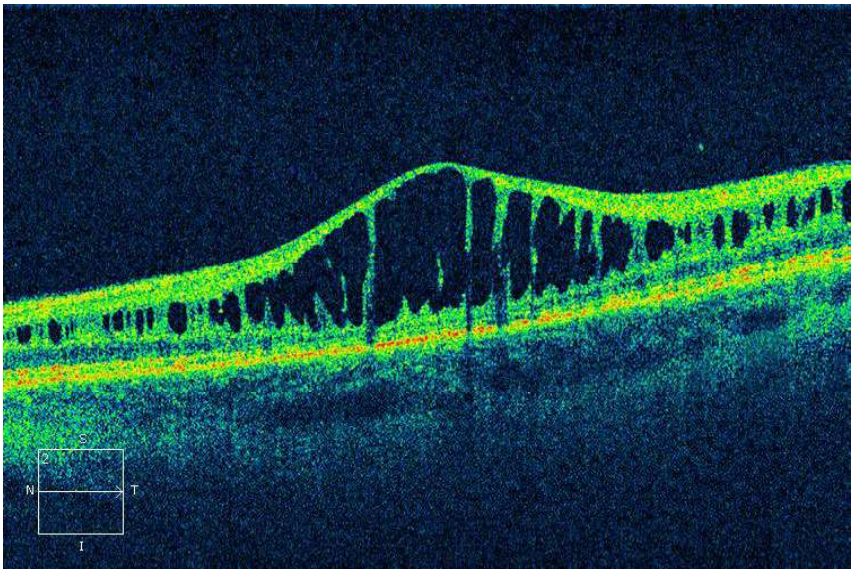
The clinical phenotype of XLRS is quite broad at the time of clinical presentation resulting from symptomatic awareness by the affected boy or by his parents [6]. Visual deterioration and central visual acuity loss typically evolve during the first two decades of life. In the mature retina, functional abnormalities also include diminished electroretinogram (ERG) responses and structural evolution of the schisis cavities. Slow progression frequently continues into the fifth and sixth decades, and, in later age, further macular degeneration commonly causes additional visual failure [2]. XLRS patients over age 50 years frequently have macular pigmentary changes and macular atrophy [7]. Progression into older age results in decreased central vision [8].

1.1.3 Retinal Structure

In boys and young men with retinoschisis, photoreceptor outer segment structure and function ranges from essentially normal to disrupted, whereas marked abnormalities are evident in the anatomy and physiology of more proximal layers of the retina which typically have large cystic cavities [5, 9]. In the foveal area, these abnormalities extend from the outer plexiform layer (the synaptic layer between photoreceptor cells and bipolar cells) to the inner plexiform layer. In the extrafoveal retina, schisis involves not only the inner nuclear layer (which contains bipolar cell nuclei) but also, and usually to a lesser degree, the outer nuclear layer (photoreceptor cell nuclei), and the ganglion cell layer [5].

The presence of schisis cavities in the plexiform and nuclear layers of the retina, as seen using OCT imaging, is the hallmark of XLRS (Figure 1).

Figure 1: OCT scan typical of patient with XLRS showing the characteristic intraretinal cysts



The best corrected visual acuity (BCVA) in this patient with XLRS is 20/125.

These cavities usually also extend into the perifovea and even peripheral retina [4]. Current commercially available spectral-domain OCT instruments allow the measurement of a retinal volume (6 mm length x 6 mm width x retinal thickness) at the fovea. Furthermore, there is software to align images at different visits to follow retinal anatomy in a specific location over time. Thus, OCT provides the means of quantifying both the size of schisis cavities and a method to judge how they change in size as a function of time.

1.1.4 Retinal Function as Measured by ERG

Patients with XLRS lack normal sensitivity for perception in dim light, as absolute threshold is impaired in these individuals consistent with loss of integrity of the rod visual pathway through on-bipolar cells. The amplitude and implicit time of the ERG a-wave, which is generated by photoreceptors, range from normal to modestly sub-normal in most boys and men up to 55 years with XLRS [9, 10]. In contrast, the amplitude of the ERG b-wave generated predominantly by the second-order retinal depolarizing bipolar cells is distinctly reduced in amplitude in this disease. The concomitant reduction in the ERG b-wave/a-wave (b/a) amplitude is one of the clinical hallmarks of XLRS. The reduction in the b/a ratio provides a quantitative measure of the disruption of photoreceptor to bipolar cell signaling in an XLRS subject. This variable has been shown to be stable in the cohort of patients followed with repeated measurements (data below).

The timing of the flicker ERG responses, generated by the cone photoreceptor system, is also frequently delayed in XLRS [9]. The function of the cone pathway through both on- and off-bipolar cells is also impaired [9], and for many XLRS-affected males, visual acuity mediated by cone circuits is reduced to a level below that required for a driver's license in most states. The acuity deteriorates further with age, albeit at a slow rate over many years. Thus, measurement of ERG amplitudes and implicit times provide quantitative measures of retinal function. Further, the ERG b/a ratio provides a quantitative measure of the degree to which photoreceptor to bipolar cell transmission is affected in retinoschisis patients.

1.2 Genetic Basis for XLRS

XLRS is a monogenic trait caused by mutations in the *RS1* gene [11, 12]. Women who carry the trait rarely experience vision abnormalities indicating that haploinsufficiency does not result in obvious visual deficiencies. The *RS1* gene product, retinoschisin, is expressed primarily in the retina [13, 14] and has also been found in the pineal gland [13]. Retinoschisin (RS1) is a 224 amino acid secretory protein that is present throughout the neural retina [14-17]. Retinoschisin gene expression is localized to photoreceptors in both human [15] and mouse retina [14-16, 18], as well as to neurons in the inner nuclear layer [14, 16], and ganglion cell layer [15, 16, 18]. Thus, retinoschisin synthesis is expressed in selective cells as regulated by the retinoschisin promoter.

Retinoschisin has a signal peptide sequence that allows for transport of the protein to the extracellular surface [19, 20]. It also contains a single discoidin domain [11, 15]. Discoidin domains are associated with a family of extracellular cell surface proteins that are involved in cell adhesion and signaling and that interact with collagens to regulate extracellular matrix modeling [21]. Ten (10) cysteine residues within the retinoschisin discoidin domain are important for appropriate protein folding and the formation and function of retinoschisin dimers and octamers [22]. For these reasons, RS1 is thought to be critical for cell adhesion during the normal

development and maintenance of retinal structure. This is consistent with other discoidin domain-containing proteins and is supported by clinical observations in patients and in mouse models [23]. Moreover, we have localized RS1 protein in the photoreceptor synapse using immune-electron microscopy and find that the retinoschisin protein lies in the synaptic cleft and post-synaptic element, consistent with its functional role as being critical to the production of the b-wave [24]. New data demonstrate that there is loss of the synaptic receptor proteins in mouse models of XLRS which lack the retinoschisin protein.

Retinoschisin expression begins during early postnatal retinal development and is maintained throughout adult life [14]. However, as the eye develops, additional retinal neurons produce retinoschisin, including the outer retinal cells, demonstrating that retinoschisin is produced by multiple retinal neuron cells, including the inner segment of rod cells. Interestingly, RS1 protein is also associated with bipolar cells, suggesting that it may be critical for maintaining the structural connection of postsynaptic and presynaptic elements in the outer plexiform layer that is necessary for the transmission of a visual signal from photoreceptors to bipolar cells [14, 16, 17]. This implies that monitoring synaptic integrity by the ERG b-wave should be a suitable means for gauging this connectivity. These findings support the hypothesis that retinoschisin plays a critical role in maintaining cell-cell adhesion and in preserving retinal structure and function.

1.3 Current Treatments for XLRS

Currently, there are no proven FDA-approved treatments for XLRS. Various experimental treatment strategies have been considered, including the use of carbonic anhydrase inhibitors, dorzolamide and acetazolamide [25, 26]. It is thought that the macular cystic spaces in human XLRS disease are filled with excess intraretinal fluid which impacts acuity. As such, carbonic anhydrases work to acidify the subretinal space and thereby increase fluid transport through the retinal pigment epithelium (RPE) by decreasing the standing potential. In contrast, data from the NIH indicate that RS1 protein directly affects asynaptic receptor protein localization to the dendritic tips, thereby explaining the deficient ERG b-wave, and, by extension, would impact acuity [27]. Oral acetazolamide was employed in a single case report [25], while topical dorzolamide has been used in several small studies beginning with a first report of eight participants [28]. In this report, a reduction in the size of macular cysts was observed and a small improvement in visual acuity was noted in most patients. Another paper studied “sustained treatment”, ranging from 4 - 41 months, with an average of 16 months of treatment with topical carbonic anhydase inhibitors [29]. While this follow-up time is relatively longer than previous studies, it is still relatively short term given the participants’ young age when these changes manifest. Additionally, this paper reported a mix of responses to therapy ranging from apparent benefit to no effect, to apparent worsening. The safety of systemic carbonic anhydrase inhibitors for retinal treatment, particularly beginning at early age, is problematic, as continued life-long use likely would be required. The topical treatment is well-tolerated for the most part and has a favorable safety profile; however, larger studies with longer duration will be needed to demonstrate efficacy.

One study conducted in RS1 knockout (*Rs1*-KO) mice indicated that treatment with carbonic anhydrase inhibitors failed to show either short-, intermediate- or long-term evidence of structural improvement [30].

A three-patient retrospective study [31] described an effect of vitreoretinal surgery on XLRS. A reduction in macular schisis was observed in all cases, with a questionable modest improvement in acuity in two of five eyes. No significant change was observed in the ERG.

None of these approaches are FDA-approved for XLRS treatment, nor is it known that any putative changes would persist beyond the acute intervention, as the underlying genetic pathology is not addressed. Further, it is worth noting that these reports describe short-term pilot studies and are therefore unable to demonstrate a sustained effect of their interventions. Thus, there is a need to identify and develop new therapies that will provide sustained benefit to XLRS individuals.

1.4 Rationale for Gene Transfer Therapy

1.4.1 Carrier State

X-linked retinoschisis carrier women have essentially no change in retinal function, and it is extremely rare to have overt symptomatic visual reduction. Instead, the primary concern for women with XLRS are the offspring of an affected man and a carrier woman, usually in the context of a consanguineous family [32]. Sparing of the heterozygous carrier female is likely due to the nature of the product of the RS1 gene, the protein retinoschisin [19].

Retinoschisin is a secreted protein, and even a reduced amount of protein synthesized in the retina of a female carrier appears to fulfill its function as a putative adhesion/stabilization molecule. As a consequence of random inactivation of one X chromosome in female XX cells, (i.e., lionization), the retina of XLRS carrier women will contain a mixture of both normal and mutant gene products, and the lack of overt retinopathy in carriers thereby argues against a dominant negative effect of mutant retinoschisin protein.

If the mutant gene product were to interfere with normal retinoschisin expression and function, heterozygous carrier women would manifest some (albeit possibly slight) XLRS phenotype. This is relevant for the gene augmentation strategy to treat XLRS-affected males. Co-expressing wild-type and mutant retinoschisin protein does not impair normal folding, sub-unit assembly, and secretion in human embryonic kidney cells stably expressing the Epstein-Barr virus nuclear antigen-1(HEK293-EBNA) [33], as demonstrated in the NIH laboratory for several mutations found in NIH XLRS patients. The absence of clinical disease in carrier women also implies that full replacement of expression levels is not requisite to promote therapeutic function. Gene replacement therapy is aimed at addressing the fundamental biological need of the retina for the retinoschisin protein and the apparent role of this protein in maintaining retina architecture, synaptic integrity and function. The general strategy and efficacy of ocular gene transfer for delivery of essential proteins to the retina to treat genetic recessive disease has been validated by three human clinical trials of RPE65 gene transfer therapy in participants with Leber Congential Amaurosis [34-36].

1.4.2 Animal Model of XLRS

As there are no natural models of XLRS, the NIH created a mouse RS1 knockout model (Rs1-KO) of XLRS using transgenic technology to substitute a neomycin resistance expression cassette in place of exon 1 and 1.6 kb of intron 1 of the mouse Rs1 gene. This truncates transcription of the gene and eliminates expression of retinoschisin protein by Western blot analysis and by immunohistochemistry of retinal sections [37].

Structural and functional changes observed in the Rs1-KO mouse eye recapitulate the findings typical for human XLRS-affected males. The Rs1-KO model displays the classical characteristics of human XLRS disease, retinal splitting within the retina through the nerve fiber layer. The Rs1-KO mouse also shows reductions in b-wave amplitudes which coincides with the clinical findings in dark-adapted thresholds for XLRS patients. There are currently no larger animal eye model systems that replicate XLRS disease in humans. Overall, studies in the Rs1-KO mouse model showed evidence for a mechanism of action for the AAV8-scRS/IRBP-hRS IP. This holds true also for additional transgenic XLRS models in mouse and rat [38]. It was also demonstrated that animals treated with AAV8-scRS/IRBP-hRS had a favorable safety profile including toxicology and has established the proposed clinical starting and maximum doses [39-41].

1.4.3 In vivo Pre-clinical Efficacy Studies of AAV8-scRS/IRBP-hRS in a Mouse Model of XLRS

The gene transfer strategy used in this proposed study utilizes a replication-deficient, AAV serotype 8 vector that contains the human retinoschisin promoter (RS/IRBP) and the complete human retinoschisin cDNA (GenBank NCBI Reference Sequence—NM_000330.3). Importantly, we have designed AAV8-scRS/IRBP-hRS with the use of the retinoschisin promoter so that gene expression is activated in selected cells (e.g., retinal cells) [13]. More information on this vector is described in Section 1.5.

In the in vivo studies with AAV8-scRS/IRBP-hRS, we examined male *Rs1*-KO mice whose eyes were injected with a single intravitreal inoculation of the investigational product. In this series of studies, vehicle or AAV8-scRS/IRBP-hRS vector at doses of 1e6, 1e7, 5e7, 1e8, 5e8 and 2.5e9 vector genome (vg)/eye, were administered by intravitreal injection to 18-34 day old *Rs1*-KO mice. The mice were then evaluated by ERG for retinal function at 11-15 weeks and 6-9 months post-injection, followed by OCT for retinal structure and immunohistochemistry for retinoschisin expression. Experiments were designed to determine the dose range over which this vector significantly preserves retinal function and structure in the *Rs1*-KO mouse and achieves significant retinal expression of protein.

Study results showed a clear dose response, with data demonstrating that doses of 1e8 to 2.5e9 vg/eye in a mouse consistently improved retinal function at “Long Term” as well as “Short Term” time points.

There was further improvement in retinal structure at doses of 1e8, 5e8 and 2.5e9 vg/eye as indicated by statistically significant reductions ($P < 0.0001$) in schisis cavity score at these doses compared to 5e7 vg/eye.

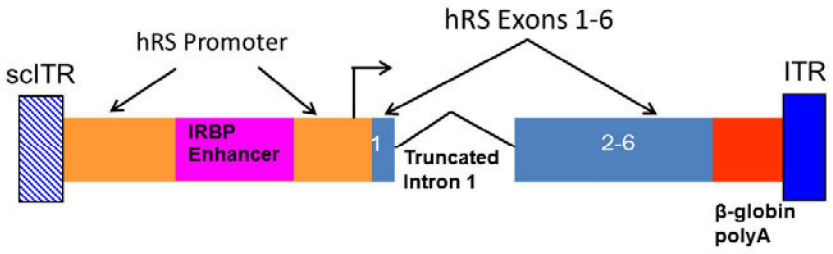
It was also shown that there was significant expression of retinoschisin compared to untreated eyes (zero) at all doses except 1e6, and the three highest doses had levels of expression that were statistically higher than expression at 5e7 vg/eye ($P < 0.05$, 0.0001, 0.0001, respectively, one-way analysis of variance (ANOVA) with Tukey's correction for multiple comparisons) and not significantly different from each other. The 2.5e9, 5e9 and 1e8 vg/eye doses were also evaluated at the Long Term time point (6-9 months, post-injection), and durability of expression was maintained at these three doses as indicated by values that are higher than at the Short Term time point.

Moreover, the retinoschisin expression studies indicate that full retinoschisin protein levels (as in a wild-type mouse eye) are not necessary for retinal restoration. As low as ~30% of the wild-type retinoschisin level is sufficient to significantly preserve retinal function and architecture. This coincides with the finding that the majority of female carriers with retinoschisin mutation(s) do not experience visual acuity loss, suggesting a full complement of retinoschisin is not required to maintain normal vision.

1.5 Description of AAV8-scRS/IRBP-hRS

AAV8-scRS/IRBP-hRS, is a replication-deficient, nonpathogenic, adeno-associated virus type 8 vector that delivers a self-complementary vector genome that contains the complete human retinoschisin cDNA. This human retinoschisin cDNA sequence is a normal sequence synthesized in the laboratory. Gene expression is driven from a tissue-selective modified human retinoschisin promoter, thus limiting gene expression. Promoter activity is augmented by including an interphotoreceptor retinoid-binding protein (IRBP) enhancer element. The expression cassette uses a truncated retinoschisin first intron, a human beta-globin 3' untranslated region, and polyadenylation site. The vector plasmid for the production of AAV8-scRS/IRBP-hRS, called pAAV scRS/IRBP-hRS, is composed of the entire human retinoschisin cDNA (six exons with a partial fragment of intron 1) as described above, bounded by AAV2 inverted terminal repeat sequences. The structure of its genome is shown below in the vector plasmid diagram (Figure 2). This vector plasmid, together with a plasmid expressing AAV2 Rep and AAV8 Cap proteins, and a plasmid carrying adenovirus genes that help AAV replication and packaging (pCCVC-AD2HPv2), were co-transfected into HEK-293 cells to produce AAV8-scRS/IRBP-hRS vector. The Children's Hospital of Philadelphia produced the GMP material for this trial. AAV8-scRS/IRBP-hRS will be formulated as a liquid for administration to the eye. It will be stored at < -60°C in cryovials until ready for use.

Figure 2: AAV8-scRS/IRBP-hRS Vector Plasmid Diagram



Abbreviations: hRS= human retinoschisin; scITR= self-complementary inverted terminal repeats; IRBP= interphotoreceptor retinoid-binding protein; ITR= inverted terminal repeats

1.5.1 Rationale for Use of Adeno-Associated Virus Serotype 8 (AAV8)

Adeno-associated virus (AAV) vectors are one of the most common virus systems under development for gene transfer. Several aspects of the natural biology of AAV make them suitable for somatic cell gene transfer: 1) adeno-associated viruses are non-pathogenic, 2) the viruses are able to transduce replicating and non-replicating cells, 3) AAV requires only terminal repeats in *cis* for packaging and particle assembly, thus eliminating viral coding DNA, 4) does not or integrates only at very low frequencies into genomic DNA (Guidance for Industry Gene Therapy Clinical Trials—Observing Subjects for Delayed Adverse Events, Table 1), 5) with a long-acting promoter can provide stable expression, and 6) compared to other viral vectors (e.g., adenoviral vectors), adeno-associated vectors induce a relatively mild immune response. For the above-mentioned reasons, AAV2 vectors were advanced into clinical testing for numerous indications, including several trials in the eye (Leber's congenital amaurosis, choroideremia, and wet age-related macular degeneration). However, several studies now demonstrate that the seroprevalence of antibodies to AAV2 in the population ranges from 30-60% [42-44] and that neutralizing antibodies potentially can interfere in vector transduction. In contrast, AAV8 vectors have a lower seroprevalance in humans [45] and transduce retinal cells, in particular photoreceptor cells, better than AAV2 vectors [46-48]. AAV8 was selected instead of AAV2 because of the lower seroprevalence of neutralizing antibodies in the human population for AAV8 and its superior tropism for retinal cells [49]. Results from extensive preclinical studies demonstrate that gene delivery by AAV8 encoding the human retinoschisin cDNA under the regulation of a retina-specific promoter leads to long-term vector–delivered expression of the transgene, and effectively reduces retinal cavities and restores b-wave function in a murine model of XLRS [37, 50] (see Section 1.4.3).

1.5.2 Rationale for Intravitreal Delivery of AAV8-scRS/IRBP-hRS

AAV vectors have been used in the eye for several ocular indications utilizing subretinal delivery. They have shown great promise and in some cases efficacy, however subretinal delivery has several limitations [34-36, 51]. In particular, subretinal delivery leads to regional retinal detachments, results in only a limited proportion of area that is transduced and is technically more challenging than intravitreal injections. XLRS participants are pre-disposed toward retinal detachments, and surgical manipulation required for subretinal administration would exacerbate this problem. With the advent of Lucentis® and other anti-angiogenic agents, intravitreal delivery is now common practice. In parallel, the investigation/development of AAV technology has progressed. Several AAVs have been shown to transduce retinal cells by intravitreal delivery, including AAV8 [47, 52]. Because of these advances, intravitreal delivery has become more feasible. Moreover, several AAV serotypes (AAV2, AAV5, AAV8) have been delivered intravitreally in a XLRS mouse model and found strong transduction in the photoreceptor neurons that lie deep in the retina [37, 50]. Intravitreal delivery of an AAV2 with an anti-angiogenic factor has advanced to clinical testing in wet age-related macular degeneration patients [MacLachlan 2011, NCT 01024998].

1.5.3 Summary of Nonclinical Studies with AAV8-scRS/IRBP-hRS

The nonclinical program for characterizing AAV8-scRS/IRBP-hRS's efficacy profile comprised pharmacology studies evaluating the pharmacodynamic properties and biological activity of AAV8-scRS/IRBP-hRS in relevant animal models of disease (Rs1-KO mice and in New Zealand White (NZW) rabbits) and the retinal transduction potential of the vector in vivo.

Using the Rs1-KO mouse model, a non-GLP study showed that intravitreal injection of AAV8-scRS/IRBP-hRS transduced retinal cells and expressed RS1 at similar levels at two- and nine-weeks post subretinal (SR) injection; the GLP lot used in the toxicology studies was confirmed to express human RS1. In a non-GLP study of NZW rabbits, transduction of AAV8-scRS/IRBP-hRS was restricted to scattered cells in the ganglion cell layer and nerve fiber layer, and overall transduction levels were lower than in the mouse model.

To determine efficacy of transduction, Rs1-KO mice were administered a single IVT injection in a non-GLP study. Significant amounts of RS1 expression were detected in the eye and statistically significant improvements in retinal function and structure were observed.

The GLP biodistribution study was conducted in Rs1-KO mice, and by six months post-injection, partial clearance of AAV8 scRS/IRBP-hRS vector was observed in most tissues, except for the injection site. There was no measurable transgene RS1 expression observed in any tissue, except the liver and harderian gland three days and six months following IVT injection.

Non-GLP toxicology studies were conducted in NZW rabbits of four and 12 weeks in duration. In the 12-week study, ocular inflammation was observed at similar frequency and severity in both dose groups tested ($2\times10_{10}$ vg/eye and $2\times10_{11}$ vg/eye). In the four-week study, the cause of ocular inflammation was determined as likely in response to the AAV8 capsid and not due to the expression of RS1.

In retinoschisin knockout (RS1-KO) mice, a GLP toxicology study showed that there were no toxicity findings over six months. The NOEL was determined to be $2\times10_{10}$ vg/eye. A GLP toxicology study in NZW rabbits showed there was no systemic toxicity after a single dose. The most common effects were localized to the injected site and consisted of uveitis and vitreal precipitates. Microscopic inflammation was observed in several ocular tissues. Ophthalmic and histopathological findings were dose-dependent and were primarily observed two weeks post-injection. By nine months post-injection, minimal inflammation was still present by microscopic evaluation in a few ocular tissues and predominately in the highest dose group. NOAEL was determined to be $2\times10_{11}$ vg/eye.

Overall results demonstrate AAV8-scRS/IRBP-hRS was efficacious in relevant animal models of XLRS and was well tolerated with no test article-related mortality, weight changes, or adverse clinical findings. No additional pharmacology studies were planned, as results have established the nonclinical efficacy and safety profiles of AAV8-scRS/IRBP-hRS to support clinical use.

The starting dose of $1e_9$ vg/eye corresponds closely with the $2e_9$ vg/eye dose delivered intravitreously into the mouse and rabbit eye. A single intravitreal injection of AAV8-scRS/IRBP-hRS at $2e_9$ vg/eye was well tolerated following delivery into the eye of two species, with no evidence of significant ocular or systemic toxicities. Safety of the additional selected doses is supported by results from two pre-clinical GLP-compliant toxicological studies.

A more detailed summary of the nonclinical development program with all relevant data can be found in the Investigator's Brochure.

1.6 XLRS Rationale for Outcome Measures

Many different *RS1* mutations have been described that cause XLRS [2, 12, 53-55] A comprehensive evaluation of the human XLRS phenotype and genotype was initiated in 2003. This study is ongoing and being conducted by the investigators who will participate in the current gene transfer protocol. To date, more than 200 XLRS-affected males and 30 female relatives have enrolled in this study which is conducted at NEI. DNA samples are obtained from participants.

Phenotype-genotype correlations in these participants have yielded observations relevant for the present protocol. In an extended human family that we studied with an insertion/deletion in exon 5, the ERG amplitude was negatively correlated with subject age [56], which suggests that some human patients with XLRS experience a progressive retinal degenerative disease that could potentially be rescued by gene delivery. A study of the association between the predicted effect of missense *RS1* mutations on retinoschisin structure and phenotype severity is ongoing [57]. Preliminary results suggest that disease severity is related to the degree of molecular abnormality of retinoschisin. A corollary of this observation is that partial restoration of normal retinoschisin activity through gene delivery has the potential of mitigating disease severity in patients with XLRS [58].

1.6.1 Test-retest Intervisit Variability of Functional and Structural Parameters in XLRS

A trial was conducted to examine the variability of four outcome measures (visual acuity, ERG, microperimetric macular sensitivity, and retinal thickness measured by OCT) that could be used to address safety and efficacy in therapeutic trials with XLRS. All outcome measures except microperimetry have been discussed above.

Microperimetry provides a means of testing central retinal sensitivity at precise anatomical locations by using computerized perimetry simultaneously with fundus imaging and eye tracking software. Microperimetry had been used in a host of macular diseases to provide functional measures of anatomic abnormalities in the macula. It has more recently been used to quantify the dysfunction of the retina associated with the central changes seen in XLRS patients [59]. Mean deviation of the microperimetry test is a single measurement that quantifies luminance sensitivity throughout the area of visual field measured. This variable is the average of differences between the values obtained in all visual field locations and their expected values in a subject with normal visual function. While this leads to a loss of spatial information, it provides a summary of all measurements in a given perimetry study. Mean deviation is less affected by test-retest fluctuations and compared with other perimetric methods, microperimetry is less dependent on a subject having central fixation. More importantly, microperimetry is little affected by inter-test differences in fixation loci between subjects. A sum of threshold values provides an equivalent summary measurement of field sensitivity. Thus, microperimetry provides the means of quantifying central retinal sensitivity in XLRS participants and how central sensitivity may change as a function of time.

In clinic, seven men aged 19-49 years with confirmed mutations *RS1* were evaluated over four visits spanning six months: baseline, months one, three and six. Eyes were separated into "Better

Eye" or "Worse Eye" groups based on visual acuity at baseline. Repeatability coefficients which define the range by which two measurements on a subject can differ due to random variability were calculated for each parameter [60, 61]. The 95% confidence interval for each repeatability coefficient was calculated using a jackknife resampling procedure [62, 63]. Table 1 below summarizes the data for the main variables to be used in the study.

Table 1: Repeatability Coefficients

Parameter (n=7 over 4 visits)	Better Eye		Worse Eye	
	Repeatability coefficient ± 95% C.I.*	Repeatability coefficient % change (% range)	Repeatability coefficient ± 95% C.I.*	Repeatability coefficient % change (% range)
Visual Acuity Letters	3.7 ± 0.9	2.8 – 4.6	5.9 ± 2.1	3.8 – 8.0
Scotopic ERG a-wave amplitude (log µV)	0.241 ± 0.116	↓43% (25-56)	0.215 ± 0.086	↓39% (26-50)
b-wave/a-wave ratio†	0.24 ± 0.20	↑74% (33-127)	0.17 ± 0.06	↑64% (35-100)
Retinal Sensitivity MP1 Macular sensitivity (dB)	2.2 ± 0.61	↓40% (31-48) ↑66% (44-91)	1.7 ± 0.50	↓33% (24-40) ↑48% (32-66)
Point-wise sensitivity (dB)	6.8 ± 3.6	↓78% (38-92) ↑351% (63-1152)	5.4 ± 1.6	↓71% (58-80) ↑245% (139-399)
Central Retinal Thickness (logOCT)	0.107 ± 0.057	↓22% (11-31) ↑28% (12-46)	0.065 ± 0.025	↓14% (9-18) ↑16% (10-22)

* C.I.: Confidence Interval.
† Linear parameter, percentage change will depend on absolute value of b/a ratio.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective is to assess the safety and tolerability of a single intravitreal administration of AAV8-scRS/IRBP-hRS. Safety will be assessed by monitoring retinal function, ocular structure, and occurrence of adverse events. Laboratory safety tests also will be monitored as part of the safety assessment.

3.0 PARTICIPANTS

Males clinically diagnosed with XLRS and verified by genetic analysis. Listed below are the inclusion and exclusion criteria.

3.1 Participant Eligibility Criteria

3.1.1 Inclusion Criteria

1. Participant is male with a mutation in the *RS1* gene identified by genotyping.
2. Participant must be 18 years of age or older.
3. Participant must be able to understand and sign the informed consent.
4. Participant must be medically able to comply with the study treatment, study testing and procedures and follow-up visits.
5. Participant has at least one eye that meets the study eye criteria listed below.
6. Participant must agree to use an effective barrier method (male or female condom) of contraception starting two weeks before and continuing until one year after gene transfer.
7. If the participant's partner is able to become pregnant, a second form of effective contraception will be required starting two weeks before and continuing until one year after gene transfer. Effective methods of contraception for this study include:
8. Hormonal contraception (birth control pills, injected hormones or vaginal ring),
    a. intrauterine device,
    b. barrier methods (condom or diaphragm) combined with spermicide,
    c. surgical sterilization (hysterectomy or tubal ligation in partner or vasectomy).

3.1.2 Exclusion Criteria

1. Participant is actively receiving another study medication/investigational product (IP).
2. Participant has previously enrolled in another gene therapy trial.
3. Participant is currently taking, or has taken in the last three months, a systemic carbonic anhydrase inhibitor prior to enrollment/baseline 1 testing.
4. Participant has any condition that significantly increases risk of systemic corticosteroids or systemic steroid-sparing immuno-modulatory agents, such as HIV, syphilis, tuberculosis, hepatitis B, hepatitis C, or diabetes mellitus (DM).
5. Participant has an underlying serious illness that impairs regular follow-up during the study.
6. Participant has had diagnosis or treatment of a malignancy (excluding non-melanoma skin cancer) within the previous five years.
7. Participant has pre-existing ocular tumors (excluding non-suspicious nevi).

8. Participant has a known allergy to fluorescein dye or other contraindications to obtaining a fluorescein angiogram.
9. Participant is on a medication that prevents safe administration of study related drugs.
10. Participant has uncontrolled hypertension. (Hypertension judged to be adequately controlled at baseline medical evaluation is not exclusionary.)
11. Participant has compromised renal function such that cyclosporine or Cellcept, which could be used to treat any manifest ocular inflammation, would be contraindicated.
12. Participant has significant liver disease with elevated liver enzymes (≥2.5 times upper limit of normal [ULN]).
13. Participant has low absolute neutrophil count (ANC<1.3 x $10^3/\mu L$).
14. Participant has used any biologic immunosuppressive agents within the last three months (within the last six months for rituximab or cyclophosphamide).

3.2 Study Eye Eligibility Criteria

The participant must have at least one eye meeting all inclusion criteria and none of the exclusion criteria listed below.

3.2.1 Study Eye Inclusion Criteria

1. The study eye must have a best-corrected E-ETDRS visual acuity letterscore of ≤ 63 (i.e., worse than or equal to 20/63). The visual acuity from the first baseline visit (Baseline 1) will be used for eligibility determination in case of a change in visual acuity at the second baseline visit (Baseline 2).
2. Electroretinogram in the study eye with a scotopic combined response demonstrating a subnormal b-wave, consistent with retinoschisis.

3.2.2 Study Eye Exclusion Criteria

1. The study eye has a history of other ocular disease likely to contribute significantly to visual loss or likely to present special risks (e.g., optic neuropathy, glaucoma, uveitis, large bullous schisis cavities or bullous retinal detachment precluding safe intravitreal injection).
2. The study eye has lens, cornea or other media opacities precluding adequate visualization and testing of the retina.
3. The study eye has undergone intraocular surgery within six months prior to enrollment.
4. The study eye is receiving topical carbonic anhydrase inhibitor or has received topical carbonic anhydrase inhibitors in the past three months.

3.2.3 Study Eye Selection Criteria

If both eyes of a participant meet the study eye eligibility criteria, the choice of study eye will be determined as follows:

1. The eye with the worse visual acuity will be selected as the study eye.

2. If both eyes have visual acuities within five letter differences of one another, the choice of study eye will be determined at the discretion of the Investigator in consultation with the participant.

4.0 STUDY DESIGN AND PROCEDURES

4.1 Recruitment

Participants will be recruited based on the inclusion and exclusion criteria. To date at the NEI, more than 100 XLRS-affected males have been evaluated under the NIH study 03-EI-0033. and DNA samples obtained from participants. Additional participants including more than 62 affected males have been entered into the NIH study 03-EI-0033 as off-site participants.

4.2 Screening

There will be an initial screening appointment at the site clinic to determine whether participants meet the eligibility criteria. If determined eligible, participants will sign the informed consent form for this protocol and undergo the additional baseline screening testing.

4.3 Dosing Scheme

This is a Phase I/IIa, prospective, single-center, dose escalation study to evaluate the safety of AAV8-scRS/IRBP-hRS gene transfer vector in male study participants with X-linked retinoschisis (XLRS). The treated eye will be unmasked. Only one eye will be treated per participant. As this is a safety study, it will be unmasked and not randomized.

Table 2: Proposed AAV8-scRS/IRBP-hRS Clinical Dose Escalation Scheme

Cohort*	Dose in Vector Genomes (vg)†
1	1e9
2	1e10
3	1e11
4**	1e11
5	Not to exceed 3e11
6	Not to exceed 6e11

* Up to six participants may be enrolled into each dose cohort based on DMC review and recommendation as described in Section 6.

† All injection volumes will be 0.05 mL to achieve the proper dose based on dilution in the pharmacy.

** The number in this cohort will be determined after review from the DMC.

The starting dose of 1e9 vg/eye corresponded closely with the 2e9 vg/eye dose delivered intravitreously into the mouse and rabbit eye. A single intravitreal injection of AAV8-scRS/IRBP-hRS at 2e9 vg/eye was well-tolerated following delivery into the eye of two species, with no evidence of significant ocular or systemic toxicities. Safety of the additional selected doses is supported by results from the pre-clinical GLP-compliant toxicological studies.

Another Phase I/II clinical trial for X-linked retinoschisis using AAV gene transfer also tested doses between 1e11 vg/eye to 6e11 vg/eye [Ye 2015; NCT 02416622].

An independent Data and Safety Monitoring Committee (DMC) will review the cumulative data through Month 1 for each participant and each cohort. Following the review of the data, the DMC may recommend enrolling additional participants in the current dose cohort or may recommend dose modification. In addition, after a participant is dosed and there is one month of follow-up for each participant, the Sponsor and the DMC will review the data to assess whether the immunomodulation regimen is appropriate. It will then be decided, upon approval of the DMC, whether additional participants may be injected, including possible modification of the immunomodulation regimen or dose.

Participants will be followed for 18 months according to the schedule in Appendix 1. Surveillance will include annual evaluations at the site for years two to five. Adjustments to the plan for ongoing participant surveillance will be made during the course of follow-up if new information warrants such change, as per FDA recommendations.

The untreated eyes will be evaluated in parallel with the treated eyes on each of the study visits to serve as an untreated control and gather data on the natural history of human XLRS.

4.4 Study Design and Procedures

4.4.1 Study Design

Upon consent and enrollment into this protocol, participants will have a baseline testing visit (baseline 1) where the study eye will be identified. A second baseline examination (baseline 2) will occur within 60 days of AAV vector injection. A third baseline (baseline 3) visit will occur within two days of AAV vector injection (Day -2, Day -1 or Day 0).

The follow-up schedule is guided by several factors including expectations of immune response, monitoring of immuno-modulatory treatment given to blunt or avoid an inflammatory response to the AAV vector, knowledge of protein expression and previous experience with ocular AAV gene transfer vector studies [34-36, 48]. The clinic will schedule follow-up evaluations post-injection according to the flowsheet in Appendix 1. In the event that testing is not completed within a day, subsequent clinic visits may be scheduled within the visit window to complete the scheduled evaluation. Additional visits will be planned according to clinical need based upon the findings at each examination. Participants may choose to return to their community ophthalmologists between the required study visits.

Additionally, the site will perform a safety assessment on each participant annually (Years 2 - 5) to comply with the long-term follow-up currently recommended by the FDA.

4.4.2 Study Procedures

Participants will undergo medical evaluations, a complete ophthalmic examination, visual function measurements and laboratory tests at scheduled visits as defined in Appendix 1. The untreated eye will be evaluated in parallel with the treated eye to serve as an untreated control and to gather data

on the natural history of human XLRS. Procedures are performed at the study visits as indicated in the study flowsheet and will include the following:

1. Medical/Ophthalmic History*
2. Physical Examination*
3. Vital Signs
4. Concomitant Medication Assessment
5. Manifest Refraction with Best Corrected Visual Acuity (BCVA)
6. Microperimetry testing
7. Electroretinogram following ISCEV standards
8. Optical Coherence Tomography
9. Intraocular Pressure Measurement
10. Slit Lamp Examination
11. Fundus Examination/color fundus photography
12. Axial Length Measurement
13. Complete Blood Count
14. Acute Care, Mineral and Hepatic Panels
15. Urinalysis
16. Adverse Event Assessment
17. Fluorescein Angiogram (FA)
18. Syphilis testing (rapid plasma reagin [RPR] and antibody tests)*
19. Purified protein derivative (PPD) (tuberculin skin test) or Quantiferon*
20. HIV antibody testing*
21. Hepatitis B antigen/antibody testing*
22. Hepatitis C antibody testing*
23. Hemoglobin A1C*
24. AAV8 Serologic Testing
25. Anti-RS1 Serologic Testing
26. Anterior Chamber (AC) Tap
27. Ozurdex Injection

*Required at baseline 1 or baseline 2 only.

4.4.2.1 Medical/Ophthalmic History

Study investigators will obtain a medical and ophthalmic history on each participant at visits indicated on the study flowsheet.

4.4.2.2 Physical Examination

A physical examination includes the measurement of vital signs and a general assessment of all body systems at visits indicated on the study flowsheet.

4.4.2.3 Vital Signs

Study investigators will assess vital signs, including blood pressure, respiratory rate and temperature, at visits indicated on the study flowsheet.

4.4.2.4 Adverse Event Assessment

Study investigators will assess and grade adverse events.

4.4.2.5 Concomitant Medication Assessment

Investigators will document all medications at baseline and any changes in medication usage over the course of the study.

4.4.2.6 Manifest Refraction and BCVA

Refraction and visual acuity measures use the EVA and ETDRS methods as described in the Manual of Procedures (MOP). The investigators may request repeat visual acuity testing, including measurements using a standard Snellen chart, at any study visit. The first EVA and ETDRS measurements will be recorded as the visual acuity measurements on study record.

4.4.2.7 Microperimetry Testing

Study investigators will assess retinal sensitivity using a Nidek MP-1 microperimeter with a 10-2 pattern.

4.4.2.8 Electroretinogram

A small metal disk electrode will be taped to the participant's forehead and the participant's eyes will be patched. The participant will then sit for thirty minutes. After 30 minutes, the eye patches will be removed. Numbing eye drops, followed by electrodes, will be placed on the cornea and the participant will be asked to avoid blinking while watching flashing lights. Additional information on the procedure can be found in the MOP.

4.4.2.9 Optical Coherence Tomography (OCT)

Spectral domain OCT utilizing the Zeiss Cirrus^{TM} machine to obtain quantitative information of the macular thickness. Heidelberg Spectralis system will be used in addition with tracking capabilities when possible to demonstrate proof of principle anatomic changes, especially to document morphologic changes in schisis cavities.

4.4.2.10 Intraocular Pressure Measurement

Study investigators will assess intraocular pressure using applanation tonometry at visits indicated on the study flowsheet.

4.4.2.11 Slit Lamp Examination

The sclera, cornea, anterior chamber and iris will be examined and abnormalities recorded.

4.4.2.12 Fundus Examination/Color Fundus Photography

A fundus camera will be used to take color pictures of the eye. Color fundus photographs involve a bright flash to take pictures of the retina. This brief flash may cause temporary discomfort but does not damage the eye.

4.4.2.13 Fluorescein Angiogram (FA)

As a safety assessment, a baseline FA will be performed, which includes the optic nerve of both eyes in the late-phase frames. Another FA will be obtained at the Month 1 and 2 visits, as the steroid dose is tapered. In subsequent visits, we will continue to pay particular attention to any evidence of inflammation, including retinitis or vasculitis. Additional FAs will be obtained if clinical evaluation indicates that it is warranted.

4.4.2.14 Axial Length Measurement

The length of the eye will be measured using optical biometry (partial coherence interferometry) and/or ultrasonography.

4.4.2.15 Laboratory Tests

Peripheral blood samples will be drawn for tests as follows: hematology (complete blood count [CBC] with differential, hemoglobin, hematocrit and platelet count), serum chemistries (including blood urea nitrogen [BUN], creatinine, alanine aminotransferase (ALT)/aspartate aminotransferase (AST) and total bilirubin), and mineral panel. Syphilis testing (RPR and antibody), Hemoglobin A1C, tuberculosis testing, HIV, HBV and Hepatitis C virus (HCV) testing are performed at baseline. Urinalysis will also be performed according to the study flowsheet.

4.4.2.16 AAV8 Serologic Testing and Research Blood Collection

At visits indicated on the flowsheet, up to 55 mL of blood will be collected from participants via phlebotomy and stored for safety testing, including neutralizing antibody assay for AAV8 and anti-retinoschisin (RS1) antibody. Research blood may also be collected as needed, but the amount of blood collected will not exceed 55 mL. Research blood collection will occur in conjunction with blood draws for other study labs when possible. Additional information for blood draw, processing, storage and shipping can be found in the Laboratory Manual.

4.4.2.17 Anterior Chamber (AC) Tap

At visits indicated on the flowsheet, AC taps will be performed using topical anesthesia, to collect a sample (intended volume of at least 100µL) to allow for RS1 protein testing as well as any desired testing for vector, capsid or immune markers. AC taps may also be performed at Investigator discretion up to three more times at study or interim visits between Week 2 and Month 18, if testing from at least one previous post-injection sample shows positive or equivocal presence of RS1

protein, or if an additional sample would be potentially helpful at characterizing the intraocular immune response or testing for RS1 protein or AAV-RS1 components. The procedure will include use of a lid speculum, topical anesthesia, and povidone iodine applied to the ocular surface and periorbital region. Additional information for AC tap procedure and sample storage and processing can be found in the Laboratory Manual.

4.4.2.18 Ozurdex Injection

Three to 14 days before the planned day of injection, participants will receive an injection of Ozurdex (dexamethasone 0.7 mg) in the study eye. The injection procedure will include use of a lid speculum, topical anesthesia, povidone iodine applied to the ocular surface and periorbital region, and discretionary use of subconjunctival lidocaine and post-procedure pressure patching. The procedure may be performed in conjunction with the pre-injection AC tap, at Investigator discretion. If done with the AC tap, the Investigator may inject sterile saline inside the eye to counteract hypotony and maintain safety, if warranted. If the participant has previously had a vitrectomy in the study eye and has a compromised lens capsule, Triesence (triamcinolone acetonide, 2-4 mg) will be substituted for Ozurdex given the possibility, in such cases, of migration of the Ozurdex implant into the anterior chamber where it can cause corneal edema.

4.4.3 Investigational Product (IP) Administration

One eye from each eligible participant will receive ocular *RS1* AAV vector (Day 0). *RS1* AAV vector will be delivered inside the eye via intravitreal injection using a modified version of the procedure used for intravitreous delivery of other medications, such as ranibizumab, bevacizumab and aflibercept. Intravitreal injection will be performed at the site operating room using the procedure detailed in Appendix 2. The procedure will be performed on an outpatient basis, without need for systemic anesthesia or intra-procedural cardiopulmonary monitoring. Participants will undergo pain assessment and vital sign measurement following the procedure and will be discharged with written post-procedure instructions.

4.4.4 Follow-up/Termination Procedures

At the conclusion of the study, follow-up care will be arranged with either an external ophthalmologist or the participant will continue to be seen at the site under another protocol if one is available and if the participant is eligible. Clinical data obtained during participation will be shared with participants and, with written permission from the participants, their private physicians. Results from the overall study will be shared once the study data have been analyzed from all the participants.

4.5 Study and Concomitant Therapies

4.5.1 AAV8-scRS/IRBP-hRS Preparation and Storage

This study will be conducted under an IND. Clinical-grade vector-gene construct was manufactured by a GMP facility, The Center for Cellular and Molecular Therapeutics (The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA). The study will employ the Center's FDA-approved standard operating procedures for the preparation, testing and storage of the construct. The vector-gene construct will be delivered to the site Pharmacy, where it will be stored frozen until dispensed to the site for use. Dilution with excipient provided by

The Center for Cellular and Molecular Therapeutics (The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA) will be used to prepare the IP for administration. This will be performed immediately after thawing the IP by trained personnel in the pharmacy before distribution for administration. The IP will be diluted to the appropriate concentration and drawn up in a 1 mL syringe.

Intravitreal injections of up to 0.1 mL have commonly been used clinically for over a decade. A recent example of a drug with FDA approval that uses a 0.1 mL intravitreal injection is for Jetrea® (Ocriplasmin) [64]. Using that volume, study investigators observed ~4% risk of increased intraocular pressure following injection of drug or placebo, which is similar to the rate reported for 0.05 mL injections. Their publication does not state whether any of the eyes with increased intraocular pressure required paracentesis, but that treatment is indicated for the relatively unusual patient with restricted ocular blood flow caused by increased intraocular pressure from the injection, whether 0.05 mL, 0.1 mL or higher. All doses in this study will be administered in 0.05 mL volume.

4.5.2 Concomitant Medications and Procedures

Information regarding medications received during the study (except for routine medications given for ocular examination and testing procedures required by the protocol, such as topical anesthetics and mydriatics), including dose, indication and start and stop dates, will be recorded in the medical record. Information about all ocular and non-ocular procedures, including the indication and date, will also be recorded in the medical record. Elective use of carbonic anhydrase inhibitors will be prohibited during study participation.

This study will use an intraocular injection of Ozurdex (dexamethasone 0.7mg) administered in a 3-14 day window prior to the planned vector injection day. The goal of the Ozurdex injection is to maximize participant safety by averting or blunting any potential inflammatory response to IP and is not expected to decrease potential efficacy of IP or to confound study results. We are using this injection prior to administration of the therapeutic AAV8-*RS1* vector starting with Participant 12. The risks of the Ozurdex injection are low and are included in Section 5: Risks/Discomforts.

Appearance of intraocular inflammation (anterior chamber or vitreous cell greater than baseline levels, or any papillitis, retinitis, vasculitis or choroiditis) will be treated medically as needed as outlined below (Section 6.1: Treatment of Endophthalmitis and Non-infectious Intraocular Inflammation).

4.6 Drug Accountability

The site Pharmacy will be responsible for the secure and stable storage of each unit dose of AAV vector material until delivery of the dose to the Investigator on the day of treatment. The Investigator will be responsible for the safe disposal of any residual AAV vector material in the manner prescribed by the Sponsor policies and guidelines. The Principal Investigator will be notified of any AAV vector material suspected to be materially defective or compromised, and such material will not be used and will be sent to the appropriate laboratory for analysis when warranted. Adequate drug accountability records will include documentation by the Pharmacy of all AAV vector material received, stored and dispensed; and documentation by clinical staff of all

AAV vector material administered, stored and discarded. The final dilution of AAV vector material will be stored in secure freezers until the end of the study.

4.7 Storage of Samples and Data

Blood, aqueous humor, serum, and peripheral blood mononuclear cells (PBMC) will be stored and used for this study. Samples will be stored in secure freezers at the site or may be shipped to a contracted laboratory for specific research testing, preparation and storage.

Data and samples may be shared with collaborating laboratories at the site or outside of the site and/or submitted to the site -designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at the site. Data and samples may be shared with investigators and institutions with federal wide assurance (FWA) or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to databases and repositories will be reported at the time of Continuing Review by the Coordinating Center on behalf of the Sponsor. Submission to databases and repositories will be submitted for prospective IRB approval by the Coordinating Center on behalf of the Sponsor

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with federal regulations.

The clinical data will be stored in the user-restricted, password-protected site Electronic Medical Record, the site patient chart and secure databases at Emmes.

5.0 RISKS/DISCOMFORTS

The anticipated discomforts and risks of this protocol are those associated with 1) the manipulations of administering the study agent, Ozurdex, and any other warranted immuno-modulatory agents (if needed); 2) AC tap; and 3) the reaction to the AAV vector.

A modified procedure for intraocular injection that incorporates a needle long enough to reach the posterior vitreous cavity and uses direct visualization of the needle tip with an operating microscope and corneal contact lens will be used to deliver the AAV construct. The risks associated with the injection procedure include intraocular hemorrhage, retinal tear or detachment, intraocular pressure elevation, lens damage and endophthalmitis. To minimize this risk, potential participants judged high-risk for these complications will be excluded.

Several risks are possibly associated with ocular gene transfer using AAV vectors. At present, the key observation is intraocular inflammation following vector dosing. To date, this has been handled using topical or systemic immune suppressing agents, principally with topical or oral steroids. In this trial to date, 12 participants have been dosed with intravitreal vector, and some

degree of ocular inflammation has been observed in all six participants receiving doses of 1 x10^{11} vg/eye or higher. In all cases the inflammation was handled medically with ocular topical or oral steroid with suitable resolution.

An immune response against the AAV capsid or the transgene product is a potential risk. There are clinical reports suggesting that, in some subjects, cells transduced by AAV vectors become the target of a cell-mediated immune response. This was observed in a hemophilia gene transfer trial by systemic administration to the liver using an AAV type 2 Factor IX vector [65], and it was also observed with systemic gene transfer for hyperlipidemia using an AAV type 1 lipoprotein lipase vector delivered to muscle [Unpublished data]. The mechanism proposed for this phenomenon was AAV capsid-directed, cell-mediated attack of recently transduced cells displaying AAV capsid antigens [66]. The immune response was asymptomatic and self-limiting in both studies. In this current trial for X-linked retinoschisis, the strategy to reduce the likelihood of such an occurrence is to use a 100-fold smaller AAV dose range, to monitor participants carefully with eye exams which will evaluate for any evidence of inflammation surrounding vector administration and to institute use of a prophylactic Ozurdex injection to lower risk of inflammation. Further, there is a theoretical advantage in the present study because the eye is a relatively immune privileged environment. The risks of Ozurdex injection include: less than 1% chance of each of the following: infection, inflammation, intraocular bleeding, retinal tear, retinal detachment, lens trauma (traumatic cataract), central serious chorioretinopathy, chronic glaucoma; approximately 5% chance of steroid-induced cataract; and approximately 25% chance of ocular hypertension. The likelihood of steroid-induced cataract or ocular hypertension is based on a combined analysis of 6-month trials which tested a single Ozurdex injection for treatment of uveitis or macular edema from retinal vein occlusion, as indicated in the package insert [67]. Risk of steroid-induced cataract or ocular hypertension will increase if additional Ozurdex or other local steroid treatments are used to treat inflammation during the study. Triesence (triamcinolone acetonide 2-4 mg) will be substituted for Ozurdex for any eye that has had a prior vitrectomy and has a compromised lens capsule, given the possibility of migration of Ozurdex into the anterior chamber in such cases, where it can cause corneal edema. Triesence injection has similar risks to Ozurdex.

Short- and long-term systemic and/or local immuno-modulatory treatment can be used to treat ocular inflammation as needed at Investigator discretion, but these agents have their own potential risks. As indicated in Section 6.1, oral corticosteroids are expected to be the initial systemic therapy of choice for manifest inflammation not controlled by locally administered medications, if necessary, with use of steroid-sparing immuno-modulatory agents if needed. Systemic corticosteroids have acute and chronic risks. Acute and sub-acute risks include fluid/electrolyte imbalances; hyperglycemia; hypertension; esophagitis, gastritis or peptic ulcer formation; elevation of liver enzymes (without hepatitis); anxiety; insomnia; striae; psychosis; aseptic necrosis of bone; tendon rupture; pancreatitis; glaucoma; cataract; impaired wound healing; and masking/promotion of infection. Chronic risks include those above, plus weight gain and fat re-distribution; diabetes mellitus; osteoporosis; hyperlipidemia; atherosclerosis; pathologic bone fracture; and muscle weakness and wasting.

AC tap risks include less than 1% chance of each of the following: infection, inflammation, corneal edema, iris trauma with pupil changes, intraocular bleeding (from iris or posteriorly due to hypotony), and lens trauma which may lead to traumatic cataract. Expected post-procedure hypotony can be managed with injection of sterile saline at the end of the procedure and/or post-procedure pressure patching.

Malignant transformation of AAV transduced ocular cells is theoretically possible. There was a report of hepatocellular carcinoma developing in mice subsequent to liver delivery of an AAV vector [68]. A large number of animal studies investigating the effects of many different AAV vectors on many tissues (including liver) have been completed without reported malignant transformation. There is a theoretical possibility of the development of an intraocular tumor, but this has not been observed in the many ocular gene augmentation trials conducted to date. However, to minimize this risk, potential participants are excluded if they have had intraocular tumor (excluding non-suspicious nevi) with potential for malignant transformation, and those with any history of diagnosis or treatment of malignancy (excluding non-melanoma skin cancer) within five years prior to enrollment.

Treatment with the vector during the current trial may result in generation of a long lasting humoral immune response [36, 65]. Consequently, participation in the current gene transfer trial may preclude successful future re-administration of AAV vector-based therapies, including a first administration to the untreated eye or repeat administration to the study eye.

Incorporation of AAV vector material into the gamete genome is not known to occur. However, as a precautionary measure, participants and partners are being requested to use double barrier contraception particular those with partners that can be childbearing starting two weeks before the initiation of the immunosuppressive regimen and continuing for one year after vector administration (~five rounds of spermatogenesis and an assumption of systemic clearance).

If participants are interested, sperm banking will be provided at no cost to the participant. Methods and procedures for sperm banking will be according to instructions from the service provider.

In the unlikely event a patient were to die from a cause suspected to be related to either the AAV vector or the gene transfer procedure, a request for a complete autopsy will be made to the patient's next of kin at the time of death. If there are any findings suggestive of vector-induced pathology, then appropriate organ samples will be processed for real-time polymerase chain reaction (PCR) assessment of the presence of vector DNA and to look for possible transgene expression. All patients will be informed that at the time of their death, no matter what the cause, a request will be made to their family for an autopsy.

The procedures for evaluating participants with these conditions carry minimal risks and are performed in the manner standard of care; but, are being performed for the research purposes of this study. Dilating drops or anesthetic drops may sting or cause an allergic reaction. These drops can also cause acute, transient pressure elevation. Rarely, the cornea is abraded during measurement of intraocular pressure or placement of the ERG contact lens, which requires treatment by patching the eye for a day.

6.0 PARTICIPANT MONITORING

Participants will be evaluated for safety over the course of the study. At each visit, participants will be asked about any new symptoms, given eye exams and samples will be taken for laboratory testing. Participants will be instructed to contact a study Investigator at any time if they experience any new concerns.

The independent Data and Safety Monitoring Committee (DMC) will review the cumulative data through Month 1 for each participant and each cohort. Following the review of the data, the DMC may recommend enrolling additional participants in the current dose cohort or may recommend dose modification. In addition, after a participant is dosed and there is one month of follow-up for each participant, we and the DMC will review the data to assess whether the immunomodulation regimen is appropriate. It will then be decided, upon approval of the DMC, whether additional participants may be injected, including possible modification of the immunomodulation regimen or dose. Additionally, the study will continue to follow participants as recommended by the FDA, which is currently up to 5 years. Participants will be monitored by study investigators and clinical staff or their ophthalmologists at each visit.

The following events are designated in advance for tracking as part of safety monitoring during the study.

Events related to natural progression of disease include:

a. Central or peripheral retinoschisis;
b. Macular atrophy;
c. Retinal degeneration;
d. Intraocular hemorrhage;
e. Retinal tear;
f. Retinal detachment;
g. Cataract.

Events relevant to monitoring for complications of IP administration (intravitreal injection) or IP action include those listed above, plus:

h. Intraocular pressure elevation or glaucoma;
i. Endophthalmitis;
j. Intraocular inflammation (including anterior uveitis, vitritis, retinitis, retinal vasculitis, choroiditis, optic neuritis, panuveitis);
k. New intraocular neoplasm;
l. Systemic immune response to constituents of the IP.

Participants will be monitored for any decline in visual function in the study eye. A noteworthy and reportable decrease in visual function is defined as:

a. Decrease in BCVA of $\geq$ 10 ETDRS letters; OR
b. Decrease in ERG response amplitude $\geq$ 75%.

Suspension Rules

Occurrence of any of the following will constitute grounds for suspending enrollment and IP administration to any further participants, pending DMC review as to continuing study enrollment:

1. A significant decrease in visual function (as defined above) in the study eye, unless this decline is attributable to ocular surface effects or due to minimal inflammation (anterior chamber pigmented or nonpigmented cells of grade 1+ or less) from the intravitreal injection procedure during the first 10 days following IP administration;
2. Endophthalmitis in the study eye;
3. Anterior chamber cellular reaction of grade 3+ or higher in the study eye [71];
4. Vitreous cellular reaction of grade 3 or greater in any portion of the vitreous cavity in the study eye [71];
5. Optic nerve edema of grade 1+ or higher in the study eye [72];
6. Retinitis, retinal vasculitis or choroiditis in the study eye;
7. Systemic response as reflected by grade 2 laboratory tests or any grade 3-4 event/laboratory test that is related to the IP (grading according to the common terminology criteria for adverse events [CTCAE] v5);
8. A suspected serious adverse reaction related to the study article.

6.1 Treatment of Endophthalmitis and Non-infectious Intraocular Inflammation

If a significant inflammatory response develops in the study eye following IP administration, measures will be taken to rule out and treat any possible infection, when warranted, and to manage uveitis using local and systemic means. Appearance of anterior chamber cellular reaction (1+ or greater), vitreous cell (1+ or greater), vitreous haze (1+ or greater), foci of retinitis or retinal vasculitis, choroiditis, optic nerve edema or other ocular inflammatory lesions, apart from the acute conjunctivitis and keratopathy that often occur immediately following intravitreal injection, will prompt appropriate treatment and close surveillance.

If features suggest a possible acute infectious endophthalmitis, treatment will be performed consistent with standard clinical practice. Generally, vitreous and/or aqueous biopsy will be obtained for culture and broad-spectrum intravitreous antibiotics will be employed, with or without vitrectomy surgery and with or without systemic antibiotics, at Investigator's clinical judgment. A portion of any intraocular fluid obtained will be sent for microbial culture, and some of the remaining fluid will be analyzed for the IP. Local and systemic corticosteroids will be given at Investigator's clinical judgment.

If features suggest a non-infectious uveitis, acute treatment of intraocular inflammation will include local and systemic corticosteroid administration as warranted, using the guiding principles of published consensus guidelines [73]. Mild inflammation, such as an anterior chamber cellular reaction of 2+ or less or 1+ vitreous cell, may be initially treated with topical corticosteroids, with addition of systemic corticosteroids at Investigator's clinical judgment. More severe inflammation, such as anterior chamber reaction of 3+ or greater, vitreous cell or haze of 2+ or greater, or appearance of retinitis, retinal vasculitis, choroiditis or optic neuritis, will warrant medical decision

on systemic corticosteroid therapy and immuno-modulatory agents guided by medical judgment and incorporating the recommendations of consensus guidelines [73]. Additional periocular or intraocular injection of corticosteroids (such as Ozurdex or Triesence) may be considered in cases of persistent inflammation in which a prolonged high local concentration is desired.

All participants receiving systemic corticosteroids will be monitored for adverse effects of corticosteroids (periodic measurement of blood pressure, weight, blood glucose, bone density) and any warranted treatment as prophylaxis for adverse effects (such as calcium and vitamin D supplementation to minimize osteoporosis), according to standard clinical care. Any participant receiving immuno-modulatory agents will undergo all customary monitoring for adverse effects. Corticosteroids and non-steroidal immuno-modulatory agents will be tapered off as the situation permits. Vitrectomy surgery may be considered as an adjunct to treatment in certain circumstances, such as for attempted removal of IP from the vitreous in cases of severe inflammation, or for removal of non-clearing inflammatory debris.

6.2 Treatment of Intraocular Pressure Elevation, Intraocular Hemorrhage and Other Possible Events

Intraocular pressure elevation causing non-perfusion of the retina immediately following administration of IP will be treated with paracentesis at Investigator's clinical judgment. Sustained intraocular pressure elevation at any time will be treated with topical and systemic pressure-lowering agents as necessary to minimize risk of glaucoma, and any glaucoma will be managed according to standards of clinical care. Intraocular hemorrhage may be observed, if judged not immediately threatening to long-term visual potential, or will be managed with standard surgical approaches. Retinal tear, retinal detachment and cataract will be managed in accordance with present practice patterns. Any vitrectomy surgery may include use of pharmacologic adjuncts as described by Wu and colleagues [74] at Investigator's clinical judgment. Any intraocular fluid or tissue sampled during intraocular surgery may be analyzed for components of the IP. Suspicion of an intraocular neoplasm will prompt referral to an ocular oncologist.

6.3 Withdrawal Criteria

Participants may choose to withdraw from this study for any reason at any time without penalty or prohibition from enrolling in other clinical protocols. The investigators will ask all study participants to continue to return for all study visits for safety follow-up.

7.0 Outcome Measures

The primary functional outcome measures were selected to assess the safety of *RS1* gene delivery with an AAV vector in participants with XLRS by evaluating possible adverse effects on retinal function. In addition, subjects also will be assessed for safety events (both ocular and systemic) with clinical and laboratory assessments. Secondary functional outcome measures were chosen based on the known effects of juvenile retinoschisis on retinal function, anatomy and physiology. XLRS mice show retinal structural abnormalities at very young age [8, 24, 37, 75] and human infants with XLRS occasionally are born with extensive retinal involvements [1].

To assess the safety of gene delivery on retinal physiology, outcome measures should evaluate the maintenance of retinal function, anatomy and physiology, which are already compromised by XLRS, and give evidence of no substantial loss of key parameters.

7.1 Primary Outcome

The primary outcome is the safety of the AAV8-scRS/IRBP-hRS vector. All ocular and systemic events will be closely monitored. The following will be used to judge the safety of the AAV vector in participants with XLRS:

1. Adverse events affecting ocular function that differ clinically from those expected in the normal course of progression of XLRS including:
2. Substantial functional change as measured by a ≥ 10 EVA letters in BCVA (> 0.2 logMAR) from average of baseline 1 and 2 BCVA.
3. Decrease in ERG response amplitude by ≥ 75% from average of amplitudes from baseline 1 and 2.
4. Severe ocular inflammation beyond that inflammation anticipated consequent to an intravitreal injection.
5. Adverse events deemed clinically-related to the intraocular administration technique will also be noted and reported, including vitreous hemorrhage, retinal detachment, intraocular pressure elevation, lens damage and endophthalmitis.
    - Abnormal findings of serum chemistry, hematology, liver function tests or urinalysis/urine chemistry that are beyond Grade 1 and/or clinically significantly different than baseline.

7.2 Secondary Outcomes

Secondary outcomes were selected to further investigate the safety of gene delivery in participants with XLRS and to investigate effects on retinal physiology and/or morphology. The secondary outcomes employ quantitative measures of ERG, visual acuity, visual sensitivity and retinal thickness. This protocol is not intended for a determination of efficacy, but evidence of efficacy will be assessed from any improvement in retinal morphology and/or physiological function and/or visual assessment with respect to baseline measurements and with respect to the fellow untreated eye. The following secondary outcomes of visual function will be assessed on both the study and control eyes according to the time schedule dictated in Appendix 1:

1. Change in ERG combined response amplitudes from an average of baseline 1 and 2 measurements and/or change in the b/a-wave amplitude ratio.
2. The mean, median and distribution of change in BCVA compared to the average of baseline 1 and 2 measurements.
3. Change in retinal structure as measured by OCT compared to the average of baseline 1 and 2 measurements. Quantitative measures of total retinal thickness will be obtained with the Cirrus™ OCT using macular cube scans. Qualitative morphologic changes to macula anatomy will be investigated using the tracking ability of the Heidelberg Spectralis™ OCT system. Since acquisition of these scans can be difficult in patients with parafoveal fixation, we will obtain Cirrus™ on all patients and Spectralis™ when possible. Length of intact ellipsoid zone on the OCT and any findings of restoration of this reflectivity line will also be analyzed.

4. Change in central visual field sensitivity as measured by microperimetry (MP-1) Visual Field testing compared to the average of baseline 1 and 2 measurements. Both mean sensitivities, number of scotomatous points and number of points with a significant change in sensitivity will be analyzed.

    - Formation of circulating systemic anti-AAV or anti-RS1 antibodies.

All adverse events will be collected throughout the duration of the study regardless of severity or potential relationship to the ocular AAV vector or administration technique. Expected transient changes that may result from the injection itself would be a change in intraocular pressure and transient changes from steroid use including elevated serum glucose.

8.0 STATISTICAL CONSIDERATIONS

No formal sample size calculation has been performed in this Phase I/IIa dose-escalation study.

8.1 Analysis

In this study, analyses will be primarily descriptive by participant and dose group. The number of participants who experienced each of the outcomes in Section 7.1 will be tabulated. Similarly, BCVA, ERG, OCT and MP-1 measurements will be presented for each participant and tabulated over time, as described in Section 7.2. Additionally, these outcomes will compare the changes within the study eye and the changes between the study eye and control eye over time.

All reported adverse events will be tabulated and presented by body system, severity and relationship to IP. Similarly, all laboratory abnormalities greater than Grade 1 abnormalities (CTCAE v5.0) will be reported regardless of potential relationship to the AAV vector.

8.2 Sample Size

This study does not lend itself to a sample size calculation but has previously been discussed with the FDA who concurred with minimally three participants in each group. The protocol has been written according to this discussion. Subsequent protocol amendments provided for additional participants to be enrolled at doses expected to be safe and potentially efficacious with concurrence of the DMC.

As of Modification Z, NEI will conclude study enrollment with a total of 12 participants. Participant(s) enrollment may proceed but total trial size is expected not to exceed 24 participants.

8.3 Interim Analysis

No formal interim analysis will take place which requires group sequential methods or adjustment of p-values to control Type I error for this Phase I/IIa clinical trial. However, the DMC will review the report of data accumulated through the Month 1 visit from all participants enrolled after Participant 009 before additional participants are enrolled. Following the review of the data, the DMC may recommend enrolling additional participants in the current dose cohort or may recommend dose modification not to exceed 6e11 vg/eye.

9.0 HUMAN SUBJECTS PROTECTION

9.1 Equitability

Accrual will be equitable for this study.

9.1.1 Justification for the Exclusion of Women

XLRS disease only manifests in males and not in female carriers, hence women are not included in this study [32].

9.1.2 Justification for the Exclusion of Children

Boys < 18 years of age will not be included in this study as this study represents the first time that this experimental agent will be used in humans and they are unable to consent themselves. If the study shows no detriment to the participants, future studies will expect to include participants under age 18.

9.1.3 Justification for the Exclusion of Vulnerable Subjects

Vulnerable subjects who lack consent capacity, are mentally ill or cognitively impaired are excluded from this study because they are frequently unable to cooperate with evaluation and testing in the manner standardized for other adult participants.

10.0 CONSENT DOCUMENTS AND PROCESS

Study investigators with consenting privileges will obtain informed consent. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study. Participants must have the ability to understand and sign an informed consent form, which must be signed prior to enrollment. Participants will have the opportunity to carefully review the consent and ask questions regarding this study prior to signing, and they will be informed that they may withdraw from the study at any time without prejudice to themselves.

If a participant requires the consent to be in larger font in order to read it well, this will be provided. If a participant is visually impaired to the point of being unable to read the consent, he can take the consent back with them to read it over with a family member or with the use of magnifying devices. If a participant chooses, the Investigator can also read the consent verbatim to the participant and answer any questions that may arise.

An Investigator present during the consent process will document the consent process in the participant's medical record. A signed copy of the informed consent form will be provided to the participant.

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study Investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study Investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other approved remote platforms used in compliance with site policy) per discretion of the designated study Investigator and with the agreement of the participant/consent designee(s). The remote consent option will allow the consent designee and participant to engage in the informed consent process in a way that is similar to what it would be if it were conducted in-person when a participant is unable to be present at the study site. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the Investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the Investigator and participant below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to the participant) or on the electronic document. The process for documenting signatures on an electronic document is described below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the following electronic platform to obtain the required signatures:

- iMedConsent platform (which is 21 CFR Part 11 compliant)

Both the Investigator and the participant will sign the electronic document using a finger, stylus or mouse. Electronic signatures (i.e., the "signature" and a timestamp are digitally generated) will not be used.

10.1 Telephone Consent

An alternative to electronic consent is phone consenting. A hard copy of the informed consent document will be sent to the participant. During the consent process, participants and investigators will view individual copies of the consent in their respective locations. An explanation of the study will be provided over the telephone after the participant has had the opportunity to read the documents. Participants will have the opportunity to carefully review the consent and ask questions regarding this study prior to signing, and they will be informed that they may withdraw from the study at any time without prejudice to themselves. The participant will sign and date the informed consent. The original signed informed consent document will be sent back to the consenting Investigator who will sign and date the consent form with the date the consent was returned. A fully executed copy will be returned via mail to the subject. The informed consent process will be documented in the participant's medical record by the consenting Investigator.

11.0 DATA AND SAFETY MONITORING

The Data and Safety Monitoring Committee (DMC) will be responsible for monitoring data and safety, and will exercise oversight of the clinical investigation independently from the study investigators.

11.1 Data and Safety Monitoring Committee

The DMC is responsible for reviewing and approving the study design and, as appropriate, recommending design changes. In addition, the DMC assesses study data with particular consideration of participant safety. The DMC will convene prior to subsequent participant enrollment to review the protocol.

The Committee will review accumulated data for each participant and cohort, on a regular basis, but will convene ad hoc meetings to address any significant problems related to participant safety brought to its attention by any study participant or Investigator. Following the review of data and/or AE materials, the DMC will determine if the information is complete, may determine if additional DMC review is required, and may make recommendations to the Sponsor concerning continuation or modification of the study. . In addition, after a participant is dosed and there is one month of follow-up for each participant, the Sponsor, and the DMC will review the data to assess whether the immunomodulation regimen is appropriate. It will then be decided, upon approval of the DMC, whether additional participants may be injected, including possible modification of the immunomodulation regimen or dose.

The Committee will also consider whether a protocol modification is necessary. If changes in the protocol are indicated, recommendations will be made to the Sponsor who will consider and act on such recommendations in a timely manner.

Suspension Rules

Occurrence of any of the following will constitute grounds for suspending enrollment and IP administration to any further participants, pending DMC review as to continuing study enrollment:

1. A significant decrease in visual function (as defined above) in the study eye, unless this decline is attributable to ocular surface effects or due to minimal inflammation (anterior chamber pigmented or nonpigmented cells of grade 1+ or less) from the intravitreal injection procedure during the first 10 days following IP administration;
2. Endophthalmitis in the study eye;
3. Anterior chamber cellular reaction of grade 3+ or higher in the study eye [71];
4. Vitreous cellular reaction of grade 3 or greater in any portion of the vitreous cavity in the study eye [71];
5. Optic nerve edema of grade 1+ or higher in the study eye [72];
6. Retinitis, retinal vasculitis or choroiditis in the study eye;

7. Systemic response as reflected by grade 2 laboratory tests or any grade 3-4 event/laboratory test that is related to the IP (grading according to the CTCAE v5);

8. A suspected serious adverse reaction related to the study article.

11.2 Coordinating Center

Emmes has been assigned as the coordinating center for this trial to conduct data collection, protocol monitoring, data analysis and reporting. The coordinating center provides routine monitoring of study participants' data. Monitoring visits will occur on a schedule depending on the status of the study.

The protocol monitor will monitor documentation of the informed consent process. In addition, the protocol monitor will review source documentation for the data collected throughout the trial to ensure that the data points are collected and reported. The coordinating center will ensure the AAV vector material is stored properly and will assess drug accountability documents throughout the duration of the study.

Although the coordinating center advises the site Clinical Director and Principal Investigator on data and statistical activities, the coordinating center staff does not have direct access to or interaction with participants. The gene transfer vector manufacturer, The Center for Cellular and Molecular Therapeutics, through The Children's Hospital of Philadelphia, will not have access to the clinical data until the completion of the trial.

12.0 QUALITY ASSURANCE

The Sponsor, the site and Emmes, the Coordinating Center, maintain quality control by adhering to standard operating procedures (the site's I QA program and the site's standard operating procedures). These procedures cover the full protocol cycle beginning with staff credentialing and training, and protocol development and approval, through database development, data collection, monitoring and analysis, and finally manuscript preparation at the conclusion of the study. Data quality assurance is of the utmost importance to the Sponsor, the site, and Emmes. These groups use a quality assurance system that relies on real-time data checks and reports throughout the course of a study to ensure the accuracy of information. This system is a secure and confidential data management system that stores data and provides quality assurance and reporting. Emmes has developed a number of routine reports specifically designed for monitors (e.g., listings of serious adverse events, etc.).

Additionally, Emmes has developed summary reports of discrepancies, as well as reports of the exceptions databases, which include requests and reasons for exceptions. The results of the reports are communicated back to site staff, and, along with protocol compliance issues, to the DMC (if applicable).

Following the monitoring plan for this study, Emmes will perform monitoring activities, including on-site audits, review of database entries and the resolution of study issues. In addition to monitoring, Emmes performs various detailed automated and manual data quality checks. The results from these checks and any protocol compliance issues are communicated back to site staff and to the Sponsor, and applicable regulatory bodies.

13.0 Reportable Events

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801, 21 CFR 56, 21 CFR 312, and appropriate FDA guidance.

14.0 Alternatives to Participation or Alternative Therapies

Currently, there are no FDA-approved treatments for XLRS. Carbonic anhydrase inhibitors (CAIs), including dorzolamide and acetazolamide, have been explored as therapeutic agents, but there is little and inconsistent evidence of benefit, and they have not been clinically proven to be therapeutic agents.

15.0 Privacy

All research activities will be conducted in as private a setting as possible.

16.0 Confidentiality

Blood, serum, aqueous humor and PBMCs will be stored for this study. The samples will be coded using a unique identifier that cannot identify the participant. These samples will be stored in secure freezers at the site. All medical records will be kept confidential and will only be reviewed by the participating investigators. Data will be kept in password-protected computers held at the site and Emmes. Only study investigators, the Sponsor, and Emmes staff will have access to the study data. The participants' names will not appear on any of the data forms reported to the coordinating center and Sponsor. A unique identifier, a study registration number, will identify the participant to the coordinating center and Sponsor. Participants' personal information will be kept as private as possible. However, records can be inspected by organizations for quality assurance and data analysis. These include the members of the IRB, the DMC, or the Sponsor.

17.0 Conflict of Interest/Technology Transfer

The NIH guidelines were distributed to all the investigators and none of the investigators had any financial or conflicts of interest. Any potential conflicts of interest are reviewed annually or at the time a new Investigator is added to the protocol by the Deputy Ethics Commissioner (DEC) in Building 1 of NIH.

The Center for Cellular and Molecular Therapeutics, through The Children's Hospital of Philadelphia, the drug manufacturer, provided only the ocular AAV vector as outlined in their contract.

18.0 Compensation

Participants are not compensated for their participation in this study. However, this protocol does include reimbursement for travel, accomodation and subsistence.

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APPENDIX 1: STUDY FLOWSHEET

VISIT SCHEDULE*	Baseline 1	Baseline 2[1]	Baseline 3	Injection (Day 0)[1]	Day 1	Day 7	Day 14	Month 1	Month 2	Month 3	Month 4	Month 6	Month 9	Month 12	Month 18	Year 2-5[3]
VISIT NUMBER	BL1	BL2	BL3	D00	D01	D07	D14	M01	M02	M03	M04	M06	M09	M12	M18	Y02 Y05
TARGET DAY FROM SURGERY	N/A	N/A	-2 to D00	N/A	1	7	14	30	60	90	120	180	272	365	545	730-1825
VISIT WINDOWS						± 3 days	± 3 days	± 10 days	± 10 days	± 10 days	± 10 days	± 10 days	± 10 days	± 10 days	± 2 months	± 2 months
GENERAL ASSESSMENTS																
Medical / Ophthalmic History	X															
Physical Examination	X[4]	X[4]														
Concomitant Medication and Procedures Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (BP, resp, temp)	X	X	X	X	X	X	X	X	X	X	X					
Telephone Follow-Up																
VISUAL SYSTEM EXAMS (both eyes unless otherwise noted)																
Manifest Refraction [2]	X	X										X		X	X	X
BCVA (EVA and ETDRS)	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Microperimetry	X	X						X		X		X	X	X	X	X
Electroretinography (ERG)	X	X						X		X		X		X	X	X
Optical Coherence Tomography (OCT)	X	X	X				X	X	X	X	X	X	X	X	X	X
Intraocular Pressure (IOP)	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Slit Lamp Examination	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X

APPENDIX 1: STUDY FLOWSHEET (CONTINUED)

VISIT SCHEDULE*	Baseline 1	Baseline 2[1]	Baseline 3	Injection (Day 0)[1]	Day 1	Day 7	Day 14	Month 1	Month 2	Month 3	Month 4	Month 6	Month 9	Month 12	Month 18		Year 2-5[3]
Fundus Examination	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X
Digital Color Fundus Photography	X											X		X			X
Fluorescein Angiogram (FA)	X[5]	X[5]						X	X		X						
Axial Length Measurement	X																
STUDY INTERVENTION																	
Ocular Gene Transfer				X													
LABORATORY																	
Complete Blood Count with Differential	X[4]	X[4]		X[8]		X		X	X	X	X	X	X	X	X		
Acute Care, Mineral, and Hepatic Panels	X[4]	X[4]		X[8]		X	X	X	X	X	X	X	X	X	X		
Antibodies to AAV Vector[9]	X[6]	X[6]				X	X	X	X	X	X	X	X	X	X		X[7]
Blood for Storage (including anti-RS1 antibody)[9]	X[6]	X[6]				X	X	X	X	X	X	X	X	X	X		X[7]
Urinalysis	X[4]	X[4]				X		X	X	X	X	X	X	X	X		
Syphilis testing (RPR and antibody)	X[4]	X[4]															
Tuberculosis testing (PPD or Quantiferon)	X[4]	X[4]															
Hemoglobin A1C	X[4]																
HIV, HBV, and HCV testing	X[4]	X[4]															
Anterior Chamber Tap[10]		X[11]					X	X		X							
Ozurdex Injection		X[12]															

* One or more unscheduled visits for safety assessment can be added based on the discretion of the investigators. In the event that testing is not completed within a day, subsequent clinic visits may be scheduled within the visit window to complete the scheduled evaluation.

APPENDIX 1: STUDY FLOWSHEET (CONTINUED)

** Baseline 3 (BL3) assessments may occur up to two days prior to vector injection or on the same day of and prior to injection.

[1] Baseline 1 (BL1) must occur within 90 days of the injection and Baseline 2 (BL2) must occur within 60 days of the injection.

[2] BCVA with manifest refraction must be performed when scheduled and when there is a change in visual acuity of □ 10 ETDRS letters (□ 0.20 logMAR) from relevant baseline.

[3] Long term follow-up is not part of the primary end point.

[4] These procedures can be performed at either baseline visit, or any day prior to injection. They can also be performed under another NEI study within the 60 days prior to injection.

[5] The baseline FA can be performed at either baseline visit, or under another site study within 9 months prior to injection, provided it includes late phase frames of the optic nerve of each eye.

[6] These procedures can be performed at either baseline visit or any day prior to injection.

[7] Blood for storage and antibodies to AAV vector testing may be drawn at Visits Y02-Y05.

[8] Day 0 laboratory assessments may be performed up to seven days prior to the D00 visit.

[9] Research blood may also be collected as needed.

[10] Anterior chamber tap may also be performed anytime at Investigator discretion up to an additional 3 times between Week 2 and Month 18.

[11] May be performed within two weeks of Baseline 2. Ozurdex injection will be given 3 - 14 days prior to planned vector administration (D00).

APPENDIX 2: INJECTION PROCEDURE FOR INTRAVITREAL DELIVERY OF INVESTIGATIONAL PRODUCT

The injection procedure is designed to maintain the sterility and activity of the IP and to minimize the risk of adverse events associated with intravitreal injection (e.g., endophthalmitis). Aseptic technique will be used in preparation of materials for the procedure and in administration of the IP. In addition to the procedures outlined below, standard safety measures conforming to site policies applicable to intravitreal injections will be observed. The injection procedure will take place at the site operating room. The procedure will be performed using local anesthesia applied by nursing staff and the Investigator, without need for systemic anesthesia and without any need for intravenous line placement or intra-procedural cardiopulmonary monitoring. The following description of events in the event room is for illustrative guidance and need not be applied literally

1. A nurse will assemble the supplies and prepare a sterile field for the injection procedure. Sterile supplies will include drapes, drape scissors, 4□4 gauze pads, cotton-tipped applicators, eyelid speculum, ophthalmic adhesive drape, towels, saline solution, 5% povidone iodine solution, measuring calipers, 0.12 forceps, corneal contact lens and lens coupling agent (such as Ocucoat Viscoelastic® [Bausch and Lomb, Inc.]), operating microscope handles, 30 gauge 1 inch needle, and standard of care methods for providing anesthesia for intravitreal injections including: 0.5% proparacaine hydrochloride or 0.5% tetracaine ophthalmic drops, 2% or 3.5% lidocaine gel, or 1 or 2% lidocaine for subconjunctival injection (at Investigator's clinical judgment). These supplies will be kept sterile for use during the procedure.

2. The site Pharmacy will prepare the IP according to a separate protocol. In brief, this will consist of aseptic dilution and transfer of the IP from its storage container to a sterile Becton-Dickinson (BD) 1 mL syringe. An adequate volume of the IP will be loaded into the syringe, and a BD syringe cap will be affixed. The syringe contents will remain sterile, but the external surface of the syringe and cap will not be considered sterile. The capped syringe will be transported on ice to the site operating room. A study Investigator will receive the capped syringe containing the IP and will confirm appropriate packaging, labeling, volume, integrity, and appearance. The capped syringe will be kept in its Pharmacy-applied packaging on ice until use.

3. The capped syringe containing IP will be removed from its packaging by the Investigator or a nurse using no-touch technique or while wearing a gown and sterile gloves (to be discarded in a medical pathological waste (MPW) box after use) and placed on a sterile field in the operating room separate from the sterile field containing all other supplies for the procedure. A sterile empty specimen cup will be placed next to the capped syringe.

4. The participant will be positioned supine in the operating room after pupil dilation and marking of the study eye in the pre-operative area.

5. An operative time-out procedure will be held to confirm the identity of the participant, the eye to be treated, and the IP to be administered, in accordance with site policy.

6. The Investigator or a nurse will instill 2 to 4 drops of proparacine or tetracaine in the study eye.

7. The Investigator or a nurse will prepare the periocular skin and eyelids of the study eye for injection, cleansing the eyelids, lashes, and periorbital skin with 10% povidone iodine swabs, starting with the eyelids and lashes and continuing with the surrounding periocular skin.

8. The Investigator or a nurse will instill 2 to 4 drops of 5% povidone iodine ophthalmic solution in the study eye, making sure the drops cover the planned injection site on the conjunctiva. Additional proparacaine or tetracaine drops may be applied if necessary to relieve any ocular discomfort, but if additional drops are used, instillation of 5% povidone iodine solution should be repeated. The Investigator and scrub nurse will put on sterile gown, sterile gloves, and eye shield (with a mask having been worn since entering the operating room). Any other assisting nurses will put on non-sterile gown, non-sterile gloves, and eye shields in addition to their masks.

9. The Investigator will drape the study eye in routine fashion for ophthalmic surgery and will place a lid speculum.

10. The Investigator or a nurse will apply another drop of proparacaine or tetracaine to the eye surface.

11. The Investigator will saturate one or more sterile cotton-tipped applicators with proparacaine hydrochloride or tetracaine drops and will hold the swab or swabs against the planned injection site for 30 to 50 seconds. The Investigator will provide standard of care anesthesia for the intravitreal injection which may involve injecting a small amount (between 0.1 and 0.4 mL) of lidocaine subconjunctivally near the injection site, using a 30 gauge 0.5 inch needle directed tangentially to the globe surface.

12. The Investigator will mark the planned injection site 3.5 to 4 mm from the limbus with sterile calipers.

13. The Investigator will dab the injection site with a sterile cotton-tipped applicator soaked in 5% povidone iodine.

14. The Investigator may supplement analgesia by adding lidocaine gel to the injection site. This should be done following the application of 5% povidone iodine in the previous step. If used, lidocaine gel will be irrigated from the eye surface using sterile saline approximately 30 seconds after its application. The calipers may be used again to confirm the injection site location, if necessary.

15. The Investigator will place a viewing contact lens and coupling agent (such as Ocucoat Viscoelastic®) on the cornea, and will visualize the posterior pole of the eye using an operating microscope with coaxial illumination.

16. The Investigator will turn to the second sterile field containing the IP and will remove the syringe cap and mount a 30 gauge 1 inch needle from the sterile field on the syringe for

injection. Taking care to depress the syringe plunger slowly and to direct the needle tip into the sterile specimen cup, the Investigator will expel excess IP down to the appropriate volume to be injected, taking care not to allow any large air bubbles to persist.

17. The Investigator will insert the needle into the eye until the tip is visible in the mid-vitreous. Under direct visualization, the needle tip will be slowly advanced to the desired location in the posterior vitreous cavity. The Investigator or an assistant will depress the syringe plunger to inject the IP into the vitreous cavity, expelling the study agent slowly. The Investigator is permitted to use a 0.12 forceps to displace conjunctiva overlying the injection site before penetration with the needle.
18. The Investigator will immediately dispose of the needle/syringe in a sharps container.
19. The Investigator will irrigate the eye surface with sterile saline.
20. The lid speculum and drape will be removed.
21. The Investigator will remove and discard his or her sterile gloves and will set aside his or her eye shield. Another set of gloves (sterile or non-sterile) will be put on and the participant's fundus visualized by indirect ophthalmoscopy to confirm retinal perfusion and absence of injection complications, taking care not to disturb the ocular surface. Visual acuity of hand motions or better will be confirmed.
22. The lids and cheek will be cleansed by the Investigator or a nurse, still wearing gown and gloves.
23. Bacitracin ointment, erythromycin ointment, or white petrolatum ointment will be placed on the eye surface, and an eye patch will be affixed to maintain eye closure.
24. The participant will be transported to the recovery area, where pain assessment and vital signs will be recorded before discharge. The participant may be asked to lie in a supine position for up to 4 hours after injection (before and/or after discharge).
25. Written post-operative instructions will be given to the participant. He or she will be asked to remove the eye patch between one and three hours after the procedure. He or she will be given a telephone number to use to contact the Investigator or other physician in the Eye Clinic immediately for any increasing eye pain, worsening vision, or any other new concerns. He or she will be asked not to place any drops in the eye or to rub or place any pressure on the eye, and to use Tylenol for any minor discomfort.
26. Non-disposable items from the surgical field will be isolated and packaged for re-sterilization. Disposable items from the procedure (apart from the unused IP which will be stored until the end of the study) will be discarded in a MPW box.